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*Editors*

# Green Materials for Energy, Products and Depollution

Springer

# Green Materials for Energy, Products and Depollution

# Environmental Chemistry for a Sustainable World

## Volume 3

*Series Editors*

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# Preface

*There are always flowers for those who want to see them.*

Henri Matisse

Nature is the best green and environmental chemist. Most biological reactions occur at room temperature and thus save energy, whereas many synthetic reactions need high temperature. Most biological reactions are quantitative and thus do not produce undesired by-products, whereas synthetic reactions are rarely quantitative and thus generate substantial amounts of undesired, sometimes toxic, by-products. Most biological reactions occur in water, a safe solvent, whereas many synthetic reactions are still often performed in hazardous solvents. Most biological products are useful, recyclable and recycled, whereas many synthetic products are not degradable and end up polluting the environment for centuries. Living organisms possess the best catalysts called enzymes, which should be awarded the Nobel Prize in chemistry if Nobel Prizes were given to natural substances, whereas synthetic catalysts almost never reach the natural efficiency and often contain toxic metals. Biological reactions use renewable products that are usually available in the very near surroundings of the living organism, whereas human chemistry often use non-renewable substances extracted from deep earth ores then shipped thousands of miles, thereby wasting time, money and energy. In short, despite several centuries of advanced scientific progress, human chemistry has still a lot to learn from nature chemistry. Therefore the positive side of actual society issues – or seeing flowers as suggested by Henri Matisse – is that there is a huge progress margin for the imagination of green chemists.

This book presents the following key examples of green materials and safe remediation:

– Biofuel from microalgae is a very promising fuel because microalgae is rapidly renewable, does not compete with food production and does not need huge surface area for production (Fig. 1, Chap. 1).
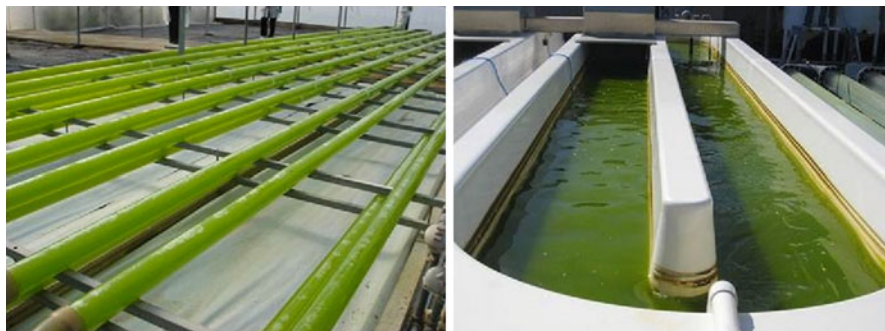
**Fig. 1** Biofuel production from microalgae is fast and does not compete with food production from agricultural crops. Source: Gonçalves et al. Biodiesel from microalgal oil extraction, Chap. 1

– Water and soil cleaning can be performed with unprecedented speed and efficiency by novel techniques of electrochemistry and photocatalysis that remove toxic metals and gas, pharmaceuticals, pathogens, chlorinated polycyclic aromatic hydrocarbons (PAH) and other pollutants (Chaps. 2–4, 10). Chapter 8 presents cheap natural materials such as rice husk, wheat straw and fly ash to remove toxic metals. Chapter 7 shows how surfactants can be used to decrease pesticide toxicity.
– Food security can be improved by a wide number of bioindicators including plants, animals and microbes, which betray the presence of pollutants in air, water and soil (Chap. 5).
– Sustainable and safe clothes can be designed using natural dyes and antimicrobials, an old practice that is regaining interest in a fossil-free society (Chap. 6).

Thanks for reading
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# Chapter 1
# Biodiesel from Microalgal Oil Extraction

**Ana L. Gonçalves, José C.M. Pires, and Manuel Simões**

**Abstract**  The rapid development of the modern society has resulted in an increased demand for energy, and consequently an increased use of fossil fuel reserves. Burning fossil fuels is nowadays one of the main threats to the environment, especially due to the accumulation of greenhouse gases in the atmosphere, which are responsible for global warming. Furthermore, the continuous use of this non-renewable source of energy will lead to an energy crisis because fossil fuels are of limited availability. In response to this energy and environmental crisis, it is of extreme importance to search for different energy supplies that are renewable and more environmentally friendly. Microalgae are a promising sustainable resource that can reduce the dependence on fossil fuel. Biodiesel production through microalgae is actually highly studied. It includes several steps, such as cell cultivation and harvesting, oil extraction and biodiesel synthesis. Although several attempts have been made to improve biodiesel yields from microalgae, further studies are required to optimize production conditions and to reduce production costs.

This chapter reviews recent developments on oil extraction for biodiesel production. Two different processes are distinguished: (i) an indirect route, in which microalgal oil is recovered in an appropriate solvent and then converted into biodiesel through transesterification; and (ii) a direct route, in which the production of biodiesel is performed directly from the harvested biomass. Both routes, direct and indirect, should be preceded by cell wall disruption because this step facilitates the access of solvents to microalgal oil. The most advantageous disruption methods for lipid extraction are enzymatic and pulsed electric field disruption because enzymes present higher selectivity towards cell walls. In addition pulsed electric field requires less energy than other disruption methods.

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For the indirect route, it is possible to use three different types of solvents to recover microalgal oil. Although extraction with supercritical fluids has higher extraction efficiencies and is safer for the environment, costs are very high. The use of ionic liquids is also safer for the environment, but their cost is also very high. An alternative is the use of organic solvents such as n-hexane because it is less harmful and has a higher selectivity for neutral oil fractions than other organic solvents. We conclude that the direct route, which involves production of biodiesel directly from the microalgal biomass, is more efficient. Indeed, the application of the direct route to the microalga *Schizochytrium limacinum* resulted in a biodiesel yield of 72.8 %, while the indirect route, in the same conditions, has resulted in a biodiesel yield of 63.7 %.

**Keywords** Biofuel • Algae • Microalgae • Oil extraction • Liquid • Transesterification • Chlorella vulgaris • Schizochytrium • Limacinum • Cell wall disruption • Pulse electric field • Enzymatic disruption

## Contents

## 1.1   Introduction

The depletion of fossil fuels reserves and the effect of exhaust gas emissions on global climate change have stimulated the search for sustainable sources of energy that are carbon neutral or renewable. As an alternative energy source, much attention has been paid to biodiesel production from vegetable oil crops, such as palm, rapeseed and soybean, and animal fats (Demirbas 2011; Ranjan et al. 2010). However, biodiesel production yields from oil crops and animal fats do not achieve the current demand for transport fuels (Chisti 2007; Demirbas 2011). Furthermore, producing biodiesel from vegetable crops is time consuming and requires great areas of arable land that would compete with the one used in food crops, leading to starvation in developing countries (Costa and de Morais 2011; Demirbas 2011; Demirbas and Demirbas 2011).

Avoiding the competition between energy and food production, attentions are now focused on evaluating the potential of microalgae as oil source for biodiesel production. Microalgae are eukaryotic photosynthetic microorganisms that can be found in aquatic or terrestrial ecosystems (Fig. 1.1). They present several advantages
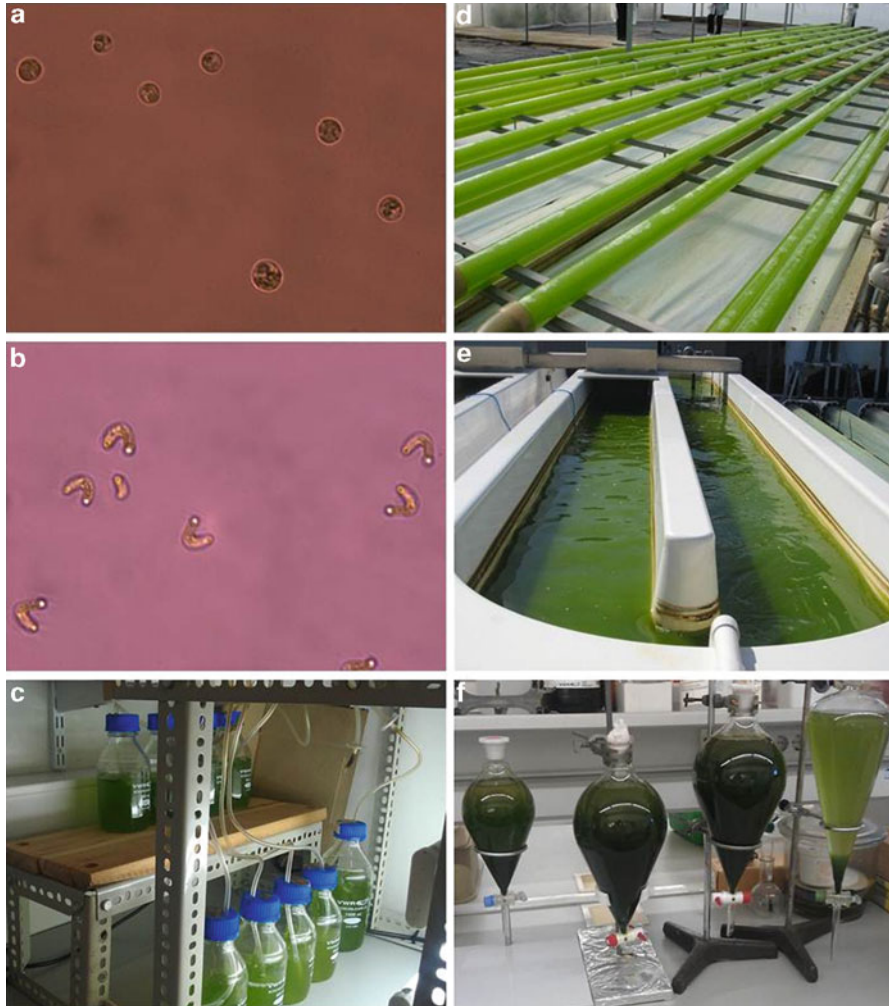
**Fig. 1.1** Microalgae: (**a**) microscopic photograph of the microalga *Chlorella vulgaris*; (**b**) microscopic photograph of the microalga *Pseudokirchneriella subcapitata*; (**c**) microalgal culturing in small flasks; (**d**) microalgal culturing in horizontal tubular photobioreactors; (**e**) microalgal culturing in raceway ponds; and (**f**) harvesting of microalgae through sedimentation. (**a**), (**b**), (**c**), and (**f**) were obtained from our research group; (**d**) from http://badger.uvm.edu; and (**e**) from http://www.abc.net.au

over oil crops, including: (i) higher oil contents; (ii) higher growth and biomass production rates; (iii) shorter maturity rates; and (iv) require far less land due to higher oil productivities (Chisti 2007; Mercer and Armenta 2011). As well as microalgae, there are other photosynthetic microorganisms with potential interest. These prokaryotic microorganisms, known as cyanobacteria, behave similarly to microalgae and present the same advantages. Despite the referred advantages, the

production of biodiesel from microalgae is not economically viable. Technological improvements should be performed to reduce the associated costs, including: (i) improvement of photosynthetic efficiency by the study of photobioreactor design; (ii) reduction of water and carbon dioxide losses in microalgal cultures; (iii) improvement of energy balance for water pumping, $CO_2$ transfer, biomass harvesting, oil extraction and biodiesel synthesis; and (iv) use of flue gas as $CO_2$ source. This review focuses on the oil extraction and biodiesel synthesis, presenting the research advances in the associated processes.

## 1.2 Lipid Recovery

After cell cultivation, the downstream process towards the production of biodiesel includes: (i) harvesting of microalgae; (ii) drying or dewatering; (iii) cell disruption and oil extraction; and (iv) transesterification reaction (Amaro et al. 2011; Brennan and Owende 2010), as it is possible to see in Fig. 1.2.

Oil extraction from biomass requires a specific solvent with great affinity to microalgal oil. Extraction procedures involving organic solvents, supercritical fluids and ionic liquids are the most common applied methods to recover oil from microalgae (Amaro et al. 2011; Mercer and Armenta 2011; Taher et al. 2011; Kim et al. 2012). Although these procedures can be applied directly to the dewatered biomass, it is reported that their efficiency is very low because microalgae present cell walls that block the access of solvents to cytosol (Cravotto et al. 2008; Lee et al. 2010), the cell compartment where the majority of microalgal lipids accumulate (Chen et al. 2009). To overcome the low efficiencies associated to the application of solvent extraction methods, some authors have reported the application of cell wall disruption methods, such as (i) enzymatic disruption; (ii) pulsed electric field; (iii) ultrasound and microwave; and (iv) expeller pressing (Amaro et al. 2011; Cravotto et al. 2008; Lee et al. 2010; Mercer and Armenta 2011; Taher et al. 2011). Table 1.1
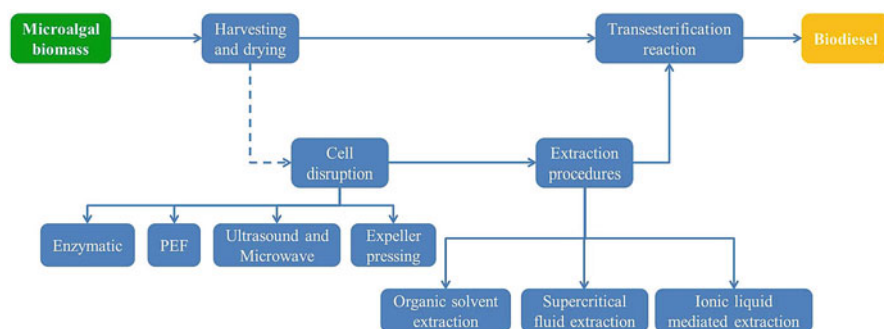


**Fig. 1.2** Steps involved in the production of biodiesel from microalgal biomass. *PEF* pulsed electric field

**Table 1.1** Effectiveness of some oil extraction methods applied in fatty acid recovery from different microalgae

| Extraction method | Microorganism | Total fatty acids[a] % ($w_{FA}/w_{DW}$) | Reference |
|---|---|---|---|
| Organic solvent (n-hexane) | *Crypthecodinium cohnii* | 4.8 | Cravotto et al. (2008) |
| Ultrasonic-assisted + Organic solvent (n-hexane) | *Crypthecodinium cohnii* | 25.9 | |
| Microwave-assisted + Organic solvent (n-hexane) | *Crypthecodinium cohnii* | 17.8 | |
| Organic solvent (ethanol:n-hexane 1:1 v/v) | *Scenedesmus dimorphus* | 6.3 | Shen et al. (2009) |
| Ultrasonic-assisted + Organic solvent (ethanol:n-hexane 1:1 v/v) | *Scenedesmus dimorphus* | 21.0 | |
| French press + Organic solvent (ethanol:n-hexane 1:1 v/v) | *Scenedesmus dimorphus* | 21.2 | |
| Organic solvent (ethanol:n-hexane 1:1 v/v) | *Chlorella protothecoides* | 5.6 | |
| Ultrasonic-assisted + Organic solvent (ethanol:n-hexane 1:1 v/v) | *Chlorella protothecoides* | 10.7 | |
| French press + Organic solvent (ethanol:n-hexane 1:1 v/v) | *Chlorella protothecoides* | 14.9 | |
| Organic solvent (chloroform:methanol, 3:1 v/v) | *Scenedesmus* sp. | 2.0 | Ranjan et al. (2010) |
| Ultrasonic-assisted + Bligh and Dyer (chloroform:methanol, 3:1 v/v) | *Scenedesmus* sp. | 6.0 | |
| Organic solvent (n-hexane) | *Scenedesmus* sp. | 0.6 | |
| Organic solvent (chloroform:methanol, 1:1 v/v) | *Botryococcus* sp. | 7.9 | Lee et al. (2010) |
| Microwave-assisted + Organic solvent (chloroform:methanol, 1:1 v/v) | *Botryococcus* sp. | 28.6 | |
| Organic solvent (chloroform:methanol, 1:1 v/v) | *Chlorella vulgaris* | 4.9 | |
| Microwave-assisted + Organic solvent (chloroform:methanol, 1:1 v/v) | *Chlorella vulgaris* | 9.9 | |
| Organic solvent (chloroform:methanol, 1:1 v/v) | *Scenedesmus* sp. | 1.9 | |
| Microwave-assisted + Organic solvent (chloroform:methanol, 1:1 v/v) | *Scenedesmus* sp. | 10.4 | |
| Organic solvent (n-hexane; 10 h) | *Scenedesmus obliquus* | 46.9 | Balasubramanian et al. (2011) |
| Microwave-assisted + Organic solvent (n-hexane, 0.5 h) | *Scenedesmus obliquus* | 77.1 | |
| Organic solvent (water:methanol:chloroform, 0.8:2:1 v/v/v; 18 h) | Marine microheterotroph | 25.8 | Lewis et al. (2000) |
| Organic solvent (chloroform:methanol:water, 1:2:0.8 v/v/v; 18 h) | Marine microheterotroph | 35.0 | |
| Organic solvent (n-hexane) | *Spirulina maxima* | 4.1 | Gouveia and Oliveira (2009) |

**Table 1.1** (continued)

| Extraction method | Microorganism | Total fatty acids[a] % ($w_{FA}/w_{DW}$) | Reference |
|---|---|---|---|
| Organic solvent (n-hexane) | *Chlorella vulgaris* | 5.1 | |
| Organic solvent (n-hexane) | *Scenedesmus obliquus* | 17.7 | |
| Organic solvent (n-hexane) | *Dunaliella tertiolecta* | 16.7 | |
| Organic solvent (n-hexane) | *Nannochloropsis* sp. | 28.7 | |
| Organic solvent (n-hexane) | *Neochloris oleoabundans* | 29.0 | |
| Organic solvent (ethanol, 96 % v/v) | *Phaeodactylum tricornutum* | 6.4 | Fajardo et al. (2007) |
| scCO$_2$ (70.0 MPa; 55 °C; 10 kg.h$^{-1}$; 1.4 h) | *Nannochloropsis* sp. | 23.0 | Andrich et al. (2005) |
| Organic solvent (n-hexane; 8 h) | *Spirulina platensis* | 7.8 | Andrich et al. (2006) |
| scCO$_2$ (70.0 MPa; 55 °C; 10 kg.h$^{-1}$; 0.75 h) | *Spirulina platensis* | 7.7 | |
| Organic solvent (chloroform:methanol, 2:1 v/v) | *Crypthecodinium cohnii* | 19.9 | Couto et al. (2010) |
| scCO$_2$ (20.0 MPa; 40 °C; 0.6 kg.h$^{-1}$; 3 h) | *Crypthecodinium cohnii* | 6.9 | |
| scCO$_2$ (25.0 MPa; 40 °C; 0.6 kg.h$^{-1}$; 3 h) | *Crypthecodinium cohnii* | 6.3 | |
| scCO$_2$ (30.0 MPa; 40 °C; 0.6 kg.h$^{-1}$; 3 h) | *Crypthecodinium cohnii* | 5.7 | |
| scCO$_2$ (20.0 MPa; 50 °C; 0.6 kg.h$^{-1}$; 3 h) | *Crypthecodinium cohnii* | 5.6 | |
| scCO$_2$ (25.0 MPa; 50 °C; 0.6 kg.h$^{-1}$; 3 h) | *Crypthecodinium cohnii* | 7.1 | |
| scCO$_2$ (30.0 MPa; 50 °C; 0.6 kg.h$^{-1}$; 3 h) | *Crypthecodinium cohnii* | 8.6 | |
| Organic solvent (n-hexane; 5.5 h) | *Chlorococcum* sp. | 3.2 | Halim et al. (2011) |
| scCO$_2$ (30.0 MPa; 60 °C; 18.5 kg.h$^{-1}$; 1.3 h) | *Chlorococcum* sp. | 5.8 | |
| scCO$_2$ (30.0 MPa; 80 °C; 18.5 kg.h$^{-1}$; 1.3 h) | *Chlorococcum* sp. | 4.8 | |
| scCO$_2$ (35.0 MPa; 70 °C; 6 kg.h$^{-1}$; 4.5 h) | *Nannochloropsis granulata* | 2.8 | Bjornsson et al. (2012) |
| IL ([Emim]MeSO$_4$) and methanol | *Dunaliella* sp. | 8.6 | Young et al. (2010) |
| IL ([Emim]MeSO$_4$) and methanol | *Chlorella* sp. | 38.0 | |
| Organic solvent (chloroform:methanol, 2:1 v/v) | *Chlorella vulgaris* | 11.1 | Kim et al. (2012) |
| IL ([Bmim]CF$_3$SO$_3$) and methanol | *Chlorella vulgaris* | 19.0 | |
| IL ([Bmim]MeSO$_4$) and methanol | *Chlorella vulgaris* | 17.4 | |

[a]Total fatty acids recovered are represented as a fraction (in %) of oil weight in the biomass dry weight

**Table 1.2** Advantages and disadvantages of the cell wall disruption methods and oil extraction procedures applied to microalgae

|  | Procedure | Advantages | Disadvantages |
|---|---|---|---|
| Cell wall disruption methods | Enzymatic disruption | Higher degradation selectivity | Enzymes are very expensive |
|  |  | Requirement of less energy than mechanical methods |  |
|  | Pulsed electric field | Requirement of less time and energy than other applied methods | Difficulties in operating at large scale |
|  |  |  | High operational and maintenance costs |
|  | Ultrasound and Microwave | Higher efficiencies and reduced extraction times | Moderate to high energetic costs |
|  |  | Increased yields |  |
|  | Expeller pressing | Simple method | High power consumption and maintenance costs |
|  |  | Useful for large scale applications |  |
| Lipid extraction methods | Organic solvent extraction | Simple and inexpensive method | The majority of organic solvents are toxic, harmful and non-reusable |
|  |  |  | Time-consuming |
|  | Supercritical fluid extraction | Supercritical fluids are non-toxic and present higher mass transfer rates | High energetic and maintenance costs |
|  |  | Recovery of fatty acids is easier | Difficulties in scale-up |
|  |  | Requirement of less time than organic solvent extraction |  |
|  | Ionic liquid mediated extraction | Ionic liquids are non-toxic and non-volatile and present higher solvatation capacities | Ionic liquids are expensive |
|  |  | Ionic liquids can be produced specifically, according to their application |  |

shows the most applied extraction methods and the achieved mass percentages of recovered oil and Table 1.2 summarizes the main advantages and disadvantages of the presented cell disruption methods and oil extraction procedures.

## 1.2.1 Cell Disruption Methods

Cell disruption methods aim to disintegrate cell walls to allow the release of intracellular components into an adequate solvent. The methods used in cell wall disruption can be classified into mechanical, where cell wall destruction is non-specific, and

non-mechanical, where methods are more specific. Mechanical methods include bead mill, French press, ultrasound, microwaves and high pressure homogenizer, whereas non-mechanical methods comprise the physical methods thermolysis, decompression and osmotic shock, the chemical methods, where antibiotics, chelating agents, solvents and detergents are applied, and the enzymatic methods, which use lytic enzymes (Geciova et al. 2002; Chisti and Moo-Young 1986).

## Enzymatic Disruption

Enzymes can be applied in oil extraction from microalgae, as they can mediate the hydrolysis of cell walls, enabling the release of their content into an appropriate solvent. Application of lytic enzymes with little volumes of organic solvent can improve oil recovery yields, as well as extraction times (Mercer and Armenta 2011). For cell wall degradation, cellulases are the most applied enzymes (Mercer and Armenta 2011; Sander and Murthy 2009). Although enzymatic extraction has not yet been applied to microalgae, it has been successfully used in oil extraction from *Jatropha curcas* L. seeds (Shah et al. 2004). Three phase partitioning (TPP) method and an enzyme-assisted TPP (EATPP) method were applied to recover oil from these seeds. The TPP consisted in the addition of three solvents to the seeds, in order to form a three phase system. The applied solvents were water, ammonium sulphate, and *t*-butanol. The three phases were separated by centrifugation and the phase containing the recovered oil was the one containing *t*-butanol, which was eliminated through evaporation. TPP and EATPP were performed at different pH conditions: 4.0, 7.0, and 9.0. Higher oil yields were obtained at pH 9.0: (i) 32.0 % (wt.) for TPP; and (ii) 36.8 % (wt.) for EATPP (Shah et al. 2004).

Despite being expensive, enzymes offer several advantages over other cell wall disruption methods. They present higher degradation selectivity than mechanical disruption methods. Furthermore, microalgal cell walls are more recalcitrant than the ones of other microorganisms, being very resistant to degradation. Thus, the use of mechanical disruption methods requires higher energy amounts (Sander and Murthy 2009).

## Pulsed Electric Field

The pulsed electric field (PEF) technology seems to be a potential alternative for oil extraction from microalgae (Taher et al. 2011). This technique applies brief pulses of a strong electric field to cells, which induces non-thermal permeabilization of membranes (Guderjan et al. 2005; Taher et al. 2011). In determined conditions, PEF can also cause significant damage in microalgal cell walls (main barrier for oil extraction in most microalgae), membrane and it can led to complete disruption of cells into fragments. PEF is a relatively new method that has not yet been applied to extract microalgal oil. However, evidence of high extraction efficiencies in plant products, such as maize, olives (Guderjan et al. 2005) and *Brassica napus* cells

(Guderjan et al. 2007), suggests that this can be a suitable method to improve the permeabilization of microalgal membranes and efficiently extract their oil (Mercer and Armenta 2011). Guderjan et al. (2005) used PEF to induce stress in plant cells and thus recover functional food ingredients, such as phytosterols and polyphenols. The authors applied 120 pulses with field strength of 0.6 and 7.3 kV.cm$^{-1}$ to maize and they added a small amount of n-hexane to perform the oil extraction. On the other hand, olives were treated using the following conditions: 30 pulses with field strength of 0.7 kV.cm$^{-1}$ and 100 pulses with field strength of 1.3 kV.cm$^{-1}$. After application of PEF the membranes were completely disintegrated and the oil content was recovered by centrifugation. Oil recovery obtained for dried maize was 23.5 and 23.9 % (wt.) for pulses with 0.6 and 7.3 kV.cm$^{-1}$, respectively. These results were obtained for maize treated with PEF and n-hexane and further drying for 24 h, at 38 °C. Application of electric pulses with field strength of 0.6 kV.cm$^{-1}$, followed by n-hexane addition, an incubation period of 24 h and drying for 24 h at 38 °C, resulted in an oil yield of 43.7 %, which means that incubation of the mixture with the organic solvent allows higher oil recovery. Regarding olives, the application of PEF with strength of 0.7 and 1.3 kV.cm$^{-1}$ followed by centrifugation resulted in an oil yield of 6.5 and 7.4 $g_{oil}$ per $g_{mash}$. Guderjan et al. (2007) applied 120 pulses with a field strength of 7.0 kV.cm$^{-1}$ and a duration of 30 µs to hulled rapeseed, followed by drying at 50 °C for 10 h and an extraction step with n-hexane. With these extraction procedures, the authors obtained an oil yield of 32 % (wt.), against 23 % obtained without PEF application.

PEF requires less time and energy than other applied methods (Guderjan et al. 2005) and its use as a pre-treatment for organic solvent extraction requires far less organic solvents (usually presenting high toxicity) than the conventional organic solvent extraction methods, which reduces the energy needs in the extraction process (Guderjan et al. 2007).

### Ultrasound and Microwave

Another method that can be used to promote cell wall disruption of microalgal cells is the application of ultrasounds and microwaves. In ultrasonic-assisted method, microalgal oil can be recovered by cavitation. This phenomenon occurs when vapour bubbles of the liquid are formed when the pressure is lower than its vapour pressure. As these bubbles grow when pressure is negative and compress under positive pressure, a violent collapse of the bubbles is promoted. When bubbles collapse near cell walls, damage can occur, leading to the release of cell contents (Mercer and Armenta 2011; Taher et al. 2011). Application of this ultrasound-assisted method to microalgal biomass can improve extraction efficiencies by reducing extraction times and increasing oil recovery yields. The experiments performed by Cravotto et al. (2008) with the microalga *Crypthecodinium cohnii* showed that cell disruption using ultrasounds increased oil extraction yields from 4.8 %, when applying Soxhlet extraction with n-hexane, to 25.9 % (wt.). Shen et al. (2009) used the microalgae *Scenedesmus dimorphus* and *Chlorella protothecoides*

to evaluate the effect of sonication before solvent extraction using a mixture of ethanol:hexane in a ratio of 1:1 (v/v). Application of ultrasound-assisted disruption increased the oil yields from 6.3 % to 21.0 % (wt.) for *S. dimorphus* and from 5.6 % to 10.7 % (wt.) for *C. protothecoides*. Additionally, Ranjan et al. (2010) compared oil extraction yields from *Scenedesmus* sp. using the following methods: (i) Bligh and Dyer's method (1959), organic solvent extraction using a solvent mixture of chloroform, methanol and water, where solvents were applied in a ratio of 3:1:0 (v/v); (ii) ultrasound-assisted extraction followed by the Bligh and Dyer's method, using the same mixture of chloroform and methanol. Results obtained with these experiments showed an increase in oil yields from 2.0 % to 6.0 % (wt.), when applying method (i) and (ii), respectively. One possible reason for this increase in oil recovery is that when both methods are applied, oil extraction is a result of the interaction between two phenomena: oil diffusion across the cell wall and disruption of the cell wall with release of its contents to the solvent (Ranjan et al. 2010).

Microwave-assisted method is supported by the principle that microwaves directly affect polar solvents and materials. Even when they are applied to dried cells, trace amounts of moisture are affected: temperature increases due to microwaves, moisture is evaporated, and pressure in the cells increases, leading to a damage or rupture of the cell wall followed by the release of its contents. Microwave theory and the extraction principle are described in detail by Mandal et al. (2007). The use of microwaves followed by organic solvent extraction using small amounts of solvent contributes to an efficient and inexpensive extraction procedure, which does not require previous biomass dehydration. Cravotto et al. (2008) applied organic solvent extraction with n-hexane and microwave-assisted solvent extraction (using the same solvent) to the microalga *C. cohnii*, achieving oil recovery yields of 4.8 % and 17.8 % (wt.), respectively. Furthermore, Lee et al. (2010) used the Bligh and Dyer's method (1959) with a mixture of chloroform:methanol in the ratio of 1:1 (v/v) preceded by the application of a microwaves treatment to the microalgae *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. With this microwave-assisted method, the oil extraction yields obtained for these organisms were 28.6, 9.9, and 10.4 % (wt.), respectively, against the 7.9, 4.9, and 1.9 % (wt.) obtained for the control method – Bligh and Dyer's method (1959; Lee et al. 2010). Recently, Balasubramanian et al. (2011) promoted cell wall disruption of *Scenedesmus obliquus* using the microwave-assisted method and compared the achieved results with organic solvent extraction with n-hexane. Disruption using microwaves was performed for 30 min, while organic solvent extraction was performed by 10 h. Oil recovery yields obtained with solvent extraction and the microwave-assisted method were 46.9 % and 77.1 % (wt.), respectively (Balasubramanian et al. 2011).

Both methods improve significantly oil extraction from microalgae, presenting higher efficiency, reduced extraction times and increased yields, as well as moderate costs and negligible added toxicity.

**Expeller Pressing**

Pressing techniques lie on the principle that when microalgal cells are submitted to high pressures, they start to crush, releasing their contents to an adequate solvent. As the methods described before, pressing techniques can be advantageous when using as a pre-treatment for organic solvent extraction. A pre-treatment using French press was applied by Shen et al. (2009) to the microalgae *S. dimorphus* and *C. protothecoides*. After pressing microalgae, the oil was recovered using a solvent system containing ethanol and n-hexane in a 1:1 (v/v) ratio. Comparing extracted oil yields with those obtained without pre-treatment, oil content achieved for *S. dimorphus* raised from 6.3 % to 21.2 % (wt.), while for *C. protothecoides* it raised from 5.6 % to 14.9 % (wt.).

Although this method is very simple and has reduced equipment costs, it presents some disadvantages when compared to other cell disruption methods, such as high power consumption and maintenance costs.

## 1.2.2 Lipid Extraction Methods

Extraction of microalgal oil can be performed directed to the harvested biomass or in addition to a cell wall disruption method. The second methodology generally presents higher lipid recoveries, as cell contents are released to the solvent applied. In the recovery of microalgal oil, it is very important to choose an appropriate solvent because this choice can improve lipid recovery yields and reduce process costs. Additionally, the majority of solvents applied are harmful and toxic, meaning that the selection of the solvents used should take into account their impact in the environment and in public health. The extraction procedures commonly applied to extract microalgal oil include the use of organic solvents, supercritical fluids and ionic liquids (Mercer and Armenta 2011; Kim et al. 2012).

**Organic Solvent Extraction**

The use of organic solvents to extract microalgal oil is the most applied extraction method. The main organic solvents applied include hexane, cyclohexane, benzene, ethanol, acetone, and chloroform (Brennan and Owende 2010; Mercer and Armenta 2011; Grima et al. 2003). These solvents have shown to be quite effective in oil extraction from microalgae. A good solvent for oil extraction may present the following characteristics: (i) to be insoluble in water; (ii) to have high affinity for oil, i.e. non-polar, to increase its permeability to cell membrane and also to solubilise the target compounds; (iii) to have a low boiling point to facilitate its removal after extraction; (iv) to have a considerably different density from that of water. Furthermore, the organic solvent applied should be inexpensive, non-toxic and reusable (Mercer and Armenta 2011).

Several studies have reported the use of a chloroform, methanol and water mixture, known as the Bligh and Dyer's method (1959), to extract microalgal oil (Mercer and Armenta 2011). Lewis et al. (2000) studied the effect of applying the solvents chloroform, methanol and water in different sequences and proportions on an oil-producing strain of a marine microheterotroph, Thraustochytrid ACEM 6063. The authors used the following sequences and ratios: (i) water:methanol:chloroform (0.8:2:1, v/v/v); (ii) chloroform:methanol:water (1:2:0.8, v/v/v); (iii) chloroform:methanol:water (1:4:0.8, v/v/v). Total fatty acids extracted with these three solvent systems were 258.5, 350.0, and 326.5 mg.g$^{-1}$$_{dry weight}$, respectively. With this study, the authors concluded that changing solvent sequence can have significant effects on extraction yields and that increasing the proportion of methanol did not significantly affect the extraction efficiency. Later, Lee et al. (2010) used a mixture of chloroform and methanol (1:1, v/v) to extract oil from the organisms *Botryococcus* sp., *C. vulgaris*, and *Scenedesmus* sp. Extraction yields obtained with this method were 12.0, 24.9, and 18.8 mg.g$^{-1}$$_{dry weight}$ for *Botryococcus* sp., *C. vulgaris*, and *Scenedesmus* sp., respectively.

Another common organic solvent applied to extract microalgal oil is n-hexane. Gouveia and Oliveira (2009) used n-hexane to determine oil contents of the microalgae *Spirulina maxima*, *C. vulgaris*, *S. obliquus*, *Dunaliella tertiolecta*, *Nannochloropsi*s sp., and *Neochloris oleoabundans*. Oil yields obtained with this solvent ranged between 4.1 % (wt.) from *S. maxima* and 29.0 % (wt.) from *N. oleoabundans* (Gouveia and Oliveira 2009). Shen et al. (2009) applied a solvent system composed by a mixture of ethanol:n-hexane (1:1, v/v) to the microalgae *S. dimorphus* and *C. protothecoides*. Oil contents obtained with this method were 6.3 % and 5.6 % (wt.) for *S. dimorphus* and *C. protothecoides*, respectively (Shen et al. 2009).

Ranjan et al. (2010) compared oil extraction from the *Scenedesmus* sp. using two organic solvent methods: Soxhlet extraction with n-hexane, and the Bligh and Dyer's (1959), using a chloroform and methanol mixture in a ratio of 3:1 (v/v). The achieved oil contents were 0.6 % (wt.) for extraction with n-hexane and 2.0 % (wt.) for extraction with the Bligh and Dyer's method, showing that the last method is most efficient. This can be explained by the non-polar character of n-hexane, which results in a lower selectivity of microalgal oil, mainly composed by unsaturated fatty acids, toward n-hexane. On the other hand, chloroform has a polar nature, which allows a higher selectivity of microalgal oil toward this organic solvent (Ranjan et al. 2010).

Fajardo et al. (2007) used ethanol as an organic solvent for oil extraction from the microalga *Phaeodactylum tricornutum*. The authors applied an ethanol solution (96 % v/v) to freeze dried biomass, obtaining a oil yield of 6.4 % (wt.) (Fajardo et al. 2007).

Although n-hexane may be less efficient than chloroform, it is less toxic and it has an apparently higher selectivity for neutral oil fractions (Amaro et al. 2011). The application of this organic solvent coupled with an efficient cell wall disruption method could be a promising alternative to avoid the harmfulness of chloroform.

## Supercritical Fluid Extraction

An alternative to the use of volatile and toxic organic solvents in microalgal oil extraction is the application of supercritical fluids as solvents (Amaro et al. 2011; Halim et al. 2011; Mercer and Armenta 2011). Supercritical fluids are compounds that behave both as a liquid or a gas when exposed to temperatures and pressures above their critical values. The most used supercritical fluid for oil extraction is $CO_2$ ($scCO_2$) because it presents low critical temperature (31.1 °C) and pressure (72.9 atm) (Mercer and Armenta 2011).

The $scCO_2$ extraction presents several advantages over the traditional organic solvent extraction procedures, such as: (i) tuneable solvating power; (ii) low toxicity and flammability; (iii) favourable mass transfer rates; and (iv) production of solvent free extracts because at room temperature $CO_2$ behaves as a gas (Amaro et al. 2011; Crampon et al. 2011; Halim et al. 2011; Macías-Sánchez et al. 2007). The main disadvantage is the associated cost, which is mainly due to the required infrastructure and operational conditions (Halim et al. 2011).

Efficiencies of oil extraction using $scCO_2$ depend on the following factors: (i) pressure; (ii) temperature; (iii) $CO_2$ flow rate; and (iv) extraction time. Furthermore, the use of modifiers or co-solvents, such as ethanol can be adjusted to optimize extractions. When ethanol is applied as a co-solvent, polarity of $CO_2$ increases and its viscosity is altered, increasing the fluid solvating power. In these conditions, lower temperature and pressure are required, improving the extraction efficiency. Another limiting factor of $scCO_2$ extraction is the level of moisture in the sample. High moisture content can reduce contact time between the solvent and biomass, making difficult the diffusion of $CO_2$ into the sample and the diffusion of oil out of the cell, because microalgal biomass tends to gain a thick consistency (Halim et al. 2011).

Studies performed by Mendes et al. (1995) showed that application of $scCO_2$ with a gas flow rate of 21.4 kg.h$^{-1}$ at 35.0 MPa and 55 °C to *C. vulgaris* cells resulted in an oil yield of 13.3 % (wt.). Application of organic solvents, such as acetone and n-hexane, resulted in an oil yield of 16.8 % and 18.5 % (wt.), respectively (Mendes et al. 1995). Andrich et al. (2005) applied different extraction conditions using $scCO_2$ and also organic solvent extraction with n-hexane to the microalga *Nannochloropsis* sp. Extraction procedures allowed the achievement of 250 mg.g$^{-1}$$_{dry\ weight}$ (23.0 %) using $scCO_2$ (gas flow rate of 10 kg.h$^{-1}$, 70.0 MPa, and 55 °C) and 120 mg.g$^{-1}$$_{dry\ weight}$ (12.0 %) using n-hexane at both 52 °C and room temperature (Andrich et al. 2005; Crampon et al. 2011). Later, Andrich et al. (2006) used *Spirulina platensis* to verify the extraction efficiency of $scCO_2$ technique using four different pressures (25.0, 40.0, 55, and 70.0 MPa) and two different temperatures (40 and 55 °C), with a gas flow rate of 10 kg.h$^{-1}$. In addition to this method, the authors also tested solvent extraction with n-hexane. Results showed that after 45 min, the amount of oil extracted reached its maximum (78.2 mg.g$^{-1}$$_{dry\ weight}$) for extraction performed at 55 °C and 70.0 MPa. The same amount of extracted oil was obtained after 2.5 h and after 3.5 h, for extraction

performed at 55 °C and 40.0 MPa and at 40 °C and 40.0 MPa, respectively. For solvent extraction with n-hexane, the highest oil recovery (77.7 mg.g$^{-1}$$_{dry\ weight}$) was achieved after 8 h of reaction (Andrich et al. 2006). Couto et al. (2010) performed supercritical fluid extraction from the microalga *C. cohnii* at temperatures of 40 and 50 °C and pressures of 20.0, 25.0 and 30.0 MPa. Gas flow rate was 0.6 kg.h$^{-1}$ and extraction time was 3 h. Optimum extraction conditions were found to be 30.0 MPa and 50 °C (8.6 %), against the 19.9 % (wt.) achieved by application of Bligh and Dyer's method (1959). With this work, it was possible to state that at the highest pressures (25.0 and 30.0 MPa) the extraction yield increases with the temperature, while at the lowest pressure (20.0 MPa), an increase in temperature leads to a decreased yield. Temperature influence on the extraction efficiency results from the combination of the following antagonic thermodynamic effects: (i) at constant pressure, an increase in temperature leads to a decrease in the density of the supercritical fluid and thus its solvatation capacity; (ii) vapour pressure of solutes increase with the temperature, resulting in an high solubility in the supercritical fluid (Couto et al. 2010). Using scCO$_2$ to extract oil from the microalga *Chlorococcum* sp., Halim et al. (2011) achieved an oil recovery of 58 mg.g$^{-1}$$_{dry\ weight}$ (5.8 %) at a flow rate of 18.5 kg h$^{-1}$, a pressure of 30.0 MPa and a temperature of 60 °C, during 80 min. By increasing temperature to 80 °C, oil yield was 48 mg.g$^{-1}$$_{dry\ weight}$ (4.8 %). The authors also applied organic solvent extraction (using n-hexane) obtaining a oil yield of 32 mg.g$^{-1}$$_{dry\ weight}$ (3.2 %) after a reaction time of 5.5 h (Halim et al. 2011). Bjornsson et al. (2012) used the microalga *Nannochloropsis granulata* to study the effect of pressure, time and temperature in oil extraction yields. Firstly, the authors evaluated the effect of pressure (35, 45, and 55 MPa; 50 °C; 6 kg h$^{-1}$; 3 h), concluding that no significant differences in oil yields were obtained by varying this process variable. Later, different extraction times were applied (3, 4.5, and 6 h), maintaining pressure, temperature and gas flow rate constant (35 MPa; 50 °C; 6 kg.h$^{-1}$). The increase of extraction time resulted in differences in oil yield that ranged from 8.67 mg.g$^{-1}$$_{dry\ weight}$ (over 180 min) to 15.56 mg.g$^{-1}$$_{dry\ weight}$ (over 270 min) and to 16.91 mg.g$^{-1}$$_{dry\ weight}$ (over 360 min). However, the differences in yields were not statistically significant. Finally, scCO$_2$ extraction was performed by keeping pressure, gas flow rate, and time constant (35 MPa, 6 kg.h$^{-1}$, and 4.5 h), and by varying temperature (50, 70, and 90 °C). The increase of extraction temperature resulted in a statistically significant increase in oil yield from 15.56 mg.g$^{-1}$$_{dry\ weigh}$ at 50 °C to 28.45 mg.g$^{-1}$$_{dry\ weight}$ at 70 °C and to 25.75 mg.g$^{-1}$$_{dry\ weight}$ at 90 °C. Although oil yield decreased by increasing temperature from 70 to 90 °C, this decrease was not statistically significant (Bjornsson et al. 2012).

Application of scCO$_2$ to extract microalgal oil is very attractive, as it is a green technology and it allows a complete characterization of the extracted oil and the resulting biofuel. However, it still needs to be improved, because of its high cost-effectiveness and high energy-consuming drying step required before supercritical fluid extraction (Crampon et al. 2011).

**Ionic Liquid Mediated Extraction**

Ionic liquids (ILs) have been reported as an attractive alternative for volatile and toxic organic solvents because of their non-volatile character, thermal stability, and high solvatation capacity (Kim et al. 2012; Lateef et al. 2009). ILs are salts of relatively large asymmetric organic cations coupled with smaller organic or inorganic anions. These organic salts can be liquid at room temperatures or low melting point solids (<100 °C) (Lateef et al. 2009; Young et al. 2010; Khodadoust et al. 2006). Cations are generally composed of a ring structure containing nitrogen, such as imidazolium or pyrimidine. On the other hand, anions can vary from single ions, such as chloride, to larger complex ions, such as $[N(SO_2CF_3)_2]^-$ (Young et al. 2010). By altering the nature of both cation and anion of the ionic liquid, either hydrophilic or hydrophobic ionic liquids can be prepared, in order to make them suitable for different applications (Lateef et al. 2009).

The use of ionic liquids for oil extraction has been reported by Young et al. (2010). The authors applied mixtures of 1-ethyl-3-methylimidazolium methyl sulphate [Ethyl-mim]$MeSO_4$ and methanol to extract oil from microalgal biomass and different seeds. Ionic liquid extraction from the microalgae *Dunaliella* sp. and *Chlorella* sp. resulted in an oil yield of 8.6 % and 38.0 % (wt.), respectively (Young et al. 2010). A wide variety of ILs has also been used by Kim et al. (2012) for oil extraction from *C. vulgaris*. Oil was extracted from harvested biomass using systems of methanol and the following ILs: [Butyl-mim]$CF_3SO_3$, [Butyl-mim]$MeSO_4$, [Butyl-mim]$CH_3SO_3$, [Butyl-mim]$BF_4$, [Butyl-mim]$PF_6$, [Butyl-mim]$MeSO_4$, [Butyl-mim]$Tf_2N$, [Butyl-mim]Cl, [Ethyl-mim]$MeSO_4$, [Ethyl-mim]Cl, [Ethyl-mim]Br, and [Ethyl-mim]Ac. Bligh and Dyer's method (1959) was also used in terms of comparison. Application of these methods resulted in a total fatty acid content of 106.2 (11.1 %), 125.4 (19.0 %), and 118.4 (17.4 %) mg.g$^{-1}_{\text{dry weight}}$ for Bligh and Dyer's method, [Butyl-mim]$CF_3SO_3$, and [Butyl-mim]$MeSO_4$, respectively. It was also shown that fatty acids profiles were very similar for the three extraction methods. The use of an IL-methanol system reduces high prices and aquatic toxicity of imidazolium-based ILs, providing an efficient and environmentally-friendly system for oil extraction from microalgal biomass (Kim et al. 2012).

Although ILs are more expensive than the conventional organic solvents, application of these compounds as solvents for microalgal oil can be a promising alternative because of their higher affinities and non-toxic character.

## 1.3  Biodiesel Production

Biodiesel is a renewable fuel produced from vegetable oils, animal fats, microorganisms' oils, or waste cooking oil (Wahlen et al. 2011). It constitutes the best candidate to substitute diesel fuel, as it can be used directly as a fuel requiring some engine

modifications, or blended with petroleum diesel and used in diesel engines with few or no modifications (Leung et al. 2010). Chemically, biodiesel is a mixture of esters with long-chain fatty acids, such as lauric, palmitic, stearic, oleic, etc. (Demirbas and Demirbas 2010). Recently, this biofuel has become more attractive due to its environmental benefits: it is biodegradable and it has lower sulphur and aromatic content than diesel fuel, meaning that it will emit less toxic gases. Furthermore, it presents several advantages over conventional petroleum diesel, such as higher combustion efficiency and cetane number. The main disadvantages of biodiesel include the high production costs, its higher viscosity, lower energy content and higher nitrogen oxide ($NO_x$) emissions (Demirbas and Demirbas 2010; Leung et al. 2010).

Biodiesel can be produced from extracted oil through four different methods: (i) direct use or blending of oils; (ii) microemulsification of oils; (iii) thermal cracking or pyrolysis; and (iv) transesterification, also known as alcoholysis (Balat and Balat 2010; Leung et al. 2010; Ma and Hanna 1999). Additional information of the referred methods can be obtained in Balat and Balat (2010) and Ma and Hanna (1999). Transesterification process, schematically represented in Fig. 1.3, constitutes the most applied method for biodiesel production, as it presents several advantages over the other methods. For example, blending and microemulsification may have some problems, such as carbon deposition and oil contamination, whereas pyrolysis is responsible for the production of low valuable products, as well as the production of gasoline instead of diesel (Sharma and Singh 2009). Therefore, transesterification, the chemical conversion of triglycerides in glycerol and esters in the presence of an alcohol, seems to be the most appropriate method for biodiesel production and it will be studied in detail in the next sections.



**Fig. 1.3** Biodiesel production process through transesterification

### 1.3.1 Transesterification Reaction

As shown in Eqs. 1.1, 1.2, and 1.3, the transesterification is a multi-step reaction where triglycerides are converted into diglycerides, monoglycerides and finally into glycerol during three reaction steps. These reactions are reversible and each one results in the formation of 1 mol of fatty acid ester (Leung et al. 2010; Ma and Hanna 1999).

$$\text{Triglyceride (TG)} + R'OH \overset{\text{catalyst}}{\longleftrightarrow} \text{Diglyceride (DG)} + R'COOR_1 \qquad (1.1)$$

$$\text{Diglyceride (DG)} + R'OH \overset{\text{catalyst}}{\longleftrightarrow} \text{Monoglyceride (MG)} + R'COOR_2 \qquad (1.2)$$

$$\text{Monoglyceride (MG)} + R'OH \overset{\text{catalyst}}{\longleftrightarrow} \text{Glycerol (GL)} + R'COOR_3 \qquad (1.3)$$
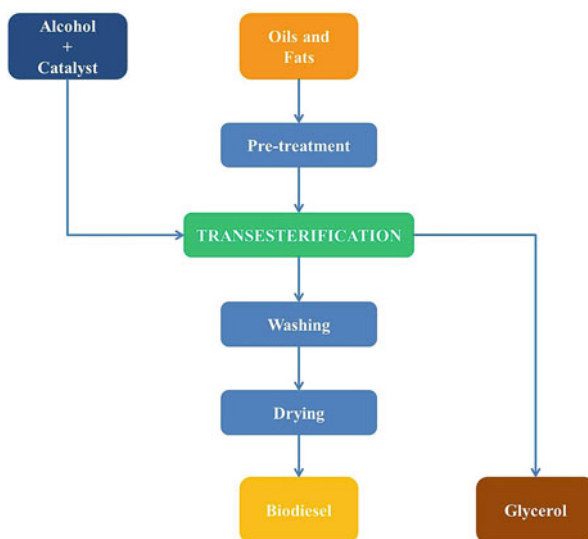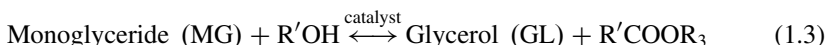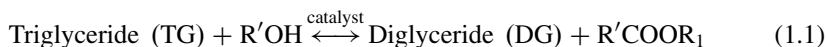
R represents a small hydrocarbon chain, whereas $R_1$, $R_2$, and $R_3$ represent long-chain hydrocarbons, also known as fatty acid chains.

Variables affecting biodiesel yields during transesterification include the alcohol and molar ratio employed, type of used catalyst, amount of free fatty acids (FFA), water content, and reaction temperature and time (Ehimen et al. 2010; Ma and Hanna 1999; Miao et al. 2009; Sharma and Singh 2009; Wahlen et al. 2011). Due to the reversibility of the above mentioned reactions, an excess of alcohol is used to shift the equilibrium towards fatty acid esters formation. In a study performed by Miao and Wu (2006), transesterification reaction was applied to oil recovered from the microalga *C. protothecoides* at 30 °C for 7 h, using 100 % (v/v) of catalyst (sulphuric acid) and the following molar ratios of methanol to oil: 25:1, 30:1, 45:1, 56:1, 70:1, and 84:1 (v/v). Results showed that the highest biodiesel yields (68.0 % and 63.0 %) were obtained using the molar ratios of alcohol to oil of 45:1 and 56:1, respectively (Miao and Wu 2006). In the transesterification process, short-chain alcohols such as methanol, ethanol, propanol, butanol, and amyl alcohol are used. However, the most used ones are methanol and ethanol. Methanol has been extensively applied in transesterification reactions because of its low cost (Gong and Jiang 2011; Leung et al. 2010; Ma and Hanna 1999) and physical and chemical properties, including its high polarity and small chain length (Ma and Hanna 1999). When methanol is used as alcohol the produced esters are known as fatty acid methyl esters (FAME). Wahlen et al. (2011) used five different alcohols (methanol, ethanol, butan-1-ol, 2-methylpropan-1-ol, and 3-methylbutan-1-ol) to produce biodiesel from the microalga *Chaetoceros gracilis* through transesterification. The assays were performed at 60 °C for 100 min, using sulphuric acid as a catalyst in a proportion of 1.8 % (v/v). The amount of fatty acid esters produced with the different alcohols was not significantly different, meaning that methanol, the cheapest one, is suitable for application in the transesterification process (Wahlen et al. 2011).

Three types of catalysts can be used in the transesterification reaction: alkalis, acids, and enzymes (Drapcho et al. 2008; Ma and Hanna 1999). Alkalis and acids are the most commonly used catalysts, both including homogeneous and heterogeneous catalysts (Drapcho et al. 2008; Leung et al. 2010). The alkalis used in this process include NaOH, KOH, carbonates and corresponding sodium and potassium alkoxides like sodium methoxide, sodium ethoxide, sodium propoxide, and sodium butoxide (Ma and Hanna 1999). The main acids used as catalysts include sulphuric acid, sulfonic acid, and hydrochloric acid. Normally, alkali catalysts are preferred over acid catalysts because reactions catalysed by alkali catalysts are faster than reactions catalysed by acids (Drapcho et al. 2008; Leung et al. 2010). However, transesterification reaction using an alkali catalyst results in the formation of small amounts of water (Sharma and Singh 2009). Water is undesirable in this process because it promotes the hydrolysis of the glycerides, forming FFA. FFA produce soap and water through a saponification reaction with the alkali catalyst (Leung et al. 2010). Soap formation must be avoided because it lowers the fatty acid esters yield and inhibits the separation of the esters from glycerol (Leung et al. 2010; Ma and Hanna 1999). Thus, when using an alkali catalyst, glycerides and the used alcohol must be substantially anhydrous (Ma and Hanna 1999) and FFA content of glycerides must be below 0.5 % (wt.) (Gong and Jiang 2011). When FFA contents in glycerides exceed 0.5 %, an acid catalyst should be employed (Drapcho et al. 2008; Leung et al. 2010; Ma and Hanna 1999). Acids catalyse the formation of water and fatty acid esters, i.e. biodiesel, from FFA and alcohol. The main disadvantage of acid catalyst application is the slow reaction rate and the high methanol to oil molar ratio that is required (Leung et al. 2010). Alternatively, triglycerides can be purified by saponification by alkali treatment before transesterification (Ma and Hanna 1999). The use of enzymes, e.g. lipases, as catalysts constitutes a promising alternative in biodiesel production. They avoid soap formation and facilitate the downstream process of purification, i.e. recovery of glycerol at the end of the reaction is easier when using an enzymatic catalyst. Furthermore, the reactions catalysed by lipases are not affected by FFA and water content (Gong and Jiang 2011; Leung et al. 2010). The main obstacles in using enzymes as catalysts are related with longer reaction times (Leung et al. 2010) and higher costs (Gong and Jiang 2011; Leung et al. 2010).

Reaction temperature and time also influence biodiesel yields through transesterification. Increasing reaction time normally increases conversion rate. Alternatively, different optimum temperatures of transesterification can be determined, depending on the used oil (Ma and Hanna 1999). It is very common to study these variables together because an increase in temperature reaction allows the production of higher amounts of biodiesel in a shorter period of time. This conclusion is supported by a study of Ehimen et al. (2010), who studied the effect of reaction temperature and time on the production of biodiesel from *Chlorella* sp. Transesterification reaction was performed at four different temperatures (23, 30, 60, and 90 °C), the used alcohol was methanol and the catalyst was sulphuric acid. Results showed faster conversion rates at higher temperatures (60 and 90 °C). FAME conversion rates reached similar asymptotic values after 2 and 4 h of reaction for 60 and 90 °C (Ehimen et al. 2010).

At the end of the transesterification process, a mixture containing essentially esters and glycerol is obtained. As the phase containing glycerol has higher density than the one containing esters, the separation of the two phases becomes easier because glycerol phase tends to settle at the bottom. However, both glycerol and esters phase may contain residues of the alcohol, catalyst and oil that did not react during the transesterification, and also soap that has been formed (Leung et al. 2010; Ma and Hanna 1999). The presence of these contaminants indicates that crude biodiesel should be purified before its use in diesel engines. It is also important to refine the crude glycerol because it has a wide variety of industrial applications, such as soaps, cosmetics, medicines, and others (Leung et al. 2010). The procedures used in the purification of crude biodiesel and glycerol were described in detail by Leung et al. (2010).

There are two types of transesterification: transesterification applied to the extracted oil and transesterification directly applied to the oil source, without previous oil extraction, also known as transesterification *in situ*. The following sections compare current attempts in producing biodiesel from microalgae using these two types of transesterification. Table 1.3 shows a resume of the research studies about transesterification reaction, presenting the achieved biodiesel yields.

**Transesterification from the Recovered Oil**

Several studies have reported the use of transesterification to convert fatty acids extracted from microalgae into fatty acid esters. The applied reaction follows the above mentioned principles and can be influenced by the referred variables. Miao and Wu (2006) have applied acid-catalysed transesterification to oil extracted from the microalga *C. prototheocoides* with n-hexane. The authors adopted an acid catalyst, e.g. sulphuric acid, because of the high acid value of microalgal oil due to high FFA content. Transesterification was performed at different conditions, to evaluate the effect of catalyst quantity, methanol to oil molar ratio, and reaction time and temperature on the yield and properties of biodiesel product. The reaction was then carried out using: (i) four levels of catalyst quantity – 25, 50, 60, and 100 % sulphuric acid based on oil weight; (ii) six different molar ratios of methanol to oil – 25:1, 30:1, 45:1, 56:1, 70:1, and 84:1 (v/v); and (iii) three different temperatures – 30, 50, and 90 °C. In each experiment, 9.12 g of microalgal oil was used. Results after oil extraction showed that the *C. prototheocoides* produced 55.2 % (wt.) of oil under heterotrophic conditions. Transesterification of this oil resulted in maximum biodiesel yields of 68.0 and 63.0 % for a molar ratio of methanol to oil of 45:1 and 56:1 (v/v), respectively (Miao and Wu 2006). Later, in a study performed by Johnson and Wen (2009), oil extracted from *Schizochytrium limacinum* was submitted to transesterification reaction using methanol and sulphuric acid. Firstly, the authors applied the Bligh and Dyer's method (1959) to extract oil from 1 g of microalgal biomass. Chloroform and methanol were added to biomass in the ratio of 1:2 (v/v). After the extraction step, oils were transesterified using a mixture of methanol, sulphuric acid and chloroform at 90 °C with the reaction time of 40 min.

**Table 1.3** Biodiesel yields as a fraction of total oil obtained after transesterification of microalgal oils or after transesterification of microalgal biomass

| Transesterification of the recovered oils | | Transesterification *in situ* | | | |
| Conditions | Biodiesel yield (%) | Conditions | Biodiesel yield (%) | Microorganism | Reference |
| --- | --- | --- | --- | --- | --- |
| Methanol (45:1), $H_2SO_4$ (100 %), 30 °C, 7 h | 68.0 | – | – | *Chlorella protothecoides* | Miao and Wu (2006) |
| Methanol (3.4 mL), $H_2SO_4$ (0.6 mL), chloroform (4.0 mL) 90 °C, 40 min | 63.7 | Methanol (3.4 mL), $H_2SO_4$ (0.6 mL), chloroform (4.0 mL) 90 °C, 40 min | 72.8 | *Schizochytrium limacinum* | Johnson and Wen (2009) |
| – | – | Methanol (60 mL), $H_2SO_4$ (2.2 mL), 90 °C, 1 h | 92.0 | *Chlorella* sp. | Ehimen et al. (2010) |
| – | – | Methanol (2 mL), $H_2SO_4$ (1.8 %, v/v), 80 °C, 20 min | 82.0 | *Chaetoceros gracilis* | Wahlen et al. (2011) |
| – | – | Methanol (2 mL), $H_2SO_4$ (1.8 %, v/v), 80 °C, 20 min | 78.0 | *Tetraselmis suecica* | |
| – | – | Methanol (2 mL), $H_2SO_4$ (1.8 %, v/v), 80 °C, 20 min | 77.0 | *Chlorella sorokiniana* | |
| – | – | Methanol (2 mL), $H_2SO_4$ (1.8 %, v/v), 80 °C, 20 min | 39.0 | *Synechocystis* sp. | |
| – | – | Methanol (2 mL), $H_2SO_4$ (1.8 %, v/v), 80 °C, 20 min | 40.0 | *Synechococcus elongatus* | |

From the total fatty acids present in *S. limacinum* representing 40–50 % of dry biomass, transesterification reaction resulted in biodiesel yields of 63.7 % (Johnson and Wen 2009).

Transesterification of microalgal oil seems to be a promising alternative in biodiesel production, as conversion rates obtained with this method are very high. However, further improvements in operation conditions are needed, to reduce production costs and increase biodiesel yields. One possible alternative is the transesterification *in situ*, as it overtakes the high-costly extraction step.

### Transesterification *In Situ*

Transesterification *in situ* is very similar to the previously referred method, but instead of being applied to oils, it is applied directly to the biomass containing the oils. As this process can produce biodiesel without the extraction step, it is thought that direct transesterification could lower the production costs of biodiesel fuel (Ma and Hanna 1999; Patil et al. 2011, 2012). Several authors have reported the use of transesterification *in situ* to produce biodiesel from micro and macroalgae. For instance, Ehimen et al. (2010) applied transesterification *in situ* to the microalga *Chlorella* sp. Different reaction conditions were applied to identify the main variables that affect biodiesel yields. Transesterification of 15 g of dried biomass was performed using: (i) 2.2 mL of sulphuric acid as a catalyst; (ii) different volumes of methanol – 20, 40, 60, 80, and 100 mL; (iii) different reaction temperatures – 23, 30, 60, and 90 °C; and (iv) different reaction times – 0.25, 0.5, 1, 1.5, 2, 4, 8, and 12 h. Conversion of microalgal biomass into biodiesel reached 92.0 % at 90 °C after 1 h of reaction, using 2.2 mL of catalyst and 60 mL of methanol (Ehimen et al. 2010). Carvalho Júnior et al. (2011) applied *in situ* methanolysis to *Nannochloropsis oculata*. The transesterification reaction of 2 g of biomass was carried out using a mixture of methanol:chlorydric acid:chloroform in a 10:1:1 (v/v/v) ratio, at 80 °C for 2 h under continuous stirring. These conditions allowed the production of 23.2 % of biodiesel in $w_{esters}/w_{biomass}$. Considering that microalgae has an oil content ranging from 20 % to 50 % (Chisti 2007), the performance obtained is quite satisfactory (Carvalho Júnior et al. 2011). Wahlen et al. (2011) used transesterification *in situ* to produce biodiesel from the microalgae *C. gracilis*, *Tetraselmis suecica*, and *Chlorella sorokiniana*, and from the cyanobacteria *Synechocystis* sp., and *Synechococcus elongatus*. Transesterification reaction was applied to 100 mg of biomass using 2 mL of methanol and sulphuric acid in a volume fraction of 1.8 %. Reaction temperature and time were 80 °C and 20 min. Levels of biodiesel per extractable oil were 82.0, 78.0, 77.0, 39.0 and 40.0 % for the organisms *C. gracilis*, *T. suecica*, *C. sorokiniana*, *Synechocystis* sp. and *S. elongatus*, respectively (Wahlen et al. 2011). To compare the two types of transesterification, Johnson and Wen (2009) also applied direct transesterification to cells of *S. limacinum*. Using a mixture of methanol, sulphuric acid, and chloroform at 90 °C and during 40 min, the authors obtained an ester yield of 72.8 %.

These studies show that biodiesel conversion yields are similar for both types of transesterification, meaning that transesterification *in situ* should be adopted instead of the double-step procedure of extraction and transesterification. However, these results also suggest that application of direct transesterification require higher volumes of alcohol and catalyst.

## 1.4 Conclusion

The extraction methods described in this review constitute promising alternatives to recover microalgal oil. Experiments conducted in the last decade have showed that organic solvent extraction is the most efficient method for microalgal oil extraction. The main drawback of applying organic solvents for oil extraction is related to the harmfulness of these compounds. The amount of organic solvent required can be reduced by previous disruption of cell walls using enzymatic, PEF, ultrasound, microwave and expeller pressing methods. These procedures are also responsible for an increase in oil extraction efficiencies. Enzymatic and PEF extraction could be promising techniques because enzymes present higher selectivity towards cell walls and PEF has reduced energetic costs compared to the other disruption methods presented in this chapter. Application of $scCO_2$ extraction has shown to have high extraction efficiencies and to be safer to the environment, but the costs associated are extremely high. Another possibility to avoid the use of organic solvents is to use ionic liquids as solvents. Although extraction efficiencies are lower, these compounds are more environmentally friendly than organic solvents.

An important lack in the research of microalgal oil extraction is the process scale-up, including the analysis of the process cost and efficiency. The majority of studies concerning oil extraction and biodiesel production from microalgae have been performed for lab-scale. As a result, little is known about the feasibility of these processes in large scale. Further studies should be conducted using larger amounts of microalgal biomass to analyse the oil extraction efficiency and to compare with results already known for lab-scale.

In the transesterification reaction, the different variables affecting this reaction must be taken into account. As referred earlier in this review, the alcohol and its employed amount, the type of catalyst and its concentration, temperature and reaction time have a great influence in the alcoholysis reaction. As well as in the oil extraction procedures, the reaction scale is also a determining factor, as it has a great impact on the volumes of alcohol and catalyst employed and on reaction time, which reflects in the total costs of the reaction. Therefore, transesterification reaction should be considered using higher volumes of oil and also different combinations of operational conditions. Additionally, conditions that maximize oil extraction efficiencies should be applied together with those responsible for higher oil to biodiesel conversion rates, to produce biodiesel able to compete with current diesel fuel with reduced costs and higher productivities.

Finally, attention should also be paid to the transesterification *in situ* process, as it eliminates the oil extraction step. Future studies should focus on this method

to verify if costs can be really reduced in the absence of oil extraction steps. Operational conditions at large scale should also be addressed in order to achieve higher productivities of fatty acids esters.

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# Chapter 2
# Electrochemistry and Water Pollution

Subramanyan Vasudevan and Mehmet A. Oturan

**Abstract**  This article reviews both the pollution by the electrochemical industry and the use of electrochemistry to clean water, air and soils. Main pollutants include Pd, Cd, Ni, Hg and other metals, $SO_2$, $CO_2$ and cyanide. The cause for water pollution by electrochemistry is due to the effluents from different electrochemical industries such as mercury from chlor-alkali industry; lead, cadmium and mercury from battery industry; heavy metals and organic contaminants from electroplating wastes; and contaminants from corrosion processes. Most pollutants can be successfully eliminated or converted to non-toxic materials by methods based on electrochemical principles. Electrochemical depolluting methods are mainly electrodialysis, electrocoagulation, electroflotation, anodic processes, cathodic processes, electrokinetic remediation, and electrochemical advanced oxidation processes.

**Keywords**  Water and soil pollution • Hg • Cd • Pd • Ni • Electrochemical remediation • Electrodialytic • Electrocoagulation • Electroflotation • Battery • Chlor-alkali

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**Contents**

## 2.1    Introduction

Water is one of the essential companion of life on earth and the nature has iterated the vitality of water for life. It is very appropriate to say that existence of life on Earth is largely owed to the presence of water, but it is fast becoming a scare commodity in most part of the world. The earliest form of life appeared in sea water about 3.5 billion years ago and was transferred to land only 380 million years ago. There is no aspect of our life that is not touched by water. Water is one of the clear signs of prosperity, health, serenity, beauty, artistry, purity and many other attributes. Leonardo Da Vinci had described water as "the vehicle of nature" ("vetturale di natura"). Water resources of a country constitute one of its vital assets. Groundwater is a more reliable and less expensive water supply than those obtained from surface water (lakes, rivers and streams), marine and coastal; therefore, it is withdrawn in large quantities for domestic and agricultural applications all over the world. It is also reported that more than 1.2 billion people lack access to safe drinking water, 2.6 billion have little or no sanitation; millions of people die annually (3,900 children a day) from diseases transmitted through unsafe water or human excreta (Mark Shannon et al. 2008; Ball 2008; Montgomery and Elimelech 2007; Kumar et al. 2005; Ayoob and Gupta 2006). As per the international norms, if per-capita water availability is less than 1,700 $m^3$ per year then the country is categorized as water stressed and if it is less than 1,000 $m^3$ per capita per year then the country is classified as water scarce Hence, there is a need for proper planning, development and management of the greatest assets of the country, viz. water and land resources for raising the standards of living of the millions of people, particularly in the rural areas (Chakraborti et al. 2009; Roy Chowdhury et al. 1999).

Though water is available in the universe in huge quantity in the order of $1,400 \times 10^6$ km$^3$, only 2.5 % of the global water resources are consisted of fresh water. Among the fresh waters, only about 5 % of them or 0.15 % of the total world waters are readily available for beneficial use, the rest being contained in the polar glaciers or glaciers in altitude. Global freshwater reserves are rapidly depleting and this is expected to significantly impact many densely populated areas of the world. Low to middle income developing regions as well as highly developed countries will face water stress in the future, unless existing water reserves are managed effectively. Although low and middle income developing countries currently have low per capita water consumption, rapid growth in population and inefficient use of water across sectors is expected to lead to a water shortage in the future. Developed countries traditionally have high per capita water consumption and need to focus on reducing their consumption through improved water management techniques and practices. By 2025, India, China and select countries in Europe and Africa will face water scarcity if adequate and sustainable water management initiatives are not implemented.

The present review article presents the causes for water pollution by electrochemistry which are due to the effluents from different electrochemical industries viz., (i) mercury from chlor-alkali industry, (ii) lead, cadmium and mercury from battery industry, (iii) heavy metals from electroplating wastes and (iv) organic pollutants from dye, pesticide, pharmaceuticals industry etc., and electrochemical treatment technologies viz., (i) electrodialysis, (ii) electrocoagulation/electroflotation, (iii) anodic processes, (iv) cathodic processes, (v) electrokinetic remediation, (vi) electrochemical advanced oxidation processes and the details of the same are described.

## 2.2 How Electrochemistry Is the Cause for Water Pollution?

The modern definition of electrochemistry, as per Bockris, is that it is the interdisciplinary activity which has grown up around phenomena connected with charge or electron transfer at interfaces (Bockris and Nagy 1974). Some of the industries like caustic soda-chlorine, aluminum, electroplating, electrowining of metals, batteries etc., are based on electrochemical science and form the typical electrochemical industries – some of them being multibillion dollar industries.

i. The battery industry can cause lead, cadmium and mercury poisoning of water.
ii. The disposal of electroplating solutions can lead to water pollution especially due to cadmium, zinc, lead, copper, chromium and cyanides.
iii. Mercury which enters water from chlor-alkali mercury cells has been found to exceed tolerable limits (this type of industry is operated still in some places).
iv. Chlorides, carbonates etc. found in water will be the cause for corrosion of metals which can get included in water and corrosion is naturally an electrochemical reaction.

These will be dealt with in a more detailed manner in the following.

### 2.2.1 Pollution from Battery Industry

There will be a possibility of one or more of the eight metals like mercury, cadmium, nickel, lead, zinc, manganese, silver are contaminating the water from battery industry. At present mercury pollution due to battery industry is very limited or otherwise almost stopped. Metal pollutants like lead, cadmium and nickel are major contaminant from battery industry rather than zinc, manganese etc., in ground water.

### 2.2.2 Lead

Lead acid batteries are considered to have one of the fastest global growth rates [16–20]. The emerging new concept of zero emission pollution control in the West, has paved way for development of electric vehicles (EVs) and Hybrid Electric Vehicles (HEVs). Usage of lead acid battery is expected to grow further with technological advancements in the electric vehicles market. Rapidly developing markets within Asia-Pacific, particularly China and India, hold ample opportunities for future growth (Wang et al. 1998; Chen et al. 2012).

But in lead acid batteries, lead poisoning is one of the most ubiquitous environmental health threats to children and a significant contributor to occupational disease (Chen et al. 2012). Lead causes a range of symptoms ranging from the loss of neurological function to death depending upon the extent and duration of exposure. In children, moderate lead exposure is responsible for a significant decrease in school performance, lower standardized test scores (including IQ test scores), and is linked with hyperactive and violent behavior. Both children and adults can suffer from a range of illnesses including effects on the central nervous system, kidneys, gastrointestinal tract, muscle and joint pains, sleep problems, blood pressure, anemia, and the reproductive system in both men and women. Fetuses, infants, and children are especially vulnerable to lead exposure compared with adults, since lead is more easily absorbed into growing bodies. Also, the tissues of small children are more sensitive to the damaging effects of lead (Srianujata 1998).

Despite the potentially harmful effects of lead, it continues to be used in a wide variety of industries in the manufacture of thousands of products. But the battery industry is by far the principle consumer of lead, using an estimated 76 % of annual primary and secondary lead (mined and recycled metallic lead) production. Lead-acid batteries are primarily used in automobiles for Starting, Lighting and Ignition purposes (SLI), but are also used as back-up power supplies. Battery composition by weight is composed as follows: 50 % lead salts and oxides, 24 % acid, 17 % metallic lead, 5 % plastics, and 4 % ebonite and separators (Dagogo 1994).

Lead-acid batteries contain concentrated sulphuric acid and large amounts of lead. The acid is extremely corrosive and also a good carrier for soluble lead and lead particulate. If the acid leaks onto the ground, it may contaminate the soil and

then the soil will become a source of lead particulate as the solution dries out and the lead becomes incorporated into soil particles which may be blown by wind or raised by vehicle transit. From the soil, the lead will go into ground water resulting the water will be contaminated with lead (Haiao et al. 2001).

### 2.2.3  Cadmium and Nickel

Cadmium is considered to be highly toxic and therefore its permitted concentration in water is limited to 5–10 ppb. In recent years cadmium has become of major environmental concern due to its introduction to natural water reservoirs. The main sources of cadmium contamination is Ni/Cd battery production which may deliver both cadmium and nickel to groundwater in untreated aqueous wastes, or because of uncontrolled- led disposal of used batteries (Friberg 1974).

### 2.2.4  Pollution from Metal Finishing Industry

The electroplating or metal finishing industry uses a wide variety of materials and processes to clean, etch, and plate metallic and nonmetallic surfaces to provide desired surface properties (Weiner 1963). The materials include solvents and surfactants for cleaning, acids and bases for etching, and solutions of metal salts and other compounds to plate a finish onto a substrate.

The electroplating or metal finishing industry has been playing a momentous role in the development and growth of numerous metal manufacturing and other engineering industries since the early part of this century. While electroplating operations have, in the course of time, become an essential and integral part of many engineering industries throughout the world, there has also been a steady growth of independent and small- to medium-scale electroplating industries, especially in the developing countries including those of South East Asia after World War II. The growth of these independent small-scale electroplating industries in the developing countries may be attributable to the growth of light and medium engineering industries which found it more convenient and economical to have their products metal plated by independent electroplaters (Nagendran and Parthasaradhy 1966).

In comparison with other industries, however, the electroplating industry uses much less water; hence the volume of the waste waters produced by this industry is also comparatively much smaller. Nevertheless, the waste waters are highly toxic in nature due to the presence of metals such as Ag, As, Be, Cd, Pb, Cr(VI), Cu, Zn and Ni etc., and of acids and the highly dangerous cyanides. The plating operations are preceded by cleaning and stripping of metal surfaces by alkali and/or other solvents and acids respectively. After plating is completed, the plated objects are rinsed with water. The cleaning, stripping and rinsing are the main sources of waste waters in

an electroplating industry. The volume and characteristics of electroplating waste waters vary widely from industry to industry, depending on the operating practices and water conservation measures adopted in the industry.

Among the cations, most heavy metal compounds such as Ag, As, Be, Cd, Pb, Cr(VI), Cu, Zn and Ni are toxic to aquatic life. Ammonia would be another cation toxic to fish even in low concentrations. As far as anions are concerned, very low concentrations of cyanides, chromic acid and soluble chromates have been found to be toxic to aquatic life. However, nitrates and phosphates are anions coming under the heading of nuisance effects rather than toxicity (Drogen and Passek 1965).

### 2.2.5   Mercury Pollution from Chlor-Alkali Industry

While about 23 % of total mercury consumption in USA is in chlor-alkali production, the consumption of mercury in United Kingdom for chlor-alkali is 49 %. Since purer and concentrated caustic soda is obtained from the mercury cells, these cells are preferred. Until 2006, in India, the production capacity of caustic soda is 2.33 million tons per year. In this total capacity, 1.90 and 0.43 million tons per year is from membrane and mercury cell technologies respectively. In general, in India the membrane and mercury cell technology share is 82 % and 18 % respectively. Mercury losses from these cells have been estimated to be of the order of 0.25 Kg $T^{-1}$ of chlorine. Mercury is lost from the cell in several phases: (a) through the caustic soda product; (b) through the overall cleaning of the mercury cell room; (c) through the brine solution (d) through the hydrogen; (e) through the brine saturation. Further, the amount of mercury (at the trace level) available in caustic soda varies depending on its degree of purification. These traces of mercury can be introduced into the human food cycle if the caustic soda produced by this method is used in processing of food e.g. peeling agent for tubers and fruits, a sour-cream-butter neutralizer, a reagent in the refining process for vegetable oils and animal fats as well as a reagent to adjust alkalinity in the canned vegetable industry. The safe limit for the mercury in water is 0.5 mg $L^{-1}$ and 0.1 μg $L^{-1}$ for India and Europe respectively.

Mercury is usually discharged into the environment as divalent mercury or elemental mercury and which has a solubility of about 0.025–0.03 mg $L^{-1}$ in water at 25°C. Mercurous mercury is readily precipitated by sulphide ions and these are inert. But mercury organic complexes can be attacked by bacteria to give methyl mercuric compound (<pH 7) $(CH_3\ Hg)^+$ or dimethyl mercury (>pH 7) $(CH_3\text{-Hg-}CH_3)$. We still have much to learn about quantitative aspects of the mercury cycle. Methyl mercury can enter lake and river food chain, from water to plants to fish, to bird and to human beings, becoming concentrated at each step. Methyl mercury is particularly toxic to animals, because it can readily pass the blood–brain barrier, causing injury to cerebellum and cortex. The clinical symptoms of this damage are numbness, awkwardness or gait and blurring or vision. However, the dreaded

problem of mercury pollution was tackled on a war footing in Japan with the sole objective of making it the 'zero' mercury process and it had already achieved a level of 0.5–0.6 g of mercury consumption per tonne of caustic soda. In view of this mercury menace, there is a big swing in favor of membrane or diaphragm cells for the production of chlor alkali (Ditri and Ditri 1977).

### 2.2.6 Water Contamination by Corrosion

Corrosion is most often considered to be an electrochemical process, and it contaminates the drinking water source in different ways.

**Corrosion of Cementations Materials**

Many drinking water distribution systems contain pollution creating materials such as cement concrete, asbestos-cement pipes and cement-asbestos-cement pipes and cement-mortar-lined steel and ductile iron pipes. There will be a possibility of asbestos fiber or sand or other inorganic materials contamination in the drinking water source by severe attack of $CO_2$ on cement-based pipe line materials. On the other hand, if there is no carbonate species, the leaching of the alkalinity from the cement can lead to increase in pH value of drinking water (Yu Peng and Korshin 2011; Elfstrom Broo et al. 1997).

**Iron Contamination**

In general failures of iron and steel pipes caused by corrosion are very rare due to the formation of protective scale on the pipes and reduce the rate of corrosion very low. But after certain period of time, corrosion will start. For example iron in contact with water, following reactions will occur,

$$\text{At anode}: \text{Fe} \rightarrow \text{Fe}^{2+} + 2\text{e}^- \qquad (2.1)$$

$$\text{At cathode}: 2\text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{H}_2 + 2\text{OH}^- \qquad (2.2)$$
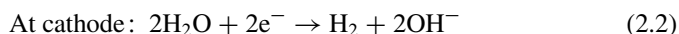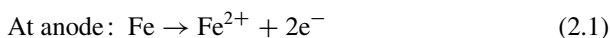
The cathodic Reaction (2.2) will generally occur slowly, but a faster alternative one will occur in the presence of oxygen and the following reaction will occur,

$$\text{H}_2\text{O} + 2\text{e}^- + \tfrac{1}{2}\,\text{O}_2 \rightarrow 2\text{OH}^- \qquad (2.3)$$

For both cathodic reactions, two hydroxide ions will be produced and an alkaline condition will result near the cathode and make the water more alkaline. However,

the ferrous ion can be further oxidized by dissolved oxygen and precipitate as ferric hydroxide (red mud),

$$2Fe^{2+} + 5H_2O + \tfrac{1}{2}O_2 \rightarrow 2Fe(OH)_3 + 4H^+ \tag{2.4}$$
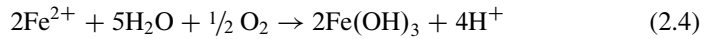
and make the water become contamination. In neutral water dissolved oxygen is necessary for appreciable corrosion of iron. The corrosion rate will be higher as the relative motion of the water increases with respect to iron surface. Increased temperature can also increase iron corrosion. Two principal types of electrochemical corrosion cell are concern for the above corrosion process. The first results from a galvanic cell, which is due to the contact of two different metals (for example metal alloy). The other and often more important corrosion cell is the concentration cell. This cell involves a single metal, but different portions of the metal are exposed to different aqueous environments (Refait et al. 1998).

## Heavy Metal Contamination

Lead may occur in drinking water by corrosion of lead plumbing or fixtures. Corrosion of plumbing is the greatest cause of concern. All type of water is corrosive to metal plumbing materials to some degree. Grounding of household electrical systems to plumbing may also exacerbate corrosion. Copper pipeline systems carrying water can be attacked by pitting corrosion. Sometimes the heavy metals come from the corrosion of internally unprotected metallic water pipelines. Heavy metals can cause a variety of adverse health effects when people are exposed to it at levels above maximum contamination level for relatively short period of time (Isaac et al. 1997).

## Corrosion Due to Microorganisms

The role of microorganisms in the corrosion of metals in the pipeline systems has been recognized in recent times. Microorganisms may influence corrosion by affecting the rate of anodic or cathodic activity producing corrosive end product and making the water become contaminated. The pipe surface, joints, valves and gates provide a variety of niches for the growth of many different microorganisms that can enhance the corrosion of pipeline (Yu Peng and Korshin 2011; Elfstrom Broo et al. 1997).

## Carbonate, Chloride as Comrades of Corrosion

Some of the ions like chromate and phosphate may help in preventing corrosion, the presence of other ions like carbonate, chloride, nitrate etc. in general and chloride in particular will accelerate the corrosion rate and resulting water will be contaminated with corrosion products (Yu Peng and Korshin 2011).

## 2.3  How Electrochemistry Is the Cure in Pollution?

There are increasing economic, social, legal, and environmental pressures to utilize the "best available technology" not entailing excessive cost and to aspire to "performance without pollution", i.e., "zero pollution processing". Electrochemical technology has an important role to play as part of an integrated approach to the avoidance of pollution, monitoring of pollution and process efficiency, cleaner processing and modern techniques for electrical energy storage and conversion (Rajeshwar and Ibanez 1997a; Genders and Weinberg 1992; Bockris 1972; Pletcher and Walsh 1993). Electrochemistry, with its unique ability to oxidize or reduce compounds at a well-controlled electrode potential or current and by just adding (at the anode) or withdrawing (at the cathode) electrons, offers many interesting possibilities in environmental pollution control. Anodic processes can be used to oxidize organic pollutants to harmless products and to remove toxic compounds from flue gases. Cathodic processes can remove heavy metal ions from wastewater solutions down to very low outlet concentrations (Rajeshwar and Ibanez 1997b; Simonsson 1997; Panizza and Cerisola 2005; Anglada et al. 2009; Chen 2004). Not only can the two electrodes of the electrochemical cell be used in purification processes, but the ion-selective membrane(s) that are often placed between the electrodes to have a selective transfer of only anions or cations can also. New electrodialytic processes using such membranes have been developed, which can solve a variety of environmental problems.

For the removal of pollutants there are several powerful tools based on principles of electrochemistry. viz,

– Electrodialytic processes
– Electrocoagulation and electroflotation
– Electro-remediation of soils
– Cathodic processes
– Anodic processes
– Electrochemical advanced oxidation processes

### 2.3.1  Electrodialytic Processes (EDP)

EDP is an electrochemical separation process in which ions are transferred through ion exchange membranes by means of a direct current (DC) voltage (Strathmann 1986). The principle that governs electrodialysis is an electrical potential difference across an alternating series of cation and anion exchange membranes between an anode and a cathode. The feed solution containing both positive and negative ions enters the membrane stack to which a voltage is applied, thus causing the migration of the ions toward their respective electrodes. The cation exchange membranes allow the transfer of cations but inhibit the transfer of anions. Conversely,
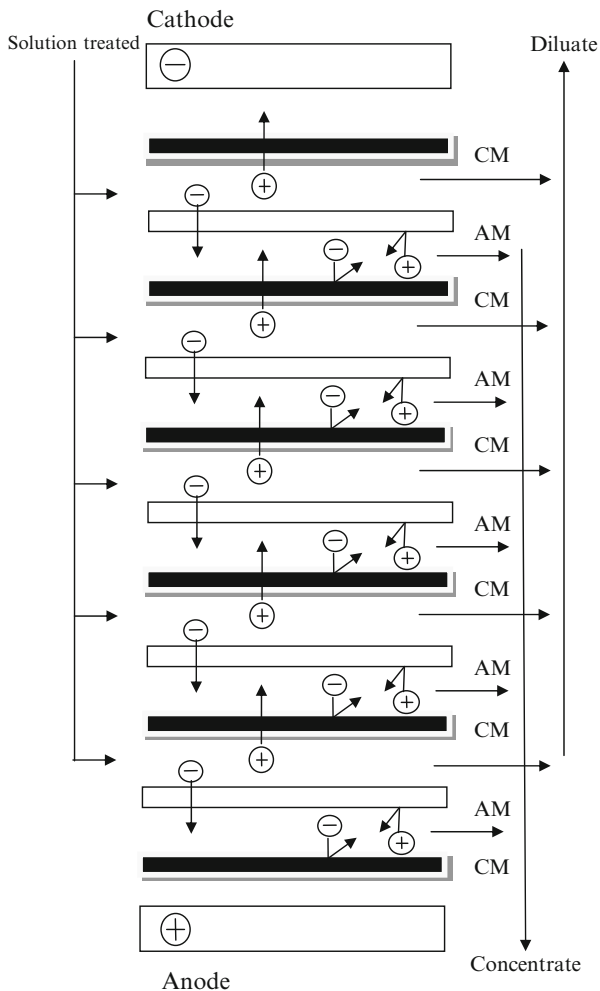
**Fig. 2.1** The working principle of electrodialysis system. *AM* anionic membrane, *CM* cationic membrane

anion exchange membranes allow the transfer of anions but inhibit the transfer of cations. The result is alternating compartments containing streams of dilute ion concentration and streams rich in ion concentration exiting the stack (Fig. 2.1). The attractive characteristics of membranes used in electrodialysis applications include selectivity between ions of opposite charge, high ionic conductivity, low electrical conduction, long-term chemical stability, mechanical strength, and resistance to fouling. Polymer materials such as polystyrene, polyethylene, and polysulfone are often chosen for the membrane matrix and are often cross-linked to ensure stability.
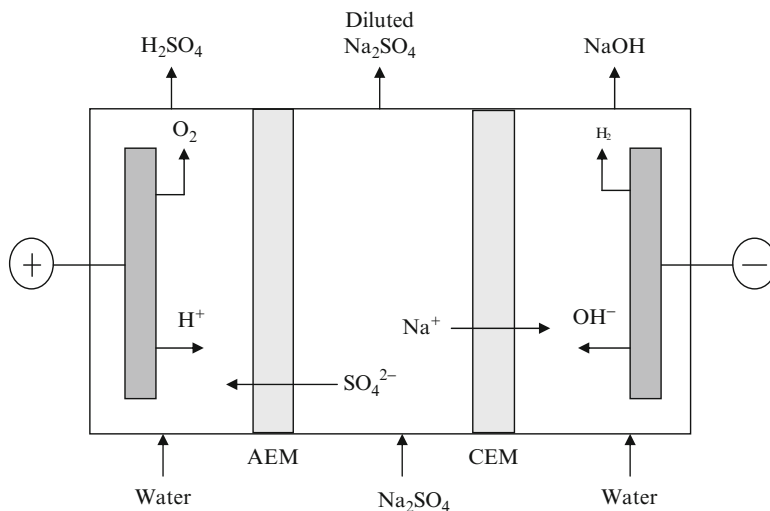
**Fig. 2.2** Schematic representation of electrodialytic processes (three-compartment cell) for splitting of sodium sulfate into sodium hydroxide and sulfuric acid solutions. *AEM* anion exchange membrane, *CEM* cation exchange membrane

Fixed ionic moieties such as $SO_3^-$, $COO^-$, $PO_3^{2-}$, $HPO^{2-}$, $AsO_3^{2-}$, and $SeO^{3-}$ are commonly used for cation exchange membranes, and $NH^{3+}$, $RNH^{2+}$, $R_3N^+$, $R_2N^+$, $R_3P^+$, $R_2S^+$, are common choices for anion-exchange membranes. Because of the corrosive nature of the anode compartments, electrodes are usually made of titanium and plated with precious metals.

In practice, it can be used in the separation and concentration of salts, acids, and bases from aqueous solutions, the separation of monovalent ions from multivalent ions, and the separation of ionic compounds from uncharged molecules. It can be used for either electrolyte reduction in feed streams or recovery of ions from dilute streams. Industrial applications encompass several industries and include the production of potable water from brackish water, removal of metals from wastewater, demineralization of whey, deacidification of fruit juices, and the removal of organic acids from fermentation broth. The best industrial important example is splitting of sodium sulfate solutions into sodium hydroxide and sulfuric acid solutions. The simplest design for this is to use a three-compartment cell with cationic and anionic selective membranes, as shown in Fig. 2.2. The main advantages are, very simple cell design, electrode products can be controlled, and concentrated solutions can be obtained. In addition, cation-selective membranes have a high selectivity and easy removal of metal deposit from cathode compartment is possible. The disadvantages are a low mass transfer coefficient, danger for poisoning, fouling, and slogging of membranes.
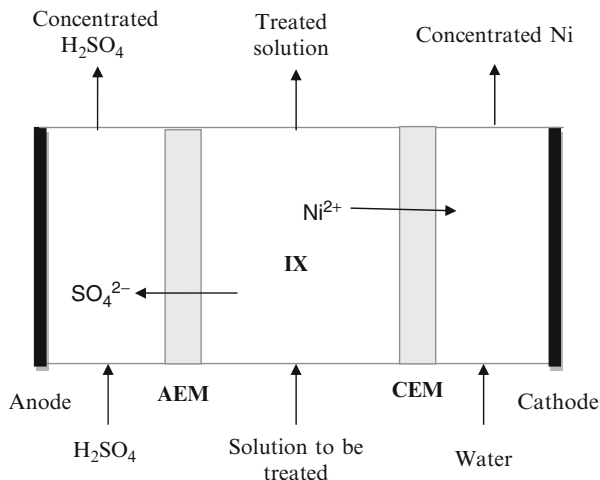
**Fig. 2.3** Schematic representation of ion exchange assisted electrodialytic processes

## 2.3.2 Ion Exchange Assisted Electrodialytic Processes

Ion exchange assisted electrodialytic processes (Fig. 2.3) is a combination of electrodialysis and ion exchange technique (Rozhdetsvenska et al. 2001). This process is similar to electrodialysis with the exception that a bed of ion-exchange particles is placed in the centre (dilute) compartment. The presence of the ion-exchange bed increases the conductivity of the centre compartment and allows for improved separation. A schematic diagram is given in Fig. 2.3 for the purification of a dilute $NiSO_4$ solution. One of the best applications in industries with processes involving heavy metal electrolyte solutions; even selective removal of metal ions will be possible. The major advantages are the continuous regeneration without extra chemicals and the relatively high current efficiency. The method is capable of cleaning dilute process liquids. The disadvantages are the need of careful ion exchange resin, and choice of process parameters. In addition, the precipitation of solids lowers the efficiency of the process.

## 2.3.3 Electrocoagulation and Electroflotation

Electrocoagulation is the process of destabilising suspended, emulsified, or dissolved contaminants in an aqueous medium by introducing an electric current into the medium (Fig. 2.4) in order to produce the coagulation of colloidal particles (Vik et al. 1984; Cañizares et al. 2005; Holt et al. 2005; Lakshmanan et al. 2009; Kobya et al. 2010). In other words, electrocoagulation is the electrochemical production of destabilisation agents that brings about neutralization of electric charge for
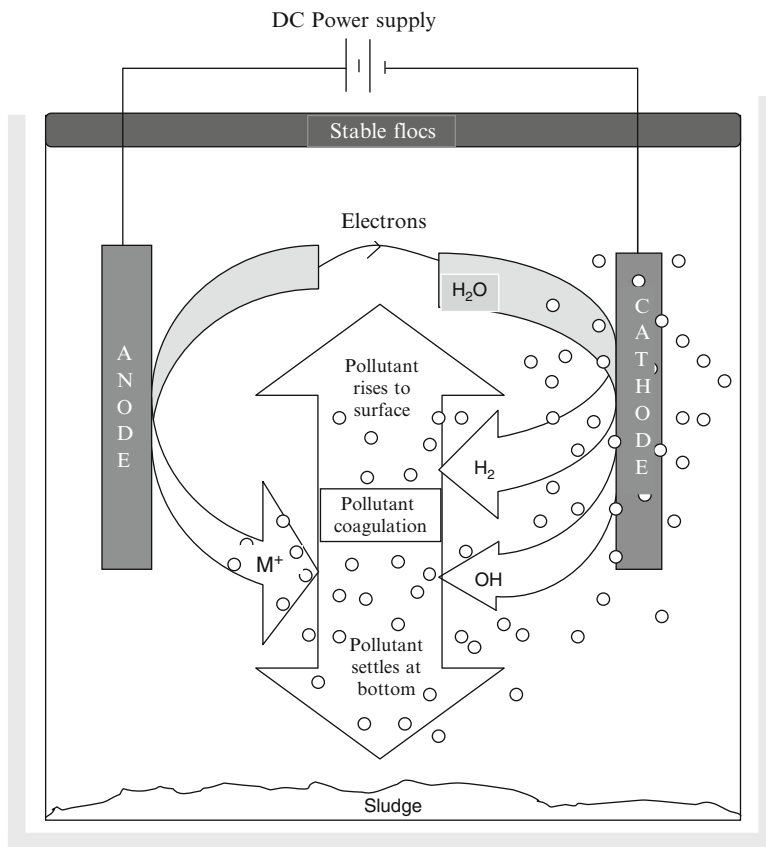
**Fig. 2.4** The working principle of electrocoagulation process

removing pollutant. Once charged, the particles bond together like small magnets to form a mass. In its simplest form, an electrocoagulation unit is made up of an electrolytic cell with one anode and one cathode. The conductive metal plates/rods are, commonly known as sacrificial electrodes, made up of the same or different materials are used as the anode and cathode. Electrocoagulation was first proposed in the nineteenth century and the first electrocoagulation plant was successfully erected in 1889 at London for the treatment of sewage wastewater.

Electroflotation is a simple process that floats pollutants to the surface of a water body by tiny bubbles of hydrogen and oxygen gases generated from water electrolysis. Therefore, the electrochemical reactions at the cathode and anode are hydrogen evolution and oxygen evolution reactions, respectively. Graphite and lead dioxide are among the most common anode used in electroflotation. Dimensionally stable anodes ($Ti/IrO_2$, $Ti/RuO_2$, $Ti/TiO_2$-$RuO_2$ and $Ti/Pt$) are also used. In recent days highly effective ($Ti/IrO_x$-$Sb_2O_5$-$SnO_2$) is used and its life is predicted to be about 20 years (Shahjahan Kaisar Alam Sarkar et al. 2010).

**Mechanism of Electrocoagulation**

This process involves applying an electric current to sacrificial electrodes inside an electrolytic cell where the current generates a coagulating agent and gas bubbles. In detail, electrocoagulation is a technique involving the electrolytic addition of coagulating metal ions directly from sacrificial electrodes (anode). These ions coagulate with pollutants in the water, similar to the addition of coagulating chemicals such as alum and ferric chloride, and allow for easier removal of the pollutants by flotation and/or filtration. For example, in the case of aluminum, magnesium, zinc or iron electrodes, aluminum in the form of $Al^{3+}$, magnesium in the form of $Mg^{2+}$, zinc in the form of $Zn^{2+}$ and iron in the form of $Fe^{2+}$ will be produced by anodic dissolution during electrocoagulation process (Vasudevan et al. 2009a, b, 2011b). The dissolution reactions taking place at aluminum, magnesium, zinc and iron electrodes are given as follows,

$$Al \rightarrow Al^{3+} + 3e^- \tag{2.5}$$

$$Mg \rightarrow Mg^{2+} + 2e^- \tag{2.6}$$

$$Zn \rightarrow Zn^{2+} + 2e^- \tag{2.7}$$

$$Fe \rightarrow Fe^{2+} + 2e^- \tag{2.8}$$
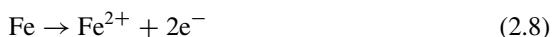
Then, $Al^{3+}$, $Mg^{2+}$, $Zn^{2+}$ and $Fe^{2+}$ react immediately with hydroxide ions in solution to produce $Al(OH)_3$, $Mg(OH)_2$, $Zn(OH)_2$ and $Fe(OH)_2$ respectively. The reactions are as follows,

$$Al^{3+} + 3OH^- \rightarrow Al(OH)_3 \tag{2.9}$$

$$Mg^{2+} + 2OH^- \rightarrow Mg(OH)_2 \tag{2.10}$$

$$Zn^{2+} + 2OH^- \rightarrow Zn(OH)_2 \tag{2.11}$$

$$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2 \tag{2.12}$$
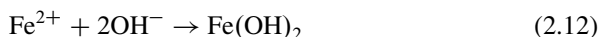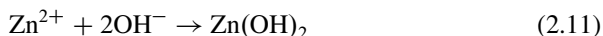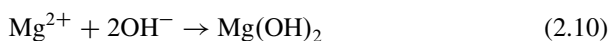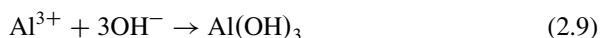
The metallic hydroxide was produced up to a sufficient concentration to initiate polymerization or condensation reactions. The polymerization reactions for aluminum and ferrous hydroxides are as follows,

$$Al(OH)_3 + Al(OH)_3 \rightarrow (OH)_2Al - O - Al(OH)_2 + H_2 \tag{2.13}$$

$$Fe(OH)_2 + Fe(OH)_2 \rightarrow (OH)\,Fe - O - Fe(OH) + H_2O \tag{2.14}$$
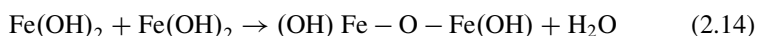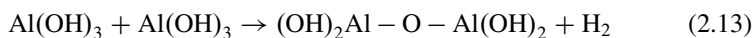
The polymeric complexes $[Al_2(O)(OH)_4]$ and $[Fe_2(O)(OH)_2]$ allows to remove pollutants from water mainly by either adsorption, surface complexation or by

co-precipitation. The following reaction explains how the pollutant removed by surface complexation,

$$M - H_{(aq)} + (HO)OFe_{(S)} \rightarrow M - OFe_{(S)} + H_2O \qquad (2.15)$$
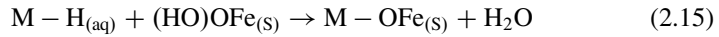
where, M represents the metal pollutant act as ligand. In general, polymeric aluminum or iron complexeses can have both positive and negative charges capable of attracting the pollutants having opposite charges and remove them from water. For instance, hydrolysis of aluminum ions may generate negative and positive species (either monomeric or polymeric) such as monomeric species like $Al(OH)^{2+}$, $Al_2(OH)_2{}^+$, $Al_2(OH)_2{}^{4+}$, $Al(OH)_4{}^-$ and polymeric species like $Al_3(OH)_4{}^{5+}$, $Al_6(OH)_{15}{}^{3+}$, $Al_7(OH)_{17}{}^{4+}$, $Al_8(OH)_{20}{}^{4+}$, $Al_{13}O_4(OH)_{24}{}^{7+}$, $Al_{13}(OH)_{34}{}^{5+}$ over a wide pH range (Cañizares et al. 2005). Similarly, ferric ions generated by electrochemical oxidation of iron electrode may form both monomeric and polymeric species like $FeOH^{2+}$, $Fe_2(OH)_2{}^{4+}$, $Fe(H_2O)^{2+}$, $Fe(OH)^{4-}$, $Fe(H_2O)_3(OH)_3{}^0$, $Fe(H_2O)_6{}^{3+}$, $Fe(H_2O)_5(OH)^{2+}$, $Fe(H_2O)_4(OH)^{2+}$, $Fe_2(H_2O)_8(OH)_2{}^{4+}$, $Fe(H_2O)_6(OH)_4{}^{4+}$ and $Fe_2(H_2O)_6(OH)_4{}^{2+}$ over a wide pH range (Lakshmanan et al. 2009). These metallic hydroxides have a strong affinity for disperse particles. Apart from the above reactions water is also electrolyzed in a parallel reaction, producing small bubbles of oxygen at the anode,

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \qquad (2.16)$$
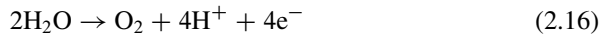
hydrogen at the cathode,

$$2H^+ + 2e^- \rightarrow H_2 \uparrow \qquad (2.17)$$

and as a result, flotation of the coagulated particles occurs.

**Electrode Materials**

The materials employed in electrocoagulation processes are usually aluminum or iron. It is usually to use iron for wastewater treatment and aluminum for drinking water treatment because iron is relatively cheaper. The aluminum electrodes are also finding applications in wastewater treatment due to the high coagulation efficiency of aluminum hydroxide. But the main disadvantage in case of aluminum electrode is the residual aluminum (maximum contamination limit is 0.05–0.2 mg $L^{-1}$) present in the treated water due to cathodic dissolution. The excess aluminum present in the water creates health problems like cancer. To overcome this disadvantage of aluminum, in recent years alternate anodes like magnesium, zinc etc., are tried for electrocoagulation processes (Vasudevan et al. 2010a, b, c, d). When there are a significant amount of $Ca^{2+}$ or $Mg^{2+}$ ions in water, the cathode material is recommended to be stainless steel or galvanized iron.
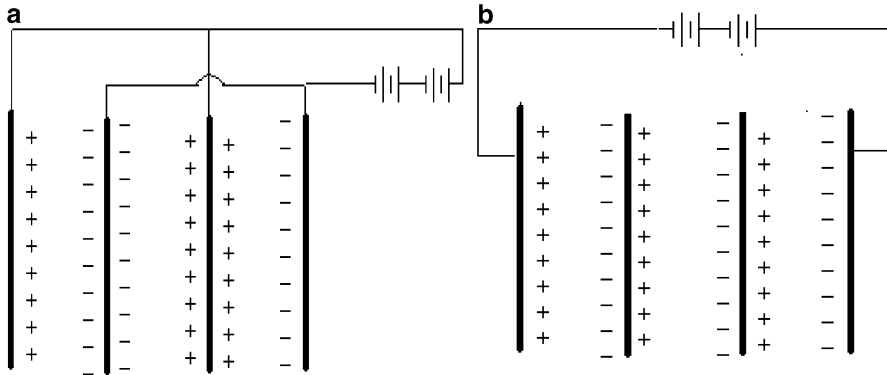
**Fig. 2.5** Schematic illustrations of (**a**) Monopolar and (**b**) Bipolar configuration of cells

## Electrode Configuration

There are two possible electrode configurations in the electrocoagulation process, which are monopolar and bipolar configuration as shown in the Fig. 2.5a, b. In the case of monopolar configuration, an electric potential (V) is applied between pairs of anodes and cathodes. The anodes and cathodes are arranged alternatively in the electrolytic cell pack. In this configuration, each unit/pair operates at the same voltage and the current being the sum of the individual unit currents. In the case of bipolar configuration, only two outer most electrodes physically connected to the power supply. Each electrode except for those at each end thus functions as anode on one face and as cathode on the other. Each unit is electrically in series with the others and the same current flows through every unit (Vasudevan and Lakshmi 2011, 2012b).

## Effect of Operating Parameters

Current Density

Current density is an important factor which strongly influences the performance of electrocoagulation (Vasudevan and Lakshmi 2012a). The current density determines the electrocoagulant dosage rate at electrocoagulation processes. For example, in the case of aluminum, the electrochemical equivalent mass is 335.6 mg/Ah and for iron, the value is 1,041 mg/(Ah). Thus, current density should have a significant impact on pollutant removal efficiency. The amount of coagulant was determined from the Faraday law,

$$E_c = I\,t\,M/(Z\,F) \tag{2.18}$$

where I is current in A, t is the time (s), M is the molecular weight, Z is the number of electron involved in the reaction, and F is the Faraday constant (96,485.3 C/mol). The suggested current density for electrocoagulation process is 20–25 A/m$^2$ but the selection should be fixed with respect to other operating parameters like solution pH, temperature and flow rate.

### Solution pH

It has been established that the influent pH is an important operating factor influencing the performance of electrochemical processes. The best pollutant removal is found near pH of 7. The power consumption is, however, higher at neutral pH due to the variation of conductivity. The solution pH after electrocoagulation treatment would increase for acidic influent but decrease for alkaline influent. This is one of the advantages of this process. The increase of pH at acidic condition was attributed to hydrogen evolution at cathodes. In fact, besides hydrogen evolution (Reaction 2.17), the formation of Al(OH)$_3$ near the anode (Reaction 2.9) would release H$^+$ leading to decrease of pH. In addition, there is also oxygen evolution reaction leading to pH decrease (Reaction 2.16). The pH decrease at alkaline conditions, is due to the formation of hydroxide precipitates with other cations and the formation of Al(OH)$_4$$^-$ in the solution (Vasudevan et al. 2012).

### Solution Temperature

In general, all electrocoagulation units will be operated between 40°C and 60°C for better removal efficiency. But at the same time, when the temperature is too high, there is a shrink of the large pores of the electrocoagulant gel resulting in more compact flocs that are more likely to deposit on the surface of the electrode resulting low removal efficiency and higher energy consumption (Vasudevan et al. 2012).

### Presence of Sodium Chloride

Sodium chloride is usually added to the water to increase the conductivity of the water or wastewater to be treated (Holt et al. 2005; Lakshmanan et al. 2009; Kobya et al. 2010). The presence of chloride ions could significantly reduce the adverse effect of other anions such as HCO$_3$$^-$ and SO$_4$$^{2-}$. The presence of the HCO$_3$$^-$ and SO$_4$$^{2-}$ ions would lead to the precipitation of Ca$^{2+}$ or Mg$^{2+}$ ions that forms an insulating layer on the surface of the electrodes resulting increase in energy consumption. To overcome this difficulty, it is recommended that the addition of 15–20 % Cl$^-$ avoids the precipitation and lower the energy consumption. Apart from the above the electrochemically generated chlorine was found to be effective in water disinfections.

**Type of Current**

In general, direct current (DC) is used in an electrocoagulation processes. In this case, an impermeable oxide layer may form on the cathode as well as corrosion formation on the anode due to oxidation. These prevent the effective current transport between the anode and cathode, so the removal efficiency of electrocoagulation processes declines. These disadvantages of DC have been overcome by adopting alternating current (AC) in electrocoagulation processes (Vasudevan et al. 2011a, c).

**Applications**

Electrocoagulation process has been widely used in textile wastewater, refractory oily wastewater, urban wastewater, restaurant wastewater, wasters loaded with phenol, laundry effluent. Likewise, it has been used to remove color, clay removal, removal of toxic metals, inorganic metals removal and drinking water treatment (Vasudevan et al. 2009a, 2010a, b, c, 2011a, b, 2012b).

### 2.3.4  How Electrochemistry Is the Cause and Cure for Soil Pollution?

One of the consequences of industrialization, in present case electrochemical industrialization, is that soil near industries tends to become contaminated. Such contamination occurs, for example, where a number of plants or industries like metal finishing and battery industries. These industries use inorganic and organic materials in their regular use. Metals like lead, chromium, cadmium, tin, arsenic, cyanide etc., are the very frequently used contaminants in these industries. Mismanagement allows the contaminating ions to reach the surrounding soils, where they gradually spread like a strain on a fabric. Electrochemical remediation is a promising remediation technology for soils contaminated with inorganic, organic, and mixed contaminants. In general, a direct-current electric field is imposed on the contaminated soil to extract the contaminants by the combined mechanisms of electroosmosis, electromigration, and/or electrophoresis.

Electrochemical remediation viz., electrokinetic technique (Fig. 2.6) has been widely used to remove inorganic and organic contaminants from wet solid matrix viz., soils and sludge (Yang and Liu 2001). This process involves the application of direct current across the contaminated soil or sludge through anode and cathode in the subsurface (Ko et al. 2000). The main electrode reactions are generally oxygen evolution at the anode and hydrogen evolution at the cathode. In this process the contaminants are removed by the combination of electroosmotic flow of the pollutant, electromigration of contaminants carrying ionic charges in the liquid phase and electrophoresis of the charged particles carrying contaminant on their surfaces. During electrokinetic process, the hydrogen and hydroxyl ions are

**Fig. 2.6** Schematic illustration of electrochemical soil remediation

transported into soil by the applied direct-current resulting a low pH environment near the anode and a high pH environment near the cathode. The acid solution will release heavy metals and other cations that are sorbed on negatively charged clay surfaces, the cations migrating towards the cathode (Yang and Long 1999). The alkaline environment causes precipitation of most heavy metals and radionuclides, unless they form negatively charged complexes. These complexes migrate towards the anode and will form free metal ions again, when they meet the acid environment. In this way species are concentrated in the region where the two pH-environments meet and pH changes abruptly (Wang et al. 2005; Yang et al. 2005).

### 2.3.5 Electrochemical Removal of Gaseous Pollutants

Gaseous pollutants may also be removed electrochemically, provided that they are first dissolved into an electrolyte (Kreysa et al. 1994; van Velzen et al. 1990). The overall process will then generally consist of at least two steps: absorption of the gaseous species in a liquid and the subsequent electrochemical conversion of them to less harmful products.

**Removal of SO$_2$ by Electrochemical Methods**

For controlling problem of acid rains resulting essentially from sulphur dioxide emissions from fluestacks, especially in coal-fired thermal stations for power production two methods are available. In the first process, molten salt electrolysis of potassium pyrosulphate is envisaged and the second process utilizes Br$_2^-$.

In the first process, the cell comprises of an immobilized molten salt electrolyte viz, potassium pyropsulphate and porous gas diffusion electrodes formed a perovskite type.

The temperature of operation is 350–400°C at a current density of 5 A/dm$^2$. The cell voltage is <0.5 V with an energy consumption of 420 KWh/T of sulphurdioxide. The sulphur dioxide removal efficiency is 99 % However SO$_3$ is the byproduct gas at the cathode chamber.

This is a continuous one step operation with the following advantages.

1. Salable by product with no waste production
2. On-line temperature operation with no cooling or reheating of the flue gas.
3. Electricity is the only added reagent.

The Second process for removing sulphur dioxide from the flue gases uses anodically generated bromine from the electrolysis of hydrogen bromide in an undivided filter press reactor. The reactor consists of 34 anode of 1 m area each. The overall reaction of the process in the conversion of SO$_2$ and water is to form H$_2$SO$_4$

$$SO_2 + Br_2 + 2H_2O \rightarrow H_2SO_4 + 2HBr \qquad (2.19)$$

The intermediate Br$_2$ is electrolytically regenerated in the membrane cell from HBr.

$$2HBr \rightarrow H_2 + Br_2 \qquad (2.20)$$

Thus the advantage of this process is the production of the hydrogen as a byproduct in addition to value added H$_2$SO$_4$ the pilot facility treat flue gas having minimum SO$_2$ content of 0.016 % by volume.

Direct electrochemical oxidation creates blocking of electrodes by sulphur. Hence the new process developed consists in dissolving hydrogen suphide in sodium hydroxide and oxidizing electrochemically in two compartment cell with Nafion membrane and carbon electrodes (Current density: 10 A/dm$^2$) to get sulphur and hydrogen. The voltage of cell is 0.5 V and a current efficiency of 94 % is achieved.

**Removal of CO$_2$ by Electrochemical Methods**

In recent years the amount of carbon dioxide in the air has been increasing, which may adversely affect the environment in the future. Carbon dioxide is the largest contributor to the greenhouse effect. Moreover carbon dioxide is a very stable, linear molecule in which the oxygen atoms are week Lewis bases and carbon is electrophilic. In general, this carbon dioxide electroreduction is of interest as a potential component of a carbon energy cycle. The electroreduction of carbon dioxide at various metal electrodes yield many kinds of organic substances, namely CO, CH$_4$, C$_2$H$_6$, EtOH, other alcohols etc., four classes of metallic electrodes in aqueous electrolytes and three classes for non-aqueous media can be distinguished. Thus in aqueous solutions:

– Metallic In, Sn, Hg and Pb are selective for the production of formic acid
– Metallic Zn, Au and Ag produce carbon monoxide
– Metallic Cu exhibits a high electrocatalytic activity for the formation of hydro-
  carbons, aldehydes and alcohols
– Metallic Al, Ga and Group VIII elements (except Pd) show electrocatalytic
  activity in carbon dioxide reduction.

In non-aqueous electrolytes:

– On Pb, Tl and Hg the main product is oxalic acid
– On Cu, Ag, Au, In, Zn and Sn CO and carbonate ions are obtained
– On Ni, Pd and Pt are selective for CO formation
– On Al, Ga and Group VIII elements (except Ni, Pd and Pt) form both CO and
  oxalic acid.

In general, metal cathodes suffer corrosion or passivation problems that avoid
its use as electrocatalysts, during long terms electrolysis. To overcome these
difficulties, a number of metal transition complexes have been investigated as
catalysts for the reduction of carbon dioxide.

## 2.3.6  Anodic Processes

Now-a-days, the presence of organic and inorganic pollutants are recalcitrant to
treat through either chemical or biological treatments together with the stringent
regulations imposed by new legislation have caused much research work to focus
on waste-water treatment by electrooxidation processes. Electrochemical oxidation
has been found to be an environmentally friendly technology able to mineralize
completely non-biodegradable organic matter and to eliminate nitrogen species.
Electrochemical oxidation of pollutants can take place through two different routes
viz., (1) direct anodic oxidation, where the pollutants are destroyed at the anode
surface; (2) indirect oxidation where a mediator (HClO, $O_3$, $H_2S_2O_8$, etc.,) is
electrochemically generated to carry out the oxidation. It has to be kept in mind that
during electro-oxidation of aqueous effluents, sometime both oxidation processes
may coexist.

**Direct Oxidation Pathways**

In a direct oxidation pathway, electrooxidation of pollutants can occur directly
on anodes by generating physically adsorbed "active oxygen" (adsorbed hydroxyl
radicals, $^\bullet$OH) or chemisorbed "active oxygen" (oxygen in the oxide lattice,
$MO_{x+1}$) (Comninellis 1994). The physically adsorbed "active oxygen" causes the

complete combustion of organic compounds, and the chemisorbed "active oxygen" ($MO_{x+1}$) participates in the formation of selective oxidation products. In general, $^\bullet$OH is more effective for pollutant oxidation than oxygen in $MO_{x+1}$. Because oxygen evolution can also take place at the anode, high overpotentials for oxygen evolution is required in order to destroy organic pollutants to proceed with high current efficiency. Otherwise, most of the current supplied will be wasted to split water.

In direct anodic oxidation processes, the important part is the anode material. Different anode materials like granular graphite (Awad and Abuzaid 1997), planar graphite (Kannan et al. 1995), Pt or Ti/Pt (Marincic and Leitz 1978), $PbO_2$ (Sharifian and Kirk 1986), $Ti/PbO_2$ (Cossu et al. 1998), $IrO_2$ (Pulgarin et al. 1994), $Ti/SnO_2$–$Sb_2O_5$ (Cossu et al. 1998), Ebonex® (Chen et al. 1999), glassy carbon (Gattrell and Kirk 1990), $Ti/RuO_2$, Ti/Pt–Ir (Murphy et al. 1992), fiber carbon (Szpyrkowicz et al. 1994), $MnO_2$ (Rajalo and Petrovskaya 1996), Pt–carbon black (Boudenne and Cerclier 1999), porous carbon felt (Polcaro and Palmas 1997), reticulated vitreous carbon (Hofseth and Chapman 1999) and stainless steel (Abuzaid et al. 1999) were investigated for their suitability in the oxidation process.

### Indirect Oxidation Pathways

The aim of indirect oxidation is to prevent fouling of electrode, avoiding direct electron exchange between the pollutant and the anode surface and to utilize the electrocatalytic potential of other anode materials. Therefore, the pollutants are oxidized through the mediation of some electrochemically generated redox reagents, which act as intermediaries for transfer of electrons between the electrode and the pollutant (Panizza and Cerisola 2009). Oxidation mediators can be metallic redox couples such as Ag(II/I), Ce(IV/III), Co(III/II), Fe(III/II), and Mn(III/II), or strong oxidizing chemicals such as active chlorine species, ozone, hydrogen peroxide, persulphate, percarbonate, and perphosphate.

### Mediated Oxidation by Metallic Redox Couples

When metallic redox couples are applied as redox reagents the process is typically termed mediated electrochemical oxidation (MEO). Metal ions in acidic solutions are anodically oxidized from their stable oxidation state ($M^{2+}$) to the higher reactive oxidation state ($M^{(2+1)+}$) in which they attack the organic feed, degrading it into carbon dioxide, inorganic salts, and water:

$$M^{2+} \rightarrow M^{(2+1)+} + e^- \tag{2.21}$$

$$M^{(2+1)+} + R \rightarrow M^{2+} + CO_2 + H_2O \tag{2.22}$$

Reaction (2.22) returns the couple back to a stable state ($M^{2+}$) and it is then recycled through the cell in order continuously to regenerate further reaction. MEO through metallic redox reagents is mainly applied for pollution abatement in divided cells, where the anode and cathode are separated by a porous separator or an ion-exchange membrane in order to prevent reduction of the oxidative agent at the cathode. The MEO process is preferably used to treat solid waste or highly concentrated solutions with an organic content higher than 20 %, in order to avoid or limit the need for implementation of a solution-solution separation step to retain the key metallic ions from the discharged solution. For total organic oxidation, a redox couple with a high oxidation potential must be chosen as for instance the Ag(I/II) ($E^0 = 1.98$ V). Other redox couples which have been demonstrated to provide full mineralization of various organic pollutants are Co(III/II) and Ce(IV/III). Redox couples more abundant in natural waters as Fe(III/II) and Mn(III/II) are not suited for total oxidation of organics, because of their low oxidation potential ($E_0 = 0.77$ V and $E_0 = 1.56$ V respectively), but they can be used for selective partial oxidation of organic compounds (Panizza and Cerisola 2009).

**Mediated Oxidation by In-Situ Generated Chemical Oxidants**

Wastewater effluents containing low concentrations of organic pollutants (organic content less than 20 %) can be treated by indirect electrolysis generating in situ chemical reactants and converting them into less harmful products (Panizza and Cerisola 2009). The oxidizing chemicals can be electrogenerated either by anodic oxidation such as formation of active chlorine, ozone, and persulphate, or by cathodic reduction such as formation of hydrogen peroxide. The oxidants like hydroxyl radical ($H_2O/^\bullet OH$), ozone ($O_2/O_3$), peroxodisulphate ($SO_4^{2-}/S_2O_8^{2-}$), hydrogen peroxide ($H_2O/H_2O_2$), permanganate ion ($MnO_2/MnO_4^{-}$), hypochlorous acid ($Cl^-/HOCl$), chlorine ($Cl^-/Cl_2$), dichromate ($Cr^{3+}/Cr_2O_7^{2-}$), oxygen ($H_2O/O_2$), hypochlorite ($Cl^-/ClO^-$) are used as oxidants and their respective standard reduction potentials are 2.80, 2.07, 2.01, 1.77, 1.67, 1.48, 1,36, 1.23, 1.23 and 0.81 V. Due to the natural abundance of chloride in most polluted waters, chlorine-mediated oxidation is the most widely employed in-situ generated chemical oxidant for wastewater treatment. The generation reactions are,

$$2Cl^- \rightarrow Cl_2 + 2e^- \tag{2.23}$$

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \tag{2.24}$$

$$HOCl \leftrightarrow OCl^- + H^+ \tag{2.25}$$

The most used electrode materials for in-situ generation of active chlorine are based on platinum or on a mixture of noble metal oxides (e.g., $RuO_2$, $TiO_2$, and $IrO_2$) that have a good electrocatalytic properties for chlorine evolution as well as long-term mechanical and chemical stability.

Ozone is another strong chemical oxidant that can be electrochemically produced in-situ by anodic oxidation. Due to its high oxidation potential it has tremendous application in water treatment. It can be generated by,

$$3H_2O \rightarrow O_3 + 6H^+ + 6e^- \tag{2.26}$$

$$O_2 + H_2O \rightarrow O_3 + 2H^+ + 2e^- \tag{2.27}$$

Persulphate ($S_2O_8{}^{2-}$), percarbonate ($C_2O_6{}^{2-}$), and perphosphate ($P_2O_8{}^{4-}$) are various other strong oxidants produced electrochemically and used in water treatment. These oxidants are produced by anodic oxidation of the corresponding anions in the solution, but they are only generated efficiently by non-active anodes as the BDD and $PbO_2$ electrodes, due to the need of high oxygen overpotentials in order to reach the formation potentials.

$$2SO_4{}^{2-} \rightarrow S_2O_8{}^{2-} + 2e^- \tag{2.28}$$

$$2CO_3{}^{2-} \rightarrow C_2O_6{}^{2-} + 2e^- \tag{2.29}$$

$$2PO_4{}^{2-} \rightarrow P_2O_8{}^{4-} + 2e^- \tag{2.30}$$

Example for Anodic Process: Destroying Cyanide Waste

Destroying cyanide waste is an example for anodic oxidation for both direct and indirect oxidation pathways (Tissot and Fragniere 1994). Different cyanide compounds are commonly used in the metal finishing industry and it is absolutely essential to destroy the cyanide in the waste water disposal. One way of accomplishing the destruction of cyanide waste is to oxidize the cyanide electrolytically at high temperature ($\sim$80°C) for several days, thereby getting the harmless products of carbon dioxide and nitrogen as given by the equation:

$$2CN^- + 8OH^- \rightarrow 2CO_2 + N_2 + 4H_2O + 10e^- \tag{2.31}$$

However, in this process the complete destruction of the cyanide may be difficult especially towards the end when cyanide concentration is low. However, it is reported that the destruction of residual cyanides at low levels can be achieved by allowing sufficient treatment time (Tissot and Fragniere 1994).

In the indirect method, which is quicker, the cyanide is oxidized to harmless nitrogen and carbon dioxide by electrolyzing the waste along with sodium chloride solution of concentration 10–70 g/1 in a cell fitted with graphite anode and stainless steel cathode; the current density being 2–13 A/dm$^2$. The hypochlorite generated electrolytically oxidizes the $CN^-$ as follows:

$$2CN^- + 5ClO^- + H_2O \rightarrow 2CO_2 + N_2 + 5Cl^- + 2OH^- \tag{2.32}$$

By this method, not only the cyanide but also the metal ion constituents of the wastes can be removed in one step. Complete destruction of $CN^-$ is accomplished in the process and the heavy metal contaminants are deposited onto the cathode resulting nontoxic product for disposal.

Ozone is also used for the destruction of cyanide (Tissot and Fragniere 1994) and the ozonation of cyanide involves two separate chemical steps, oxidation of cyanide to cyanate and oxidation or hydrolysis of cyanate to nitrogen or ammonia.

$$CN^- + O_3 \rightarrow OCN^- + O_2 \tag{2.33}$$

$$OCN^- + OH^- + H_2O \rightarrow CO_3 + NH_3 \tag{2.34}$$

### 2.3.7 Cathodic Processes

Cathodic processes (Drogui et al. 2007) can be divided into two categories viz., electrodeposition and cathodic electrochemical dechlorination. Electrodeposition refers to electrochemical removal or recovery of metal contaminants from water and waste water. This technology is widely used either to remove heavy/toxic metals like Cd, Pb, Cu, Cr, etc., or recover precious metals like Pt, Au etc., from wastewater. The principle behind for the metal recovery is basically the cathodic reduction process. Metals are formed and deposited on cathode according to the following reaction,

$$M^{n+} + ne^- \rightarrow M_{(s)} \tag{2.35}$$

where 'M' represents the metal, 'n' represents the valence of the metal and '$M_{(s)}$' is the metal deposited on the cathode surface. Under some conditions, the system favors the formation of metal hydroxides instead of metal deposition due to local alkaline pH near the cathode surface. These metal hydroxides can be removed from the solutions by adsorption on the electrode as charged colloidal particles. The metal deposition is always depends on the thermodynamics and the kinetics of the system. Certainly, the ranking of the potential on the electrochemical series determines the relative reduction power of the species. For example, Cr, Pb, Cd, Zn and Cu are present in the wastewater, the metal deposition follows the electromotive force (EMF) series as: Cu > Pb > Cd > Cr > Zn. Apart from the EMF, other parametric conditions like metal concentration, electrode material and affinity existing between the pollutant influence the deposition. The main advantage of the electrodeposition is the deposited metals can be easily recycled by electrometallurgical process. The disadvantage of the process is the surface of the cathode is modified during electrodeposition and it needs additional process control.

Dechlorination of organic toxicants can occur not only by anodically but also cathodically, proved using graphite/carbon as electrode material. It was concluded that the dehalogenation results in a decreased toxicity and an increased biodegradability,

thus enabling further biological treatment. It is concluded that, energy consumption and conversion rates are such that a technically and economically viable method for the detoxifation of waste waters can be developed.

### 2.3.8 Aerogel and Electrochemistry for Cleaning Contaminated Water

The method called capacitive deionization (CDI), consumes only 16–32 Wh/gal. In CDI, contaminated water flows in a serpentine manner down stack of electrochemical cells. Attached to each side of the electrodes is a sheet of carbon aerogel, a highly porous material that has very large surface area. The aerogel is made by dipping a thin carbon sheet in a mixture of resorcinol and formaldehyde and then carbonizing at around 1,000°C in a vacuum or inert atmosphere. A prototype system has reduced salt concentration 100 mg/L <1 mg/L a single pass. The aerogel is regenerated by switching off the voltage, which put the ions back into solutions (Wang et al. 1993).

### 2.3.9 Photo-Electrochemical Methods

In recent years, photo-electrochemistry has led to a new and interesting possibility for treatment of pollutants from wastewater. In this case, suspensions of semiconductor particles (mostly $TiO_2$) can be used to harness the light with production of electrons and holes in the solid, which can destroy pollutants by means of reduction and oxidation, respectively. In this way, water containing organic, inorganic or microbiological pollutants can be effectively treated (Malato et al. 2009).

### 2.3.10 Electrochemical Advanced Oxidation Processes (EAOPs)

In the case of industrial and/or agricultural wastewater treatment, chemical oxidation is often necessary to remove organic matter (biodegradable or not) that consumes oxygen dissolved in water. The oxidations by ozone or hydrogen peroxide ($H_2O_2$) are methods used as complement or in competition with the activated carbon filtration or nanofiltration. But in some cases, the conventional oxidation is still inadequate and remains inefficient. Moreover, the conventional oxidation treatments as well as biological treatments prove ineffective against certain types of organic micropollutants. To eliminate these kind pollutants from water, more powerful processes, namely advanced oxidation processes (AOPs) have been developed. The AOPs are based on the in situ generation of strong oxidizing agent hydroxyl radicals ($^\bullet OH$) and their high oxidation power. The use of $^\bullet OH$ in the

treatment/remediation of contaminated water is justified by a number of advantages: (i) they are not toxic (very short lifetime); (ii) they are simple to produce and use; (iii) they are very efficient to remove organic pollutants: the main feature of these powerful species is their ability to transform refractory inorganic compounds to biodegradable products; (iv) they are not corrosive to the equipment; (v) they do not induce secondary pollution: the final products of oxidation are $CO_2$ and $H_2O$, and inorganic ions (mineralization).

The term "AOPs" have been firstly defined by (Glaze et al. 1987) as "near ambient temperature and pressure water treatment processes which involve the generation of a very powerful oxidizing agent such as hydroxyl radical ($^\bullet$OH) in solution in sufficient quantity to effective water purification". This concept has been further extrapolated to electrochemical systems generating heterogeneous $^\bullet$OH on the anode surface under the application of high current density. AOPs are applied whenever conventional oxidation techniques are insufficient, when process kinetics becomes very slow, or because contaminants are refractory to chemical oxidation in aqueous medium or partially oxidized yielding stable byproducts showing even greater toxicity than the starting pollutants. Indeed conventional oxidation techniques (use of chemical oxidants like $H_2O_2$, $Cl_2$, $ClO_2$, $O_3$, etc. . . . ) prove ineffective against persistent organic pollutants (POPs) such as pesticides, polycyclic aromatic hydrocarbons (PAHs), synthetic dyes, polychlorobipenyles (PCBs), etc. (Pera-Titus et al. 2004). Contrariwise, the AOPs are able to oxidize any organic and organometallic substances until the final stage of oxidation, i.e., their transformation into $CO_2$, water and inorganic ions. They constitute a means to effectively treat effluents containing toxic and biorefractory pollutants. However, it must also be taken into consideration that the oxidation ability of most AOPs diminishes considerably when treating high organic matter contents ($>5.0$ g $L^{-1}$), thereby requiring the consumption of excessive amounts of expensive reactants that makes the treatment less cost affordable.

Electrochemistry constitutes one of the clean and effective ways to produce in situ hydroxyl radical ($^\bullet$OH), a highly strong oxidizing agent of organic matter in waters. Due to its very high standard oxidation power ($E°($$^\bullet$OH/$H_2O) = 2.80$ V/SHE), this radical species is able to react non-selectively with organic or organometallic pollutants yielding dehydrogenated or hydroxylated derivatives, which can be in turn completely mineralized, i.e., converted into $CO_2$, water and inorganic ions.

Recently, the electrochemical advanced oxidation processes (EAOPs) have received great attention by their environmental safety and compatibility (operating at mild conditions), versatility, high efficiency and amenability of automation (Brillas et al. 2009).

## Anodic Oxidation

Anodic oxidation constitutes a direct way to generate hydroxyl radical by electrochemistry. This technique does not use any chemicals, hydroxyl radicals are formed

by oxidation of water on high $O_2$ evolution overvoltage anodes (Pera-Titus et al. 2004; Martínez-Huitle and Brillas 2008; Oturan 2000a). Firstly Pt and $PbO_2$ anodes were investigated and on the obtained experimental results (Marselli et al. 2003) proposed the following mechanism for the oxidation of organics with concomitant oxygen evolution, assuming that both formation of $^{\bullet}OH$ and oxygen evolution take place on such anodes from the discharge of water (Reaction 2.36). Formed $^{\bullet}OH$ are heterogeneous since they are adsorbed on anode material (M) and react with organic matter (R) leading to its mineralization, i.e. transformation of R to $CO_2$ and $H_2O$ (Reaction 2.37), when R does not contain heteroatom.

$$M + H_2O \; \rightarrow \; M(^{\bullet}OH) + H^+ + e^- \qquad (2.36)$$

$$M(^{\bullet}OH) + R \; \rightarrow \; M + mCO_2 + nH_2O \qquad (2.37)$$
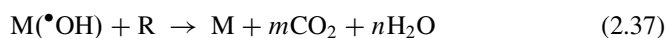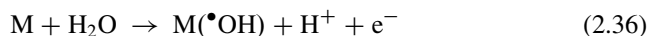
Recently a new and powerful anode material, the boron-doped diamond (BDD) thin film anode appeared as an emerging anode material. This anode possesses very good properties for the electrochemical treatment of wastewaters contaminated by organic pollutants. It has a great chemical and electrochemical stability, a wide electrochemical working range. It has been shown that this new anode has an $O_2$ overvoltage much higher than that of conventional anodes such as Pt, $PbO_2$, doped $SnO_2$, and $IrO_2$ (Canizares et al. 2004). Thus the BDD anode is able to produce larger amount of $^{\bullet}OH$ physisorbed on anode surface, $BDD(^{\bullet}OH)$, from Reaction (2.36) that are more labile and reactive compared other anode materials, leading to a rapid and efficient destruction of organic pollutants. The effectiveness of the BDD anode was shown for the oxidation of a wide range of pollutants (Ozcan et al. 2008; Canizares et al. 2008) with almost mineralization of treated solutions. These studies were highlighted that the current efficiency is influenced by applied current density and initial pollutant concentration, low organic concentration and high applied current density favoring the mineralization degree.

**Electro-Fenton Process**

The second EAOPs is the electro-Fenton process in which hydroxyl radicals are generated indirectly via Fenton's reagent (mixture of $H_2O_2$ and iron(II)) in homogeneous medium. $H_2O_2$ is electrogenerated in-situ by 2-electron reduction of dissolved $O_2$ in acidic medium (Reaction 2.38) in presence of a catalytic amount of ferrous ions (Oturan et al. 2000). The most widely applied cathode materials for the effective $H_2O_2$ are based on carbonaceous materials (carbon felt, reticulated vitreous carbon (RVC), carbon sponges, carbon nanotubes (NT), or graphite).

$$O_2 + 2H^+ + 2e^- \; \rightarrow \; H_2O_2 \qquad (2.38)$$
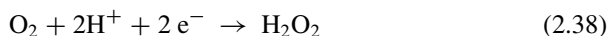
Compared to other chemical oxidant, $H_2O_2$ is a weak oxidant, but its oxidation power is enhanced in presence of $Fe^{2+}$ ions (Fenton 1894) via Fenton's reaction:
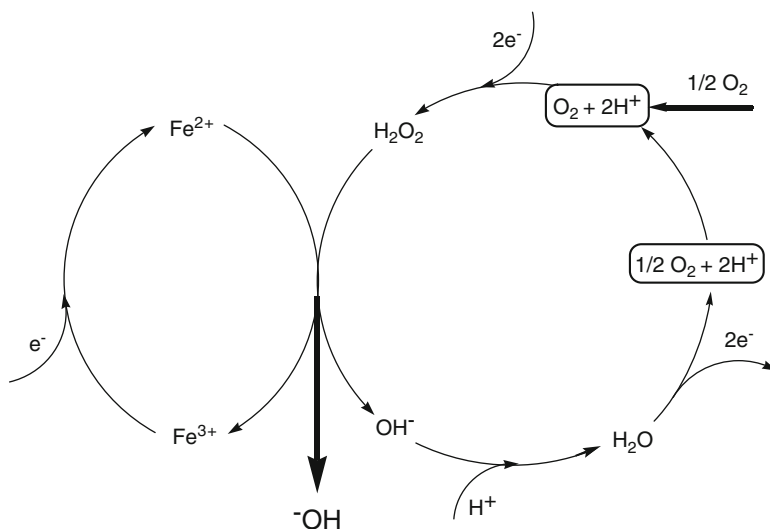
**Fig. 2.7** Schematic representation of the electrocatalytic production of hydroxyl radicals by the electro-Fenton process (Oturan et al. 2000)

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + {}^\bullet OH \qquad (2.39)$$

This enhancement is attributed to the formation of highly powerful oxidant hydroxyl radical via Reaction (2.39). $Fe^{3+}$ formed by this reaction is reduced electrochemically at the cathode to regenerate ferrous iron in order to catalyse Fenton's reaction:

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \qquad (2.40)$$

The simultaneous generation of $H_2O_2$ and regeneration of ferrous iron at the cathode allow continuous and catalytic formation of hydroxyl radicals that react on organic pollutants by hydrogen atom abstraction or addition reactions:

$$\text{Organic pollutants} + {}^\bullet OH \rightarrow \text{oxidation intermediates} \qquad (2.41a)$$

$$\text{Intermediates} + {}^\bullet OH \rightarrow \rightarrow \rightarrow CO_2 + H_2O + \text{inorganic ions} \qquad (2.41b)$$
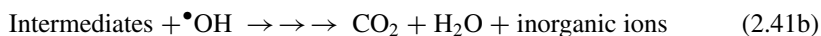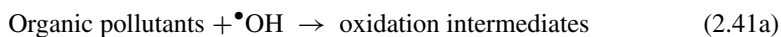
When the anode is Pt, the $O_2$ needed for the Reaction (2.38) to produce $H_2O_2$ is formed on the anode by oxidation of water (Oturan et al. 2001). Thus, the electro-Fenton process constitutes an overall catalytic system. Both catalytically cycle taking place during this system for the continuous regeneration of $Fe^{2+}$ and $H_2O_2$ in order to produce hydroxyl radicals via Fenton reaction are schematized in Fig. 2.7.
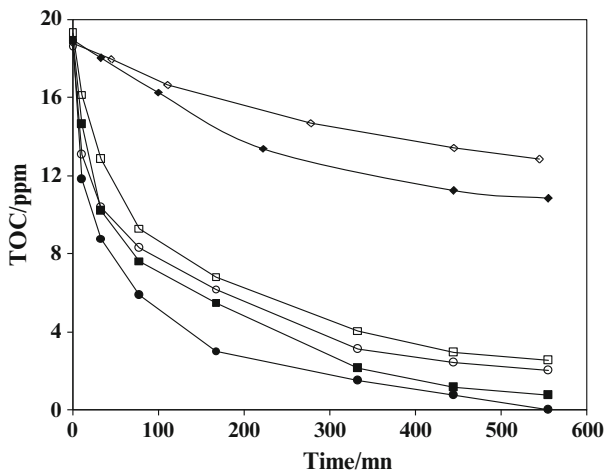
**Fig. 2.8** Effect of pH and medium on TOC removal efficiency during degradation of 0.2 mM methyl parathion (insecticide) aqueous solution by electro-Fenton process at I = 150 mA. pH: 1 ($\Diamond$); 3 ($\circ$) and 4 ($\square$) in $H_2SO_4$ medium; pH: 1 ($\blacklozenge$); 3 ($\bullet$) and 4 ($\blacksquare$) in $HClO_4$ medium (Diagne et al. 2007). *TOC* total organic carbon

### Effect of Operating Parameter on the Process Efficiency

The electro-Fenton process is governed by a number of operating parameters, the most important being solution pH, applied current, catalyst concentration, medium (supporting electrolyte) and initial organic content.

Effect of Solution pH and Medium

The solution pH is one of key parameter in electro-Fenton process. Now the optimal value of this parameter is well known. The value of pH 3 was suggested firstly (Sun and Pignatello 1993) and confirmed thereafter by other groups (Diagne et al. 2007). This value is valid for the Fenton process and all related process including electro-Fenton process (Fig. 2.8). However electro-Fenton process can be carried out more or less effectively in the range of 2.5–3.5. It is suggested that iron ions (catalyst) are lost from the solution by precipitation for pH > 4. For pH < 2.5 the efficiency of the process decreases; the lower effectiveness can be attributed to the formation of the peroxonium ion ($H_3O_2^+$) from the following reaction (Feng et al. 2003) making $H_2O_2$ electrophilic, enhancing its stability and reducing its reactivity with $Fe^{2+}$ in the Fenton reaction:

$$H_2O_2 + H^+ \rightarrow H_3O_2^+ \tag{2.42}$$

The lower pH values promote also the waste Reaction (2.43) and decreasing process efficiency (Tang and Huang 1996):

$$H_2O_2 + {}^\bullet OH \rightarrow HO_2{}^\bullet + H_2O \tag{2.43}$$
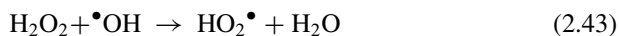
Figure 2.8 shows also the effect of the supporting electrolyte. $HClO_4$ is not a complexant for iron ions. When the electrolyte anions acts as complexing agent (such as $SO_4^{2-}$ or $Cl^-$) of iron ions, the free iron ion concentration decreases in the solution as in the case of $H_2SO_4$, leading to a decrease in the process efficiency.

Effect of Applied Current

The applied current (or current density) is another key parameter of electro-Fenton process, because the formation rate of $H_2O_2$ (Reaction 2.38) as well as the regeneration rate of ferrous iron (Reaction 2.40), and consequently the generation rate of ${}^\bullet OH$ are governed by the value of the applied current. In a general way, the process efficiency increases with applied current till a given value, since more ${}^\bullet OH$ are generated. Then the increase of applied current does not results better results because of enhancement of wasting reactions such as $H_2$ evolution at the cathode (Fig. 2.9) so the optimal current value should be determined experimentally for degradation kinetics or for mineralization efficiency before each trial (Özcan et al. 2010). As shown on Fig. 2.9a, the total disappearance of 0.5 mM (96 ppm) clopyralid is reached at 20 min under 300 and 500 mA applied current. Thus the optimal current value can be taken as 300 mA under operating conditions of Fig. 2.9. The Fig. 2.9b indicates the effect of initial organic matter on the mineralization efficiency. At the same experimental conditions the almost mineralization of 1.5 mM (288 mg $L^{-1}$) clopyralid solution was reached at 120 min while that of 3 mM (576 ppm) solution needed more longer time of 240 min at 300 mA applied current.

Effect of Catalyst Concentration

The catalyst concentration becomes an influent parameter particularly in the case of ferrous ($Fe^{2+}$) or ferric ($Fe^{3+}$) iron as catalyst. As shown on the Fig. 2.10 for the case of phenylurea herbicide fenuron, the degradation rate increases by decreasing the catalyst ($Fe^{3+}$) concentration from 0.1 to 1.0 mM (Oturan et al. 2010). The treatment time needed for complete destruction of 0.2 mM fenuron is three fold higher in the case of 1.0 mM catalyst concentration compared to 0.1 mM. This behavior can be explained by the parasitic reaction between $Fe^{2+}$ and ${}^\bullet OH$.

$$Fe^{2+} + {}^\bullet OH \rightarrow Fe^{3+} + OH^- \tag{2.44}$$
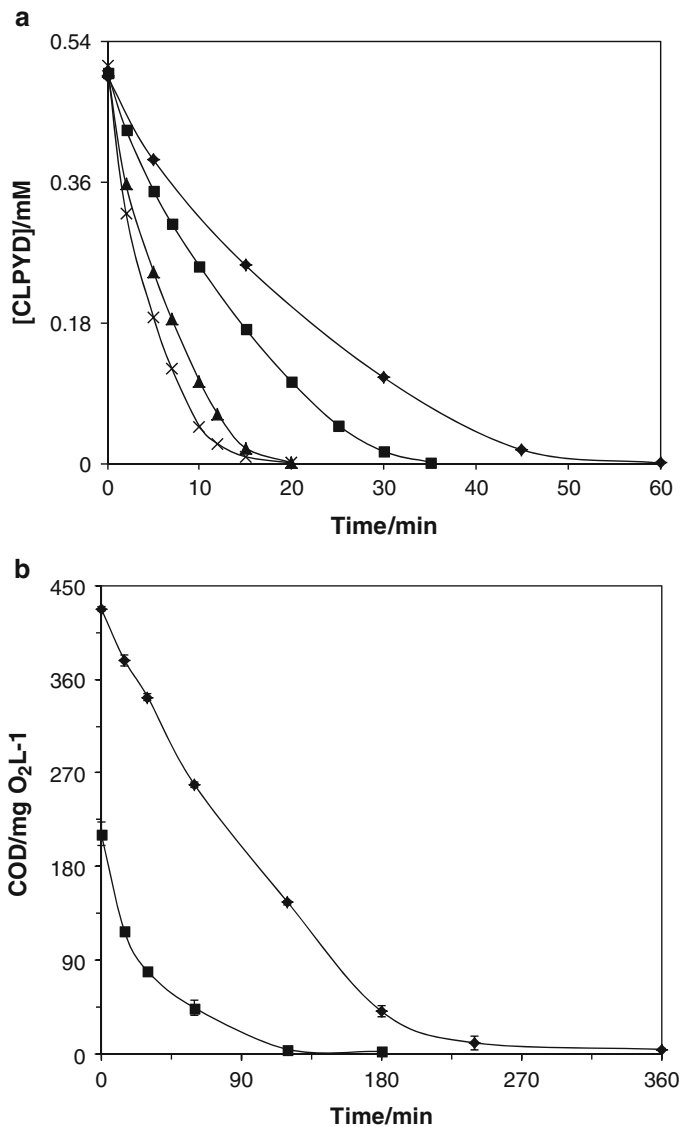
**Fig. 2.9** Oxidative degradation/mineralization of 0.5 mM Clopyralid (systemic herbicide) aqueous solution by electro-Fenton process. (**a**) effect of applied current on degradation kinetics, I: 60 (◆), 100 (■), 300 (▲) and 500 (×) mA, and (**b**) Time course of COD removal for initial concentrations of 1.5 mM (■) and 3.0 (◆) mM aqueous clopyralid solutions at I = 300 mA. Operating conditions: [Na$_2$SO$_4$]: 0.05 M, [Fe$^{3+}$]: 0.1 mM, V: 0.15 L, pH: 3, T: 35 °C
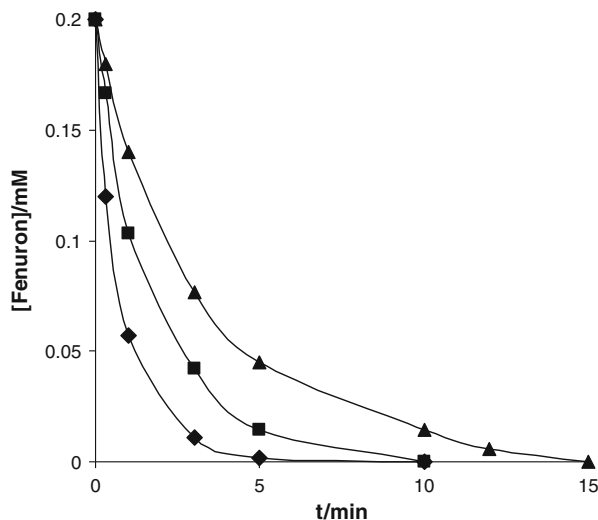
**Fig. 2.10** Effect of the catalyst ($Fe^{3+}$) concentration on the degradation kinetics of 0.20 mM fenuron (phenylurea herbicide) solution during the electro-Fenton process at 100 mA and room temperature [243]. $C_0$ (mM): 0.2 (♦); 0.5 (■); 1 (▲)

Reaction (2.44) becomes competitive with organic pollutants to be treated for ·OH in the case of high ferrous iron concentration. In the case of ferric iron as catalyst, it is quickly reduced at the cathode to ferrous iron, so a high initial concentration of $Fe^{3+}$ results in higher $Fe^{2+}$ concentration in the solution leading to decrease of the process efficiency.

**Application to the Removal of Organic Micropollutants from Water**

Electro-Fenton process was successfully applied to removal of organic pollutants from water with high oxidation and/or mineralization efficiency, mainly by Oturan's and Brillas' groups. The mineralization of several organic pollutants such as pesticide active ingredients (Oturan et al. 1999; Özcan et al. 2008), pesticide commercial formulations (Oturan and Oturan 2005), synthetic dyes (Guivarch et al. 2003; Sirés et al. 2008; Marco and Oturan 2011), pharmaceuticals (Dirany et al. 2012), industrial pollutants (Oturan et al. 2000), landfill leachates (Trabelsi et al. 2012) etc., was thoroughly studied with almost mineralization efficiency in each case, showing that the electro-Fenton process can be constitutes an alternative when conventional treatment processes remain inefficient.
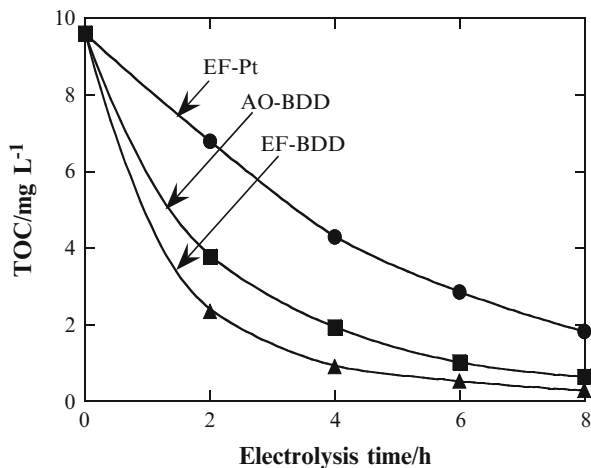
**Fig. 2.11** TOC abatement in function of the electrolysis during the mineralization of 0.1 mM atrazine solution by electro-Fenton process using Pt or BDD and a carbon-felt cathode. *AO-BDD* anodic oxidation with BDD anode, *EF-Pt* electro-Fenton process with Pt anode, *EF-BDD* electro-Fenton process with BDD anode (Following the reference Oturan et al. 2012)

The performance of electro-Fenton process can be significantly enhanced by the replacement of the classical anode Pt by the emergent anode BDD (boron doped diamond). This configuration offers two main advantages: (i) generation of supplementary heterogeneous BDD($^{\bullet}$OH) by Reaction (2.36) in addition those produced homogeneously in bulk solution, (ii) high oxidizing power of BDD($^{\bullet}$OH) than others M($^{\bullet}$OH) since they are physisorbed, and (iii) direct oxidation of organics because of high oxidation window (about 3 V). A striking example of the performance of electro-Fenton with BDD anode (Oturan et al. 2012) concerning the mineralization of herbicide atrazine. A large variety of AOPs have been applied to remove atrazine from aqueous medium for avoiding its negative impact on ecosystems and human health. However, chemical, photochemical and photocatalytic AOPs yielded the persistent cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine) as predominant by-product (De Laat et al. 1999). These studies reached to 40–60 % mineralization degree corresponding to the mineralization of side chains of atrazine. The anodic oxidation with a Pt anode converted atrazine into cyanuric acid (Mamián et al. 2009), whereas AO with a DSA anode allowed 46 % of total organic carbon (TOC) decay (Malpass et al. 2006). On the other hand, the electro-Fenton with BDD anode allowed not only almost total mineralization (97 %) of atrazine (Fig. 2.11), but also a high mineralization degree (90 % TOC removal) of cyanuric acid (Fig. 2.12) which is considered long ago as the end-products of atrazine mineralization and defined as refractory to $^{\bullet}$OH produced by AOPs. As can be seen on Fig. 2.12, electro-Fenton process with Pt anode leads only 4 % of TOC removal, evidencing the interest to use of BDD anode in electro-Fenton process.
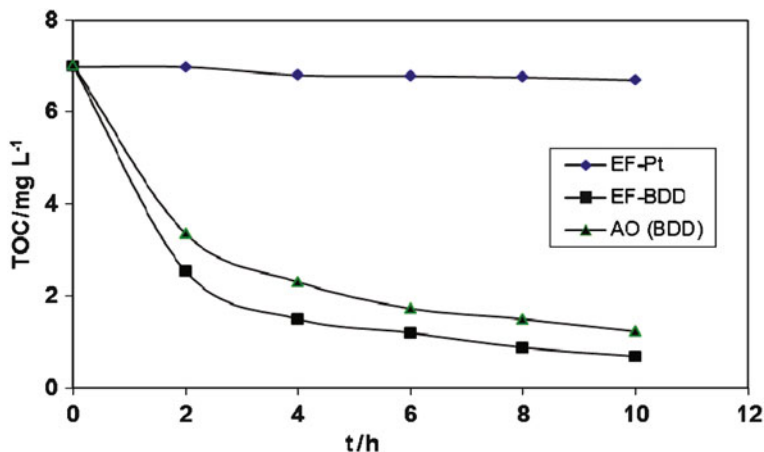
**Fig. 2.12** Mineralization of cyanuric acid by anodic oxidation with BDD anode and electro-Fenton using Pt (EF-Pt) and BDD (EF-BDD) anodes. [cyanuric acid]0 = 0.2 mM (25.8 ppm), I = 1 A

## Integrated Processes: Coupling of Electro-Fenton with Other AOPs

In order to enhance the efficiency of electro-Fenton process its coupling with other AOPs has been carried out. One of these integrated processes is the sonoelectro-Fenton in which the solution electrochemical reactor is simultaneously irradiated by ultrasounds (Oturan et al. 2008). Compared to the electro-Fenton sole, a significant synergetic effect was obtained by this coupling due to additional effect of enhancement of mass transfer rate to the electrode.

The most developed integrated process is the photoelectron-Fenton in which the electro-Fenton reactor is irradiated by an UV lamp (Ricardo et al. 2012). The interest is double: Firstly the generation of supplementary $^{\bullet}OH$ by irradiation of iron(III) $Fe(OH)^{2+}$ which is the predominant form at pH 3 (Reaction 2.45) and $H_2O_2$ (Reaction 2.46).

$$Fe(OH)^{2+} + hv \ \rightarrow \ Fe^{2+} + {}^{\bullet}OH \tag{2.45}$$

$$H_2O_2 + hv \ \rightarrow \ 2\,{}^{\bullet}OH \tag{2.46}$$
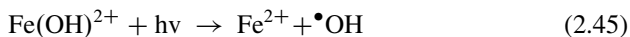
And secondly, the destruction of Fe(III)-carboxylic acid complexes (Reaction 2.47) resulting in ferrous ion regeneration and also in a significant decay in solution TOC content of the system due to the decarboxylation reaction (Oturan et al. 2011):

$$[Fe(RCO_2)]^{2+} + hv_{-} \rightarrow Fe^{2+} + CO_2 + R^{\bullet} \tag{2.47}$$
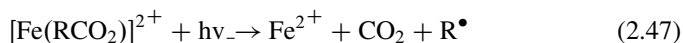
Recently solar photoelectro-Fenton process has attracted much attention (Ruiz et al. 2011). In this process, the electrical energy needed by electro-Fenton reactor was produced from solar energy in addition of natural solar irradiation of the solution. The process is particularly interesting for countries benefiting strong sunlight.

Peroxi-coagulation (or peroxi-electrocoagulation) constitutes another integrated method. This process involve an electro-Fenton like process using a sacrificial iron anode in order to generate iron(II) ions (Boye et al. 2003; Brillas et al. 2003) that react with cathollically generated $H_2O_2$ following Reaction (2.38). Then Fenton reaction (Reaction 2.39) takes place and produces $Fe^{3+}$ ions that induce the coagulation. Thus the pollutants are removed either by oxidation with $^\bullet OH$ produced from Fenton reaction (Reaction 2.39) and by coagulation.

## 2.4 Conclusion

Although electrochemistry, on one hand is the cause for pollution by the discharge of pollutants from electrochemical industries, its successful application to combat pollution supersedes the former and becomes really a cure in pollution prevention. Electrochemical technologies are vital and enabling discipline in many areas of environmental treatment, including: (a) clean synthesis, (b) monitoring of process efficiency and pollutants, (c) removal of contaminants, (d) recycling of pollutants, (e) water sterilization, and (f) clean energy conversion. The field of environmental electrochemistry has witnessed many progresses and has demonstrated many successes, as evidenced by the increasing literature. In this review, a variety of selected electrochemical processes for environmental protection of water, soil and air have been presented through electrodialytic processes, ion exchange assisted electrodialytic processes, electrocoagulation and electroflotation, electro remediation of soils, electrochemical removal of gaseous pollutants, anodic and cathodic processes, aerogel and electrochemistry for cleaning contaminated water, photo-electrochemical methods and electrochemical advanced oxidation processes. Finally, due to the specific advantages in a number of applications, electrochemical processes for the treatment of waste and prevention of pollution will find increasing acceptance in future developments.

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# Chapter 3
# Heterogeneous Photocatalysis
# for Pharmaceutical Wastewater Treatment

**Devagi Kanakaraju, Beverley D. Glass, and Michael Oelgemöller**

**Abstract** Environmentally sustainable solutions for wastewater management including the improvement of water quality and water recycling are considered key priority areas globally. The challenges facing our water resources are unprecedented due to the presence of organic and inorganic pollutants derived from numerous anthropogenic activities. This situation has been further complicated by emerging persistent contaminants such as pharmaceuticals which possess low biodegradability and resistance to chemical and biological treatments. Excretion of pharmaceuticals and their metabolites via human waste, improper disposal and veterinary applications is recognized as the principal sources of pharmaceuticals ending up in various compartments of the environment. Heterogeneous photocatalysis using semiconductor titanium dioxide ($TiO_2$) has proven to be a promising treatment method for the degradation of pharmaceuticals.

Here, we review recent research concerning the application of $TiO_2$ photocatalysis for the removal of selected pharmaceuticals belonging to different therapeutic drug classes. These classes of pharmaceuticals were chosen based on their environmental prevalence and potential adverse effects. The highlighted conclusions from this review are that (1) $TiO_2$ photocatalysis may play a major role in the degradation of pharmaceuticals; (2) various factors such as catalyst loading, initial concentrations, and water matrices significantly influence both the degradation rate and kinetics of degradation; (3) mineralization remains incomplete, despite complete abatement of the parent pharmaceutical due to the formation of stable by-products; (4) structures or number of intermediates formed differ due to the variation of photocatalytic experimental parameters, and (5) laboratory-scale experiments with artificial pharmaceutical solutions, in particular single compounds, are more common than pilot-scale or real wastewater samples. The main conclusion is

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that the use of heterogeneous photocatalysis can be considered a state-of-the-art pharmaceutical wastewater treatment. Further studies are needed to optimize the operating conditions for maximum degradation of wastewater containing multiple pharmaceuticals under realistic conditions and on industrial scales.

**Keywords** Pharmaceutical pollutants • TiO$_2$ photocatalysis • Wastewater • Antibiotic • Analgesic • Drugs • Advanced oxidation processes

## Contents

## Abbreviations

| | |
|---|---|
| AAS | Atomic absorption spectrometry |
| AMX | Amoxicillin |
| AN | Analgesic |
| AOP | Advanced oxidation processes |
| API | Active pharmaceutical ingredient |
| BOD | Biochemical oxygen demand |
| CBZ | Carbamazepine |
| CIP | Ciprofloxacin |
| COD | Chemical oxygen demand |
| CPC | Compound parabolic collector |
| DCF | Diclofenac |
| DO | Dissolved oxygen |
| DOC | Dissolved organic carbon |
| DOM | Dissolved organic matter |
| $E_g$ | Band gap energy |
| ESI-MS | Electrospray ionization mass spectrometry |
| FIA | Flow injection analysis |
| FQ | Fluoroquinolone |
| GC-MS | Gas chromatography mass spectrometry |
| HPLC | High performance liquid chromatography |

| IBP | Ibuprofen |
|---|---|
| IC | Ion chromatography |
| ICP-AES | Inductively coupled plasma atomic emission spectroscopy |
| IUPAC | International union of pure and applied chemistry |
| LC/ESI-MS-MS | Liquid chromatography-electrospray ionization tandem mass spectrometry |
| LC/ESI-TOF-MS | Liquid chromatography-electrospray ionization time-of-flight mass spectrometry |
| LC-MS | Liquid chromatography mass spectrometry |
| LED | Light emitting diode |
| L-H | Langmuir-Hinshelwood |
| Log $K_{ow}$ | Octanol/water partition coefficient |
| MOX | Moxifloxacin |
| NHE | Normal hydrogen electrode |
| NOM | Natural organic matter |
| NOR | Norfloxacin |
| NPX | Naproxen |
| NSAIDs | Non-steroidal anti-inflammatory drugs |
| OTC | Oxytetracycline |
| OX | Oxolinic acid |
| QTOF-MS | Quadrupole time of light-mass spectrometry |
| SMX | Sulfamethoxazole |
| $t_{1/2}$ | Half-life time |
| TC | Tetracycline |
| TOC | Total organic carbon |
| TOF-MS | Time-of-flight mass spectrometry |
| UPLC-ESI/MS | Ultra performance liquid chromatography-electrospray mass spectrometry |
| USEPA | United States environmental protection agency |
| UV | Ultraviolet |
| WWTP | Wastewater treatment plant |

## 3.1 Introduction: Advanced Oxidation Processes

Since water is essential to life, it is not surprising that there are a number of issues related to this essential resource. Poor sanitation, water scarcity, deteriorating water quality, waterborne diseases and lack of clean water supply are all posing global challenges due to the rising demand by an increasing world population. Further pressure on water resources has resulted from the introduction of emerging recalcitrant contaminants or xenobiotics, such as pharmaceuticals, endocrine disruptors, surfactants and personal care products. These substances display much more complex characteristics and resistance to degradation. Numerous studies highlighting the prevalence of pharmaceuticals in water environments has increased
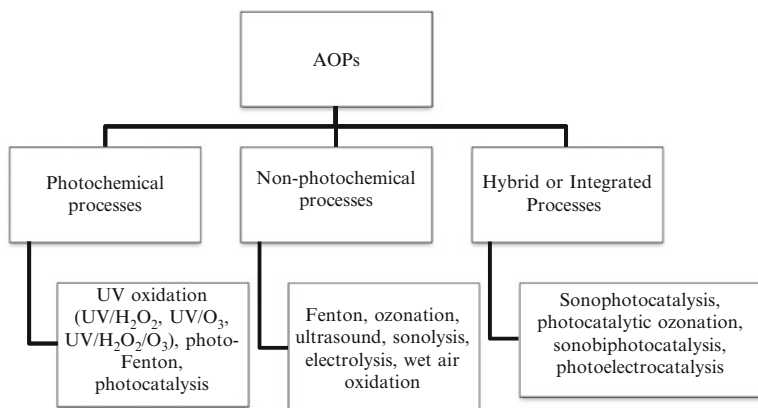
**Fig. 3.1** Types of destructive advanced oxidation processes (AOPs) for wastewater purification

significantly since 2000, Heberer (2002a), Tixier et al. (2003), Fent et al. (2006), Choi et al. (2008) and Miège et al. (2009). This development has raised widespread concern of poorly known potential effects of pharmaceutical compounds and their metabolites on human and aquatic organisms, despite occurring in trace quantities ranging from ng/L to µg/L, Kümmerer (2009) and Mompelat et al. (2009).

Currently existing sewage treatment technologies can only be considered as a compromise for emerging micropollutants, although they have proven efficient for microbial, carbon, heavy metals and nitrogen removal. Complex pharmaceuticals with various physical and chemical properties in wastewater, lead to inadequate removal by existing wastewater treatment technologies which are not designed to handle this specific class of pollutants, Suárez et al. (2008). Incomplete removal of pharmaceutical residues clearly supports the urgent need for innovative technologies that can deal with their presence, either by improving existing or engaging in alternative technologies. Advanced Oxidation Processes (AOPs) are regarded to be appropriate to degrade pollutants which are known to be non-biodegradable or have low biodegradability, persistent, and possess high chemical stability. AOPs for water and wastewater treatment include photolysis and photocatalysis, ozonation, Fenton and photo-Fenton, ultrasound radiation, sonolysis, electrochemical oxidation, and wet air oxidation. These advanced technologies can be grouped into photochemical, non-photochemical and hybrid techniques (Fig. 3.1).

In principle, all AOPs are characterized by a common chemical feature known as reactive oxygen species which can react with non-biodegradable or recalcitrant compounds in water or wastewater, Dalrymple et al. (2007). Reactive oxygen or free radical species are strong oxidants required to initiate AOPs in order to mineralize pollutants to simpler and nontoxic molecules. These free radical species are based on atoms or molecules consisting of one or more unpaired electrons such as the hydroxyl radical (HO$^\bullet$), superoxide anion radical (O$_2^{\bullet-}$), hydroperoxyl radical (HO$_2^\bullet$) or alkoxyl radical (RO$^\bullet$). Of these, the HO$^\bullet$ has attracted the most

attention. The appealing characteristics of HO$^\bullet$ radicals are its non-selective nature, high oxidation potential (2.8 V) when compared to other oxidants and its ability to react with a wide range of contaminants without any additives, with rate constants normally in the order of $10^6$–$10^9$ mol L$^{-1}$ s$^{-1}$, Andreozzi et al. (1999). When HO$^\bullet$ radicals reacts with organic molecules, they can either abstract a hydrogen atom or add to multiple bonds yielding oxidized intermediates or, in the case of complete mineralization, produce carbon dioxide, water and inorganic acids (3.1).

$$HO^\bullet + \text{Pollutant} \rightarrow CO_2 + H_2O + \text{inorganic acids} \qquad (3.1)$$
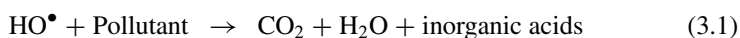
During an ideal treatment by AOPs, contaminants are structurally altered forming smaller and more biodegradable compounds until complete mineralization is achieved. For the application of AOPs in wastewater treatment, the objectives can be either to (i) increase the biodegradability of wastewater before applying conventional biological process, (ii) reduce the level of toxicity and micropollutants in the effluent or (iii) disinfect the wastewater instead of applying traditional disinfection methods such as chlorination, which is known to generate carcinogenic and mutagenic by-products such as trihalomethanes and haloacetic acids, Rizzo (2011). AOPs have been proven to be efficient for the treatment of industrial wastewater; however, due to the high chemical oxygen demand (COD) levels in industrial wastewater, AOPs are only considered efficient for a low COD level of <5 g/L. When dealing with high levels of COD, techniques such as incineration or wet oxidation are preferred, Malato et al. (2002). Semiconductor material mediated photocatalysis in particular with titanium dioxide (TiO$_2$), is distinctive compared to other AOPs for the removal of persistent pollutants from wastewaters.

## 3.2 Heterogeneous Photocatalysis

The IUPAC defines photocatalysis as a "change in the rate of a chemical reaction or its initiation under the action of ultraviolet, visible or infrared radiation in the presence of a substance, the photocatalyst that absorbs light quanta and is involved in the chemical transformation of the reaction partners", Braslavsky (2007). Heterogeneous photocatalysis may be also termed as a process which uses a semiconductor metal oxide as catalyst and oxygen as an oxidizing agent, Andreozzi et al. (1999).

In 1972, Fujishima and Honda discovered the possibility of water splitting by means of a photoelectrochemical cell consisting of a rutile TiO$_2$ photoanode and a Pt counter electrode, Fujishima et al. (2008). This discovery opened ways for new applications such as air purification, self-cleaning surfaces application, organic synthesis, disinfection, and anti-cancer therapy. An interesting application of this breakthrough is in water purification. Water purification by means of illuminating TiO$_2$ was first proposed by Frank and Bard for cyanide and sulfite removal,

Fujishima et al. (2008). Since then, degradation of various organic target compounds such as dyes, pesticides and pharmaceuticals has been demonstrated.

A conventional heterogeneous photocatalysis process can be divided into five individual steps, Herrmann (1999):

  i. Diffusion of the reactants from the bulk phase to the surface of the catalyst;
 ii. Adsorption of at least one of the reactants;
iii. Reaction in the adsorbed phase;
iv. Desorption of the product(s);
 v. Removal of the product(s) from the interface region.

An ideal photocatalyst should be chemically and biologically inert, photoactive, photostable, inexpensive, non-toxic, and should be excited with visible and near/or ultraviolet (UV) light. Despite the existence of various chalcogenide semiconductor photocatalysts (oxides and sulfides) such as zinc oxide (ZnO), zinc sulfide (ZnS), ferric oxide ($Fe_2O_3$), cadmium sulfide (CdS), cerium dioxide ($CeO_2$), tungsten trioxide ($WO_3$), tin dioxide ($SnO_2$) and titanium dioxide ($TiO_2$), none of these fulfils all the characteristics of an ideal photocatalyst. The choice of catalyst in photocatalytic studies is frequently narrowed to $TiO_2$, CdS and ZnO. Most studies involve $TiO_2$ as a semiconductor candidate despite its limitations, such as a low efficiency and a narrow light response factor, Leary and Westwood (2011). CdS and ZnO are known to undergo self-oxidation, which leads to lower photoactivity, and also the release of potentially dangerous metals such as $Cd^{2+}$, which makes these photocatalysts unattractive, Fox and Dulay (1993). Notably, ZnO has been reported to be a better photocatalyst in photocatalytic degradation than $TiO_2$ due to its broader absorption within the solar spectrum, Elmolla and Chaudhuri (2010a).

### 3.2.1  Mechanisms of TiO₂ Photocatalysis

Mechanistic processes of $TiO_2$ induced photocatalytic degradation of organic pollutants have been well described in the literature, Fox and Dulay (1993), Hoffmann et al. (1995), Gaya and Abdullah (2008), Chong et al. (2010) and Augugliaro et al. (2012). Photocatalysis occurs due to absorption of a photon with sufficient energy either equal or higher than the band gap energy ($E_g$) of the catalyst. The $E_g$ represents the difference between the valence band (vB) and the conduction band (cB) of $TiO_2$. The vB and cB energies of $TiO_2$ are about $+3.1$ eV (vs. NHE) and $-0.1$ eV (vs. NHE), respectively. Consequently, the $E_g$ between the vB and cB is 3.2 eV for anatase $TiO_2$. When $TiO_2$ is excited with UV light ($\lambda < 380$ nm for anatase $TiO_2$ which is more common or $\lambda < 400$ nm for rutile $TiO_2$), an electron ($e^-$) is promoted from the vB to the cB of $TiO_2$ generating a hole ($h^+$) in the vB and an electron ($e^-$) in the cB (3.2). The photogenerated $e^-$-$h^+$ pair or charge carriers can either migrate to the surface of the $TiO_2$, where they can actively perform their roles in oxidation-reduction reactions with the adsorbed pollutant (P) or recombine in the
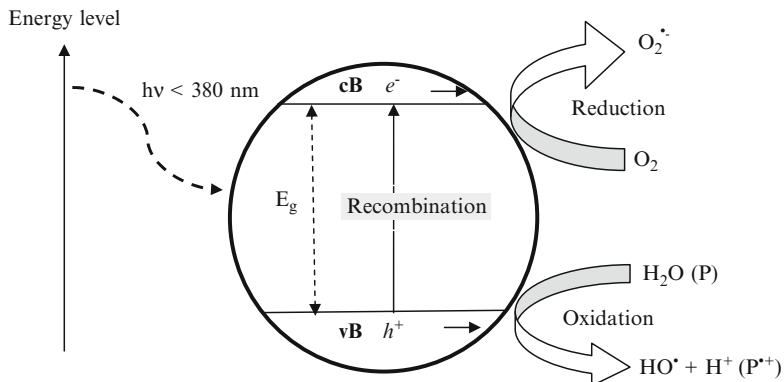
**Fig. 3.2** Photogenerated $e^-$-$h^+$ pair on the $TiO_2$ surface and oxidation-reduction reactions

bulk or on the surface of the $TiO_2$. The schematic representation of photogeneration of the $e^-$-$h^+$ pair and of the subsequent oxidation-reduction is presented in Fig. 3.2.

$$\text{Excitation of } TiO_2 \text{ with photon } (\lambda \leq 380 \text{ nm}): \quad TiO_2 + h\nu \rightarrow e^-_{cB} + h^+_{vB} \tag{3.2}$$
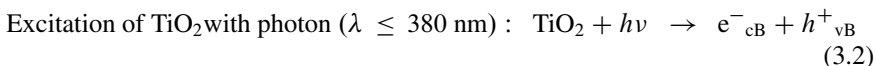
A series of oxidation-reduction reactions may occur during $TiO_2$ photocatalysis (3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 3.10, and 3.11). A generated $h^+$ can react with an adsorbed water molecule, which is an essential process in $TiO_2$ photocatalysis, or reacts with $OH^-$ anions to form powerful $HO^\bullet$ radicals (3.3 and 3.4). The $HO^\bullet$ radicals can subsequently oxidize water pollutants P to complete mineralization (3.5). When molecular oxygen is available, it is adsorbed onto the surface of $TiO_2$ and can scavenge an electron to form the superoxide anion radical ($O_2^{\bullet-}$) (3.6).

$$h^+_{vB} + H_2O_{ads} \rightarrow HO^\bullet_{ads} + H^+ \tag{3.3}$$

$$h^+_{vB} + OH^-_{ad} \rightarrow HO^\bullet_{ads} \tag{3.4}$$

$$HO^\bullet_{ads} + P \rightarrow \rightarrow \rightarrow H_2O + CO_2 \tag{3.5}$$

$$(O_2)_{ads} + e^- \rightarrow O_2^{\bullet-} \tag{3.6}$$
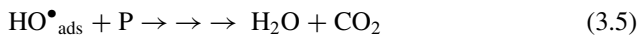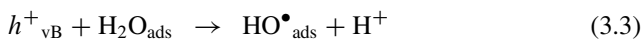
Holes can also directly oxidize pollutants P by electron transfer (3.7 and 3.8).

$$h^+_{vB} + P_{ads} \rightarrow P_{ads}^{\bullet+} \tag{3.7}$$

$$HO^\bullet_{ads} + P_{ads} \rightarrow \text{intermediates/degradation products} \tag{3.8}$$
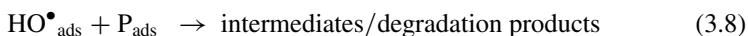
The photogenerated $e^-$-$h^+$ pairs can undergo rapid recombination within nano seconds in the absence of electron scavengers, such as oxygen, releasing heat

without favouring any reactions (3.9, 3.10, and 3.11). The $e^-_{TR}$ and $h^+_{TR}$ (3.9, 3.10, and 3.11) represent the surface trap valence band electron and conduction band hole, respectively.

$$\text{Charge carrier trapping}: \quad e^-_{cB} \quad \rightarrow \quad e^-_{TR} \tag{3.9}$$

$$\text{Charge carrier trapping}: \quad h^+_{vB} \quad \rightarrow \quad h^+_{TR} \tag{3.10}$$

$$e^- \text{-} h^+ \text{recombination}: \quad e^-_{TR} + h^+_{vB}\left(h^+_{TR}\right) \quad \rightarrow \quad e^-_{cB} + \text{heat} \tag{3.11}$$
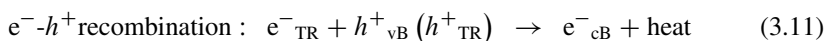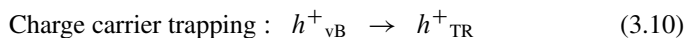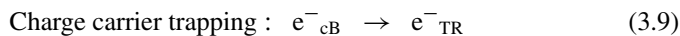
Wavelengths between 300 and 400 nm (near UV range) are of interest in $TiO_2$ photocatalysis, which are provided either by artificial UV lamps or by a small section of the solar spectrum. Sunlight-induced photocatalytic processes have been the characteristic domain of $TiO_2$ as a photocatalyst candidate. Demonstration-scale solar treatments of emerging contaminants using compound parabolic collectors (CPC) underpin this, Pérez-Estrada et al. (2005) and Miránda-García et al. (2011). However, a limitation of solar photocatalysis is the poor overlap of the solar spectrum with the absorption spectrum of $TiO_2$ (<5 %). Thus, only 5 % of solar irradiation comprises the UV light that can be harvested by $TiO_2$ photocatalysis. $TiO_2$ doping with non-metals, metal-ion implantation, co-doping and sensitizing $TiO_2$ with dyes has been applied to address this limitation and to improve the $TiO_2$ photocatalytic efficiencies, Kumar and Devi (2011). The main objective of employing doping is to increase absorption in the visible region as it induces an optical response change i.e. decrease in band gap. One major drawback of doping is significant increase in the cost of the photocatalyst due to expense of ion implantation facilities, Zhang et al. (2009).

## 3.3   Titanium Dioxide: Structure and Properties

One most prevalent application of $TiO_2$ is as a white pigment in paints, plastics, paper, fibres, foods, pharmaceuticals, and personal care products mainly due to its light scattering properties and high refractive index. Worldwide production of $TiO_2$ powder has been reported as five million tons in 2005, with projections up to a 10 % increase by 2015, Skocaj et al. (2011). Additionally, production of nano-sized $TiO_2$ which permits more applications such as anti-fogging and self-cleaning coatings has also increased from 2,000 t in 2005 to 5,000 t in 2010, Weir et al. (2012). However, the concern on safety and health aspects which arise from the usage of nano-$TiO_2$ needs to be addressed. A recent study was conducted to assess the amount of $TiO_2$ in various food, personal care products and pharmaceuticals and predict adverse effects of $TiO_2$ nanoparticles on the environment, Weir et al. (2012).

$TiO_2$ exists in three crystalline forms namely anatase, rutile and brookite with the anatase form more commonly used as an active photocatalyst than pure rutile phase. In contrast, brookite $TiO_2$ is not commonly applied in photocatalysis. The

anatase form tends to be the most photoactive and the most stable form at low temperatures ($<700$ °C). Anatase and brookite are however, thermodynamically metastable and they can be irreversibly converted to the rutile form, the most stable form at high temperatures, Silva and Faria (2009). Surface and structural properties such as crystal structure, surface area, particle size distribution, porosity, band gap and surface hydroxyl density control the efficiency of $TiO_2$ as photocatalyst. Of these, crystallinity and specific surface area have great effect on the photocatalytic activity of $TiO_2$.

The more common application of pure anatase compared to the pure rutile $TiO_2$ is attributed to a higher density of superficial hydroxyl groups, which leads to an improved capacity of anatase to adsorb oxygen, a larger specific surface area compared to pure rutile samples, and a lower recombination velocity of $e^--h^+$ pairs in anatase, Hoffmann et al. (1995) and Achilleos et al. (2010a). Combinations of anatase and rutile have demonstrated better photocatalytic activity due to the promotion of charge pair separation and inhibition of $e^--h^+$ recombination, Pelaez et al. (2012).

The most prominent $TiO_2$ photocatalyst, Degussa P25 (now known as AEROXIDE® $TiO_2$ P25) contains 80:20 anatase to rutile weight ratio and has demonstrated good performance in photocatalytic applications due to its large surface area of 50 $m^2/g$, with a particle size range of 20–30 nm. Although $TiO_2$ P25 has been widely used as a benchmark photocatalyst, its effectiveness has been limited by poor light absorption in the visible region, as a result of its large band gap.

The sol–gel method has been one of the widely used synthetic methods for the preparation of $TiO_2$ nanocrystalline forms. Several synthetic pathways are available for the preparation of the photocatalyst from precursor chemicals, for example, titanium alkoxide or titanium halogenide. The precursors are normally calcined at very high temperatures to obtain the desired crystal form and strong adhesion to surface materials or solid support such as activated carbon, zeolite, silica and glass. Hydroxyl groups from the catalyst surface and the support can react upon loss of a molecule of water, creating an oxygen bond thus increasing the adherence of the catalyst to the support during calcinations, Shan et al. (2010). The rate of hydrolysis is difficult to control due to the affinity of the $TiO_2$ precursors for water leading to poor modification of $TiO_2$'s intrinsic properties such as surface structure, structural properties and porosity. Nitric acid, hydrochloric acid and complexing reagents such as oxalate and citrate have also been commonly used in the sol–gel synthesis. Weak acids such as acetic acid also exhibit excellent control of the hydrolysis rate of $TiO_2$, as they maintain pH due to the chelating effects of anions formed (e.g. acetate ion), generation of pH buffer, and stabilization of the sol, Shamaila et al. (2010).

## 3.4 Pharmaceuticals in the Environment

The United States Environmental Protection Agency's (USEPA) priority pollutants such as volatile organics, semivolatile, pesticides and polyvinylchloride biphenyls, metals and inorganics have been the main focus of water pollution over the past

30 years. However, in recent years, concerns in water pollution have shifted towards emerging contaminants. In particular, pharmaceutical and personal care products have become pollutants of interest due to their notable detection and potential detrimental impact on aquatic environments, Khetan and Collins (2007). Pharmaceuticals, classified as environmental emerging contaminants because of their endocrine-disrupting properties are either of natural origin or are produced synthetically, Oller et al. (2011).

The term 'pharmaceutical' or active pharmaceutical ingredient (API) for the purpose of this review refers to chemicals used for diagnosis, treatment, alteration, and prevention of disease, health conditions or functions of the human body and also includes veterinary pharmaceuticals and illicit or recreational drugs, Daughton and Ternes (1999). APIs are complex molecules with various functionalities and physicochemical and biological properties, balanced in terms of hydrophilicity and lipophilicity with molecular weights ranging from 200 to 1,000 Da, Kümmerer (2009). Pharmaceuticals are generally designed with high stability for their intended effects on humans and are eventually metabolised in the body by biochemical processes. They can be eliminated from the human body either after being partially or completely converted to water soluble metabolites or, in some cases, without being metabolized, Khan and Ongerth (2004). Their classifications are generally based on their therapeutic uses and those of environmental interest can be categorized into eight groups, namely non-steroidal anti-inflammatory drugs, antibiotics, beta-blockers, antiepileptics, blood lipid-lowering agents, antidepressants, hormones and antihistamines, Khetan and Collins (2007).

Inappropriate disposal and excretion of consumed medications by households, pharmaceutical manufacturers, hospitals, pharmacies and animal farming or aquaculture have been identified as the major sources of pharmaceuticals either as the parent drug or their metabolites in water courses (Fig. 3.3). Wastewater treatment plants (WWTPs) are thus a major source of the presence of this class of micropollutants in the environment. Various studies have reported on the discrepancies in efficiency and variation of WWTPs in the removal of pharmaceuticals due to their stability and differences in physicochemical properties. In fact, studies have also confirmed the detection of pharmaceuticals in effluents and influents from WWTP samples collected, in trace concentrations, Rodil et al. (2012).

A significant contribution of pharmaceuticals in surface water also comes from inappropriate disposal of expired or unused medications from households. Occurrence of pharmaceuticals in groundwater is related to a leaching process from landfills and soil and from sludge or manure spreading. Groundwater, being the extraction source of drinking water, has also been affected by pharmaceutical pollution, which can cause long term accumulation of trace amounts in humans. Finally, the accumulation of pharmaceuticals can be also expected in aquatic and terrestrial organisms via the food chain, Hoeger et al. (2005) and Deegan et al. (2011).

Monitoring studies conducted thus far have verified the occurrence of pharmaceutical in trace amounts in surface waters, groundwater and also sewage effluents, Andreozzi et al. (2003), Carballa et al. (2004), Lishman et al. (2006) and Moldovan
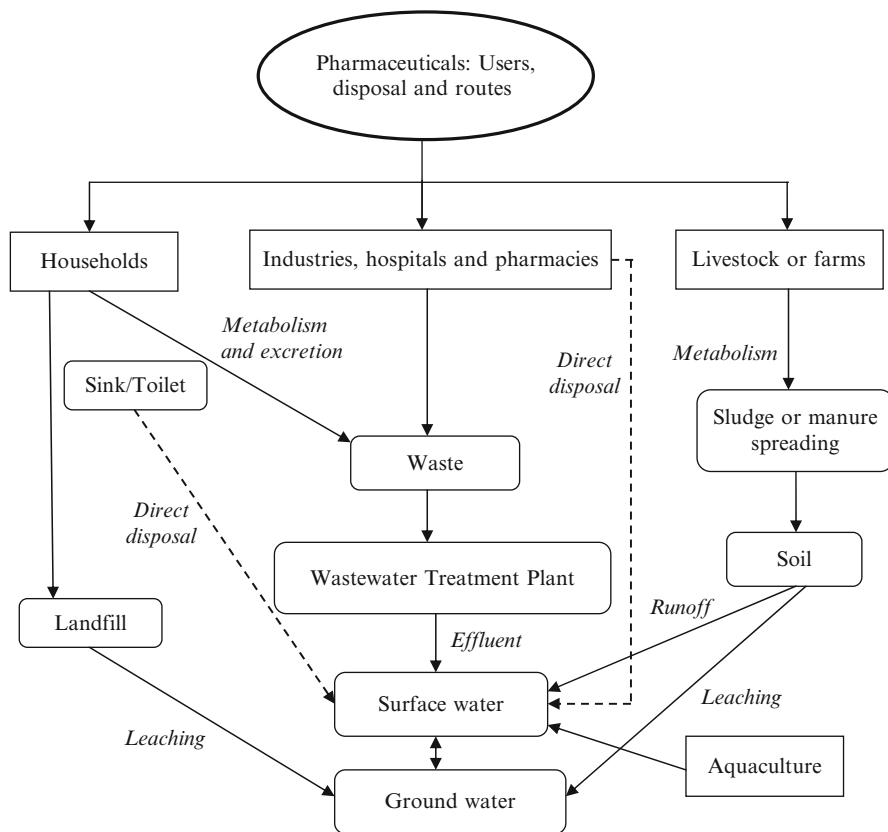
**Fig. 3.3** Sources and routes of pharmaceuticals in water (Modified from Nikolaou et al. (2007) and Mompelat et al. (2009))

(2006). Moreover, concentrations of pharmaceuticals in the water environment have also been established based on their therapeutic use, Nikolaou et al. (2007), Pal et al. (2010) and Ziylan and Ince (2011). The occurrence of pharmaceutical residues in drinking and tap water has also been reported, Ternes et al. (2002) and Rodil et al. (2012). A study reported levels of numerous pharmaceuticals and endocrine disruptors in the finished water of 19 full-scale water utilities in the United States, Benotti et al. (2009).

Once released into the environment, APIs are exposed to different natural conditions and resulting transformations, which is not only affected by their physicochemical properties, but also their structure. These changes create further complexities and concerns regarding the identity, toxicity and potential risk of any new products, which may be formed.

Although studies are generally focused on a wide spectrum of pharmaceuticals, those frequently prescribed have gained importance amongst researchers.

**Table 3.1** Relative annual per capita consumption (in mg/cap/y) of selected pharmaceuticals in Australia and some European countries, data taken from Carballa et al. (2008)

| Pharmaceuticals | Australia (1998) | France (1998) | Germany (2001) | Spain (2003) | Sweden (2005) |
|---|---|---|---|---|---|
| Ibuprofen | 720.6 | 2,841.0 | 1,553.4 | 6,391.2 | 7,864.3 |
| Naproxen | 1,159.9 | 646.2 | n.a | 986.1 | n.a |
| Diclofenac | 222.8 | 254.7 | 594.7 | 747.7 | 375.9 |
| Sulfamethoxazole | n.a | 382.9 | 571.4 | 294.0 | 160.4 |
| Trimethoprim | 136.8 | n.a | n.a | 85.6 | n.a |
| Metoprolol | 313.5 | n.a | 631.1 | 53.2 | n.a |
| Carbamazepine | 506.3 | 601.7 | 946.6 | 463.0 | 820.2 |
| Diazepam | 371.7 | 6.8 | 5.3 | 20.8 | 19.8 |
| Bezafibrate | n.a | 589.7 | 315.5 | 92.6 | 66.7 |

*n.a* not available

For example, acidic pharmaceuticals such as ibuprofen, ketoprofen, bezafibrate, and naproxen have been frequently monitored in receiving waters and WWTPs, Lindqvist et al. (2005) and Wang et al. (2010). Selected therapeutic classes such as antibiotics, NSAIDs and analgesics have also been reviewed, Kümmerer (2009) and Ziylan and Ince (2011). Accurate statistics on sales and consumption of pharmaceuticals present a challenge, due to difficulty in monitoring of prescription medication, in addition to non-prescription medicines, which can be obtained over-the counter or through the internet. Statistics on global consumptions and production of pharmaceuticals are not available in the literature. In addition, about 3,000 new APIs are introduced each year as a result of advancements in medicine, Beausse (2004).

The annual per capita consumption of selected pharmaceuticals in some European countries and Australia are presented in Table 3.1. The consumption patterns vary according to countries and demand or popularity of a drug. In Australia, 262 million prescriptions were dispensed via community pharmacies in 2008 which accounts for about 12 prescriptions per person, AIHW (2011). This figure represents an increase of 24 % over prescriptions dispensed in 2001. Among the mostly prescribed medicines are cholesterol-lowering drugs, antibiotics and drugs for the treatment of high blood pressure. Amoxicillin (3.9 %), an antibiotic, emerged in the top 10 most commonly prescribed medicines in 2009–2010 followed by paracetamol (3.2 %), an analgesic and cephalexin (3.2 %), a cephalosporin antibiotic. The presences of amoxicillin and cephalexin were confirmed in WWTP influents and surface water (ng/L) in a monitoring study of 28 antibiotics in watersheds of south-east Queensland. None of these drugs was, however, detected in drinking water, Watkinson et al. (2009).

Although the fate of many pharmaceuticals released into the environment remains unknown, three possible scenarios have been proposed: (i) the pharmaceutical is mineralized to carbon dioxide, water, and other inorganic ions or acids such nitrate, sulfate and phosphate ions, (ii) the pharmaceutical is not readily degradable

and will be retained in sludge due to its lipophilic nature or (iii) the pharmaceutical will be metabolized to a more hydrophilic form than the parent compound, Halling-Sørensen et al. (1998) and Klavarioti et al. (2009).

The main concern from the presence of pharmaceuticals and their metabolites is the potential adverse effects that they may pose on human health and to other living organisms, which thus far remains unknown and unclear. Due to their complex physicochemical properties as a result of mixtures of various pharmaceuticals and other pollutants in the environment, advanced treatment such as $TiO_2$ photocatalysis undoubtedly can be applied for their degradation and a substantial amount of work has been published in this field, Abellán et al. (2007), Yurdakal et al. (2007), Giraldo et al. (2010) and Tong et al. (2012).

## 3.5 Degradation, Mineralization and Transformation Pathways of Pharmaceuticals Through Heterogeneous Photocatalysis

Positive developments in the photooxidation efficiency of $TiO_2$ based photocatalysis (UV/$TiO_2$) of pharmaceuticals have lead to an increasing interest in its application for the removal of micropollutants in spiked wastewaters and also real wastewater such as wastewater effluents, river water and drinking water. Determination of optimal operating conditions to yield the highest performance in terms of degradation and mineralization has been investigated in various heterogeneous photocatalytic studies on pharmaceuticals. The disappearance of the parent API over the treatment time has been the primary focus. However, studies have reported that kinetic assessment of APIs during UV/$TiO_2$ degradation can often be complex, due to the formation of various transformation products. Interferences between the disappearance of the parent API and newly formed transformation products may also occur. The non-selective nature of hydroxyl radicals contributes to this complexity, Oller et al. (2011).

Another general observation is that while high removal rates of the parent API can be accomplished by UV/$TiO_2$, mineralization remains incomplete in most cases. The degree of mineralization also tends to vary according to the nature of the water matrices. In general, a solution of the API prepared in the laboratory with distilled water or deionized water generally yields higher mineralization compared to that of doped effluent or influent samples from WWTPs, groundwater or river water.

The interference provided by the presence of organic matter such as humic and fulvic acids and radical scavengers such as carbonate species, $HCO_3^-$ and $CO_3^{2-}$ cannot be ruled out, as these species retard photocatalysis by competing for active sites and radiation attenuation. Organic matter present in water can be classified into two major classes namely dissolved organic matter and particulate organic matter. The existence of organic matter can be naturally occurring organic matter, such as humic and fulvic acids, or that derived from anthropogenic activities, Oppenländer (2003).

The ultimate aim of TiO$_2$ photocatalysis (and also for other AOPs) is threefold, to degrade pollutants, to reduce toxicity and to enhance the biodegradability of the treated wastewater. Global parameters such as total organic carbon (TOC), COD, biochemical oxygen demand (BOD), dissolved organic carbon (DOC) and BOD/COD ratio are also often measured. The ratio of BOD$_5$/COD is commonly used in this context and ratio values of above 0.4 are regarded as highly biodegradable in wastewater, Abellán et al. (2007). Toxicity assays are used to indicate the toxicity of photocatalytically treated wastewater and various types of bioassays based on microorganisms, plants and algae, invertebrates and fish have been applied to measure the response, Rizzo (2011).
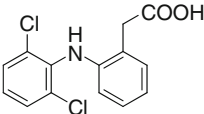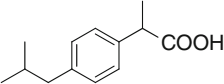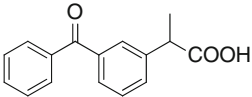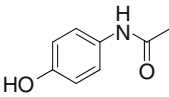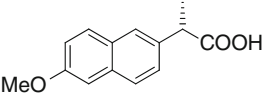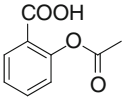
In terms of the API selection, it is either driven by their consumption which can correlate to the high probability of detection in the environment or due to an existing gap of information on the degradation rate of the particular API. Frequently investigated operational parameters include catalyst load, initial concentration, type of photocatalysts, pH of the solution, wavelength and light intensity. Other operational parameters for enhancement of the degradation rate by the addition of co-oxidants and the effect of the concentration of dissolved oxygen (DO) have also been incorporated in various TiO$_2$ photocatalysis studies. These variables are chosen in order to establish their relationship with respect to the API degradation. However, it is also widely accepted that the design and geometry of the photoreactor dictates the optimal degradation rate of APIs, Malato et al. (2009) and Friedmann et al. (2010).

### 3.5.1 Non-steroidal Anti-inflammatory and Analgesic Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) primarily reduce inflammation, while analgesic (AN) drugs are widely used to relieve pain, Ziylan and Ince (2011). Examples of NSAIDs include naproxen, ketoprofen, diclofenac, fenoprofen, indomethacin, and ibuprofen, whereas ANs are acetaminophen (paracetamol), acetylsalicylic acid (aspirin) and the opioid analgesics such as morphine. Chemical structures of selected NSAIDs and ANs are presented in Table 3.2. One of the most prominent characteristics of NSAIDs is the carboxylic acid moiety which contributes to both its activity and side effects.

Photocatalytic degradation of NSAIDs and ANs has been conducted by various researchers primarily aiming to explore the degradation kinetics and degradation efficiency of APIs under selected experimental conditions. Among the most frequently studied NSAIDs and ANs are diclofenac, ketoprofen, ibuprofen, naproxen and paracetamol. Photocatalytic studies of NSAIDs and ANs are summarized in Table 3.3 and detailed discussions of selected studies are provided to highlight both the significant findings and differences in the experimental conditions.

**Table 3.2** Chemical structures of selected non-steroidal anti-inflammatory drugs (NSAID) and analgesics



Diclofenac
  ($C_{14}H_{11}Cl_2NO_2$)

2-(2-(2,6-dichloro-
  phenylamino)phenyl)
  acetic acid

Ibuprofen($C_{13}H_{18}O_2$)

(*RS*)-2-(4-(2-methylpropyl)phenyl)
  propanoic acid

Ketoprofen ($C_{16}H_{14}O_3$)

(*RS*)-2-(3 benzoyl-phenyl)
  propanoic acid

Acetaminophen
  ($C_8H_9NO_2$)

*N*-(4-hydroxyphenyl)
  acetamide

Naproxen ($C_{14}H_{14}O_3$)

($+$)-(*S*)-2-(6-methoxynaphthalen-
  2-yl) propanoic acid

Acetylsalicylic acid($C_9H_8O_4$)

2-acetoxybenzoic acid

## Diclofenac

Diclofenac (DCF) has been identified as one of the most recognized pharmaceuticals in the aquatic environment as a result of its elevated use worldwide, Heberer (2002b). The global consumption of DCF was reported to be 940 t per year with a daily dose of 100 mg. These numbers clearly demonstrate its significance as an environmentally important pharmaceutical, Zhang et al. (2008a). Threats on fish such as rainbow trout, and birds, specifically the vulture populations, have been reported, Oaks et al. (2004). NSAIDs such as DCF (and NPX) with a partition coefficient (log $K_{ow}$) greater than 3 are generally capable of bioaccumulating in the tissues of organisms, Rizzo et al. (2009a). The concentration of DCF has been reported to be less than 10 ng/L in a drinking water sample from tap water in Berlin and ranged between 0.14 and 1.48 µg/L in WWTPs effluents and surface water, Zhang et al. (2008a). As for the removal efficiency by WWTPs, there has been a wide range varying from 0 % to 80 % with typical ranges between 21 % and 40 %, which clearly indicates insufficient removal of this pharmaceutical, Zhang et al. (2008a).

One of the most comprehensive studies of the effect of UV/TiO$_2$ on DCF to date was performed by Calza and his group (2006). The study included the identification of intermediates formed, mineralization and also degradation rate controlling parameters. The photocatalytic efficiency increased with the amount of TiO$_2$, but high loadings (0.8–0.9 g/L) showed no positive effect on the degradation due to scattering of light. The optimum combination of TiO$_2$ loading/initial

**Table 3.3** Highlights of the heterogeneous photocatalytic studies on non-steroidal anti-inflammatory drugs and analgesics

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| *Diclofenac* | | | | | |
| | Distilled water | Solar simulator suntest CPS + (Xe arc lamp, 1,500 W, 750 W/m$^2$) Initial concentration: 0.76–9.24 mg/L TiO$_2$ P25 loading: 104–896 mg/L | HPLC, TOC, LC-MS, IC, toxicity measurement on bacterium, *Vibrio fischeri* | Factorial design was used to investigate effect of TiO$_2$ loading and DCF initial concentration on reaction rate. Toxicity and TOC decreased. Complete DCF elimination after 1 h of irradiation | Calza et al. (2006) |
| | Milli-Q water | Black light fluorescent lamp (125 W, 300 and 420 nm, intensity $4.7 \times 10^{-7}$ Einstein/s) Initial concentration: 5–80 mg/L TiO$_2$ P25 loading: 0.2–1.6 g/L | UV–vis (254 nm, 276 nm), COD, bioassays on *Daphnia magna*, *Pseudokirchneriella subcapitata*, *Artemia salina* | Degradation data fitted pseudo-first order kinetic model. High DCF concentrations (40 and 80 mg/L) and 1.6 g TiO$_2$/L followed second-order kinetic model | Rizzo et al. (2009a) |
| | Deionized water, groundwater and treated municipal wastewater | UV-A lamp (9 W, 350–400 nm, intensity $3.37 \times 10^{-6}$ Einstein/s) Initial concentration: 5–20 mg/L TiO$_2$ loading: 50–800 mg/L Type of TiO$_2$: TiO$_2$ Degussa P25, Hombikat UV 100, Aldrich, Tronox AK-1, Tronox TRHP-2, Tronox TR Temperature: 23 °C H$_2$O$_2$ concentration: 0.07–1.4 mM | UV (276 nm), TOC, toxicity measurement with *Daphnia magna* | TiO$_2$ Degussa P25 performed best for DCF decomposition which was affected by water matrix and increased initial concentration. Irradiated solution, in particular after 2 h irradiation, showed high toxicity | Achilleos et al. (2010a) |

| Milli-Q water | UV (254 nm; low pressure Hg lamp; photon flux: $8.33 \times 10^{-8}$ Einstein/s) and near UV-vis (366 nm; medium pressure Hg lamp; photon flux: $2.38 \times 10^{-6}$ Einstein/s) Initial concentration: 8 mg/L $TiO_2$ loading: 0.1–2 g/L Type of $TiO_2$: $TiO_2$ Degussa P25, synthesized $TiO_2$ anatase and rutile, and composite multi-walled carbon nanotube Gas flow rate: 200 mL/min $O_2/Ar$ (50 % vol of oxygen) DO % (v/v): 21–100 $H_2O_2$ concentration: 3 and 5 mM pH: 6 | HPLC, UV-vis, TOC, LC-MS | Optimum degradation was obtained with synthesized anatase (0.5 g/L) and 50 % $O_2$ (v/v) under UV irradiation with rate constant 0.9 $min^{-1}$ | Martínez et al. (2011) |
| Urban wastewater and Milli-Q water | High pressure Hg lamp ($\lambda = 313$ nm; intensity $3 \times 10^{-5}$ Einstein/s) Initial concentration: 30–80 mg/L $TiO_2$ loading: 0.5–2.5 g/L Gas flow rate: 30–50 L/h Ozone concentration: 5–30 mg/L pH: 7 | HPLC, COD, BOD, dissolved ozone, TOC | Concentrations of ozone gas and initial DCF concentrations greatly affected DCF and TOC removals | Aguinaco et al. (2012) |

(continued)

**Table 3.3** (continued)

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| *Diclofenac mixture* | Milli-Q water | High pressure Hg lamp (313 nm; intensity $3 \times 10^{-5}$ Einstein/s) Initial concentration: $10^{-4}$ M TiO$_2$ P25 loading: 1.5 g/L Temperature: 20 °C Oxygen flow rate: 30 L/h Ozone gas concentration: 10 mg/L pH: 5, 7 and 9 (buffering conditions) | HPLC, TOC, dissolved ozone | Efficiency of TiO$_2$ photolytic systems (O$_2$/UV-A/TiO$_2$, O$_3$/UV-A/TiO$_2$, UV-A/TiO$_2$) and single ozonation were compared. DCF was completely removed under photocatalytic process and TOC removed up to 80 % which was higher than single ozonation | Garciá-Araya et al. (2010) |
| Diclofenac (amoxicillin, carbamazepine) *Naproxen* | Distilled water, urban wastewater effluent | Black light fluorescent lamp (125 W, 300 and 420 nm, intensity $4.7 \times 10^{-7}$ Einstein/s) Initial concentration: 2.5 mg/L TiO$_2$ P25 loading: 0.2–0.8 g/L | UV–vis (DCF: 276 nm), TOC, bioassays on *Daphnia magna*, *Pseudokirchneriella subcapitata*, *Lepidium sativum* | DCF degradation was examined as single compound and in mixtures with amoxicillin and carbamazepine. Degradation rate was faster in spiked distilled water compared to real wastewater | Rizzo et al. (2009b) |
| | Milli-Q water | Solar simulator (Xe lamp, 1 kW) Initial concentration: 2–1,000 mg/L TiO$_2$ P25 loading: 0.1–1 g/L Temperature: 20–40 °C DO concentration: $40 \pm 2$ mg/L Volumetric rate: 0.1–0.4 L/min | HPLC, DOC, COD, BOD$_5$, ESI-MS, toxicity assay on *Vibrio fischeri* | Degradation of NPX mainly contributed by photolysis although photocatalysis was more efficient for DOC removal. BOD$_5$/COD ratios were found to be below 0.05 suggesting no-biodegradable product formed during photocatalysis. The photoproducts formed through decarboxylation showed a high cytotoxicity | Méndez-Arriaga et al. (2008a) |

*Naproxen mixture*

| Compound | Water | Conditions | Analysis | Results | Reference |
|---|---|---|---|---|---|
| Naproxen (Diclofenac and ibuprofen) | Milli-Q water | Solar simulator (Xe-OP lamp, 1 kW; photon flux: 6.9 µEinstein/s) Initial concentration: 200 ppm $TiO_2$ loading: 0.1–2 g/L Temperature: 20, 30 and 40 °C Circulation flow rate: 0.1, 0.2 and 0.4 mL/min DO concentration: 8, 40, 8 (initial)–2 (final) mg/L | HPLC, TOC, $BOD_5$, COD, DO, ESI/MS, toxicity assay on *Vibro fisheri* | Increase in $TiO_2$ loading showed no improvement on degradation rate and the maximum $TiO_2$ loading was 0.1 g/L. Flow rate had no effect on degradation rate while increase in temperature to 40 °C yielded 99 % conversion | Méndez-Arriaga et al. (2008b) |
| Naproxen (carbamazepine, clofibric acid, phenazone, ofloxacin, furosemide, ranitidine) | Ultrapure water | Medium pressure Hg lamp (125 W) Initial concentration: 5 mg/L $TiO_2$ P25 loading: 1 g/L Temperature: 30 °C Oxygen concentration: 22 mg/L pH: 3 and 11 | UV-vis (NPX: 230 nm) | Adsorption of NPX increased with decreasing pH. NPX of 5 mg/L decreased with increasing radiation time and in accordance to pseudo-first order kinetics in experiments conducted in batch photoreactor without membrane | Molinari et al. (2006) |
| *Ibuprofen* | | | | | |
| Solar photocatalysis | Water type not provided | Three solar pilot plant reactors (Two CPCs and one axis-parabolic collector) Initial concentration: 20–200 mg/L $TiO_2$ loading: 0.1–1.0 g/L $H_2O_2$ concentration: 900 and 1,028 mg/L | HPLC, TOC, $BOD_5$, COD | IBP and TOC removal increased proportionally with $TiO_2$ concentration and addition of $H_2O_2$ accelerates the degradation process. Solar photocatalysis was efficient to degrade IBP | Méndez-Arriaga et al. (2009a) |

(continued)

**Table 3.3** (continued)

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| Hybrid method | Milli-Q water | Initial concentration: 0.024–0.044 mM; TiO$_2$ P25 concentration: 10 mg/L; Temperature: 25 °C; H$_2$O$_2$ concentration: 2.15 μmol/L/min | HPLC, DOC | Individual and hybrid processes of Fenton, sonolysis, and photocatalysis were compared. Combination of TiO$_2$/Fe$^{2+}$/sonolysis yielded faster degradation for DOC removal (98 %) and complete degradation of IBP in 240 min | Méndez-Arriaga et al. (2009b) |
| Hybrid method | Milli-Q water | Initial concentration: 0.02–0.12 mM; TiO$_2$ P25 loading: 1 g/L; Temperature: 25 ± 2 °C; Ultrasonic power: 16, 35 and 55 mW/mL; Fe$^{3+}$ concentration: 0.045 mM | HPLC, UV–vis, ESI/MS, TOC | Compared to sonolysis and photocatalysis, addition of Fe$^{3+}$ and TiO$_2$ showed better degradation of IBP. Combination of UV, TiO$_2$ and ultrasound produced highest degradation rate of $108 \times 10^{-7}$ min$^{-1}$ | Madhavan et al. (2010) |
| *Ibuprofen mixture* | | | | | |
| Ibuprofen (and carbamazepine) | Milli-Q water and wastewater from WWTP | Solar simulator (1,000 W Phillip Xe lamp, intensity 272.3 W/m$^2$) and UV-A lamp (9 W Radium lamp, photon flux $3.37 \times 10^{-6}$ Einstein/s); Initial concentration: 5–20 mg/L; TiO$_2$ loading: 50–3,000 mg/L; Type of TiO$_2$: TiO$_2$ Degussa P25, Hombikat UV 100, Aldrich, Tronox AK-1, Tronox TRHP-2, Tronox TR; H$_2$O$_2$ concentration: 0.07–1.4 mM; pH: 3–10 | UV (IBP: 220 nm), DOC | Simulated solar and UV-A light were efficient on IBP degradation in particular in the presence of TiO$_2$ Degussa P25 | Achilleos et al. (2010b) |

| | | | | |
|---|---|---|---|---|
| Ibuprofen (diclofenac and naproxen) | Milli-Q water | Solar simulator (Xe-OP lamp, 1 kW; photon flux: 6.9 μEinstein/s) Initial concentration: 200 ppm TiO$_2$ loading: 1 g/L Temperature: 20, 30 and 40 °C Circulation flow rate: 0.1 and 0.4 mL/min DO concentration: 8, 40, 8 (initial)–2 (final) mg/L | HPLC, TOC, BOD$_5$, COD, DO, ESI/MS, toxicity assay on *Vibro fisheri* | DO influenced the degradation of IBP and the optimum TiO$_2$ loading for maximum degradation was 1 g/L | Méndez-Arriaga et al. (2008b) |
| *Paracetamol* | Milli-Q water | Black light blue UV-A (8 W) and UV-C (15 W) Initial concentration: 2.0–16.0 μM TiO$_2$ loading: 0.04–5.0 g/L Temperature: 26 °C Oxygen concentration: 8.2–36.3 mg/L pH: 3.5–11.0 | HPLC, TOC, GCMS | Rapid paracetamol degradation and mineralization occurred in the presence of TiO$_2$ under UV-C irradiation. Also, direct photolysis under UV-C radiation was more effective compared to that of UV-A | Yang et al. (2008) |
| | Milli-Q water | UV lamp (254 nm) Initial concentration: 4.0 mM TiO$_2$ loading: 0.4 g/L Temperature: 26 ± 2 °C H$_2$O$_2$ concentration: 48 mM Oxygen concentration: 8.2 mg/L (saturated) or 1.3–37.1 mg/L | HPLC, GCMS, IC | HO$^{\bullet}$ radical played an important part in the degradation of paracetamol with a second-order rate constant ($1.7 \times 10^9$ M$^{-1}$ s$^{-1}$) | Yang et al. (2009) |

**Table 3.3** (continued)

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| | Double-distilled water | Metal halide lamp (250 W)<br>Initial concentration: 20–200 $\mu$M<br>TiO$_2$ loading: 0.25–1.0 g/L<br>Temperature: 15 °C<br>pH: 3.0–11.0 | HPLC, GCMS | Initial concentration of 100 $\mu$M was degraded by 95 % in the presence of 1.0 g/L TiO$_2$ within 100 min of irradiation. TiO$_2$ photocatalysis is efficient on paracetamol degradation without generating any toxic compound other than the parent drug | Zhang et al. (2008b) |
| | Milli-Q water | UV-A/LED (365 nm)<br>Light intensity: 1–4 mW/cm$^2$<br>Initial concentration: 100–385 ppb<br>TiO$_2$ P25 loading: 0.005–0.06 g/L<br>Temperature: 25 $\pm$ 2 °C | RP-LC, LC-MS-MS | Addition of TiO$_2$ accelerates the paracetamol degradation and complete conversion was obtained within 20 min. Smaller amounts of TiO$_2$ and stronger UV light helps for more light to penetrate into sample | Xiong and Hu (2012) |

concentration, 0.6 g/L/8.17 mg/L yielded the highest degradation rate with a residual DCF percentage of 0.4 % after 30 min of irradiation. Longer irradiation times of up to 2 h were required to achieve complete mineralization. The toxicity of irradiated solutions was monitored with a microtox bioassay (*Vibrio fischeri*) and showed a decrease in the inhibition percentage to less than 1 % in 2 h, thus demonstrating the efficiency of photocatalytic detoxification. A total of 11 transformation products with four products with similar m/z ratios of 312 were generated from hydroxylation of DCF. This was attributed to the non-selective nature of the $HO^\bullet$ radical. Hydroxy and bishydroxy-DCF derivatives were formed with subsequent formation of other chloro and hydroxy-phenol derivatives.

UV/$TiO_2$ degradation of DCF alone and in mixture solutions was examined in two studies by Rizzo et al. (2009a, b). For the investigation of DCF as a single compound in Milli-Q water, the authors investigated two operational parameters under oxygen supply, the initial concentration (5–80 mg/L) and loading of $TiO_2$ Aeroxide P25 (0.2–1.6 g/L) with a batch reactor. All degradation data followed pseudo-first order kinetics, but at higher initial concentrations, 40 mg/L and 80 mg/L and 1.6 g $TiO_2$/L, DCF degradation was reported to follow second-order kinetics. COD removal increased from 78 % to 85 % proportionally with $TiO_2$ loading from 0.4 to 0.8 g/L and a DCF initial concentration of 15 mg/L, but declined when DCF concentrations were increased to 40–80 mg/L, Rizzo et al. (2009a).

The degradation of DCF in the presence of two other APIs, namely amoxicillin (AMX) and carbamazepine (CBZ), was investigated in a subsequent study by the same group, Rizzo et al. (2009b). In this study, spiked samples in Milli-Q water and wastewater samples collected from a WWTP in Italy downstream of the biological process were used. The experimental set-up was similar to that in their previous study. Photocatalytic oxidation displayed higher removal from the sample mixture prepared using pure water after 30 min of irradiation, than the sample mixture in real wastewater. This difference is attributed to the existence of other oxidizable species and radical scavengers such as carbonates in the wastewater samples, which are able to compete with the APIs during the photocatalytic treatment. The following Eqs. (3.12) and (3.13) show the scavenging effects of carbonate species on hydroxyl radicals:

$$HCO_3^- + HO^\bullet \;\rightarrow\; H_2O + CO_3^{-\bullet} \quad k = 8.5 \times 10^6\,M^{-1}\,s^{-1} \qquad (3.12)$$

$$CO_3^{2-} + HO^\bullet \;\rightarrow\; OH^- + CO_3^{-\bullet} \quad k = 4.0 \times 10^8\,M^{-1}\,s^{-1} \qquad (3.13)$$

TOC removal of up to 80 % after 2 h and a rapid increase after 30 min in the sample mixtures in pure water was observed. However, TOC $t_{1/2}$ (half-life time) was found to be significantly higher in the sample mixtures in both water matrices compared to that of the single compound samples investigated.

UV-A photocatalytic irradiation was applied to the decomposition of DCF with different types of $TiO_2$, water matrices, $H_2O_2$ concentrations, $TiO_2$ loadings and
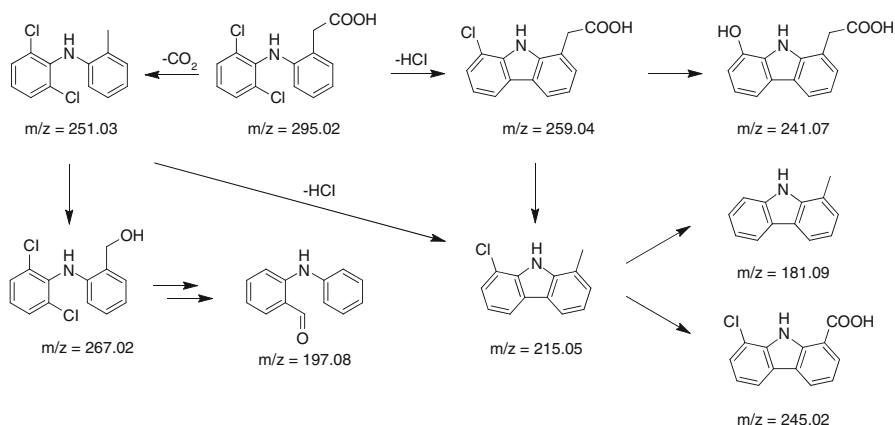
**Scheme 3.1** Photocatalytic degradation pathways and degradants of diclofenac (DCF), Martínez et al. (2011)

initial concentrations investigated, Achilleos et al. (2010a). An immersion-well type reactor equipped with a UV-A lamp (350–400 nm, 9 W) was used in this study. All experiments were conducted at a nominal pH of 6 with continuous oxygen flow. Among six $TiO_2$ samples tested, the $TiO_2$ Degussa P25 experiment showed the highest conversion of 85 % (initial concentration 10 mg/L and $TiO_2$ 250 mg/L) after 4 h of irradiation due to the better photocatalytic activity of anatase. Nevertheless, the P25 $TiO_2$ loading resulted in no improvement of DCF conversion and in all cases only 85 % conversion was attained. The results demonstrated that UV-A/$TiO_2$ treatment is effective for DCF abatement as well as mineralization. Another major finding was that photocatalytically treated 10 mg/L DCF (500 mg/L $TiO_2$) samples taken after 120 min were highly toxic to the freshwater species *Daphnia magna*.

An attempt was made to compare the effect of the irradiation source (near UV–vis and UV) on the photocatalytic degradation of DCF with commercial $TiO_2$ P25 and synthesized $TiO_2$, Martínez et al. (2011). This study highlighted that UV radiation (254 nm) was found to be superior to near UV–vis (366 nm) for DCF photocatalytic degradation. The optimum conditions for DCF removal included 0.5 g/L of synthesized anatase in the presence of 50 % (v/v) DO. In terms of mineralization, irradiation of aqueous DCF in the presence of 1 g/L $TiO_2$ P25 with near UV–vis caused about 40 % reduction in TOC in 60 min where the concentration declined from 112 mg/L to 68.6 mg/L. Eight photoproducts were identified regardless of reaction conditions. The main photoproduct formed was identified as a monohalogenated carbazole with a m/z of 259 g/mol as a result of photocyclization of DCF which was also in accordance with a DCF photolysis study, Agüera et al. (2005). Another important pathway, decarboxylation generated two other intermediates corresponding to a m/z of 251 g/mol and 215 g/mol (Scheme 3.1), respectively.

More recently, the combination of heterogeneous photocatalysis with other AOPs for DCF degradation has been studied. The effect of photocatalytic ozonation ($O_3$/UV-A/$TiO_2$) on DCF was assessed, Aguinaco et al. (2012). Irradiation was performed using a high pressure Hg lamp which was placed in the centre of a borosilicate-type glass photoreactor. Commercial $TiO_2$ Degussa P25 and ozone generated from pure oxygen were used. One interesting finding was that the wastewater sample of DCF showed no differences in terms of degradation rate and TOC removal for a DCF concentration of 30 mg/L, whereas differences were observed when Milli-Q water was used under the same conditions indicating a significant effect of the water matrix. The study reported negligible differences when investigating $TiO_2$ loadings (0.5–2.5 g/L) on 30 mg/L DCF and in all cases complete degradation was achieved in 5.5 min.

From the results above on the UV/$TiO_2$ degradation of DCF, it is evident that attempts have been made to correlate selected operational parameters with DCF degradation in pure water and also wastewater. It can be concluded that the choice of initial concentration of DCF, $TiO_2$ loading and irradiation time differs between the studies. However, there is no single study which exclusively covers all the operating parameters such as pH (from acidic to basic) and $TiO_2$ loading (low to high range). The effects of radiant flux and wavelength are not clearly understood despite the fact that UV-A type of irradiation has been chosen for the majority of the studies reviewed.

**Naproxen**

The occurrence of naproxen (NPX) has been reported both in water and wastewater. A recent review has also indicated its continuous presence in the water bodies, Ziylan and Ince (2011). Concentrations of NPX in WWTP effluents and surface water ranged between 0.1–2.6 µg/L and 0.01–0.1 µg/L respectively, Boyd et al. (2005). However, there is still a considerable lack of information on its photocatalytic degradation compared to its direct photolysis which has thus far been performed with artificial UV light and direct sunlight, Felis et al. (2007) and Packer et al. (2003). NPX photodegradation products were found to be more toxic than the parent compound based on a toxicity studies conducted using *Daphnia magna* and *Vibrio fischeri*, Isidori et al. (2005).

Only one study has been conducted on the photocatalytic and photolytic degradation of NPX, Méndez-Arriaga et al. (2008a). In this study, 3 h of photolysis and photocatalysis with a solar simulator produced 90 % and 40 % NPX removal, respectively. Lower percentage removal during the photocatalytic process resulted from either low adsorption of NPX onto the $TiO_2$ surface or possible recombination and deactivation of the HO• radical. A higher mineralization (20 %) level was however achieved with photocatalysis compared to photolysis (5 %). Initial degradation rate of NPX increased with $TiO_2$ loading (0.1–1 g/L) for 0.8 mM/L NPX. The by-products formed from the 180 min of treatment were identified
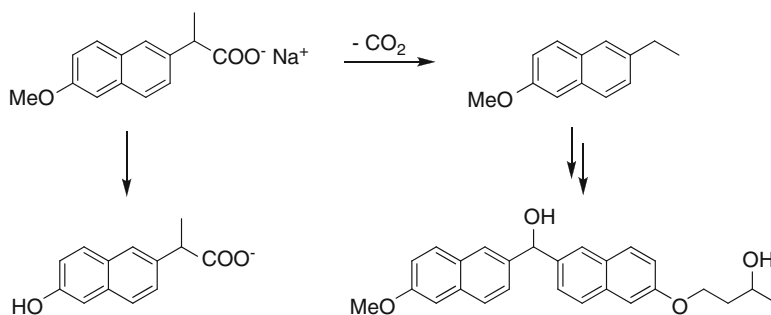
**Scheme 3.2** The proposed degradation pathway of naproxen (NPX) by $TiO_2$ photocatalysis, Méndez-Arriaga et al. (2008a)

by means of liquid chromatography-electrospray ionization-time of flight mass spectrometry (LC/ESI-TOF-MS). Two major pathways including demethylation and decarboxylation were thus suggested to occur on the photocatalytic treatment of NPX (Scheme 3.2).

In another study, the photocatalytic degradation of NPX was evaluated together with two other NSAIDs, namely DCF and ibuprofen (IBP), Méndez-Arriaga et al. (2008b). A Duran tubular photoreactor was placed in a solar simulator and irradiated with a Xe-OP lamp (290–400 nm; photon flux 6.9 µEinstein/s). A maximum degradation of NPX was obtained at a $TiO_2$ loading of 0.1 g/L for 200 mg/L NPX (30 °C and flow rate of 0.2 mL/min) with a kinetic rate constant of $7.0 \times 10^{-3}$ $min^{-1}$. An increase in $TiO_2$ (0.1–1 g/L) did not improve the degradation rate. NPX degradation and TOC conversion however showed a slight increase at 40 °C. The reaction rate for each NSAID did not vary significantly. Biodegradability ($BOD_5$/COD) showed no improvement after photocatalytic treatment of NPX, implying that the by-products formed affect post-biological treatment.

Another study was carried out to evaluate the feasibility of commercial nanofiltration membranes and $TiO_2$ P25 Degussa for NPX and six other pharmaceuticals (furosemide, rantinide, ofloxacine, phenazone, carbamazepine and clofibric acid) degradation, Molinari et al. (2006). For irradiation purposes, a medium pressure Hg lamp (125 W) was used under continuous oxygen bubbling. NPX showed a 95 % adsorption at pH 3 and only 3 % at pH 11 due to high solubility. Degradation of NPX was only performed in a batch photoreactor without any membrane. A comparison with the membrane photoreactor for NPX removal was thus not possible. In a batch photoreactor, the concentration of NPX decreased with increasing irradiation time following pseudo-first order kinetics. The rate constants for 5 mg/L NPX at pH 3 and pH 11 were $7.86 \times 10^{-2}$ and $4.91 \times 10^{-1}$ $min^{-1}$, respectively.

Although NPX is known to undergo direct photolysis under natural sunlight, Packer et al. (2003), various aspects of the efficiency of photocatalytic NPX degradation remain to be investigated. Further research needs to be undertaken in order to gain sufficient knowledge on the degradation kinetics and toxicity outcome of photocatalytically treated samples, in addition to the by-products generated.
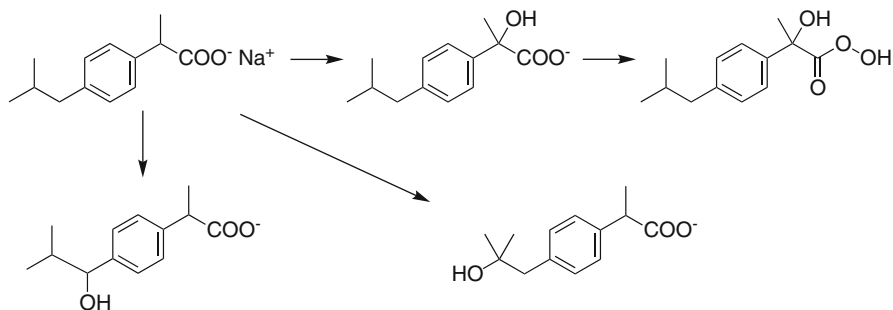
**Scheme 3.3** Photocatalytic degradation pathways of ibuprofen (IBP), Méndez-Arriaga et al. (2008b)

## Ibuprofen

As for the other NSAIDs, ibuprofen (IBP) has been found in water, wastewater and wastewater effluents due to only partial removal in WWTPs. For example, the concentrations of IBP and one of its metabolites was recorded to be more than 500 ng/L in the effluent of a primary WWTP, Sabri et al. (2012). Elimination of IBP in WWTPs varies between 75 % and 90 %, Tixier et al. (2003). The most prominent metabolites of IBP, namely carboxy and/or hydroxy IBP, have been reported to be present after biological treatment as toxic by-products, Mozia and Morawski (2012). Despite being one of the most consumed pharmaceuticals, there has been little attention devoted to the photocatalytic oxidation of IBP thus far.

A systematic study of IBP (and CBZ) photocatalytic degradation and mineralization spiked in Milli-Q and wastewater samples was performed under UV-A and simulated solar irradiation, Achilleos et al. (2010b). The study concluded that heterogeneous photocatalysis with both, UV-A and solar irradiation, was effective for IBP removal in the presence of $TiO_2$ in particular Degussa P25. The study proposed that this treatment can be employed as a post-secondary treatment in WWTPs.

UV/$TiO_2$ degradation of IBP and two other NSAIDs, DCF and NPX, revealed that IBP can be completely removed, Méndez-Arriaga et al. (2008b). A maximum conversion of 200 mg/L IBP was achieved in the presence of 1 g/L $TiO_2$ after 240 min of irradiation with a first-order kinetic rate constant of $9.1 \times 10^{-3}$ $min^{-1}$. A low initial concentration of IBP (25 ppm) resulted in maximum degradation within 60 min and 50 % mineralization. Further investigation showed that biodegradability ($BOD_5$/COD index) of IBP was enhanced in the presence of excess DO. The study thus proposed the feasibility of post-biological treatment. Electrospray ionization/mass spectrometry (ESI/MS) analysis at the end of 4 h of photocatalytic treatment revealed that hydroxylation or HO$^\bullet$ attack on the propanoic moiety or/and isobutyl side chain is the dominant pathway for IBP degradation. Demethylation and decarboxylation processes resulted in the formation of other organic acids such as propionic, formic or hydropropionic acid or their sodium salts (Scheme 3.3).

Solar photocatalysis has proven to be an effective method for IBP degradation based on a study conducted in three solar pilot plants, Méndez-Arriaga et al. (2009a). TOC and IBP removal were enhanced in the presence of $H_2O_2$. In contrast, no improvement was found on inclusion of $Na_2S_2O_8$ as the pH decreased up to 4.5 which correlates to the $pK_a$ of IBP (4.5) and thus leads to precipitation. Improvement in the biodegradability ($BOD_5/COD$) of the treated solution suggested coupling potential with biological treatment.

Effective photocatalytic degradation rates have also been reported when hybrid AOP methods were applied to IBP, Méndez-Arriaga et al. (2009b) and Madhavan et al. (2010). One study confirmed that AOP hybrid processes (sonophotocatalysis with $TiO_2$, sonophoto-Fenton and sonophotobicatalysis with $TiO_2$ and $Fe^{2+}$) improved the degree of mineralization of IBP in parallel with IBP removal. The combination of $TiO_2/Fe^{2+}$/sonolysis turned out to be the most beneficial application, owing to its highest mineralization (98 %), Méndez-Arriaga et al. (2009b). The combination of UV and ultrasound with $TiO_2$ (UV/$TiO_2$/ultrasound) enhanced the degradation of 18.5 mg/L IBP (in 1 g/L $TiO_2$ P25) from 61 % (photocatalysis only) to 85 % after 15 min of irradiation, Madhavan et al. (2010).

## Acetaminophen

Acetaminophen commonly known as paracetamol is the API in a variety of over-the-counter analgesic and antipyretic drugs. This drug is extensively used around the globe. In England, it was used in more than 400 t in 2000 while the export quantity from China was estimated to be 15,348 t in 2001, Zhang et al. (2012). Its occurrence in levels up to 6 ppb and 10 ppb, respectively, in natural waters, in European sewage treatment plant effluents and in the United States has been reported. However, only few studies deal with the removal of paracetamol by means of UV/$TiO_2$.

A study dedicated to explore the effect of various operating parameters on photocatalytic degradation for paracetamol in Milli-Q water was reported in 2008, Yang et al. (2008). Comparing UV-A/$TiO_2$ and UV-C/$TiO_2$ irradiation, the latter was found to be more effective to degrade 4.0 mM (0.4 g/L $TiO_2$) of the parent compound as well as reducing TOC to about 60 % in 300 min. Increasing $TiO_2$ loading from 0.04 g/L to 5.0 g/L caused the degradation rate to increase from $4.9 \pm 0.5$ to $14.7 \pm 1.7 \times 10^{-3}$ min$^{-1}$ until it levelled off to $13.7 \pm 1.2 \times 10^{-3}$ min$^{-1}$ at 0.8 g/L. Insignificant effects were found at 7.0 g/L due to scattering phenomena. Increase in pH from 3.5 to 9.5 also increased the degradation rates from 11.0 to $16.5 \times 10^{-3}$ min$^{-1}$. However, a higher pH of 11 significantly slowed down the degradation rate due to repulsion between negatively charged $TiO_2$ and paracetamol. A similar pH effect was also observed by Zhang et al. (2008b). Addition of oxygen showed a significant impact on paracetamol degradation compared to argon as the former inhibits $e^- $-$h^+$ recombination.

In order to establish the nature of the most active species ($e_{cb}^-$, $h_{vb}^+$, HO$^•$, HO$_2^•$, $O_2^{•-}$ and $H_2O_2$) for paracetamol degradation, a subsequent study was undertaken by Yang et al. (2009) using UV-A irradiation. It was proposed that the HO$^•$ radical plays a prominent role in the photocatalytic degradation of paracetamol.
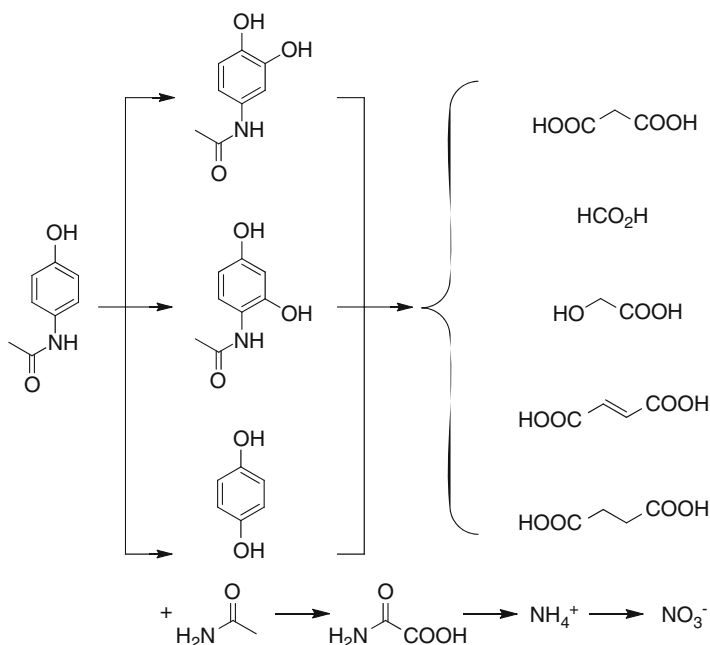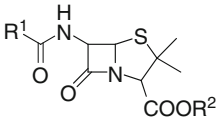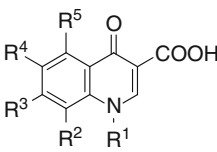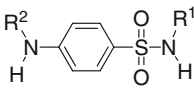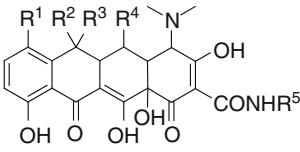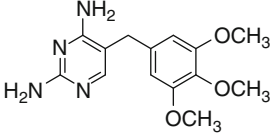
**Scheme 3.4** Photocatalytic degradation pathways and degradants of paracetamol, Yang et al. (2008, 2009)

Both studies identified 11 common intermediates, including aromatic compounds, carboxylic acids, and nitrogen containing aliphatic compounds from photocatalytic degradation of paracetamol (Scheme 3.4). Two additional inorganic intermediates, namely ammonium and nitrate, were proposed as a result of successive mineralization of the intermediates. The degradation is initiated by hydroxylation through HO$^{\bullet}$ addition onto the aromatic ring, which subsequently results in further oxidation to form carboxylic acid derivatives, Yang et al. (2009).

Recent advances in the photocatalytic degradation of paracetamol revealed that UV-A/LEDs (Light Emitting Diode) can be used instead of the traditional Hg lamps. Conventional lamps are known for their high energy cost and the incorporation of toxic mercury, Xiong and Hu (2012). Negligible amounts of paracetamol were removed with UV-A/LED photolysis. In contrast, TiO$_2$ P25 caused a significant level of degradation with complete degradation within 20 min. An increase in light intensity from 1 to 4 mW/cm$^2$ significantly enhanced the degradation rate of paracetamol. One important advantage demonstrated in this study is the possibility to employ low average amount of TiO$_2$ (0.01 g/L) due to the high light intensity from LED lamps. This finding has important implications for pursuing the applications of UV-A/LED light sources in water and wastewater treatment.

**Table 3.4** Classifications of antibiotics (Adapted from Homem and Santos (2011))

| Classes | Features of structures | Chemical structure |
|---------|------------------------|--------------------|
| Penicillins | Thiazolidine ring connected to a ß-lactam ring, to which a side chain is attached |  |
| Imidazoles | Heterocyclic compounds of 5 member di-unsaturated ring structure with two nitrogen atoms at nonadjacent positions |  |
| Quinolones | Two fused rings with a carboxylic acid and a ketone group. When $R^4$ is F, then it is a fluoroquinolone |  |
| Sulfonamides | Sulfonyl group connected to amine group |  |
| Tetracyclines | Consists of an octahydrotetracene ring skeleton and four fused rings |  |
| Trimethoprim | Diaminopyrimidine, analogue of the pteridine moiety of folic acid |  |

### 3.5.2 Antibiotics

In recent years, there has been an increasing amount of literature on the photo-catalytic treatment of antibiotics, due to their extensive use in both humans and animals as agents to prevent and treat microbial infections. Classifications of the major antibiotics are presented in Table 3.4, according to importance attached to their chemical structure and mechanism of action.

The annual consumption of antibiotics for human and veterinary usage is estimated to be about 100,000–200,000 t, Xu et al. (2007). The first detection of antibiotics in river water was reported in 1982 in England, where macrolides, tetracycline (TC) and sulphonamides at levels of 1 µg/L were found, Homem and Santos (2011). Since then, numerous studies have reported the presence of antibiotics in water courses around the world, Watkinson et al. (2009). The polar and non-volatile nature of antibiotics contributes significantly to their accumulation

in the environment due to their persistence either as the parent compound or metabolites after conventional water treatment. One of the major effects of the continuous accumulation is the emergence of antibiotic resistance strains of bacteria, Elmolla and Chaudhuri (2010a). Accumulation of small quantities of antibiotics in receiving water over a long term has been the main concern, as they may have adverse effects on both humans and aquatic organisms.

Studies conducted to assess the potential of UV/TiO$_2$ degradation on four important classes of antibiotics namely β-lactam antibiotics, quinolones, tetracyclines and sulphonamides are summarized in Table 3.5.

## β-Lactam Antibiotics

Amoxicillin (AMX) (C$_{16}$H$_{19}$N$_3$O$_5$S) has been one of the most popular candidates amongst the β-lactam antibiotics selected for UV/TiO$_2$ treatment. This API has three p$K_a$s namely 2.7, 7.5 and 9.6, Dimitrakopoulou et al. (2012). In water, AMX shows absorption up to 290 nm (Fig. 3.4). Oral consumption of 500 mg of AMX has resulted in $86 \pm 8$ % of AMX unchanged in human urine within 2 h of consumption, Martins et al. (2009). Excretion of unchanged AMX due to a slow rate of metabolism in humans has led to its discharge into the environment. AMX has also been detected in effluents from drug manufacturing facilities with levels reaching several hundreds of milligrams per liter, Mavronikola et al. (2009). The possibilities of AMX elimination by means of UV/TiO$_2$ have been investigated by several research groups, Martins et al. (2009) and Dimitrakopoulou et al. (2012).

AMX spiked ultrapure water and secondary effluent water were used in a UV-A/TiO$_2$ degradation comparative study, Dimitrakopoulou et al. (2012). Of the various catalysts tested, TiO$_2$ Degussa P25 (250 mg/L) was found to be the most efficient TiO$_2$ catalyst, yielding complete degradation after 25 min and 93 % mineralization within 90 min of irradiation for 10 mg/L of AMX. Under the same conditions, TiO$_2$ (Anatase) required at least 45 min to cause a complete removal. The other six TiO$_2$ catalysts needed much longer reaction times and in most cases lead to incomplete degradation. In addition, the degrees of mineralization failed to rise above 75 % after 90 min of irradiation. Direct photolysis with a UV-A light source had little effect on AMX degradation (only 4 %) due to its poor absorbance within the emission spectrum of the irradiation source. The comparison between ultrapure water and secondary effluent showed that the latter hindered the degradation due to the presence of organic matter and scavenging effects of bicarbonates and chlorides.

AMX spiked hospital wastewater samples yielded complete degradation with TiO$_2$ Degussa P25 photocatalysis (800 mg/L at pH 4 and 30 °C) after 30 min of irradiation while only 85 % degradation was achieved using the photo-Fenton process after an extended period of 60 min, Martins et al. (2009). The lower degradation in the latter case was explained by the pH conditions as it has a significant effect on the treatment. At pH 4 and beyond, the formation of Fe$^{2+}$ hydroxide complexes and Fe$^{3+}$ species retard the production of HO$^{\bullet}$. In contrast, the

**Table 3.5** Highlights of heterogeneous photocatalytic studies on antibiotics

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| *Amoxicillin* | | | | | |
| | Ultrapure water and treated wastewater (secondary effluent) | 9 W Radium Ralutec Initial concentration: 2.5–30 mg/L TiO$_2$ loading: 100–750 mg/L Type of TiO$_2$: TiO$_2$ Degussa P25 (21 nm), Hombikat UV 100 (5 nm), Millennium PC50 (20–30 nm), Millennium PC100 (15–25 nm), Millennium PC105 (15–25 nm), Millennium PC500 (5–10 nm), Tronox AK1 (20 nm), Aldrich Anatase AA (15 nm) pH: 5 and 7.5 | TOC, HPLC, toxicity on *Escherichia coli*, *Klebsiella pneumonia* and *Enterococcus faecalis* | UV-A/TiO$_2$ is an effective abatement method for AMX in water in particular with TiO$_2$ Degussa P25. Antibiotic activity of irradiated solution showed that *Escherichia coli* and *Klebsiella pneumonia* were highly resistant to AMX up to 25 mg/L | Dimitrakopoulou et al. (2012) |
| | Hospital wastewater | Medium pressure Hg lamp (125 W; intensity: 401 W/m$^2$) TiO$_2$ P25 loading: 400–1,200 mg/L Temperature: 20–40 °C pH: 3–11 | HPLC, COD, toxicity on *Artemia salina* | Response surface methodology was used to study the combined effect of pH and TiO$_2$ loading on COD reduction and AMX photocatalysis. Maximum COD removal was achieved with 800 mg/L TiO$_2$ and pH 3. Toxicity inhibition of 46.3 % was obtained | Martins et al. (2009) |

| | Water type | Experimental conditions | Analytical methods | Results | References |
|---|---|---|---|---|---|
| | Water type not provided | 15 W low pressure luminescent Hg lamp (UV, 365 nm) and 15 W daylight fluorescent lamp (Vis, 0.6 mW/cm$^2$) Solar radiation (16 mW/cm$^2$) Initial concentration: 20–100 mg/L TiO$_2$ concentration: 1 g/L Temperature: 20 ± 1 °C pH: 3–9 | UV–vis (230 nm), IC, UPLC-ESI/MS, QTOF/MS, COD | Carbon and iron doped titania and Degussa P25 was tested for AMX degradation under artificial visible light and solar radiation. Doped titania yielded comparable degradation with that of Degussa P25 under UV light | Klauson et al. (2010) |
| *Amoxicillin mixtures* | | | | | |
| Amoxicillin (ampicillin and cloxacillin) | Distilled water | UV lamp (6 W, 365 nm) Initial concentration: 104 mg/L TiO$_2$ (Anatase) loading: 0.5–2.0 g/L ZnO loading: 0.2–2.0 g/L Temperature: 22 ± 2 °C pH: 3–11 H$_2$O$_2$ concentration: 50–300 mg/L | HPLC, COD, BOD$_5$, DO, DOC | All treatments degraded the antibiotics and also improved biodegradability except for UV/ZnO which did not show any effect on the latter. Photo-Fenton showed highest rate constant (0.029 min$^{-1}$) and was most cost-effective | Elmolla and Chaudhuri et al. (2010b) |
| Amoxicillin (ampicillin and cloxacillin) | Distilled water | UV lamp (6 W, 365 nm) Initial concentration: 104 mg/L TiO$_2$ (Anatase) loading: 0.5–2.0 g/L Temperature: 22 ± 2 °C pH: 3–11 H$_2$O$_2$ concentration: 50–300 mg/L | HPLC, COD, BOD, DO, DOC | Addition of H$_2$O$_2$ at pH 5 with TiO$_2$ 0.1 g/L led to complete degradation of all antibiotics. Mineralization was also observed through DOC removal and formation of nitrate, ammonia and sulphate | Elmolla and Chaudhuri et al. (2010c) |

(continued)

**Table 3.5** (continued)

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| Amoxicillin (Diclofenac) | Deionized water | Solar simulator (250–765 W/m² ) Initial concentration: 200 mg/L TiO₂ loading: 100 mg/L | HPLC, UV-vis | Degradation of AMX was more efficient in the presence of TiO₂ where about 70 % removal was achieved after 6 h of irradiation at 400 W/m² | Kockler et al. (2012) |
| *Sulfonamide and other sulfa antibiotics* | | | | | |
| Sulfamethoxazole | Milli-Q water | Solar simulator Xe lamp (1,000 W) Initial concentration: 100 ppm TiO₂ concentration: 0.1–2.0 g/L Temperature: 25 ± 0.1 °C Oxygen concentration: 8 mg/L Initial pH: 5 (not buffered) pH: 2, 5, 7 and 11 | HPLC, TOC, BOD₅/COD, FIA, IC, LCMS | Increase in TiO₂ amount improved SMX degradation and TOC removal. Change in pH showed more effect on TOC removal than that on the degradation of SMX | Abellán et al. (2007) |
| Sulfamethoxazole (and trimethoprim) | Milli-Q water | Solar simulator (Xe lamp, 1,500 W) Initial concentration: 100 mg/L TiO₂ loading: 0.1–2.0 g/L Temperature: 25 °C | HPLC, TOC, BOD, COD | Increase in the amount of TiO₂ has great influence on the reaction rate. The optimum catalyst concentration was between 0.5 and 1.0 g TiO₂/L for SMX degradation | Abellán et al. (2009) |
| Sulfamethoxazole | Reverse-osmosis water | Hg-Ar (Germicidal UV-C) lamp Initial concentration: 3–12 mg/L TiO₂ P25 loading: 0.5 g/L Temperature: 25 ± 0.1 °C DO concentration: 8.8 mg/L Pure oxygen concentration: 42 mg/L | HPLC, COD, DO, pH, UV–vis, LCMS, toxicity test on *Daphnia magna* | Kinetic rate constant decreased with increasing SMX concentration. DO did not favour removal of SMX | Nasuhoglu et al. (2011) |

| Compound | Water matrix | Conditions | Analytical method | Results | References |
|---|---|---|---|---|---|
| Sulfamethoxazole | Ultrapure water, municipal wastewater and ground water | UV-A Radium lamp (9 W) Initial concentration: 2.5–30 mg/L TiO$_2$ loading: 100–750 mg/L Type of TiO$_2$: TiO$_2$ Degussa P25, Hombikat UV 100, Millennium PC50, Millennium PC100, Millennium PC105, Millennium PC500 Temperature: 25 °C pH: 4–8 | HPLC, TOC | Degradation of SMX was described by L-H model. Excess DO showed large effect on TOC removal rather compared to SMX degradation. Initial reaction rate for SMX decomposition increased from 0.428 mg/(L × min) to 1.510 mg/(L × min) when SMX concentration was increased from 2.5 mg/L to 20 mg/L | Xekoukoulotakis et al. (2011) |
| Sulfachlorpyridazine, sulfapyridine and sulfisoxazole | Milli-Q water | High pressure Hg lamp (125 W) Initial concentration: 50–200 µM TiO$_2$ P25 loading: 0.25–3.0 g/L Initial pH: 3–11 Radical quencher: isopropanol and potassium iodine | TOC, HPLC, HPLC/MS/MS | All three sulfa drugs, sulfachlorpyridazine, sulfapyridine and sulfisoxazole can be effectively degraded in aqueous system with removals of 85.2 %, 92.5 % and 85.0 % after 1 h of irradiation respectively. Effect of pH varied between the drugs | Yang et al. (2010) |
| Sulfamethoxazole and sulfonamide analogues (sulfisoxazole, sulfathiazole, sulfamethizole) | Deionized water | Xe arc lamp (450 W) Initial concentration: 5–480 µM TiO$_2$ P25 loading: 0.01–1.0 g/L Hombikat UV-100 loading: 0.1 g/L Temperature: 25 °C pH: 3–11 | HPLC, LC/MS/MS, DOC | UV-A/TiO$_2$ photocatalysis is very effective for degrading sulfonamides particularly in natural waters exhibiting either alkaline pH or low concentrations of NOM, or both conditions | Hu et al. (2007) |

**Table 3.5** (continued)

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| *Quinolones* | | | | | |
| Oxolinic acid | Water type not provided | Annular reactor with black light (36 W) Initial concentration: 18 ppm TiO$_2$: 1 g/L (suspension) and 8.79 g for immobilized form pH: 9 | Spectrophotometer, TOC, COD, antibacterial activity with *Escherichia coli* | Treated solutions showed no antibiotic activity. TiO$_2$ coated on sintered glass cylinder efficient to remove OX acid | Palominos et al. (2008) |
| Oxolinic acid | Ultrapure water | Black lamp (14 W/m$^2$; 365 nm) Initial concentration: 20 mg/L TiO$_2$ P25 loading: 0.2–1.5 g/L pH: 7.5–11 | LC with fluorescence detector, HPLC-MS DOC, COD, antimicrobial activity on *Escherichia coli* | Experimental conditions of pH 7.5 and 1.0 g/L of TiO$_2$ favoured between 80 % and 100 % of OX acid degradation. About 20 % of OX acid was adsorbed on TiO$_2$ under dark conditions | Giraldo et al. (2010) |
| Ciprofloxacin | Double distilled water | High pressure Hg lamp (0.38 mW/cm$^2$) Initial concentration: 100 µM TiO$_2$ P25 loading: 1.5 g/L Temperature: 25 °C pH: 3.0–11.0 | HPLC, LC/MS/MS | Oxidative and reductive processes are important for CIP degradation. Hydroxyl and azide radicals can react with CIP at high rate constants, $(2.15 \pm 0.10) \times 10^{10}$ and $(2.90 \pm 0.12) \times 10^{10}\ \mathrm{m}^{-1}\ \mathrm{s}^{-1}$ respectively | An et al. (2010) |

| Compound | Water | Conditions | Analysis | Observations | References |
|---|---|---|---|---|---|
| Ciprofloxacin | Deionized water | $O_3$ free Xe arc lamp (450 W) Initial concentration: 100 $\mu$M $TiO_2$ and Hombikat UV 100 loading: 0.5 g/L Temperature: 25 °C pH: 6 | HPLC, HPLC/UV/FLD, LC/MS/MS, antibacterial activity with *Escherichia coli* | Degradation rate of CIP under comparable solution conditions (100 mM CIP, 0 or 0.5 g/L $TiO_2$, pH 6, 25 °C) followed the trend UV-A/$TiO_2$ > Vis-$TiO_2$ > UV-A | Paul et al. (2010) |
| Ciprofloxacin | Hospital wastewater | Medium pressure Hg lamp (125 W) Initial concentration: 200 $\mu$g/L $TiO_2$ P25 loading: 400 mg $TiO_2$/700 mL | LC/FLD, UV–vis, COD | Heterogeneous photocatalysis and ozonation were compared for CIP degradation in hospital wastewater. Both treatments produced complete degradation in 60 min and degradation products generated were similar | Vasconcelos et al. (2009) |
| Moxifloxacin | Deionized water | UV-A (485 $\mu$W/cm$^2$) Initial concentration: 12.5–124.6 $\mu$M $TiO_2$ P25 loading: 0.25–8 g/L Temperature: 25–65 °C Oxygen concentration: 0–100 % pH: 7 Stirring speeds: 2.3, 7.9 and 13.2 rps Effect of radical inhibitors: isopropanol and potassium iodide | HPLC | Degradation kinetics was affected by the variables investigated. Oxidative holes play more important role than hydroxyl radicals as it led to 63 % MOX degradation | Van Doorslaer et al. (2012) |

(continued)

**Table 3.5** (continued)

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| Norfloxacin | Double distilled water | Medium pressure Hg lamp (125 W) Initial concentration: 0.15–0.50 mM $TiO_2$ loading: 1 g/L Type of $TiO_2$: Degussa P25, Hombikat UV100, PC500 $H_2O_2$ concentration: 10 mM pH: 4.2, 6.3, 8.1 and 10.4 | UV–vis, TOC | $TiO_2$ Degussa P25 was found to be more efficient than other catalysts. Degradation rate increased with initial concentrations at optimum pH 10.4 from $0.009 \times 10^{-3}$ mol $L^{-1}$ $min^{-1}$ (0.15 mM) to $0.017 \times 10^{-3}$ mol $L^{-1}$ $min^{-1}$ (0.50 mM) | Haque and Muneer (2007) |
| Levofloxacin | Reverse osmosis water | Hg-Ar Germicidal UV-C lamp (254 nm; intensity: $1.3 \times 10^{-3} \pm 0.3$ Einstein/min/L) Initial concentration: 20 mg/L $TiO_2$ P25 loading: 0.05–0.5 g/L | HPLC, COD, antibacterial activity on *Escherichia coli* and *Pseudomonas fluorescens* | First study to report photocatalysis and ozonation under UV-C radiation for levofloxacin removal. About 97 % levofloxacin was removed after 2 h under photocatalysis. No antibacterial activity found under both treatments | Nasuhoglu et al. (2012) |
| *Fluoroquinolone mixtures* | | | | | |
| Ofloxacin norfloxacin, ciprofloxacin and enrofloxacin | Distilled water | Solar irradiation (800 W Xe lamp) Initial concentration: 0.028 mM (ofloxacin), 0.031 mM (norfloxacin), 0.027 mM (ciprofloxacin) and 0.028 mM (enrofloxacin) $TiO_2$ loading: 0.1–1.5 g/L pH: 2–9 $H_2O_2$ concentration: 41.25–330 mg/L | HPLC, TOC, antibacterial activity with *Bacillus subtilis* | Photocatalytic reactions of the four FQs could be described by a pseudo-first order kinetic model. Complete removal of all FQs at 0.5 g/L $TiO_2$ and 82.5 mg/L of $H_2O_2$ at pH 6 after 90 min of irradiation | Li et al. (2012) |

| Ciprofloxacin, danofloxacin, enrofloxacin, levofloxacin, marbofloxacin and moxifloxacin | River water | Natural sunlight (290–470 W/m²) Phosphorus-covered low pressure Hg lamp (12 W/m², 310 nm) Initial concentration: 20–50 μg/L TiO₂ P25 loading: 0.5 g/L | HPLC with fluorescence detector, ESI-MS/MS | About 15 min sufficient to remove the FQs under natural sunlight | Sturini et al. (2012) |
| --- | --- | --- | --- | --- | --- |
| Ciprofloxacin and moxifloxacin | Deionized water | UV-A (485 μW/cm²) and UV-C (389 μW/cm²) Initial concentration MOX: 37.4 μM Initial concentration CIP: 45.3 μM TiO₂ P25 loading: 0.5 g/L Temperature: 25 °C pH: 3–10 | HPLC | The highest removal rates were obtained at pH 7 in the presence of TiO₂ (0.5 g/L) as a photocatalyst were as follows: $k_1$, CIP, UV-A = 0.137 min$^{-1}$, $k_1$, CIP, UV-C = 0.163 min$^{-1}$, $k_1$, MOX, UV-A = 0.227 min$^{-1}$, $k_1$, MOX, UV-C = 0.236 min$^{-1}$ | Van Doorslaer et al. (2011) |
| *Tetracyclines* | | | | | |
| Tetracycline | Water type not provided | Solar simulator (300–800 nm, Xe lamp, 250 W/m²) TiO₂ P25 loading: 0.5–1.5 g/L ZnO loading: 0.2–1.5 g/L pH range for TiO₂: 3–10 pH range for ZnO: 6–11 | Spectrophotometer, TOC, AAS, antibacterial activity with *Staphylococcus aureus* | Irradiation in the presence of catalyst completely removes the solution's antibacterial activity. The catalytic activity of both catalysts indicates that ZnO presents a slightly higher oxidation rates than TiO₂ | Palominos et al. (2009) |

**Table 3.5** (continued)

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| Tetracycline | Deionized water | UV lamp (125 W), UV-A lamp (black light, 160 W), solarium device (6 W × 20 W lamps, 300–400 nm) Initial concentration: 40 mg/L $TiO_2$ P25 loading: 0.5 and 1 g/L Oxygen gas flow: 200 mL/min | HPLC, TOC, UV–vis, antibacterial activity on Staphylococcus aureus strain | In the absence of $TiO_2$, no degradation was observed. Degradation of TC up to 50 % was obtained after 10, 20 and 120 min under UV lamp, solarium device and UV-A lamp respectively | Reyes et al. (2006) |
| Tetracycline | Water type not provided | Medium pressure Hg lamp (700 W) Initial concentration: 67 mg/L $TiO_2$ loading: 30 mg/L | HPLC, DOC, LC-ESI/MS/MS, toxicity on pseudomonas | Toxicity test carried out on Pseudomonas showed decrease in toxicity during tetracycline photocatalysis. Irradiation of 90 min is not sufficient to generate biodegradable by-products | Mboula et al. (2012) |
| Oxytetracyline | Deionized water | Solar simulator (Xe-OP lamp 1 kW) and CPC reactor Initial concentration: 20 mg/L $TiO_2$ P25 loading: 0.1 and 0.5 g/L pH: 3, 9, 11 and free initial pH 4.4 | HPLC, TOC, BOD, COD, UV-vis, LC-MSD-TOF, ESI-MS, toxicity test on Vibrio fischeri | Combination of 0.5 g/L $TiO_2$ at nominal pH (pH ∼ 4.4) showed complete removal after 40 min of irradiation with 7.5 kJ/L of UV dose | Pereira et al. (2011) |
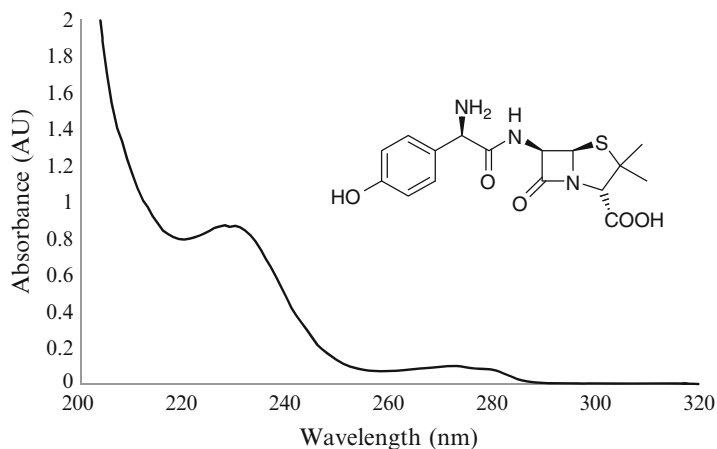
**Fig. 3.4** Absorbance spectrum of amoxicillin in water

percentage COD removal was higher for photo-Fenton than for photocatalysis with 64.6 % versus 44 %, respectively. Toxicity inhibition studies with *Artemia salina* showed that both treatments were efficient in removing toxicity and reductions of 43.5 % and 46.3 % were obtained in photo-Fenton and UV/TiO$_2$ processes, respectively.

Another study compared the efficiency of four AOPs, namely Fenton, photo-Fenton, TiO$_2$ photocatalysis (UV/TiO$_2$ and UV/TiO$_2$/H$_2$O$_2$) and ZnO photocatalysis (UV/ZnO) on AMX (104 mg/L) in distilled water in combination with two other antibiotics, ampicillin (105 mg/L) and cloxacillin (103 mg/L) under UV-A irradiation (365 nm), Elmolla and Chaudhuri (2010b). Based on the pseudo-first order rate constants under optimum operating conditions, photo-Fenton demonstrated the highest rate constant of 0.029 min$^{-1}$, followed by Fenton with 0.0144 min$^{-1}$ with t$_{1/2}$ of 49.5 min and 69.3 min, respectively. Although the performances of UV-A/TiO$_2$/H$_2$O$_2$ and UV/ZnO were similar in terms of the reported rate constants of 0.0005 min$^{-1}$ and 0.00056 min$^{-1}$, respectively, the rate constant for UV/TiO$_2$ was not reported therefore preventing a meaningful comparison. With the exception of UV-A/ZnO, biodegradability (BOD$_5$/COD) was improved for all treatment methods investigated.

A separate study reported the efficacy of TiO$_2$ (Anatase with purity >99 %) on AMX (and ampicillin and cloxacillin) degradation in distilled water, irradiated with UV-A lamp (365 nm, 6 W) which was further enhanced by the addition of H$_2$O$_2$, Elmolla and Chaudhuri (2010c). These results, somewhat contradictory with those reported by Martins et al. (2009), may be due to differences in the photocatalytic activity of the different TiO$_2$ used, the irradiation source, addition of H$_2$O$_2$ and also the water matrix. None of these studies described the degradation products formed during the photocatalytic treatment.
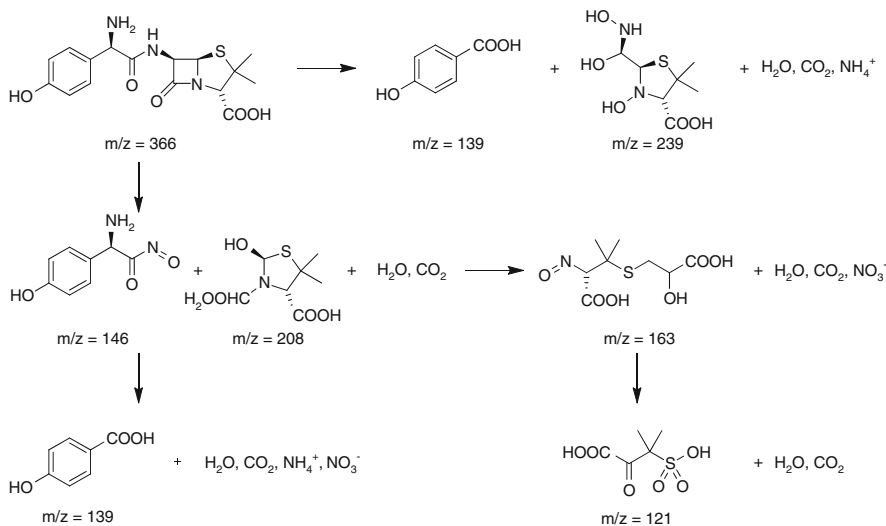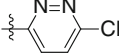
**Scheme 3.5** Proposed photocatalytic degradation pathway for amoxicillin (AMX) (10 mg/L AMX; pH 6; 20 °C), Klauson et al. (2010)

A comparison between photocatalysis using doped and undoped titania with artificial UV-A and sunlight showed that that latter produced a three times faster degradation for AMX than artificial UV, Klauson et al. (2010). The improved efficiency of 2 h of solar (16 mW/cm$^2$) photocatalytic illumination with TiO$_2$ Degussa P25 over 6 h irradiation with artificial UV-A light (365 nm, 0.5 mW/cm$^2$) for AMX was apparent. The faster degradation occurred at pH 6 (20 °C) and a concentration of 50 mg/L and yielded the highest photocatalytic efficiency of 80 %. Doped catalysts with Fe and C also showed comparable efficiencies to that of Degussa P25. With artificial UV light, COD was removed to a percentage between 10 % and 40 % in all experiments. This study revealed the superiority of solar photocatalysis for AMX conversion. Photocatalytic degradation pathways were proposed to occur for different AMX concentrations, 10, 25 and 100 mg/L (pH 6, 20 °C) at three different reaction rates of control. The majority of the degradation products differed under these three reaction conditions, with only a few common products identified. Only small amounts of ammonia, nitrate and sulphate were detected, which implies that the heteroatoms N and S remained in the organic by-products. *p*-Hydroxybenzoic acid (m/z 139) was a common degradation product found under all photocatalytic conditions. It is proposed to form through the fragmentation of the peptide bond, which is in close proximity to the aromatic ring in AMX. Scheme 3.5 shows the proposed degradation pathway for AMX.

It can be concluded that artificial UV and solar photocatalysis with TiO$_2$ facilitated efficient AMX degradation. ZnO was also effective, albeit only one study used this photocatalyst. As a result of this, more research is needed to evaluate the

**Table 3.6** Selected sulfonamides and their structures

| Common name | -R |
|---|---|
| Sulfathiazole |  |
| Sulfisoxazole |  |
| Sulfapyridine |  |
| Sulfamethoxazole |  |
| Sulfachloropyridazine |  |

potential of ZnO for AMX degradation by optimizing the parameters influencing its degradation as this catalyst has higher photocatalytic activity than $TiO_2$. There are also similarities expressed between studies where the UV-A type of irradiation was sufficient to cause AMX degradation.

**Sulfonamide and Other Sulfa Antibiotics**

Sulfonamides and sulfanilamides are derived from sulfanilic acid and their common features are the sulfanilamide group and a five or six member heterocyclic ring (shown as R in the general structure) and are represented in Table 3.4. Selected sulphonamides, with their common names and the nature of their R groups are shown in Table 3.6. Sulfonamides and sulfanilamide derivatives are antibacterial agents used both in animals and humans for the treatment of infections. Almost 80 % of all sulfonamides have been detected in the environment, which could easily infiltrate groundwater, and have shown high toxicity to microorganisms, algae and plants, Baran et al. (2011).

Sulfamethoxazole (SMX) ($C_{10}H_{11}N_3O_3S$), being the most commonly used member of the group of sulfonamide antibiotics, is prescribed for urinary tract infections and has been detected in the environment. This synthetic antibiotic is used either alone or in combination with trimethoprim. SMX has been detected in plant effluents at concentrations ranging between 0.01 and 2.0 µg/L in countries such as Germany, Spain and Italy and in surface waters between 0.03 and 0.48 ug/L, Trovó et al. (2009). SMX has two $pK_a$ values, $pK_{a1} = 1.6$ and $pK_{a2} = 5.7$, and thus exists predominantly in the cationic form at pH < 1.6 and in the anionic form at pH > 5.7. There have been reports on UV/$TiO_2$ treatment of this pharmaceutical using various lamp sources, Abellán et al. 2009 and Nasuhoglu et al. (2011).

SMX has been reported to be susceptible to direct photolysis with a Xe lamp resulting in significant degradation, but poor mineralization, Abellán et al. (2007, 2009). In water, SMX is capable of absorbing light up to 310 nm. When irradiated
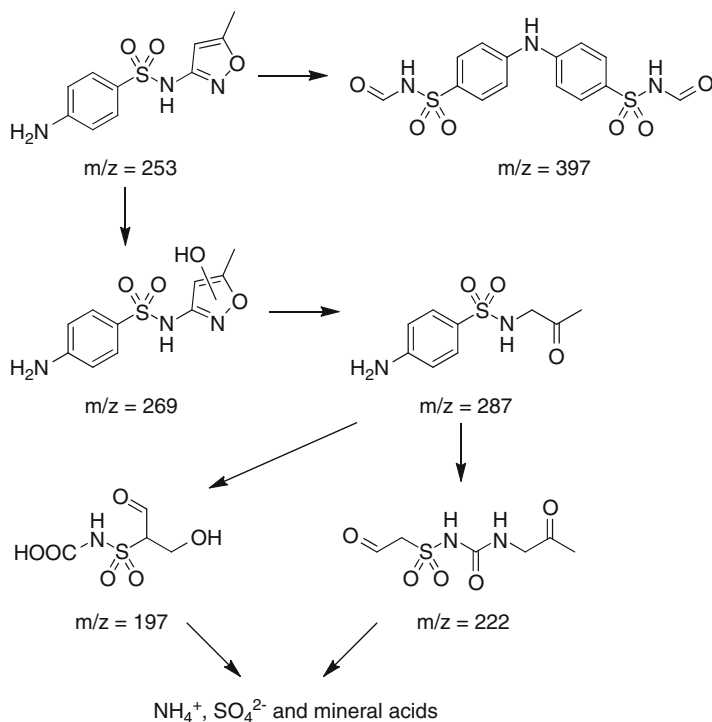
**Scheme 3.6** Degradation pathways of sulfamethoxazole (SMX) with TiO₂ photocatalysis, Abellán et al. (2007)

without the use of a filter and photocatalyst, SMX degraded up to 80 % but only resulted in a reduction of 14 % of TOC, Abellán et al. (2007). In contrast, the photocatalytic degradation of 100 ppm SMX (irradiation of 6 h) in the presence of 1.0 g/L TiO₂ yielded 88 % reduction in SMX. With 2.0 g/L TiO₂, the degradation marginally increased to 91 %. The optimum TiO₂ P25 concentration was found between 0.5 and 1.0 g/L. Variations in pH (2–11) resulted in very minor changes and the degradation remained almost constant at 85 %, with a very small increase at lower pHs. A total of five intermediates were identified using mass spectrometry. Of these, the intermediate with a molecular weight of m/z = 397 was found to be the major product and was assigned to a dimer product of the parent SMX. Other intermediates possessed molecular weights of 222 in ES positive ion mode and 197, 269 and 287 in ES negative ion mode, Abellán et al. (2007) (Scheme 3.6).

UV-C irradiation (254 nm) was employed to study the photolytic and photocatalytic degradation of SMX in pure water, Nasuhoglu et al. (2011). Irradiation was carried out in a cylindrical acrylic photoreactor equipped with an Hg-Ar lamp source. The results suggest that photolysis is an important removal mode for SMX (12 mg/L) which corroborates the findings of Abellán et al. (2007). The high tendency for direct photolysis was explained based on the matching of the SMX

absorbance with the highest intensity emission of the UV-C lamp. UV-C radiation yielded complete degradation of 12 mg/L SMX within 10 min, while UV/TiO$_2$ required 30 min to achieve a similar level of degradation. However, mineralization measured as COD was more efficient on photocatalysis treatment with 87 % removal, compared to only 24 % removal upon photolysis. Photoproducts generated as a result of both treatments were reported to be more toxic than the parent SMX, as demonstrated by the acute toxicity *Daphnia magna* testing.

SMX was reported to undergo UV-A (350–400 nm) induced photocatalytic degradation with TiO$_2$ Degussa P25, Xekoukoulotakis et al. (2011). A 9 W lamp (photon flux $2.81 \times 10^{-4}$ Einstein/min) in an immersion well batch photoreactor was used under continuous oxygen sparging. Complete degradation of 10 mg/L SMX with 250 mg/L TiO$_2$ P25 was achieved in 30 min. Likewise, a TOC reduction of up to 90 % was observed. The photocatalytic degradation at different initial concentrations (2.5–30 mg/L) followed the Langmuir-Hinshelwood (L-H) kinetic model.

The behaviour of three sulfa pharmaceuticals, namely sulfachlorpyridazine, sulfapyridine and sulfisoxazole with similar structures towards UV/TiO$_2$ treatment were investigated by Yang et al. (2010). A Pyrex reactor with a high pressure Hg lamp ($\lambda_{max} = 365$ nm) was chosen. Photocatalytic treatment proved generally effective on these sulfa drugs, with removal efficiencies varying between 85.2 % and 92.5 % after 60 min of irradiation. TOC was concomitantly removed in high percentages (81.5–90.8 %) within 240 min with all three drugs showing complete mineralization to CO$_2$, H$_2$O and inorganic ions. Photocatalytic degradation rate constants of these three sulfa drugs also increased with the amount of TiO$_2$ from 0.25 to 3 g/L. It was proposed that $h^+$ and HO$^{\bullet}$ play the most important role in the disappearance of sulfa pharmaceuticals. Remarkably, different photocatalytic degradation rate constants for the three drugs were obtained at different pH values despite their similarity in structure. This was attributed to changes in their zwitterionic nature in different pH environments. Various intermediates formed rapidly within 15 or 20 min of irradiation and then slowly disappeared. The two major pathways for photocatalytic degradation of the sulfa drugs were hydroxylation and cleavage of the S-N bond by the photohole (Scheme 3.7).

Another study employed a UV-A light source to determine the degradation of SMX and a few other sulphonamides, namely sulfamethizole, sulfathiazole and sulfisoxazole, Hu et al. (2007). The effects of natural organic matter (NOM) and bicarbonate ion on the degradation of SMX were investigated. The occurrences of NOM and bicarbonate ions in water are known to impede the degradation of compounds by scavenging HO$^{\bullet}$. However, the results showed that an increase in bicarbonate concentrations generated a higher degradation rate for SMX. This was attributed to considerably higher HCO$_3^-$ and CO$_3^{2-}$ concentrations compared to SMX, which is likely to induce a stronger interaction with Ti (IV) than between SMX and Ti (IV). As a result, these ions efficiently scavenged the adsorbed HO$^{\bullet}$ radicals, which in turn reduced the recombination of the charge carriers. The CO$_3^{-\bullet}$ radicals generated were proposed to react with SMX in the bulk rather than on the
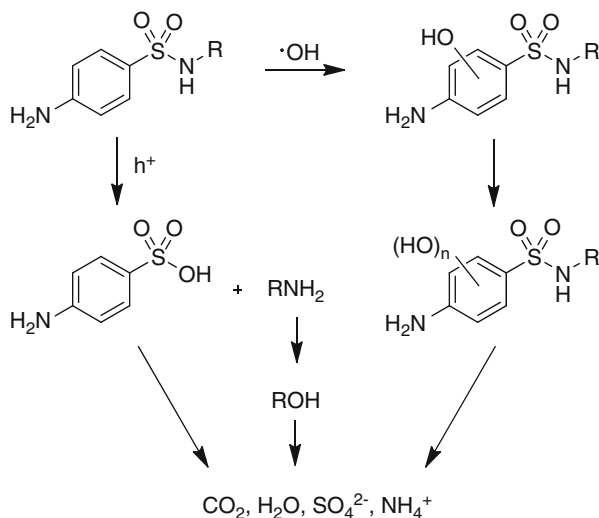
**Scheme 3.7** Photocatalytic degradation pathways of sulfa pharmaceuticals, Yang et al. (2010)

surface because they are more stable and posses longer lifetimes. In contrast, an increase in NOM concentrations (2–20 mg/L) inhibited the SMX degradation, in particular at higher concentrations of NOM.

## Quinolones and Fluoroquinolones

Quinolones are synthetic antibacterial agents which are widely used in both humans and livestock. The basic structure of quinolones contains two fused rings with a carboxylic acid and a ketone group (Table 3.4). First and second generation quinolones are active against gram-negative bacteria, while the third and fourth generations have extended activity against gram-positive bacteria as well, Nasuhoglu et al. (2012). Fluoroquinolones (FQs) have attracted substantial attention due to their incomplete metabolism and frequent detection in the environment. A FQ compound contains a fluorine substituent in position $R^4$ of the quionolone ring as shown in Table 3.4.

FQs are excreted to between 20 % and 80 % and their persistence has led to detection in surface waters, municipal wastewaters and tertiary treated effluents, Michael et al. (2010). As with other synthetic antibiotics, FQs are not fully metabolized and thus excreted unchanged. The persistent nature of FQs is associated with the quinolone ring, which results in high chemical stability and contributes additionally to their resistance towards hydrolysis, Sturini et al. (2010). Only a few studies dealing with the degradation of FQs by $TiO_2$ mediated photocatalytic treatment have been reported thus far. In general, incomplete mineralization of FQs results in the generation of a range of intermediates which still contain the quinolone ring structure and as a result continue to exhibit antibacterial activity.

The performance of immobilized $TiO_2$ integrated into an annular reactor was tested with black light (360 nm) using 18 ppm oxolinic acid (OX) at pH 9 under oxygenated conditions. A comparison was made with a 1 g/L titania suspension, using the same reactor. The coated titania was capable of removing OX acid to over 90 % after 60 min, while TOC was reduced by 50 % after 100 min of irradiation. In suspension, 90 % removal was achieved after 20 min whereas total removal took 40 min. The superior performance of the suspended $TiO_2$ was explained by the superior adsorption, which subsequently increased the reaction rate, Palominos et al. (2008).

A systematic evaluation of OX acid removal in ultrapure water was conducted by Giraldo et al. (2010). The optimum conditions for complete degradation of 20 mg/L OX acid after 60 min of irradiation were with 1.0 g/L $TiO_2$ and pH 7.5. COD was reduced by 50 % and the degradation followed pseudo-first order kinetics. The study also revealed that OX acid intermediates, which were identified by high performance liquid chromatography-mass spectrometry (HPLC/MS) exhibited lower toxicity than the parent compound in addition to a loss of antimicrobial activity based on an *Escherichia coli* assay.

UV-A/$TiO_2$ is also an effective technology for oxidation of ciprofloxacin (CIP) in pure water, An et al. (2010) and Paul et al. (2010). UV-A and Vis light sources were employed to study the photolytic and photocatalytic treatment of CIP, Paul et al. (2010). Based on the pseudo-first order rate constants, the efficiency of CIP degradation increased in the order UV-A/$TiO_2$ > Vis/$TiO_2$ > UV-A only. Degradation of CIP by UV-A/$TiO_2$ is proposed to occur via three mechanisms: direct UV-A photolysis, charge transfer and charge separation involving the semiconductor. Various piperazine ring transformation products were formed upon UV-A/$TiO_2$ and Vis/$TiO_2$ treatment and their proposed structures are shown in Scheme 3.8. Energy efficiencies for the three treatment protocols were compared and the UV-A/$TiO_2$ turned out to be the most energy efficient process.

Degradation of 100 μM CIP and a short $t_{1/2}$ of 1.9–10.9 min were furthermore achieved at different pH ranges (3.0–11.0) using 1.5 g $TiO_2$/L Degussa P25 and UV-A irradiation (365 nm) emitted from a high pressure Hg lamp. The degradations followed pseudo-first order kinetics and the rate constant increased from $0.06 \pm 0.01$ $min^{-1}$ at pH 3 to $0.38 \pm 0.01$ $min^{-1}$ at pH 9. At a higher pH value of 11, the degradation rate decreased to $0.07 \pm 0.01$ $min^{-1}$ due to the repulsive effect between the negatively charged compound and the negatively charged $TiO_2$ surface. Addition of hydroxyl radicals to CIP and photohole attack were established as the two predominant reaction pathways, An et al. (2010).

Photocatalytic degradation of four FQs, namely ofloxacin, norfloxacin, ciprofloxacin and enrofloxacin, was examined simultaneously using a solar simulator, Li et al. (2012). Alkaline conditions (pH 9) resulted in almost complete degradation (98–100%) for all four FQs after 150 min of irradiation. Likewise, TOC was reduced by 58 %. The threshold $TiO_2$ load was found to be 0.5 g/L at pH 9 to achieve 96–100% of degradation of the four FQs. An increase in the $TiO_2$ load from 0.1 g/L to 1.5 g/L did not produce a direct correlation with TOC removal. The effects of pH changes on these four FQs were rather complex, due

m/z = 332

**Photolysis**

m/z = 330          m/z = 316          or          m/z = 316

**Photocatalysis**

m/z = 263          m/z = 291          m/z = 334

or

m/z = 362          m/z = 364          m/z = 334
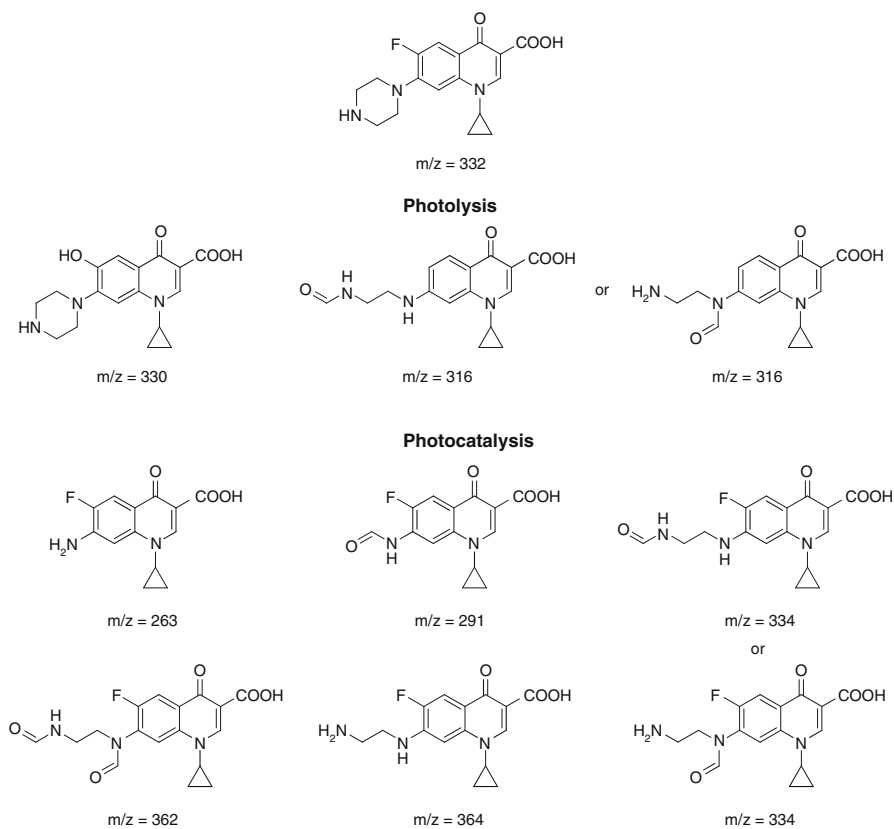
**Scheme 3.8** Possible degradation products of ciprofloxacin (CIP) from photolysis and TiO$_2$ photocatalysis, Paul et al. (2010)

**Table 3.7** Molecular formulas and p$K_a$ values of four fluoroquinolones (FQ), Li et al. (2012)

|                  | Ofloxacin            | Norfloxacin          | Ciprofloxacin        | Enrofloxacin         |
| ---------------- | -------------------- | -------------------- | -------------------- | -------------------- |
| Chemical formula | $C_{18}H_{20}FN_3O_4$ | $C_{16}H_{18}FN_3O_3$ | $C_{17}H_{18}FN_3O_3$ | $C_{19}H_{22}FN_3O_3$ |
| p$K_a$           | p$K_{a1}$: 6.05      | p$K_{a1}$: 6.20      | p$K_{a1}$: 6.00      | p$K_{a1}$: 6.03      |
|                  | p$K_{a2}$: 8.11      | p$K_{a2}$: 8.70      | p$K_{a2}$: 8.80      | p$K_{a2}$: 8.70      |

to different ionization states of the catalyst and the substrates (two p$K_a$ values for each). In particular, these FQs can either exist in their cationic (pH < p$K_{a1}$), or anionic (pH > p$K_{a2}$) or in zwitterionic form (p$K_{a1}$ < pH < p$K_{a2}$) (Table 3.7) due to the presence of a carboxylic acid group in the quinolone structure and an additional amine group from the piperazinyl ring.

A third generation FQ, moxifloxacin (MOX) was subjected to UV/TiO$_2$ and the influence of various parameters was examined using a batch photoreactor equipped with a UV-A lamp (300–440 nm, 485 $\mu$W/cm$^2$), Van Doorslaer et al. (2012). A catalyst loading of 5 g/L, temperature of 25 °C and air sparging at 60 mL/min were found optimal for removing 37.4 $\mu$M of MOX. The initial degradation rate and the t$_{1/2}$ were determined to be 16.2 $\pm$ 0.3 $\mu$M/min and 1.6 min, respectively. A stirring speed of 13.2 rps produced a higher degradation rate. The TiO$_2$ P25 loading ranged from 0.1 to 5.0 g/L.

An independent study highlighted that TiO$_2$ Degussa P25 was more effective than two other common photocatalysts, Hombikat UV 100 and PC 500 for the degradation of 0.25 mM of norfloxacin (NOR) (pH 6.3) with 1 g/L of photocatalyst, Haque and Muneer (2007). Almost complete degradation was achieved in 80 min of irradiation from a medium pressure Hg lamp (125 W) under oxygen purging. Likewise, the TOC content was depleted as a function of irradiation time. The efficiency of the photocatalytic degradation of NOR was subsequently correlated with pH, substrate concentration, catalyst concentration, and the addition of electron acceptor, H$_2$O$_2$. The results revealed that addition of 10 mM of H$_2$O$_2$ significantly enhanced the degradation rate and mineralization of 0.25 mM NOR (pH 6.3 and 1 g/L TiO$_2$ P25).

A recent study was conducted with unfiltered river water to explore the photocatalytic decomposition of six FQs, namely ciprofloxacin, danofloxacin, enrofloxacin, levofloxacin, marbofloxacin and moxifloxacin, under natural sunlight. Compared to direct photolysis under natural sunlight, all of the tested drugs except CIP underwent faster degradation with degradation rate constants ranging from 0.22 to 2.78 min$^{-1}$ in the presence of TiO$_2$ (0.5 g/L). The results demonstrated the effectiveness of solar TiO$_2$ photocatalysis for the removal of FQs from river water matrix despite the presence of other non-target matrix components, Sturini et al. (2012). CIP in hospital wastewater was also satisfactorily removed with UV/TiO$_2$ using a medium pressure Hg lamp. For comparison, ozonation was used as an alternative AOP. Both processes generated similar degradation products and oxidation of the piperazine group was found to be the major degradation pathway, Vasconcelos et al. (2009).

The results presented for quinolones and FQs are significant and demonstrate the potential removal from wastewaters not only for single compounds but also for mixtures.

**Tetracyclines**

A comparison of photocatalytic degradation and mineralization of oxytetracycline (OTC) in a laboratory and at pilot-scale was reported by Pereira et al. (2011). Laboratory scale experiments with a solar simulator showed that photocatalysis of 20 mg/L OTC with 0.5 g/L TiO$_2$ at free initial pH produced 95 % degradation. In contrast, only 36 % degradation was achieved upon direct photolysis after 60 min of irradiation. Solar photocatalysis in a CPC pilot plant reactor showed complete

**Fig. 3.5** Comparison of pseudo-first order constant for tetracycline (TC) degradation (TC: 40 mg/L; TiO$_2$ P25: 0.5 g/L) (Data taken from Reyes et al. (2006))
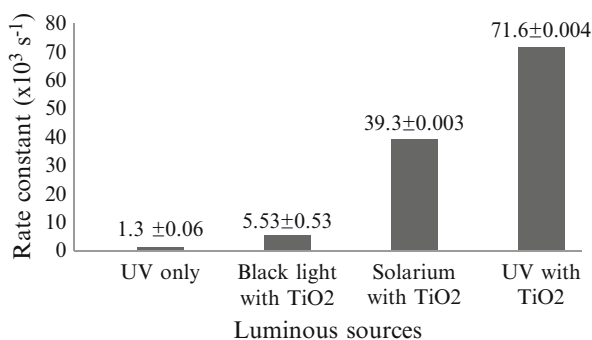


removal of OTC with energy consumption 10 times less than solar photolysis which was also performed by means of CPC reactor. Solar photocatalysis in the pilot plant reactor yielded 80 % mineralization. All experiments followed pseudo-first order kinetics, but the rate constants increased from the photolytic to photocatalytic operation modes. Solar photocatalysis in a CPC reactor produced the highest pseudo-first order rate constant of $2.63 \pm 0.03$ L/kJ, indicating the efficiency of such a photoreactor to harvest and utilize solar photons.

Several studies involving tetracycline (TC) have been carried out to determine its photocatalytic degradation, Reyes et al. (2006), Palominos et al. (2009) and Mboula et al. (2012). Catalyst performance and biodegradability or antibacterial efficiency arising from the treated solutions differed for all studies reported. Using a multivariate and response surface method approach, the optimum degradation rate for each catalyst was investigated. ZnO exhibited a slightly higher oxidation rate than TiO$_2$ P25. The optimum oxidation conditions for TiO$_2$ were established as 1.5 g/L and at pH 8.7. For ZnO, the most appropriate conditions were 1.0 g/L and pH 11, Palominos et al. (2009).

The TC disappearance over the irradiation time, at different pH values and with three different light sources, namely UV lamp ($\lambda > 254$ nm), UV-A lamp ($\lambda = 365$ nm) and a solarium device ($\lambda = 300$–400 nm), was carried out by Reyes et al. (2006). Based on the pseudo-first order rate constants, direct photolysis was regarded negligible for TC. In contrast, faster degradation rates were achieved with the solarium and UV light in the presence of TiO$_2$ (Fig. 3.5). These findings were supported by the high irradiance intensities measured from the UV lamp (1,210 $\mu$W/cm$^2$ at 365 nm) and the solarium lamp (1,980 $\mu$W/cm$^2$ at 365 nm), respectively. Rapid disappearance of TC and subsequent complete mineralization was achieved after 2 h of irradiation.

### 3.5.3 Antiepileptics

The most commonly investigated antiepileptic is the dibenzazepine derivative, carbamazepine (CBZ) (Fig. 3.6). CBZ is also used as sedative for numerous mental

**Fig. 3.6** Chemical structure of carbamazepine ($C_{15}H_{12}N_2O$) (5*H*-dibenzo [*b,f*]azepine-5-carboxamide)
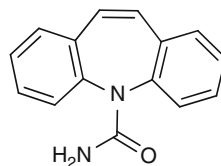


disorders, can cause toxic effects in the liver and is a haematopoietic. Only 72 % of CBZ is absorbed when orally administered, while 28 % remains unchanged and is finally excreted through faeces. CBZ has been known to persist in the environment and studies have reported the removal efficiency of CBZ in WWTPs as below 10 %, Andreozzi et al. (2002) and Zhang et al. (2008a). Thus far, CBZ has been detected in various water compartments around the world. In Germany, CBZ was detected in surface water samples at a concentration up to 1,075 ng/L, Heberer (2002b) and also in groundwater and rivers in the USA, Canada and Germany, Khetan and Collins (2007). These observations clearly confirm the CBZ presence in the aquatic environment. Although the effects of CBZ are not clearly known or confirmed, its toxic effect on aquatic organisms has been already published. There is also evidence of specific chronic effects of CBZ on the oligochaete *Chiromus*, Oetken et al. (2005). These facts show that this antiepileptic needs to be removed with effective remediation technology. In this context, photocatalysis has been shown to be effective for CBZ degradation. A summary of UV/TiO$_2$ studies of CBZ is tabulated in Table 3.8.

There has been an insufficient amount of information available pertaining to photocatalytic degradation of CBZ. Photocatalytic degradation of CBZ has however been examined in deionized water, Dai et al. (2012) and Im et al. (2012), real wastewater samples from WWTPs, Achilleos et al. (2010b) and formulated hospital wastewater, Chong and Jin (2012). In general, there are some discrepancies in the photocatalytic efficiency on CBZ.

A comparative study on the effect of different on CBZ (4.2 µM) degradation efficiencies showed the following trend: UV/Fenton (86.9 %) > UV/TiO$_2$ (70.4 %) > Fenton (67.8 %) > UV/H$_2$O$_2$ (40.6 %) > UV (14.2 %). On the basis of operating costs, the Fenton process was the most cost-effective in comparison with the other four AOPs (Table 3.9), Dai et al. (2012). As shown in Table 3.9, UV/TiO$_2$ and UV can be considered as costly approaches for wastewater treatment. However the authors reported that this statement can be challenged on the grounds that the cost of photocatalytic treatment can be reduced by applying sunlight as the irradiation source.

The removal efficiency by UV-A (350–400 nm) and solar irradiation provided by a solar simulator on CBZ was investigated along with another API, IBP, Achilleos et al. (2010b). Greater degradation was attained with UV-A irradiation than with artificial solar radiation for both APIs. Degradation of CBZ in pure water was found to be more dependent on changes in the loading of Degussa P25 TiO$_2$ compared to IBP. Conversion of 74 % CBZ was achieved after 120 min of reaction with 100 mg/L

**Table 3.8** Highlights of heterogeneous photocatalytic studies on antiepileptics

*Carbamazepine*

| Compound(s) | Water matrix | Experimental features | Analytical methods | Findings | Reference |
|---|---|---|---|---|---|
| | Deionized water | Medium pressure Hg lamp (30 W) Initial concentration: 4.2 $\mu$M TiO$_2$ loading: 0.5–2.0 g/L Temperature: 25 °C pH: 6.5 | HPLC | Comparison of UV/TiO$_2$ with UV, UV/H$_2$O$_2$ and UV/Fenton showed the UV/TiO$_2$ yielded 70.4 % degradation which was slightly lower than UV/Fenton at 86.9 % degradation. All processes followed pseudo-first order kinetics | Dai et al. (2012) |
| | Hospital wastewater | Annular slurry photoreactor UV-A black light (8 W) Initial concentration: 5,000 $\mu$g/L TiO$_2$ Anatase particles | HPLC, COD | Photodegradation at high concentration of CBZ in synthetic hospital wastewater was fitted to the L-H kinetics model | Chong and Jin (2012) |
| | Milli-Q water | UV-A lamp: 2.17 mW/cm$^2$ UV-C lamp: 3.56 mW/cm$^2$ Initial concentration: 0.021 mM TiO$_2$ loading: 0.5 g/L pH: 5.4 | HPLC, IC, TOC | UV-A and UV-C irradiation efficient to degrade CBZ | Im et al. (2012) |
| *Carbamazepine mixtures* | | | | | |
| Carbamazepine (and ibuprofen) | Milli-Q water and wastewater from WWTP | Solar simulator (1,000 W Phillip Xe lamp) and UV-A lamp (9 W Radium lamp) Initial concentration: 5–20 mg/L TiO$_2$ loading: 50–3,000 mg/L Type of TiO$_2$: Degussa P25, Hombikat UV100, Aldrich, | UV-vis (CBZ: 284 nm), DOC | Degradation under UV-A irradiation in pure water was sensitive to TiO$_2$ P25 loading. Solar and UV-A photocatalysis appears to be efficient on CBZ degradation | Achilleos et al. (2010b) |

| Compound | Water | Conditions | Analysis | Observations | Reference |
|---|---|---|---|---|---|
| Carbamazepine (Clofibric acid, iomeprol) | Milli-Q water | Tronox AK-1, Tronox TRHP-2, Tronox TR; $H_2O_2$ concentration: 0.07–1.4 mM; pH: 3–10; Solar simulator Xe short arc lamp (1,000 W) (cut-off 296 nm); Initial concentration: 1–5.5 mg/L; $TiO_2$ loading (P25 and Hombikat UV100): 500 mg/L | UV–vis, HPLC, DOC | Degradation of CBZ dependent on the concentration of NOM as it greatly influences its kinetics | Doll and Frimmel (2005a) |
| Carbamazepine (clofibric acid, iomeprol) | Ultrapure water | Solar simulator Xe short-arc lamp (1,000 W); Initial concentration: 4.3 mg/L; $TiO_2$ and Hombikat UV100 loading: 0.1 g/L; Temperature: 20 ± 2 °C | HPLC, DOC, ICP-AES, HPLC/TQMS | Pseudo-first order degradation rate of CBZ for $TiO_2$ P25 and UV100 were $4.7 \times 10^{-3}$ $s^{-1}$ and $0.13 \times 10^{-3}$ $s^{-1}$ | Doll and Frimmel (2005b) |
| Carbamazepine (Clofibric acid, iomeprol, iopromide) | Milli-Q water | Solar simulator Xe short arc lamp (1,000 W); Initial concentration: 1.0 and 4.3 mg/L; $TiO_2$ P25 loading: 0.01–1,000 mg/L; Temperature: 20 ± 2 °C; Hombikat UV100 loading: 0.1–1,000 mg/L | HPLC, DOC, ICP-AES, HPLC/ESMS, DOC | Degradation of CBZ was faster with $TiO_2$ P25 due to higher adsorption | Doll and Frimmel (2004) |

**Table 3.9** Comparison of operating costs of advanced oxidation processes (AOP) studied (Data taken from Dai et al. (2012))

| Process | Treatment cost (US$/kg) |
|---|---|
| UV | 210.4 |
| UV/$H_2O_2$ | 42.6 |
| Fenton | 5.4 |
| UV/Fenton | 26.7 |
| UV/$TiO_2$ | 70.2 |

P25 under UV-A irradiation, but it decreased to 35 % upon solar irradiation, which was attributed to completely different reactor set-ups. Wastewater samples spiked with 10 mg/L CBZ had a detrimental effect on DOC removal, due to the presence of scavengers of HO$^\bullet$ radicals and naturally occurring DOC in the raw wastewater sample. Nevertheless, the study proved that UV-A and solar irradiation can be applied to CBZ removal (and also for IBP). Direct photolysis with a UV-A lamp produced negligible degradation and DOC removal for CBZ.

The results from another study confirmed that direct photolysis supplied by a UV-C irradiation source is not effective for CBZ elimination, Im et al. (2012). An important contribution of the study is that UV-C irradiation increased the removal efficiency compared to that of UV-A irradiation employed on the 0.021 mM CBZ in the presence of 0.5 g/L $TiO_2$ P25. Addition of radical scavengers decreased the removal rate, implying that HO$^\bullet$ has a crucial role to play in the photocatalytic degradation of this compound. Addition of oxygen also enhanced the mineralization.

A $TiO_2$ nanofiber was evaluated as a pre-treatment option and for improvement of biodegradability of 5,000 µg/L CBZ in synthetic hospital wastewater, Chong and Jin (2012). This application was found to be efficient for CBZ abatement in synthetic hospital wastewater and also for COD removal. High CBZ degradation rate of 48.33 µg/L min was obtained, which also resulted in 40 % COD reduction after 4 h of treatment. The study highlighted the fact that the $TiO_2$ based system has a good potential as a sustainable pre-treatment system for hospital wastewater.

Doll and Frimmel (2004, 2005a, b) conducted multiple photocatalytic studies on CBZ under various conditions, which revealed that $TiO_2$ photocatalysis is a promising abatement method for CBZ degradation. When a comparison was made with Hombikat UV100, CBZ was degraded more efficiently with $TiO_2$ P25 under simulated solar irradiation (1,000 W), Doll and Frimmel (2004). In the subsequent study, photocatalytic degradation of CBZ was investigated with two types of $TiO_2$ photocatalysts in the presence and absence of NOM under simulated solar UV light, Doll and Frimmel (2005a). It was noted that different concentrations of NOM had different effects on the kinetics of CBZ degradation. In general, NOM retarded the photocatalytic degradation rate of CBZ. This was attributed to competitive inhibition as NOM scavenged the holes. The research group also suggested possible photocatalytic degradation products for CBZ, Doll and Frimmel (2005b) (Scheme 3.9).
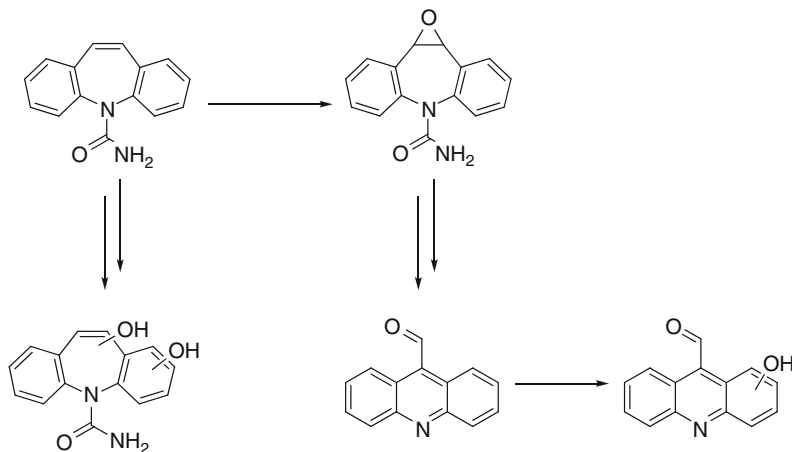
**Scheme 3.9** Photocatalytic degradation pathways of carbamazepine (CBZ), Doll and Frimmel (2005b)

## 3.6 Conclusion

A typical UV/TiO$_2$ investigation is shown in Fig. 3.7. After one or more model compounds have been chosen, the next step is the photocatalytic experimental set-up or the choice of the photoreactor and light source, which will have a significant effect on the outcome of the degradation rate, Legrini et al. (1993). There are various factors that need to be taken into consideration with respect to photoreactors, namely the material it is made of, shape, geometry, radiation path lengths, and the cooling apparatus. Similar issues have been highlighted in a review which has commented on this lack of information, when explaining photoproducts formed from the photocatalytic and photolytic studies, Fatta-Kassinos et al. (2011a).

When considering light sources, artificial light sources have been employed in most studies. These can be grouped into monochromatic light sources such as low pressure Hg lamps and polychromatic light source namely medium pressure Hg lamps and high pressure Hg lamps. Other irradiation devices include Xe, Xe-arc, Hg and Ra lamps. Solar simulators have been commonly applied to simulate solar irradiation. LED based light sources are also emerging as an alternative light source, due to advantages which include no mercury waste, longer life time compared to Hg based lamps and improved flexibility in terms of reactor design, Landgraf (2001) and Chen et al. (2007). Very few studies have made a comparison between the lamp's spectral domain and the absorption spectrum of the reactant. Photon flux, intensity and irradiance emitted from the light source also need to be clearly stated as these values determine the rate of photons emitted, which has a direct correlation to the degradation rate. Selection of a radiation source (shape and dimension) is also greatly influenced by the type of photoreactor.
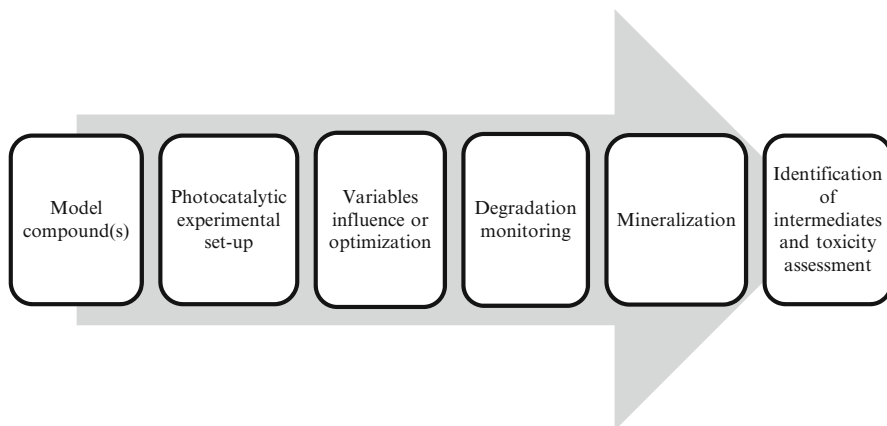
**Fig. 3.7** Typical steps involved in heterogeneous photocatalytic degradation

Reaction rates are known to be dependent on operation conditions. Optimization of these variables thus allows for maximum degradation. Statistical approaches such as multivariate analysis and response surface methodology have also been applied to establish the optimal degradation rate. One of the most commonly investigated and important factors is the $TiO_2$ loading. It has been shown that the degradation rate is not always proportional to the catalyst load, but an optimum load needs to be determined. Difficulty arises when comparing studies for a particular compound of interest. In general, optimal catalyst load is influenced by the photoreactor design including its diameter and source of irradiation. Commercially available $TiO_2$ such as Degussa P25, Hombikat UV 100, PC 500 and Aldrich have been used in various photocatalytic degradation studies of pharmaceuticals. Among them, $TiO_2$ Degussa P25 has demonstrated its superiority in removing various APIs. A concentration below the optimum catalyst loading is typically chosen for further investigation, due to the fact that catalyst deposition might occur during the experiment. For example, 0.4 g/L $TiO_2$ was chosen instead of 0.5 g/L which gave the best results for metoprolol and propranolol degradation, Romero et al. (2011).

Initial concentrations of APIs applied in photocatalytic studies tend to be higher than the environmental level. Furthermore, in most cases single pharmaceuticals are investigated despite the fact that real wastewaters contain complex mixtures of pharmaceuticals. Although most studies have demonstrated efficiency for API in spiked distilled water or ultrapure water, real wastewater samples are rarely used. The presence of radical scavengers such as $HCO_3^-$, $CO_3^{2-}$ ions and NOM in real wastewater typically impacts on degradation efficiencies. Radical scavengers or inhibitors are frequently studied to determine the role of reactive intermediates, e.g. the $HO^\bullet$ radical, $O_2^{\bullet-}$ anion and holes in the photocatalytic degradation. Isopropanol, benzoquinone and iodide ions are commonly used for this purpose.

In general, laboratory scale experiments are more common than pilot scale operations due to a more controllable environment. There is usually no direct

correlation between the effectiveness of photodegradation in the laboratory with that under real environmental conditions. Conducting photodegradation studies on pilot scales will provide more realistic environmental conditions such as season, latitude, and hardness of water. These are generally not encountered when working in the laboratory. Trials using solar energy have been reported for pharmaceutical degradation, although the results thus far vary between compounds of interest. Commercial and industrial scale applications of solar energy have been established in a few European countries, the USA and Canada.

In the subsequent step, degradation monitoring of pharmaceuticals is generally carried out by using HPLC detection or UV–vis spectrophotometry. The latter has been reported to be a fast and useful tool of monitoring although it is not the state-of-the-art technique for API detection. The kinetics of degradation in terms of percentage API removed or API disappearances versus irradiation time or concentration change versus irradiation time ($C/C_o$ vs time) are commonly reported. The L-H model has been often applied to describe photocatalytic mineralization of pharmaceutical degradation.

Apart from determining the removal rate of APIs with photocatalytic treatment, the degree of mineralization is a critical parameter in heterogeneous photocatalysis. DOC has been frequently measured as an indication of the formation of $CO_2$. In most cases, complete degradation of the pharmaceutical does not correspond directly with the mineralization rate mainly due to due to the formation of more stable compounds or transformation products during the degradation. A non-biodegradable fraction frequently remains in the treated solution. Mineralization can be also determined by measuring the formation of inorganic ions such as chlorine, sulphate and nitrate.

Although the ultimate goal of heterogeneous photocatalysis is to achieve complete mineralization of parent compounds, it is also important to evaluate the by-products generated to ensure that there are no toxic or more persistent compounds produced during the treatment. This will contradict the ultimate goal of the application of such an AOP. However, photodegradation products are not commonly studied. A few possible reasons for this are: (i) difficulties in separating and identifying a large number of these transformation products formed; (ii) lack of or non-existent analytical standards to determine the identity of these transformation products; and (iii) requirement for more than one analytical technique or sample preparation technique due to their diverse physicochemical properties, Agüera et al. (2005) and Fatta-Kassinos et al. (2011b). Moreover, studies have also assessed the toxicity of the photocatalytically treated samples by applying toxicity assays on various microorganisms and invertebrates.

In conclusion, despite most promising findings reported, the lack of compatibility in terms of degradation schemes and photoproducts formed, removal rates and degree of mineralization of APIs highlights the complex behaviour of the various APIs towards UV/$TiO_2$. Meaningful comparison between studies for scaling-up and application to real wastewater treatment at a pilot scale is limited by all these factors. Additional studies are thus urgently required to eliminate these shortcomings.

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# Chapter 4
# Water Depollution Using Ferrites Photocatalysts

**Virender K. Sharma, Chun He, Ruey-an Doong, and Dionysios D. Dionysiou**

**Abstract** The presence of organic pollutants and pathogenic microorganisms in water has become an increasing concern throughout the world. Heterogeneous photocatalytic technologies have been applied to control the organic pollutants and microorganisms in water. Development of narrow band-gap photocatalysts which function in the visible light remains a challenge in the wastewater treatment processes. Spinel ferrites has attracted a remarkable attention because of a relatively narrow band gap of about 2.0 eV, which has considerable photo-response in the visible light region. This chapter reviews recent advances in ferrites and the application of visible light photocatalysts to the remediation of contaminants such as $H_2S$, phenols, and dyes in water. Recent development in synthesis and characterization of ferrite and hybrid ferrites with other semiconductors is reviewed. The applications of ferrites in photocatalytic conversion of visible solar energy to generate $e^-/h^+$, which in turn produce reactive oxygen species through redox processes for the degradation of the pollutants in water, are demonstrated. We discuss the enhanced visible photocatalytic activity of ferrites by doping with metals and combing with

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other photocatalysts. Moreover, the addition of $H_2O_2$ to ferrite either in dark or visible light irradiation indicates the enhanced degradation efficiency for organic pollutants.

**Keywords** Water pollution • Photocatalyst • ferrite • $H_2S$ • Phenols • Dyes • $H_2O_2$ • Surfactant • Agrochemical • Glyphosate

**Contents**

## 4.1   Introduction

Freshwater is a precious resource on earth and is critical to sustain life. In many regions of the world, daily need of water is not met. According to World Health Organization (WHO), more than 880 million people in the world do not have access to potable water (WHO 2010). Water contamination caused death to 1.8 million children from diarrhea every year (WHO 2010). Water scarcity can also affect ecosystem as numerous species might not be able to cope with decrease in availability of freshwater. One of the greatest challenges in this century is to provide access to clean water. The development of nanotechnology in the past decade offers prospects of meeting challenges of safe and sustainable water demand (Di Paola et al. 2012; Qu et al. 2012). Furthermore, a combination of nanotechnology and solar energy may lead to innovative water purification technologies.

One of the well studied technologies to clean water is the photocatalytic remediation (Di Paola et al. 2012; Rajeshwar 2011; Rajeshwar et al. 2012). The photocatalytic processes on bare semiconductor titanium dioxide ($TiO_2$) have been studied for several decades, but $TiO_2$ is only active under UV irradiation ($\lambda < 400$ nm) (Abe 2011; Chen et al. 2011; Hoffmann et al. 1995; Li and Liu 2011; Serpone et al. 2012; Tao et al. 2011). The solar spectrum has very small fraction of incoming light in the UV region (ca. 4 %). A use of all the UV light would result in only 2 % solar conversion efficiency (Abe 2011). Comparatively, the portion of visible light ($400 < \lambda < 800$ nm) in the solar spectrum is much more abundant (ca. 46 %). A use of visible light up to 600 nm increases the efficiency to 16 % and further improvement to 32 % is achievable if visible light use is extended to 800 nm (Kubacka et al. 2012; Linic et al. 2011; Paracchino et al. 2011; Serpone and Emeline 2012).

Currently, the efficiency for degradation of pollutants under visible-light irradiation is still low due to the fast charge recombination and backward reactions of photocatalysts (Chen et al. 2011). The efficient visible-light-driven photocatalysts requires closing the band gaps to harvest visible light in the long-wavelength regions as well as to improve the separation of photogenerated electrons ($e^-$) and holes ($h^+$) efficiently. Dopants into $TiO_2$ have been added to narrow the band gap in order for $TiO_2$ to be appropriate for absorbing visible light (Kamat 2012; Kubacka et al. 2012; Serpone et al. 2009). However, doped $TiO_2$ materials under visible light have lower chemical activity of surface active centers and decreased photoactivities compared to those formed under UV light irradiation (Serpone and Emeline 2012). Moreover, commonly used dopants are rare, expensive and/or toxic metals, which may not fulfill the principle of sustainable chemistry. Identification of suitable candidates capable of degrading pollutants under visible light is essential to provide clean remediation processes. The objective of this article is to provide information on the progress made in remediation of water using environmentally benign visible-light active iron-based oxides, ferrites.

Ferrites have a molecular formula $M-Fe_2O_4$ in which $Fe_2O_3$ is combined with a metal oxide (M-O). Ferrites may provide an alternative to $TiO_2$ because they have shown to be efficient in the visible light region (Han et al. 2007). Metals used in ferrites include $Ca^{2+}$, $Zn^{2+}$, $Mg^{2+}$, $Ni^{2+}$, $Co^{2+}$, and $Mn^{2+}$ (Benko and Koffyberg 1986; Borse et al. 2008; Dom et al. 2011; Han et al. 2007; Tamaura et al. 1999). These ferrites, when prepared with a spinel structure, show promising photocatalytic activity (Kim et al. 2009). Spinel structures have the general formula $AB_2X_4$, where, in the case of ferrites, A is a metal, B is $Fe^{3+}$ and X is oxygen (Burdett et al. 1982). As shown in Fig. 4.1 for example for $ZnFe_2O_4$, the spinel structure usually consists of both tetrahedral and octahedral sites, where the metal atoms occupy one-eighth of the tetrahedral sites and $Fe^{3+}$ occupies one-half of the octahedral sites (Degueldre et al. 2009). The lattice parameter a and the cation-oxide distance $R_{ZnO}$ and $R_{FeO}$ in the octahedral and tetrahedral substructures were related by Eq. 4.1 (Degueldre et al. 2009)

$$A = 2(2)^{1/2}[R_{FeO} \cos(\psi/2) + R_{ZnO} \sin(\varphi/2)] \tag{4.1}$$

The tetrahedron internal angle ($\varphi$) and the octahedron equatorial angle ($\psi$) were identified as 109.471° and 95.375° for O-Zn-O and Fe-O-Fe, respectively.

The ferrites of $Zn^{2+}$, $Ca^{2+}$, and $Mg^{2+}$ seem to be favorable as they are more relatively environmentally friendly. The energy diagram of these ferrites is shown in Fig. 4.2 (Dom et al. 2011). The band gaps of ~2.0 eV show the potential of ferrites to absorb visible light absorption and also the possibility for degrading pollutants in water (Casbeer et al. 2012; Hou et al. 2010; Ida et al. 2010; Li et al. 2011; Su et al. 2012).

The present chapter first describes briefly the synthesis and characterization of ferrites, followed by application of ferrites to degrade contaminants. Most of studies in literature have been performed on $ZnFe_2O_4$ and hence examples are presented using this form of ferrite.
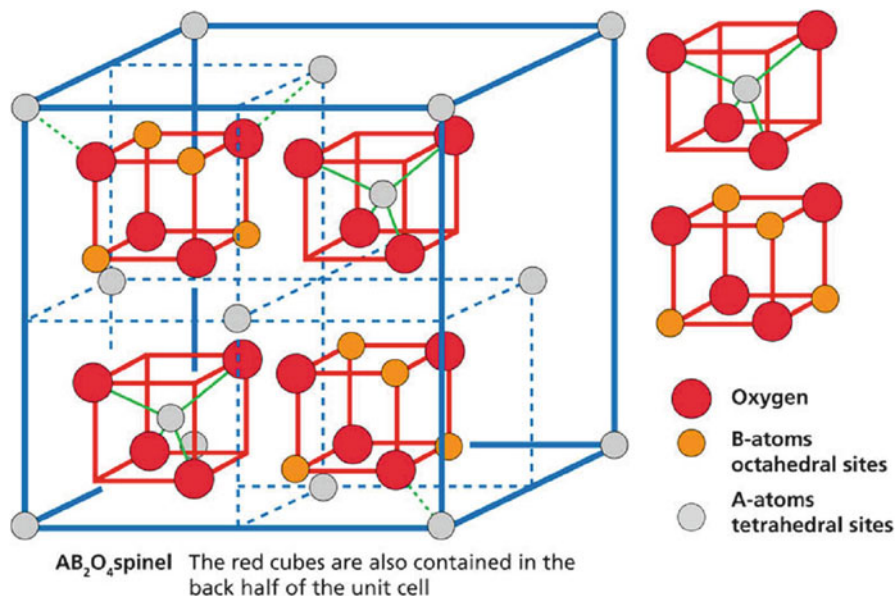
**Fig. 4.1** Schematic description of the crystallographic structure for bulk $ZnFe_2O_4$. Occupied tetrahedral site in spinel sub-cell, Zn is *gray* and O is in *red*. Occupied octahedral site in spinel sub-cell, Fe is *yellow*, and O is in *red*. The arrangement in one unit cell with 3D succession of octahedral and tetrahedral sub-cells (Adapted from (Degueldre et al. 2009) with permission from Elsevier Ltd.)
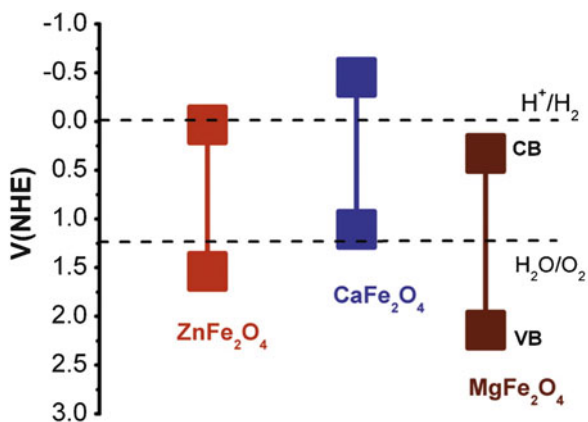


**Fig. 4.2** Schematic diagram of $MFe_2O_4$ (M: Mg, Ca, Zn) showing the feasibility of materials as visible light photocatalysts. The thickness of the band edge (CB-*conduction*/VB *valence band*) indicates the possible variation in the value depending on various physico–chemical parameters of electrolyte and environmental conditions viz. *p*H, temperature, concentration etc. and there possible effects on the Fermi energy (Adapted from (Dom et al. 2011) with the permission of Elsevier Ltd.)

## 4.2   Synthesis of Ferrites

### 4.2.1   Spinel Ferrites

A number of methods used in synthesis of spinel ferrites are shown in Fig. 4.3. These method include thermal, sol-gel and citrate, co-precipitation, and solid-state reactions (Casbeer et al. 2012; Dom et al. 2011; Hou et al. 2011; Jadhav et al. 2012; Li et al. 2011; Pardeshi and Pawar 2011; Pradeep et al. 2011; Salunkhe et al. 2012; Su et al. 2012; Zhang et al. 2010a). In synthesis of ferrites, salts of Fe(III) and M(II) were used as precursors. Synthesis methods have recently been summarized (Casbeer et al. 2012). Highly visible-light active $ZnFe_2O_4$ nanotube array has also been prepared using sol-gel methods (Li et al. 2011). Microwave sintering process may be advantageous due to its shorter synthesis time compared to conventional methods (Dom et al. 2011).

### 4.2.2   Composite Ferrites

In composite ferrites, the emphasis has been on the coupling of titania and zinc ferrite through different approaches. Ferrite is sensitive to visible light while titania has high photoactivity (Chen et al. 2010; Cheng et al. 2004) and composites showed increased photocatalytic activity (Tan et al. 2012). Sol-gel and hydrothermal methods were successfully used to prepare either titania doped ferrite or ferrite
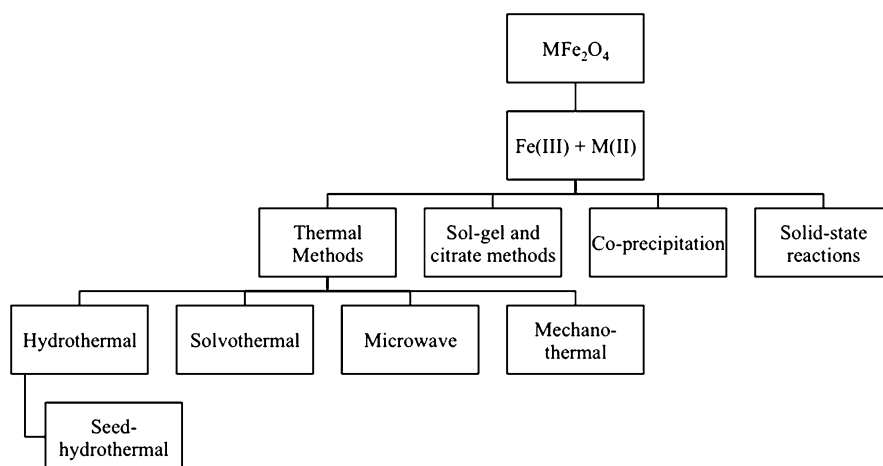


**Fig. 4.3**   Preparation methods of ferrites using Fe(III) and M(II) salts as precursors (Adapted from (Casbeer et al. 2012) with the permission of Elsevier Ltd)

doped titnia (Cheng et al. 2004; Liu et al. 2004; Moreira et al. 2012). More recently, $TiO_2$-$ZnFe_2O_4$ with an intermediate layer of alumina has been prepared by a multistep wet chemical method (Hankare et al. 2011). Other methods include the liquid catalytic phase transformation at low temperature and the two-step electrochemical processes (Hou et al. 2010; Shihong et al. 2009). Nanocomposites of $ZnFe_2O_4$ nanoplates and Ag nanoparticles and $ZnFe_2O_4$/multi-walled carbon nanotubes (MWCNTs) have also been prepared (Cao et al. 2011; Chen et al. 2010).

## 4.3  Characterization

Figure 4.4 shows the optical properties of ferrites, analyzed by UV–vis absorption spectroscopy. Ferrites have strong absorption in the visible range of 400–700 nm (Dom et al. 2011; Liu et al. 2009; Subramanian et al. 2004; Valenzuela et al. 2002). This is significant because of performing photocatalytic activity under visible light. Comparatively, $TiO_2$ does not show any significant absorption in the visible region. The electron excitation from the O-2p level into the Fe 3d level for ferrites may be causing absorption in the visible region (Lv et al. 2010). The two persistent absorption bands of spinel structures of nano $ZnFe_2O_4$ at $v_1 = 545$ and $v_2 = 292$ cm$^{-1}$ in the Fourier Transform Infrared (FTIR) spectrum were observed (Pradeep et al. 2011). These bands could be related to the oxygen-metal cation complex presence in the tetrahedral and octahedral sites of the ferrite. Other surface techniques have also been applied to characterize ferrite, which include X-ray diffraction (XRD), X-ray spectroscopy (XAS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), neutron diffraction analysis, and
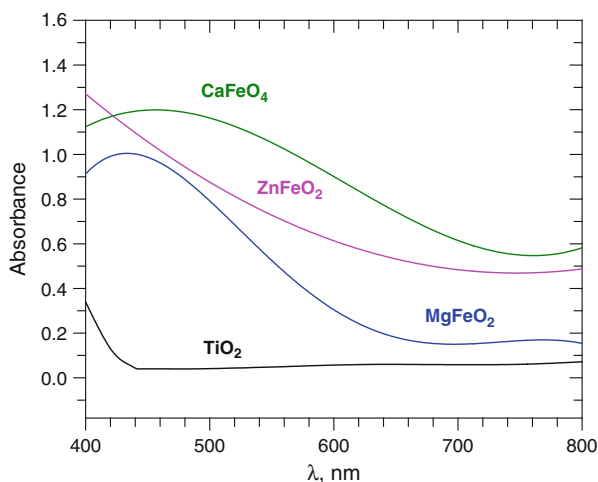


**Fig. 4.4**  Visible light spectra of metal ferrites (Adapted from (Dom et al. 2011; Liu et al. 2009; Subramanian et al. 2004; Valenzuela et al. 2002))
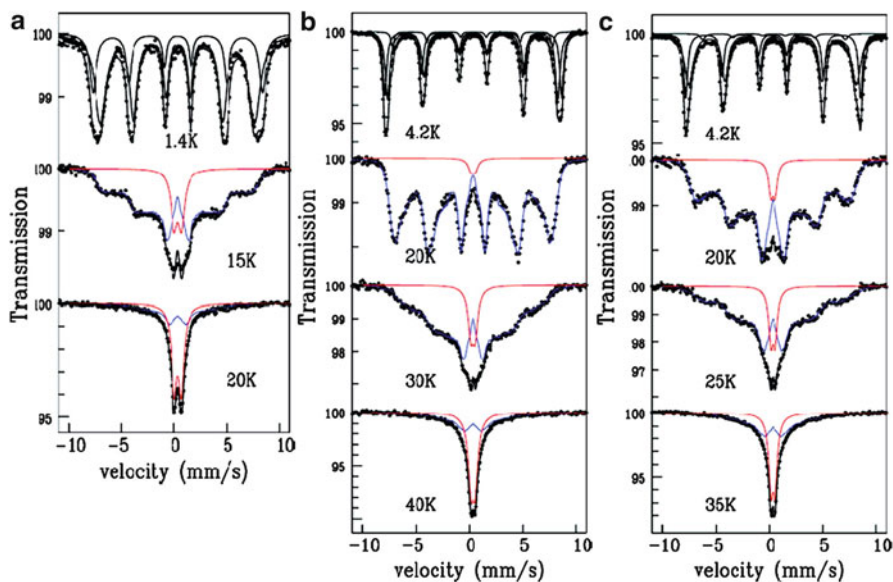
**Fig. 4.5** Mössbauer spectra obtained at different temperatures for Z3 (**a**), Z11 (**b**), and Z19 (**c**) samples (Adapted from (Blanco-Gutiérrez et al. 2011) with the permission of the American Chemical Society)

Brunauer-Emmett-Teller (BET) surface area (Blanco-Gutierrez et al. 2011; Hankare et al. 2011; Hou et al. 2011; Li et al. 2011; Moreira et al. 2012; Nilsen et al. 2007).

Mossbauer spectroscopy has also been used to characterize $ZnFe_2O_4$ samples (Blanco-Gutiérrez et al. 2011). The zero-field Mossbauer spectra of samples, prepared differently are presented in Fig. 4.5 (Blanco-Gutiérrez et al. 2011). Samples Z3 and Z19 were synthesized using solvothermal method while Z11 was prepared by means of sol-gel method. All samples had different treatment time while temperature was ranged from 160 °C to 200 °C. Treatment times were 2 and 288 h for Z3 and Z19 samples, respectively. Sample Z11 was treated for 24 h. The mean particle sizes of samples were 3, 11, and 19 nm for Z3, Z11, and Z19, respectively. Spectra of samples at 1.4 and 4.2 K had two broadened sextets in which suggested the presence of $Fe^{3+}$. The non-Lorentzian shape of lines gave mean hypefine parameters as 47.2, 50.2, and 49.9 T for Z3, Z11, and Z19, respectively. The characteristic doublets appeared with increase in temperature (Fig. 4.5). This is indicative of superparamagnetic behavior of particles of such samples.

## 4.4   Oxidation by Ferrites

The absorption of light by semiconductor photocatalysis results in several processes (Fig. 4.6) (Teoh et al. 2012). In the first step, formation of electron–hole (e-h$^+$) occurs through bandgap excitation. A number of reactions can take place in absence
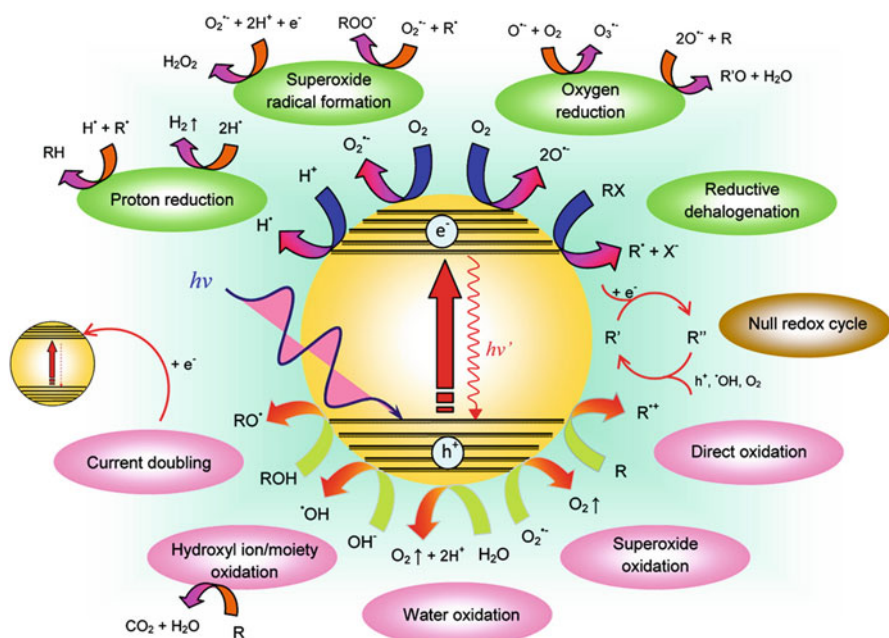
**Fig. 4.6** Possible reaction pathways arising from the excitation of photocatalyst (Adapted from (Teoh et al. 2012) with the permission of American Chemical Society)

and presence of contaminants in water (Fig. 4.6). Both electron and hole can diffuse to the surfaces of semiconductors and reduce and oxidize the adsorbed contaminants, respectively (Fig. 4.6). Other possibility is the recombination of electron and hole, which decreases the efficiency of the photocatalysts to react with contaminants in water. Hydroxyl radicals ($^\bullet$OH) have been suggested to be dominated species to oxidize contaminants. Hydroxyl radicals are efficient in abstracting hydrogen atom and attaching to electron-rich moieties (Park and Choi 2005; Turchi and Ollis 1990). Superoxide species ($O_2^{\bullet-}$) are generated by the reaction of $O_2$ with electron (Gerischer and Heller 1991; Schwitzgebel et al. 1995). Other reactive oxygen species such as singlet oxygen ($^1O_2$), hydrogen peroxide ($H_2O_2$), and hydroperoxyl radicals ($HO_2^\bullet$) may also be produced and be involved in the photocatalytic oxidation reactions. In the next section, applications of ferrites (e.g., $ZnFe_2O_4$) as a photocatlysts in degrading contaminants are presented.

## 4.4.1 Degradation of Contaminants

Ferrites have been useful in decontamination of inorganic compounds and disinfection (Li et al. 2008; Liu et al. 1996; Rana et al. 2005; Rawat et al. 2007; Zhang et al. 2010b; Zhao et al. 2010). Examples include photodehydrogenation

**Table 4.1** Degradation of phenols by ferrites

| Contaminant | Catalyst | Irradiation | Reference |
|---|---|---|---|
| Phenol | Zn-Al ferrite | No | Xu et al. (2007) |
| 4-Chlorophenol | $ZnFe_2O_4$-modified $TiO_2$ nanotube array electrode | Visible light | Hou et al. (2011) |
| 4-Chlorophenol | $ZnFe_2O_4$ nanotube | Visible light | Li et al. (2011) |
| Phenol | $TiO_2/CoFe_2O_4$ composite | UV-visible light | Li et al. (2012) |
| 2,4-Dichlorophenol | N-doped titania supported *on* $SrFe_{12}O_{19}$ | Sunlight | Aziz et al. (2012b) |
| 2,4-Dichlorophenol | $TiO_2$ nanocomposites/$SiO_2$ coating supported on $NiFe_2O_4$ | Sunlight | Aziz et al. (2012a) |
| Phenol o-Nitrophenol p-Nitrophenol Picric acid | Multi-walled carbon nanotube supported on $NiFe_2O_4$ | UV light | Xiong et al. (2012) |

of $H_2S$ using $ZnFe_2O_4$ and inactivation of *Escherichia coli* by composite ferrites, $Ag/MgFe_2O_4$, $Ag/Ni_2Fe_2O_4$, $Ag/Zn_2FeO_4$, and $Ag/CoFe_2O_4$. Ferrites have also shown effectiveness in degrading aliphatic compounds such as methanol, ethanol, isopropanol, acetaldehyde, oxalic acid, and butenes (Gibson and Hightower 1976; Manova et al. 2004, 2011; Shchukin et al. 2004; Tsoncheva et al. 2010). Most of studies on the remediation by ferrites are on the oxidation of phenols and dyes and are discussed below.

**Phenols**

A few studies on the oxidation of phenols by ferrites have been performed and are summarized in Table 4.1 (Aziz et al. 2012b; Hou et al. 2010; Li et al. 2012, 2011; Valenzuela et al. 2002; Xu et al. 2007). The concentrations of phenols in these studies were generally from 10 to 50 mg l$^{-1}$. These studies have demonstrated that phenols could be removed successfully using ferrite alone and composite ferrites. The current focus is on the composite ferrites to enhance the photocatalytic oxidation of phenols through synergistic effects. The presence of ferrites in the $TiO_2$-ferrite composite not only increases absorbance of visible light, but also increases absorbance in the UV light in order to assist photooxidation of phenol (Li et al. 2012, 2011). Furthermore, composites could be magnetically separated (Aziz et al. 2012a, b; Li et al. 2012). The photocatalytic oxidation of phenols using a magnetically recyclable photocatalyst, multi-walled carbon nanotubes (MWNT) supported $NiFe_2O_4$ is shown in Fig. 4.7 (Xiong et al. 2012). Figure 4.7a shows the degradation of phenol using $NiFe_2O_4$/MWNT nanocomposites having different MWNT content. The degradation of phenols increased with increasing amount of MWNT in the nanocomposites, however, the degradation rate had no such relationship. The degradation of phenol under UV radiation followed pseudo-first-order kinetics and obtained rate constant ($k$) as a function of content of MWNT are
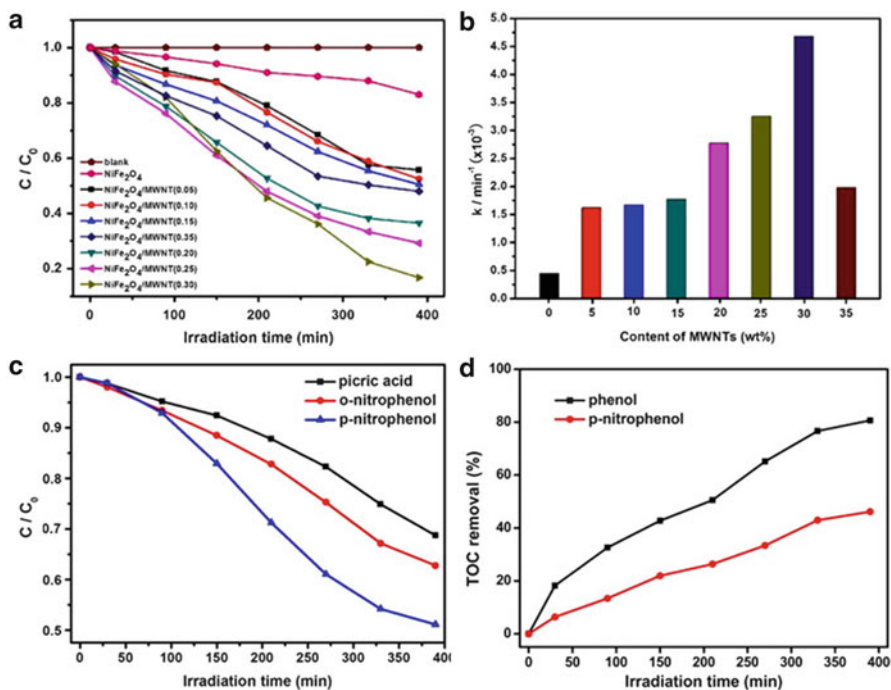
**Fig. 4.7** (**a**) Photocatalytic degradation of phenol by $NiFe_2O_4$/multi-walled carbon nanotubes (MWNT) photocatalysts with differing MWNT contents under UV light irradiation; (**b**) The pseudo-first-order rate constant *k* as a function of MWNT content; (**c**) Photocatalytic degradation of nitrophenols (o-nitrophenol, p-nitrophenol, picric acid) by $NiFe_2O_4$/MWNT(0.30) nanocomposite photocatalysts under UV light irradiation; (**d**) Evolution of TOC removal of phenol and p-nitrophenol with irradiation time (Adapted from (Xiong et al. 2012) with the permission of Elsevier Ltd.)

shown in Fig 4.7b. $NiFe_2O_4$/MWNT nanocomposite with 30 wt % MWNT exhibited the best photocatalytic activity. Degradation of picric acid, o-nitrophenol, and p-nitrophenol are shown in Fig. 4.7c. Most of the phenol degraded significantly under UV light in 400 min. This study also determined the total organic carbon (TOC) for the degradation of phenol and p-nitrophenol (Fig. 4.7d). Removals of TOC were ~80 % and ~40 % for phenol and p-nitrophenol, respectively. Incomplete removal of TOC suggests that the oxidation of phenol and p-nitrophenol resulted in simple organic compounds in the $NiFe_2O_4$/MWNT/UV radiation (Xiong et al. 2012).

## Dyes

Numerous studies on the oxidation of degradation of dyes by ferrites alone and composite ferrites have been carried out (Baldrian et al. 2006; Casbeer et al. 2012; Li et al. 2011; Moreira et al. 2012). Dyes studied include methyl orange (MO),
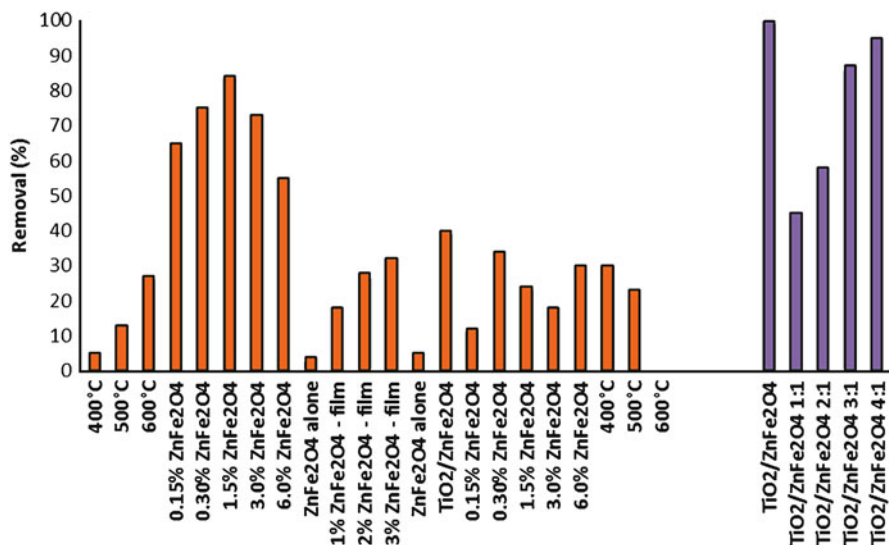
**Fig. 4.8** Degradation of methyl orange (MO) and rhodamine B (RhB) dyes by TiO$_2$/ZnFe$_2$O$_4$ composite photocatalysts. All listed reactions contain the composite unless otherwise noted. MO solutions irradiated with visible light, while RhB solutions are irradiated with UV light (Adapted from (Casbeer et al. 2012) with the permission of Elsevier Ltd.)

methylene blue (MB), rhodamine B (RhB), bromophenol blue, Chicago sky blue, eosine yellow, evans blue, naphthol blue black, phenol red, poly B-411, and reactive orange 16. The concentration levels of dyes were usually in the range of 50–500 mg L$^{-1}$. In a recent work, results of the studies have been summarized (Casbeer et al. 2012). Ferrites as photocatalyst alone were effective in degrading dyes, but the efficiency of degradation was enhanced when ferrites were used as composite photocatalysts. This is shown in Fig. 4.8. The degradation of RhB by ferrites was examined by UV light while visible light was applied in irradiating the MO dye. The temperature of ferrite synthesis influenced the degradation of RhB. Increase in the amount of TiO$_2$ in the composite ferrites increased the degradation efficiency of dyes (Fig. 4.8). Decrease in crystal size of ferrites also increased the degradation efficiency of dyes (Fan et al. 2009).

In a recent work, the photocatalytic degradation of acid orange II (AOII) was studied by combining ZnFe$_2$O$_4$ with H$_2$O$_2$ under visible light ($\lambda > 400$ nm) (Su et al. 2012). Significantly direct photolysis of AOII by visible light was difficult, but oxidation of AOII was possible due to generation of $^\bullet$OH radicals in the system (Fig. 4.9). The Fenton-type reaction can produce $^\bullet$OH radicals (Path A). The formation of $^\bullet$OH radicals is also due to oxidation of water by holes on the surface of the ferrite (Path B). The capture of electron by H$_2$O$_2$ gives $^\bullet$OH radicals (Path C). The Path C is advantageous because it decreases the recombination of electron and hole and hence enhance the formation of $^\bullet$OH radicals. The mechanism of the reactions was examined by adding scavengers of reactive species, oxalate,
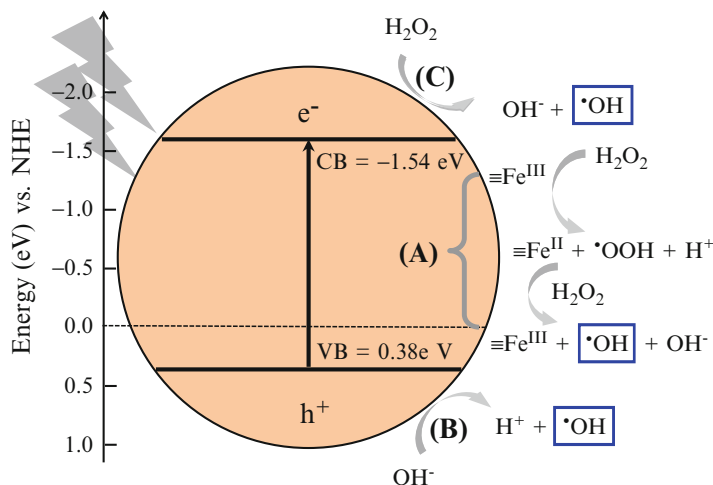
**Fig. 4.9** A hypothetical scheme for the generation of OH radical in $H_2O_2$-$ZnFe_2O_4$-visible light system (Adapted from (Su et al. 2012) with the permission of Elsevier Ltd.)

iso-propoanol, Cr(VI), and KI, into the system. Degradation rate did not show any influence by adding oxalate. This suggests that oxidation of AOII by hole was less likely to be involved because hole is highly reactive with oxalate. Cr(VI) can react rapidly with electron and the degradation rate decreases in the presence of Cr(VI), which suggest that electron formed at the surface of the ferrite is involved. Both iso-propoanol and KI are good scavengers of $^{\bullet}$OH radicals and degradation of AOI decreased very significantly. This confirmed the dominant role of the $^{\bullet}$OH radicals in degradation of AOII. Basically, Paths, A, B, and C enhanced the degradation of AOII (Su et al. 2012). Degradation of AOII in the $ZnFe_2O_4/H_2O_2$/visible light was almost complete within 2 h. The amount of $ZnFe_2O_4$ and the concentration of $H_2O_2$ influenced the degradation of AOII.

## 4.5 Conclusion

$ZnFe_2O_4$-mediated photocatalytic oxidation of organic contaminations is a promising alternative technology for remediation of contaminants in water treatment. Pioneering works indicate that $ZnFe_2O_4$ possesses a relatively low visible-light-driven activity for organic pollutants, however, the technology is a potentially economical and benign process due to its capability to absorb the visible light of solar energy, stability against photo- and chemical- corrosion, low cost, and non-toxicity. In this case, it is still a challenge to explore the highly-efficient modified ferrites for remediation of contaminants under visible light. The size and morphology control of ferrites is one of the most important approaches that

can enhance the photocatalytic activity. Compared with a traditional synthesized method, i.e. co-precipitation and sol-gel techniques, resulting in large particles and a broad size distribution, some innovative methods should be developed which can synthesize smaller size $ZnFe_2O_4$. An alternative promising approach is to modify ferrites with an appropriate matching-band-gap-energy semiconductor in a way that the photogenerated $e^-/h^+$ can be transferred between ferrites and matching-band-gap-energy semiconductor. This effective charge suppresses the combination of the photoinduced electrons and holes, which is beneficial to improve the photocatalytic efficiency. Further improvement in the modification of ferrites and ferrite composite for photocatalytic degradation of organic pollutants is still needed.

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# Chapter 5
# Bioindicators of Toxic Metals

**Slavka Stankovic and Ana R. Stankovic**

**Abstract** Growing social concern about environmental quality has been observed in recent years, on global and local scales. As the world's population continues to grow, it becomes imperative to understand the dynamic interactions between human activities and the environment. With the growth of all aspects of the activities of modern man, warning signs appeared that the sources of the basic environmental components, air, water, and land are not unlimited. The advent of an increasing number of contaminants reduces the power of self-purification of these media, and degradation of nature and biosphere continues infinitely, consequently acting on the man.

Toxic heavy metals in air, soil, and water are global problems that are a growing threat to the environment. There are hundreds of sources of heavy metals pollution. Metals in the environment arise from natural sources directly or indirectly from human activities such as rapid industrialization, urbanization, and other anthropogenic sources. They are potential hazards to aquatic, animal, and human life because of their toxicity and bioaccumulative and nonbiodegradable nature. Non essential metals such as Hg, Cd, Cr, Pb, and As are toxic in their chemically combined forms as well as the elemental form. The major impacts of pollution can be stated as health problems with exposed human populations and ecosystems contamination. Those problems have been a cause of increasing public concern throughout the world.

During the last two decades the interest in using bioindicators as monitoring tools to assess environmental pollution by toxic metals has steadily increased. Bioindicators are flora and fauna members or groups of them which are used indirectly to measure the levels of metal contaminants in their environment, systematically collected and analyzed to identify potential health hazards to biota and humans. In

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practice, microbial systems, fungi, animal and plants are used as bioindicators to formulate conclusions regarding the environmental conditions. Similarly, different kind of living organisms, plants and animals, and even humans, can be used as bioindicators for environmental pollution by toxic metals because of their ability to absorb them from air, water, sediment, soil, and food chain.

Problems concerning toxic metals are very wide and encompass all parts of the nature. In this review the recent studies of organisms used as a toxic metals bioindicators in air, water, and soil ecosystems are presented. Also, the related toxicity, storage or defense mechanisms of living organisms and ecological effects of heavy metals pollution will be considered.

**Keywords** Heavy metal • Bioindicator • Soil • Water • Air • Pollution • Hg • Cd • Cr • Pb • As

## Contents

## Abbreviations

| | |
|---|---|
| BLB | Bioluminescent Bacteria |
| Bw | Body Weight |
| CAFE | Clean Air for Europe |
| CAT | Catalase |

| COM | European Commission |
|---|---|
| d.w. | Dry Weight |
| DNA | Deoxyribonucleic Acid |
| EC | European Commission |
| EEA | European Environment Agency |
| EEC | European Economic Community |
| Eh | Soil Redox Potential |
| EPA | Environmental Protection Agency |
| EU | European Union |
| FAO | Food and Agriculture Organization of the United Nation |
| GPX | Glutathione Peroxidase |
| GSH | Glutathione |
| GST | Glutathione S-transferases |
| HBM | Human Biomonitoring |
| IARC | International Agency for Research on Cancer |
| ICES | International Council for the Exploration of the Sea |
| LP | Lipid Peroxidation |
| MAP | Mediterranean Action Plan |
| MED POL | Marine Pollution Assessment and Control Component of MAP |
| MeHg | Methylmercury |
| MG | Methylglyoxal |
| MSFD | Marine Strategy Framework Directive |
| MT | Metallothionein |
| NCM | Nordic Council of Ministers |
| OECD | Organization for Economic Cooperation and Development |
| OSPAR | Oslo/Paris Convention (for the Protection of the Marine Environment of the North-East Atlantic) |
| PC | Phytochelatin |
| PTWI | Provisional Tolerable Weekly Intake |
| RNA | Ribonucleic Acid |
| ROS | Reactive Oxygen Species |
| SOD | Superoxide Dismutase |
| SPCS | Soil Pollution Control Site |
| T | Tonne |
| t/yr | Tonne per Year |
| TRI | Toxic Release Inventory |
| UK | United Kingdom |
| UNEP/MAP | United Nations Environment Programme/Mediterranean Action Plan |
| USA, US | United States of America, United States |
| USEPA | United States Environmental Protection Agency |
| WFD | Water Framework Directive |
| WHO | World Health Organization |
| wt% | Weight Percent |
| ww | Wet Weight |

## 5.1   Introduction

Increasing industrialization and urbanization have been accompanied throughout the world by the extraction and distribution of mineral substances from their natural deposits. The pressure from the activities of the urban population is intense, as anthropogenic emissions of potentially toxic trace metals have accelerated considerably.

Metals are in the environment occurring in varying concentrations in parent rock, soil, water, air and all biological matter. In natural systems, potentially toxic heavy metals can originate from rocks, ore minerals, volcanoes, Szyczewski et al. (2009), and weathering releases metals during soil formation and transports them to surface and/or aquifer waters, Siegel (2002). Natural metal loading is aggravated by anthropogenic sources. A wide spectrum of anthropogenic sources include smelting of metallic ores, industrial fabrication and commercial application of metals, traffic, agro-chemicals pesticides as well as burning of fossil fuels. Anthropogenic impacts of toxic metals are related mostly to the mining, extraction and refining stages and can be the cause of substantial air, water and soil pollution, Norgate et al. (2007).

Metal uptake by organisms occurs through diffusion as well as ingestion of food and particulate matter, Szyczewski et al. (2009). The metals serving as micronutrients in living organisms usually occur in trace amounts that are precisely defined for each species. Both their deficiency and high excess badly affect living organisms. They, also directly or indirectly, throughout air, water and food (plants, animals and humans) get into human bodies. The excessive content of metals in the human body may in many ways affect the body and psychophysical development Stankovic et al. (2011a), Jovic et al. (2012), Stankovic and Jovic (2012). Therefore, the content of heavy metals needs to be known not only in water, air, soil and sediment, but also in biological samples: plants, animals, and on the end, in the human population.

Traces of heavy metals in living organisms are not just a reflection of human activity. Together with essential nutrients, living organisms also take up heavy metals and their compounds and can accumulate them. Metals fall into one of two categories, essential and non-essential. Essential metals are required for the optimal functioning of biological and biochemical processes in organisms; non-essential elements have no known biological function in organisms and exert their toxicity by competing with essential metals for active enzyme or membrane protein sites, Stankovic et al. (2011a). Even metals that are biologically essential have the potential to be harmful to humans and other living organisms at high levels of exposure, Hu (2002).

In the world today there are different methods of monitoring the concentration of pollutants, mostly as instrumental physicochemical measurements. The use of living organisms as indicators for environmental stability has long been widely recognized. Furthermore, exposure can be measured by either levels or effects. Plants and

animals are commonly known as toxic metals bioindicators. Higher plants, animals, alga, fungi, bacteria and lichen have been used as bioindicators in air, soil and water pollution surveys over the past few decades.

Bioindicators are a good tool for monitoring the state of the environment. The widespread development and application of bioindicators has occurred primarily since the 1960s, Holt and Miller (2011), using various animals like birds, mollusks and mammals. Bioindicators are living organisms that are particularly sensitive to the environment, and provide information about ecosystem health. Indicator species respond well both, to the presence or absence of other species as well as the presence of pollutants.

The application of living organisms has many advantages over standard methods of chemical analysis of trace metals in air and water. For example, in water samples the concentration of metals are often below the detection limit of the instrument that is used, Morillo et al. (2005), while the concentration of metals in water living organisms are significantly higher (102–106 times) compared to the water, and allow determination of biologically available elements and/or metal compounds in aqueous media, Roméo et al. (2005). Hence, using live organisms as analysis samples instead of sampling directly air and water reduces the possibility of measuring mistakes.

Various aquatic organisms occur in rivers, lakes, seas and marines potentially useful as metal pollutant bioindicators, including fish, shellfish, oyster, mussels, Jovic et al. (2011), Joksimovic et al. (2011a), Stankovic et al. (2011a), Stankovic and Jovic (2012), clams, aquatic animals and aquatic plants and macroalgae, Joksimovic et al. (2011b), Akcali and Kucuksezgin (2011), Rybak et al. (2012), Luy et al. (2012).

Metal content in bioindicators depends not only on the concentration of metals in air, water, soil and sediment, but also depends on environmental conditions, biological factors of the organisms themselves. It is therefore necessary to monitor the impact of all these factors in this complex system.

Heavy metals are released into the environment – the air, surface water, soil and hence into the groundwater and crops: once in the environment, they do not disappear, but accumulate in soils, sediments and biomass.

Although the adverse effects of heavy metals on human health have long been known, exposure of humans to heavy metals unfortunately continues, especially in less developed countries. The main threats to human health from heavy metals are associated with exposure to Pb, Cd, Hg and As, Schwartz et al. (2011), Stankovic et al. (2011a), Stankovic and Jovic (2012).

One of the largest problems associated with the persistence of toxic metals is the potential for bioaccumulation and biomagnification causing heavier exposure for some organisms than toxic metals present in the environment alone. In this review bioindicators of toxic metals will be presented in biotic material of the following ecosystems: air, water, and soil/sediment.

## 5.2 Metals and Environmental Pollution

### 5.2.1 Toxic Metals

Toxic metals are redistributed in the biosphere and dispersed in the air, soil, water, plants and animals throughout bioaccumulation, and consequently, in human beings through food chain bio-magnification causing chronic ailments. Environmental problems and toxic metal pollutions are serious problem in many parts of the world. Much has been done globally in the past few decades to improve human health related to environmental pollutants: on the Earth Summit in Rio de Janeiro, Brazil in 1992 at the World Conference on Environment and Development was convened to address urgent problems of environmental protection and socio-economic development. The world leaders adopted Agenda 21, a plan for achieving sustainable development in the twenty-first century.

Heavy metals normally occur in nature and some are essential to life, but can become toxic through accumulation in organisms. Only a few of the many metals present in the environment are essential and necessary in very small amounts to all living organisms, so-called micronutrients. Essential micronutrients are involved in the functional activities of living organisms. Pb, Cd, Cr, Cu, Zn, Ni, As and Hg are the most common heavy metal pollutants, and Hg, Pb and Cd are of the greatest concern because of their ability to transport long distances in the atmosphere. Dissolved Cu can affect lower trophic levels such as phytoplankton. Pb, Cd, and Hg bioacumulate through the food-chain posing a toxic risk to species higher in the food chain and to humans, Stankovic et al. (2011a), Stankovic and Jovic (2012).

For *heavy metals* many different definitions have been proposed – some based on density, some on atomic number or atomic weight, and some on chemical properties or toxicity. There is an alternative term *toxic metal*, for which no consensus of exact definition exists either. Depending on context, heavy metals can include elements lighter than carbon and can exclude some of the heaviest metals, Duffus (2002). Heavy metals occur naturally in the ecosystem with large variations in concentration. In modern times, anthropogenic sources of heavy metals pollutions have been introduced to the ecosystem. The classification of elements from the periodic table, according to the toxicity and their uptake, where easily exposed to organisms or not, is presented in Table 5.1.

**Table 5.1** Classification of elements according to toxicity and their uptake, Wood (1974)

| Not critical | Toxic, partially dissolved or easily exposed | Very toxic and easily exposed |
|---|---|---|
| Na, C, F, K, S, Sr, H, Cl P, Li, Mg, Al, O, Br, Si Fe, Rb, Ca, N | Ti, Ga, Hf, Rh, Nb, Ir La, Zr, Os, Ta, Ru, Re, W | Be, As, Au, Cu, Pd, Pb Co, Se, Hg, Zn, Ag, Sb Ni, Te, Tl, Pt, Sn, Cd, Bi |

Metals fall into one of two categories, essential and non-essential. Essential metal micronutrients, such as Cu, Zn, Fe, Mn, Co, Mo, Cr and Se, are required in small quantities, a few mg or µg per day, and Ca, Mg, Na, P and S, are also required, but in larger quantities, 100 mg or more per day, for the optimal functioning of biological and biochemical processes in humans. Non-essential elements, such as Cd, Pb, Hg and As, have no known biological function in organisms and exert their toxicity by competing with essential metals for active enzyme or membrane protein sites, Valavanidis et al. (2006), Zhou et al. (2008).

Very toxic metals (Table 5.1) are potential hazards to aquatic, animal, and human life because of their toxicity and bioaccumulative and nonbiodegradable nature. Nonessential metals such as Hg, Cd, Pb, As, and Sb are toxic in their chemically combined forms as well as the elemental form, Ahmad (2006), Peralta-Videa et al. (2009), Tangahu et al. (2011). The elements can interact and be interdependent. The strongest toxic properties are characteristic for inorganic metals compounds, which dissociate well and are easily soluble because they can easily penetrate through cell membranes and get into organisms, Szyczewski et al. (2009).

Together with essential nutrients, living organisms also take up toxic metal compounds and can concentrate them. Not all the traces of toxic metals in plants and animals are the results of human activity. The circulation and migration of metals in the natural environment are related to rock decay, volcano eruptions, evaporation of oceans, forest fires and soil formation processes too, Szyczewski et al. (2009). Some metals arise through the absorption processes in organisms of naturally occurring elements in soil components. Purely theoretically every 1,000 kg of "normal" soil contains 200 g Cr, 80 g Ni, 16 g Pb, 0.5 g Hg and 0.2 g Cd, CAOBISCO (1996). Therefore it is not always easy to assign a definite cause for increased toxic metal content.

Toxic metals are commonly considered as simulators or inhibiting factors of life processes, due to which they may appear toxic for living organisms. This depends on their concentration, ability to form complexes and degree of oxidation.

The excessive content of metals in living organisms and in the human body may in many ways affect the body and psychophysical development, Szyczewski et al. (2009). As certain heavy metals such as Pb, Cd, As and Hg have been recognised to be potentially toxic within specific limiting values, a considerable potential hazard exists for human nutrition.

## 5.2.2  Toxic Metals Production and Emission

Metal pollution has increased with the technological progress of human society. The Industrial Revolution brought an exponential increase in the intensity of metal emissions released, both, in absolute masses and in the number and type of toxic metal compounds: about 90 % of mine outputs of Cd, Cu, Zn, Ni, and Pb were consumed in the last century, Nriagu (1996), Fig. 5.1. World mining
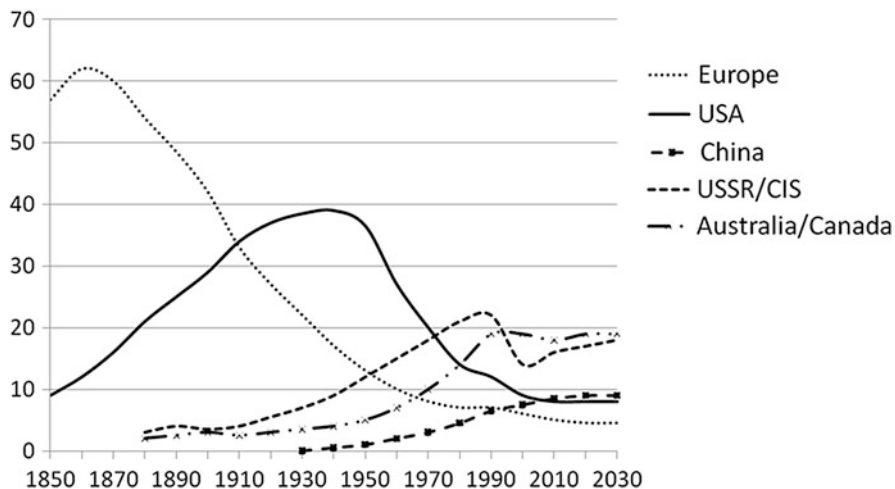
**Fig. 5.1** Distribution of global mine production among regions in %, Östensson (2006)
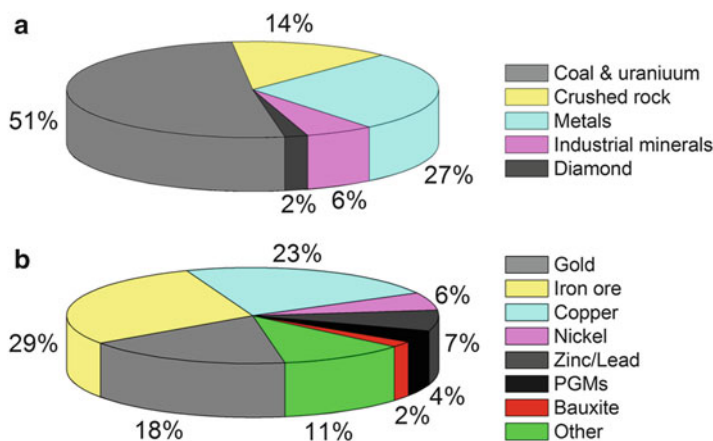


**Fig. 5.2** (**a**) Value of world mining activities (**b**) world metals value at mine

activities (Fig. 5.2a) are known to release significant amounts of toxic metals into the surrounding environment. Some toxic metals, including Hg, As, and Pb, are frequently associated with mining. These elements are present at low levels in soil, rock and water, but the process of world metals mining may release quantities harmful to the health of people and the ecosystem.

The cumulative releases of heavy metals into our environment are indeed massive and overwhelmed the natural biochemical cycles of the ecosystems. The levels of the toxic metals such as Hg, Pb, Cd, Cu, As, Zn, and Cr are increasing cumulatively in the environment due to atmospheric emissions from human activities. For example,

Hg levels in Minnesota, Wisconsin, Canada, and Sweden were found to be increasing in sediments, soils, and fish at rates between 2 % and 5 % per year. The four major industrial sources of total metal emissions as reported by USEPA Toxic Release Inventory (EPA TRI 2002–2005) include the electric power industry, the primary metals industry, hazardous waste disposal facilities and world metal mining operations, Östensson (2006) (Fig. 5.2b).

The toxicity impacts mostly occur in the mining, smelting and refining stage via emissions of contaminants in the ores, the fossil fuels or of the auxiliary materials used in these processes. In waste management, metals may end up in landfills and there form a source of slow pollution. Also, metals often occur as ore contaminants in fossil fuels or phosphate rock but also in other metals, and are emitted to the environment together with their host material. Metals are elements not degradable: once in the environment, they do not disappear and their environmental impact is dominant: they tend to accumulate in soils, sediments and biomass, der Voet et al. (2000).

The sources of anthropogenic contamination or pollution of the environment by heavy metals include different branches of industry, the power industry, transport, municipal waste, and fertilizers used to fertilize soil, Szyczewski et al. (2009). The heavy metals from these sources are dispersed in the environment and they contaminate air, water and soil. They, directly or indirectly, throughout air, water, plants and animals get into human bodies. As already mentioned, the metals classified as heavy metals are: Cu, Co, Cr, Cd, Fe, Zn, Pb, Sn, Hg, Mn, Ni, Mo, V and W, Szyczewski et al. (2009). Within the group of heavy metals one can distinguish both, the elements essential for living organisms (microelements) and the elements whose physiological role is unknown, and thus they are "inactive" towards plants, animals and people. The metals serving as microelements in living organisms usually occur in trace amounts that are precisely defined for each species. Both, their deficiency and excess badly affect living organisms, Szyczewski et al. (2009).

The most common heavy metal environmental pollutants are Cu, Cd, Cr, As, Ni, Pb, and Hg, especially in locations where metals tend to accumulate, der Voet et al. (2000). However, metals can become biologically unavailable via geological routes or via soil processes. Also, their hazardous potential can be reduced by transformations in nature, but also in technological processes to a less harmful state. The free-metal ionic activity may be more important in producing metal toxicity than the total concentration of a metal.

Potentially toxic metals follow natural pathways and cycles through the biosphere. Terrestrial and aquatic life forms can suffer short-or long term perturbations if these pathways or cycles are interrupted by natural events or impacted by human activities. Mobilization of suspect metals, resulting in their bioavailability and hence access to a food web, can impact the integrity of a web. Conversely, immobilization of specific metals can have a negative impact if it causes a deficiency of an essential micronutrient within an ecosystem.

The chemical form of a potentially toxic metal determines its bioavailability to a food web. When a heavy metal enters a food web, organisms can react to

its bioavailability in different ways. Some organisms may discriminate against the uptake of one or more potentially toxic metals. Others may incorporate the metal in their soft or hard parts in proportion to the concentration in the growth environments, excreting any excess. Still other organisms may be tolerant of heavy metals and will accumulate concentrations greatly in excess amounts in a growth environment without any damage, Siegel (2002). Currently microorganisms or plants which exhibit hyper accumulation can be used to remove heavy metals from water and soils by concentrating them in their bio matter, Stojanović et al. (2012).

Trace element pollution results from various sources, mostly: (i) industrial wastewaters such as Hg from chlor-alkali plants, (ii) mining and smelter wastes, As and Cd, (iii) urban run-off, particularly Pb, (iv) agricultural run-off (where Cu is still used as a pesticide), (v) atmospheric deposition, and (vi) leaching from solid waste dumps. Essentially, the toxic metals have become a focus of public interest since analytical techniques have made it possible to detect them even in very small traces, Szyczewski et al. (2009).

## Mercury (Hg)

Mercury is an element that occurs naturally in the environment. However, human activity has significantly changed its cycling in recent centuries. The main natural sources of Hg are diffusion from the Earth's mantle though the lithosphere, evaporation from the sea surface, geothermal and volcanic activity WHO (2007).

The largest anthropogenic source of Hg on a global scale is the combustion of coal and other fossil fuels. Others sources include metal and cement production, forest fires and waste disposal.

Hg, one of the most toxic trace metals, is spread to the atmosphere at significant amounts. On a global scale, the estimated natural emission of Hg represents about one-third of the total, and anthropogenic emissions represent about two-thirds, WHO (2007). Total values of global Hg emission to the air from both anthropogenic and natural sources vary from 4,000 to 9,230 t/year, UNEP (2008) and it is in inorganic form that can be converted biologically to methylmercury (MeHg) in soil and water. MeHg bioaccumulates and enters the human body readily via the dietary route, Stankovic et al. (2011a, b), Stankovic and Jovic (2012).

This element is used in many industries, including pharmaceutical and chloralkali manufacturing industries, electrical engineering, mining, metallurgy and agriculture. Annually, anthropogenic pollution reaches approximately 2,000 t of this metal, most of which comes from burning coal, milling of nonferrous metals and gold mining by amalgamation, Kalisinska et al. (2012). In Europe, the majority of Hg emitted into the environment comes from the combustion of solid fuels, mainly coal (47 %), cement production (13 %), Fe milling (5 %), Zn and Pb milling (7 %) and incineration of waste (5 %), Kalisinska et al. (2012). According to official emission data the total Hg anthropogenic emissions in Europe was 413 t/year in 1990 and 195 t/year in 2003, WHO (2007).

The total concentrations of Hg in natural waters are normally very low, below 1 ng/L. The concentration range for mercury in drinking-water is, in the average 25 ng/L. The WHO guideline value is 1 μg/L, WHO (2007). Representative values for dissolved total mercury are: open ocean 0.5–3 ng/L; coastal seawater 2–15 ng/L; freshwater lakes and rivers 1–3 ng/L. In the European topsoils, Hg concentrations range from 10 to 160 μg/kg, reaching a median value of 40 μg/kg, WHO (2007).

**Arsenic (As)**

Arsenic is one of the few elements for which a large proportion of the world-production was 30,453 t in 1995 and 40,000 t in 2008 and ends more or less in the environment, Reimann et al. (2009). This is due to its wide-spread use in pesticides, fungicides, herbicides and insecticides and especially as a wood preservative. Further uses are in the glass and electronics industry, in metal treatment, alloys and bronze, in the production of dyes and colours, and in some pharmaceuticals. Another important source of anthropogenic As emissions is the burning of coal and oil. The mean As concentration of coal is given as 10 mg/kg, Reimann et al. (2009), but coals containing up to 3.5 wt% As have been reported from China. Severe health problems in populations living close to power plants burning such coal are well documented, Bencko (1997).

The total annual anthropogenic As emissions were estimated as 28,000–54,000 t/year, but in this range is not included natural As emissions to the atmosphere, including volcanism, forest fires and soil dust. For example, significant amounts of As species are released through hydrothermal activities in Yellowstone National Park. Such sources have not previously been assessed nor their contribution to the global As cycle, Reimann et al. (2009). Plants generally show low As concentrations, much lower than in the supporting soils and the suggested value for the world reference plant of As is 0.1 mg/kg, Nagajyoti et al. (2010). This is clear indication that the majority of plants have mechanisms for avoiding As uptake, but mosses and lichenes consistently show higher As values than other terrestrial plants, Reimann et al. (2009), Serbula et al. (2012).

The most carcinogenic of all substances named in current drinking water regulations is As. Values up to 3,200 μg/L for As have been reported from in India and Bangladesh water wells. The drinking water action limit for As was quite recently lowered to 10 μg/L (from 50 μg/L). A lower As limit of 2 μg/L was suggested for a better safety margin but rejected due to the financial implications, Reimann et al. (2009). Concentrations of As in air is generally very low and ranges from 0.4 to 30 ng/m$^3$; in seawater from 1.0 to 8.0 μg/L, while in marine sediments As concentration ranges from 1.0 to 60.0 mg/kg, depending on the sediment kind. The world-average value for As in soil is 5 mg/kg, but varies considerably among geographic regions, Reimann et al. (2009). The toxicity of As has been well known at least since Roman times, when arsenolite ($As_2O_3$) was often used as a poison. Trivalent ($As^{3+}$) compounds are usually more toxic than $As^{5+}$ compounds, Tangahu et al. (2011), Stankovic et al. (2011a).

## Lead (Pb)

The current annual worldwide production of Pb is approximately 5.4 million tonnes and continues to rise. Pb is used in the automobile batteries production (6 %), while the remainder is used in the production of pigments, glazes, solder, plastics, cable sheathing, ammunition, weights, gasoline additive, and a variety of other products, WHO (2007). Pb from soil tends to concentrate in root vegetables and leafy green vegetables (onion and spinach, respectively). Individuals will absorb more Pb in their food if their diets are deficient in Ca, Fe, or Zn, Hu (2002). In general, ingestion of Pb through food and water is the major exposure pathway for Pb in humans.

According to Krystofova et al. (2009) levels of Pb in the environment are not stable and vary according to industrial production, urbanization, climate changes and many other factors. The levels of Pb in the environment vary between 4 and 20 mg/g of dust. Uncontaminated waters contain Pb in concentrations ranging from 0.001 to 0.06 mg/L, while in seawater to 0.03 µg/L, Bardi (2010). Levels of Pb in soils are between 5 and 30 mg/kg. Pb is released into the atmosphere from natural and anthropogenic sources. Natural emissions are from wind resuspension and from sea salt, volcanoes, forest fires and biogenic sources. Major anthropogenic emission sources of Pb on a global scale include the combustion of fossil fuels, from traffic, non-ferrous metal production and iron and steel production, WHO (2007). Pb levels in air in Europe fell by 50–70 % between 1990 and 2003 because of restrictions on the use of leaded petrol, WHO (2007).

The natural Pb content in soil typically ranges from below 10 mg/kg soil up to 30 mg/kg soil. In Europe, Pb concentrations in topsoils are spatially heterogeneous and vary from below 10 mg/kg up to >70 mg/kg. The median value is estimated to be 22.6 mg/kg. In addition to atmospheric deposition, agricultural practices are a source of Pb input to soils from mineral and organic fertilizers and other sources, WHO (2007). Pb is incorporated into several crops through absorption, by the roots, from soil and through direct deposition on plant surfaces. The Pb levels in various food crops amounted to 2–136 mg/kg for grains and cereals, 5–649 mg/kg for vegetables and 6–73 mg/kg for fats and oils, WHO (2007). The uptake of soil-borne Pb by vegetables is very low. This explains the very low Pb concentrations in root vegetables, seeds and fruit. In leafy vegetables, the accumulation of airborne Pb largely exceeds the soil-borne part taken up via the roots. Airborne Pb is mainly accumulated at the leaf surface and can be removed to a large extent by thorough washing of the crop. There is, however, a relationship between the Pb content of the soil and the Pb content of the atmospheric deposition, WHO (2007).

The EU ambient air quality guideline for Pb is 0.5 µg/m$^3$ and in the immediate vicinity of specific industrial sources, the value was 1 µg/m$^3$ until 2010, WHO (2007). With regard to drinking-water, Pb contamination of the water can occur from, for example, industrial discharges or highway run-offs. Furthermore, drinking-water can be contaminated with Pb from lead pipes, lead-soldered copper pipes, lead-containing brass joints in plastic pipes, or from other parts of the water system. In particular, soft water has the potential for dissolving Pb. The limit value for Pb in drinking-water in the EU is currently 25 µg/L and will be reduced to 10 µg/L by 2013, WHO (2007).

Within the EU, the maximum permissible level of Pb in foods ranges from 0.02 mg/kg ww for milk and milk supplements for infants up to 1 mg/kg for mussels, Stankovic et al. (2011b). A PTWI of 25 µg/kg bw/week has been established for all age groups by the Joint FAO/WHO Expert Committee on Food Additives, Stankovic et al. (2011b). Pb accumulates in bone and bone-Pb concentrations are associated with the Pb concentration in the whole blood and plasma, WHO (2007).

### Cadmium (Cd)

Cd is released to the biosphere from both natural and anthropogenic sources. The major natural sources for mobilisations of Cd from the earth's crust are volcanoes and weathering of rocks, than airborne soil particles, sea spray, biogenic material and forest fires. The total Cd emission to air from natural sources is estimated at about 150–2,600 t, WHO (2007). The weathering of rocks releases Cd to soils and aquatic systems. Within the biosphere Cd is translocated by different processes.

The anthropogenic sources of Cd include non-ferrous metal production, stationary fossil fuel combustion, waste incineration, iron and steel production, and cement production, WHO (2007). World Cd mine production was 19,700 t in the year 2000, 73 % was for automobile batteries. In the US, combustion of coal and oil is assumed to be responsible for around 76 % of the total emission of Cd to the atmosphere. The global Cd production increased with a factor of four from 1950 to 1990 and in the recent decade the production has slightly decreased, NCM (2003). Cd emissions in Europe constituted 485 t in 1990 and 257 t in 2003. Emissions from EU countries have decreased by 50 %. In European countries the dominant sources of Cd are atmospheric deposition and commercial phosphate fertilizers, OECD (1994). Global emission of Cd to air until the year 2000 was closed to 3,000 t/year, NCM (2003).

On the basis of the Cd contents in surface soils from many parts of the world, the average value lies between 0.07 and 1.1 mg/kg; values above 0.5 mg/kg usually reflect anthropogenic inputs, WHO (2007). There are three main anthropogenic sources of terrestrial Cd: atmospheric deposition, agricultural application of phosphate fertilizers, and use of municipal sewage sludge as a fertilizer on agricultural soils. It has been reported that 90 % of Cd in the soil remains in the 15 cm of the top. Accumulation of Cd in the soil depends on the soil properties: generally clay soils retain more Cd than sandy soils, WHO (2007). Cd levels of up to 5 mg/kg have been reported in sediments from river and lakes, and from 0.03 to 1 mg/kg in marine sediments. The average Cd content of seawater is about 5–20 ng/L in open seas, while concentrations of 80–250 ng/L has been reported in French and Norwegian coastal zones. A concentration measured in European rivers roughly varies from 10 to 100 ng/L, OSPAR (2002).

Drinking-water generally contains low Cd levels, and a value of 1 µg/l or less is often assumed to be a representative content in most situations. Thus, Cd exposure from drinking-water is relatively unimportant compared with the dietary contribution, WHO (2007). Drinking-water contains very low concentrations of Cd, usually between 0.01 and 1 µg/L. The average daily intake of water is about 2 L per person, WHO (2007).

**Chromium (Cr)**

Cr (VI) exists in soils naturally and is the sixth most abundant element in the Earth's crust. It is also present as a result of human practices, and mostly from those practices associated with industry. Cr occurs naturally in Irish agricultural soils in concentrations between 5 and 250 mg/kg and in various soil types ranging from 1.04 to 3,015 mg/kg worldwide. Cr has been extensively shown to induce general environmental toxicity, as well as more specific effects of an acute and chronic nature such as neurotoxicity, dermatoxicity, genotoxicity, carcinogeneticity, and immunotoxicity. It is believed that Cr inflicts most damage during reduction of Cr (VI) to Cr (III), a process considered to be initiated in the cell by glutathione, Boyle and Kakouli-Duarte (2008). Cr is found in all phases of the environment, including air, water and soil. Naturally occurring in soil, Cr ranges from 10 to 50 mg/kg. In fresh water, Cr concentrations generally range from 0.1 to 117 mg/L, whereas values for seawater range from 0.2 to 50 µg/L. In the atmosphere Cr concentration vary widely, from background concentrations of 0.0012 µg/m$^3$ in the air sample, Shanker et al. (2005).

## 5.3    Metal Pollution

### 5.3.1    Air

Air pollution is certainly not a new phenomenon and the recognition thereof was marked as early as during the 1930s catastrophe in the Meuse Valley, Belgium, where $SO_2$ and particulate matter, combined with a high relative humidity, caused 63 excess deaths in five days. In the year 1948 similar conditions in Donora, Pennsylvania, a small industrial city, caused 20 excess deaths in five days, and in the early 1950s in London, England, two episodes of "killer fogs" claimed the lives of more than 6,000 people.

Atmospheric pollution causes serious damage for human health and to all natural ecosystems. The composition of the air pollutants can be inorganic, organic, or a complex mixture of both. Air pollutants also are classified as primary or secondary. Primary pollutants are those that are emitted directly into the atmosphere, CO and $SO_2$, and secondary pollutants are those that are produced in the atmosphere by chemical and physical processes from primary pollutants and natural constituents. Nowadays, when the biggest provocative of atmospheric pollution is anthropogenic human activities and transport sector, main pollutants are toxic metals, Blagnytė and Paliulis (2010). Monitoring toxic air pollutants is needed for understanding their spatial and temporal distribution and ultimately to minimize their harmful effects. Among the many inorganic air pollutants originating from anthropogenic activities, heavy metals such as As, Cd, Cr, Hg, and Pb are of a major concern due to their toxic and potentially carcinogenic characteristics. One of the most important sources of Cd, Cr and Pb in the urban environment is road traffic, Melaku et al. (2008).

Environmental sources for pollutants could include construction and agricultural activities, mining and mineral processing, wind-blown dust, traffic activities on the road. Power plants are the largest source of several harmful pollutants. They are responsible for 50 % of Hg and about 25 % of toxic metal emissions in the United States. Coal-fired power plants are responsible for 99 % of Hg emissions and the bulk the other pollutants from the power sector. Thus, power plants are the largest source of Hg emissions to the air. The Hg concentration in air is in most areas close to the mean global background value, which is 1.5–2.0 ng/m$^3$, WHO (2007). The total concentration of Hg is 2 ng/m$^3$, which is valid for the northern hemisphere. In recent years, emissions of Hg into the air in Europe have been declining. The decrease in Hg emissions in 1990–1996 in 39 countries ranged from 0 % to 56 %, WHO (2007). According to EEA (2003), the global anthropogenic emissions of Hg may amount to 30,000 t/year.

According to Harmens et al. (2008) in some European countries during 1990–2005/6 the main sources of Pb emissions were from manufacturing industries and construction (41 %) and road traffic has become the second source of Pb emission (17 %). Pb levels in ambient air in Europe have decreased in recent decades: Pb levels in air in Europe between 1990 and 2003 fell by 50–70 %. Low concentrations of Pb in the air rural areas are between 0.05 and 0.1 μg/m$^3$. Natural Pb content is estimated to be 6.0 ng/m$^3$ in the atmosphere, WHO (2007).

Cd pollution levels exhibit about a twofold decrease for Europe as a whole in the period 1990–2003, WHO (2007). The WHO 2000 air quality guideline has been recommended 5 ng/m$^3$ for Cd in order to prevent any further increases in Cd levels in agricultural soils, WHO (2007).

In addition to direct physical and chemical methods of air pollution monitoring, bioindicators have also been used to evaluate air pollution risk, Dmuchowski and Bytnerowicz (2009). Biomonitoring based on plant as heavy metal air pollution indicators has been widespread all over the world since the 1970s. Biomonitoring of environmental pollution with toxic metals using vascular plants, mosses, and lichens have been used for many years in Europe and North America. Foliage and bark of trees has also been widely used in this regard, Dmuchowski and Bytnerowicz (2009).

## 5.3.2 Water

Metals have many sources from which they can flow into the water. Heavy metals in aquatic system can be naturally produced by the slow leaching from soil/rock to water, which are usually at low levels, causing no serious effects on human health, Zhou et al. (2008). Nowadays, the industry and agriculture development promotes the rapid increase of water metal pollution. Industry, mining, advanced agriculture, household waste, and motor traffic are all among the activities considered to be major sources of aquatic metal pollution. Metals can accumulate in aquatic organisms and persist in water and sediments, Sevcikova et al. (2011).

Aquatic heavy metal pollution usually represents high levels of Hg, Cr, Pb, Cd, Cu, Zn, and Ni in water system, Zhou et al. (2008). Cu, Ni, Cr, and Zn are essential trace metals to living organisms, but become toxic at higher concentrations. Toxic elements Pb, Hg, As, and Cd have no known biological function. The aquatic environment with its water quality is considered the main factor controlling the state of health and disease in man and biota, Markovic et al. (2012).

Some heavy metals including Hg, Cr, Cd, Ni, Cu, As, and Pb introduced into environmental water system may pose high toxicities on the aquatic organisms, Zhou et al. (2008), Stankovic et al. (2011a), Stankovic and Jovic (2012). Concentrations of inorganic Hg in surface and groundwaters are generally below concentrations known to cause adverse health effects. MeHg in freshwater fish originates from inorganic Hg in the soil and direct atmospheric deposition, Chen et al. (2008). Although, the anthropogenic Hg emission in Europe decreased approximately by 50 % after 1990, but MeHg concentration decreasing has not been observed in freshwater fish WHO (2007). In fishes as a predatory animals Hg levels can be biomagnified. In general, fish as food has important beneficial effects on human health, Chen et al. (2008).

The physicochemical properties of water play an important role in metal solubility. The amount of dissolved metal strongly depends on water pH and metal can accumulate in aquatic organisms. Organic substances found in water can bind metal ions influencing their lower bioavailability to aquatic organisms and thereby somewhat reduce metal toxicity. Some metals can rapidly be bonded to organic substances and cannot be detected in water, but they can later become accessible to water organisms and their food, Sevcikova et al. (2011).

### 5.3.3   Soil

Soil is one of the principal regulatory compartments of all terrestrial and benthic ecosystems. Soil is a fundamental natural resource for agriculture. Preservation of healthy, high-quality soil is essential to maintain the health of plants, animals and human beings.

Soil is not only subjected to physical degradation due to deforestation, flooding, and wind erosion, it is recently intensively polluted by various toxic metals and other pollutants as well. The contamination of soil occurs through air pollution, wastewater intake and fertilizers in agriculture. A special type of soil contamination is related to the accumulation of industrial and municipal wastes, especially those that are hard to break. Soil pollution by heavy metals which are released into the environment through various anthropogenic activities such as mining, energy and fuel production, electroplating, wastewater sludge treatment and agriculture is one of the world's major environmental problem. An extensive literature review of trace metal contamination of the urban terrestrial environment, in particular of urban soils, in Europe, America, Africa, and Asia region is given by Wong et al. (2006).

Heavy metals refer to a large group of elements which are both industrially and biologically important. Initially, heavy metals are naturally present in soils as natural components but as of now, the presence of heavy metals in the environment has accelerated due to human activities. This is a widespread problem around the world where excessive concentration of heavy metals such as Ni, Pb, Zn, Cr, Cu, Cd, Hg, and As can be found in soils, Wong et al. (2006).

The soil environment is a major sink for multitude of chemicals and heavy metals, which inevitably leads to environmental contamination. Millions of tonnes of trace elements are produced every year from the mines in demands for newer materials. On being discharged into soil, the heavy metals get accumulated and may disturb the soil ecosystem, plant productivity, and also pose threat to human health and environment, Musarrat et al. (2011).

In areas of agricultural and industrial activity, higher concentrations of heavy metals, in comparison with background levels, can be detected. Especially, soils near heavy metals mines are exposed to metalloids pollution by Zn, Pb, Cr, Mn, Fe, Tl, In or As, Babula et al. (2008). Landfilling is still the main waste disposal method in Europe, Paoli et al. (2012). Consequence of the waste disposal in landfills is the emission of pollutants via the leachate and gas generation. Recent EU legislation prescribes strict rules for waste disposal in landfills, EU Directive 2008/98/EC, Paoli et al. (2012).

Cadmium is accumulating in soils and catchments under certain environmental conditions, thus increasing the risk of future exposure through food. The transfer of Cd from soil to the food-chain depends on a number of factors, such as the type of plant, the type and pH of the soil and the zinc and organic matter content in the soil, WHO (2007). Soil pH is the principal factor governing the concentration of Cd in the soil solution. As soil pH consequence, Cd plant uptake decreases as the soil pH increases.

Cd normally occurs in low concentrations in soils and is a nonessential element for plants and potentially toxic pollutant all over the world. The toxicity of Cd to plant growth, phytosynthesis, carbonhydrate metabolism, and enzyme activities is well documented, Javed and Greger (2011). The cropping land with total soil Cd concentration exceeding 5 mg/kg would be announced as Soil Pollution Control Site (SPCS) and all farming activities were not allowed, since elevated levels of Cd in soil may potentially enter a food chain, Meharg and Hartley-Whitaker (2002) and increase the risk of cancer development in humans, Stankovic et al. (2011a).

Trace metal assessments of urban soils frequently examined trace metals that were traditionally significant for the environment and health, particularly Cd, Cu, Pb, and Zn, Wong et al. (2006). In the EU the limit value for Cu in soil is 50–140 mg/kg of dry weight (Council Directive, 86/278) and the mean levels for Cu in soils vary from 13 to 24 mg/kg; the Zn soil levels usually fall in the range of 10–300 mg/kg and in the EU the limit value for Zn in soils from 150 to 300 mg/kg of dry weight (Council Directive, 86/278); the limit values in the EU are: for Pb from 50 to 300 mg/kg, for Cd from 1 to 3 mg/kg and for Hg from 1 to 1.5 mg/kg dry weight (Council Directive, 86/278), Serbula et al. (2012).

The mean Pb concentration for surface soils on the world scale is estimated at 25 mg/kg; the average contents of Cd in soils are between 0.06 and 1.1 mg/kg; for various soils on a world level mean concentrations of Hg in surface soils do not exceed 0.4 mg/kg, Serbula et al. (2012). The terrestrial abundance of As is around 1.5–3.0 mg/kg. The world-average value for As in soils is 5 mg/kg, Reimann et al. (2009) and EU limit for As in agricultural soil is 20 mg/kg, Bhattacharya et al. (2010). In well-aerated soils, arsenate (As(V)) is the predominate form. Lai et al. (2010) showed that As(V) in aerated soils will be reduced to more mobile and toxic As(III) in paddy soils and transferred to plants. As(III) is more soluble and more mobile, and is much more toxic than As(V), Lai et al. (2010), Tangahu et al. (2011).

Traffic-related activities continue to exert a widespread influence on the urban environment contaminating urban land. Nowadays, platinum group elements, and manganese (Mn) have been recommended as environmental indicators of traffic and other "urban" activities, especially where leaded gasoline is no longer in use, Wong et al. (2006). Also, soil pollution problems arise from agrochemicals being used and dispersed into the environment, Reijnders and Huijbregts (2009). Currently about half of the world's land and 70 % of total water are used for agriculture. In recent years, agriculture materials come out as high priority from an environmental impact point of view, Reijnders and Huijbregts (2009).

Since metals are not degradable they accumulate in soils and sediments. This affects human and ecosystem health. Their hazardous potential can be reduced by transformations via geological routes or via soil processes to a less harmful state.

Heavy metals as soil contaminants typically occur in low concentrations: 10,000 mg $kg^{-1}$ dry soil, Schwartz et al. (2011). The contaminants from soil are able to infiltrate deep into the layer of underground waters and pollute the groundwater as well as the surface water. Also, toxic metals in the soil subsequently enter the human food web through plants and animals and they constitute risk to the ecosystem as they tend to bioaccumulate and can be transferred from one food chain to another.

### 5.3.4   Biota

Flora, fauna and other forms of life such as fungi are collectively referred to as biota. Fauna is all of the animal life of any particular region or time. The corresponding term for plants is flora. Biota is the total collection of organisms of a geographic region or a time period, from local geographic scales and all the way up to whole-planet and whole-timescale spatiotemporal scales. The biota or biotic components of the Earth makes up the biosphere.

Biota growth and development are essential processes of life and propagation of the species. They are continuous and mainly depend on external resources present in water, soil and air. Growth is chiefly expressed as a function of genotype and environment, which consists of external and internal growth factors, Shanker et al.

(2005). Presence of toxic metals in the external environment leads to changes in the growth and development pattern of the biota. Metals are commonly considered as simulators or inhibiting factors of life processes, due to which they may appear toxic for living organisms, Szyczewski et al. (2009).

The first reports started to appear in 1950–1970s on the toxic effects of Hg and Cd contamination on wild animals, including foxes, seafood and cultivated rice in Japan, Basu and Head (2010), when countries with highly industrialized agriculture were widely using organic Hg in chemicals for plant protection and seafood and rice as food. Some of the toxic metals such as Cd, Pb, Hg and As are strongly poisonous to metal-sensitive enzymes, resulting in growth inhibition and death of organisms, Nagajyoti et al. (2010), Stankovic et al. (2011a, c).

Various metals play a key role to maintain and control various vital functions of living organisms. The essential metals include, among others, Cu, Zn, Fe, Mn, Cr, Co, Mo, and Ni and play biochemical and physiological functions in living organisms. Two major functions of essential metals are the following: (a) participation in redox reaction, and (b) direct participation, being an integral part of several enzymes, Szyczewski et al. (2009), Nagajyoti et al. (2010). However, in the literature there are several references regarding the essential characters of Ni, Nagajyoti et al. (2010), but at the some Ni is classified as 'probably essential', Szyczewski et al. (2009). Too large doses of essential metals can damage living organisms or in some cases the organism can be injured or die, Szyczewski et al. (2009). The availability of metals in medium varies, and metals such as Cu, Zn, Fe, Mn, Mo, Cr, Ni and Co are essential micronutrients whose uptake in excess results in toxic effects, Nagajyoti et al. (2010).

## Fauna

Metals uptake by fauna occurs through diffusion as well as ingestion of food. Metals can be stored in the skeletal structure and intracellular matrices of an organism, and excreted in feces and eggs. Fauna organisms have additional defense mechanisms: metallothioneins and other heavy metal-binding proteins bind metals within the organism helping them to control toxic metal levels, Garcia et al. (2008). In comparison to plants, animals have generally developed a greater arsenal of defense mechanisms; in addition, non-sessile animals can avoid a certain number of environmental or anthropogenic stressors by their mobility.

Prey, predatory birds and mammals, are accumulators of significant amounts of organic Hg by eating beans and small warm-blooded vertebrate herbivores contaminated with Hg, Basu and Head (2010). Toxicity of methylmercury (MeHg) and Cd in mammals has been well documented, Basu and Head (2010). Metals are toxic to mammalian renal cells, causing damage that leads to leakage of phosphates, Ca, glycogen and proteins from the kidney, Lavery et al. (2009).

Terrestrial vertebrates, including wild animals, absorb Hg via food, water, air and through the skin. Organic Hg is strongly absorbed from the gastrointestinal tract (>90 %) and accumulates in internal organs, mainly in the liver, kidney and also

in the brain, Kalisinska et al. (2012). In contrast, elemental Hg and its inorganic compounds are very poorly absorbed in the gastrointestinal tract (<10 %), and additionally their harmful effects on warm-blooded vertebrates, including man, is much lower than MeHg, which is widespread in the environment, especially in water ecosystem, Kalisinska et al. (2012).

Birds are primary and secondary consumers, feeding on plants, invertebrates and vertebrates alike. Colonial waterbirds (herons, storks, ibises, pelicans, cormorants, gulls, terns, and marine birds) often have been used or proposed as indicators of Pb, Cd, As, Se, and Hg. Many toxic metals (Cr, Mn, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Hg) have been studied in several terrestrial, aquatic and seabirds, as birds are ordinarily at the top of the food web, they are valuable for environmental monitoring, Horai et al. (2007), Hargreaves et al. (2011).

Heavy metals such as Hg, Cd, As, and Pb are toxic metals that have no known vital or beneficial effect on organisms and their accumulation over time in the bodies of animals can cause serious illness, Stankovic et al. (2011a), Stankovic and Jovic (2012). Certain elements that are normally toxic are, for certain organisms or under certain conditions, beneficial: examples include V, W, and even Cd, Hogan (2010). Kidneys and liver are important sites for metal accumulation and toxicity in vertebrates, Adham et al. (2011). Metals accumulate initially in the liver where they form a complex with metal-binding proteins, such as metallothionein, until an accumulation threshold is reached. Then the metal protein complex is transported via the plasma to the kidney, where they accumulate over time, Adham et al. (2011).

## Flora

Uptake of elements into plants can happen via different ways. The elements can be taken up via roots from soil and transported to the leaves. Also, they may be taken up from the air or by precipitation directly via the leaves. Trace elements may be also taken up via both mentioned ways. Many factors influence the plants metals uptake and include the growing environment, such as temperature, soil pH, soil aeration, Eh condition (particularly of aquatic environment) and fertilization, competition between the plant species, the type of plant size, the root system, the availability of the elements in the soil or foliar deposits, the type of leaves, soil moisture and plant energy supply to roots and leaves, Nagajyoti et al. (2010).

Plants dig their roots in soils, sediments and water, and roots can take up organic compounds and inorganic substances. Roots can stabilize and bind substances on their external surfaces. Uptaken substances may be transported, stored, converted, and accumulated in the different cells and tissues of the plant. Finally, aerial parts of the plant may exchange gases with the atmosphere allowing uptake or release of molecules, Marmiroli et al. (2006). Accumulation and distribution of heavy metals in the plant depend on the plant species, the levels of the metals in the soil, water and air, the element species and bioavailiability, pH, cation exchange capacity, climacteric condition, vegetation period and multiple other factors, Filipovic-Trajkovic et al. (2012).

Metal toxicity can inhibit protein activity or disrupt their structures in plant. Metal detoxification mechanism in plants involves the activation of antioxidative enzymes systems that are responsible for protecting the plant cells from the toxic effects during metal contamination, Garcia et al. (2008). Plants' response to the presence of heavy metal ions includes synthesis of plant thiol compounds, namely phytochelatins, Babula et al. (2008). Some plants, called metallophytes, can demonstrate tolerance–hypertolerance to heavy metals, Babula et al. (2008) and hyperaccumule one or more metals/metalloids, Stojanovic et al. (2012).

Plants might react to environmental stress on various levels: on the cellular biochemical, or morphological scale, and at species or population level. The phototoxic effects typically cause physiological, morphological and ecological changes, expressed in many different ways, from reduced photosynthetic activity to changes in plant species distribution, the establishment of adapted species, and the dying-off of whole plants or communities, Johnson et al. (2011). However, stress can be induced in vegetation by a large number of other factors, including water deficiency, poor soil drainage, poor soil aeration, soil salinity, weed competition, nutrient deficiency, or nutrient poisoning, Schwartz et al. (2011).

For their development, plants require not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients such as Fe, Zn, Mn, Ni, Cu, and Mo. Most of these micronutrients accumulate in the plant tissues for their metabolic needs, but they never exceed 10 mg, except Fe, Mn and Zn, Nagajyoti et al. (2010). Some metals which are considered contaminants are also essential micronutrients for all vegetation species; in particular Cu and Zn. Higher levels of these metals, and other heavy metals, in plant tissue might have phytotoxic effects, sometimes resulting in death, Szyczewski et al. (2009). The levels of heavy metals in plants, both terrestrial and aquatic, vary widely because of the influence of environmental factors and the type of plant itself. The metals range observed in plants was presented in the review paper of Nagajyoti et al. (2010).

The main sources of toxic metals to plant may be atmospheric or terrestrial, including mining and metal working industries, combustion of fossil fuels, disposal of ash residues from coal combustion, vehicular traffic and use of pesticides and fertilizers in agriculture. A few heavy metals such as Co, Mn, Fe, Cu, Se, Mo and Ni are essential, whereas As, Cd, Cr, Hg, Pb are nonessential elements and toxic to plants. Besides their toxicity, heavy metals are also quite persistent in the environment, Szyczewski et al. (2009).

Babula et al. (2008) claim that essential elements are important constituents of pigments and enzymes, mainly Cu, Ni, Zn, Co, Fe and Mo for algae and higher plants, but all metals/metalloids, especially Cd, Pb, Hg and Cu are toxic in higher concentrations because of disrupting enzyme functions, replacing essential metals in pigments or producing reactive oxygen species (ROS). The similarity of certain heavy metals to essential heavy metals, for example couples Cd–Zn, Se–S or As–P, predestinates their high toxicity due to the possibility to replace essential metals in enzymatic systems. The toxicity of less common heavy metals and metalloids (Tl, As, Cr, Sb, Se, and Bi) are still under investigation. The important factor of

bioavailability of metals/metalloids is their presence in soil and water, although there are not many plants that are able to uptake them from an air, Babula et al. (2008).

Plants contribute to the circulation of heavy metals in the food chain through their active and passive absorption, accumulation in tissues as well as subsequent grazing by animals or consumption by humans. All plants have the ability to accumulate all metals, especially essential for their growth and development: Mg, Fe, Mn, Zn, Cu, Mo and Ni. Plants absorb metals through the root from the soil, water and through over ground vegetative organs from the atmosphere, Filipovic-Trajkovic et al. (2012). Levels of heavy metals in various species of wild plants growing in the same habitats may vary considerably, Wisłocka et al. (2006).

### 5.3.5 Human-Health Implications

Humans can be exposed to metals through inhalation of dust or gaseous particles, or ingestion through food and drink, Hu (2002). Once a metal is incorporated, it is distributed to tissues and organs. Although excretion typically occurs through the kidneys and digestive tract, metals tend to persist in sites like the liver, bones, and kidneys for years or decades. Low-level metals exposure likely contributes to chronic disease and impaired functioning, Hu (2002).

Living organisms, and as humans as well, require varying amounts of "heavy metals": Fe, Ni, Cu, Zn, Co, Mn, and Mo, Wong et al. (2006), Joksimovic et al. (2011a, 2012). Their excessive levels can be damaging to the organism. Other heavy metals such as Hg, Cd, As, and Pb are toxic metals that have no known vital or beneficial effect on humans, Stankovic et al. (2011a), Stankovic and Jovic (2012), and their accumulation over time in the bodies can cause serious illness.

Within the European community the eleven elements of highest concern are: As, Cd, Co, Cr, Cu, Hg, Mn, Fe, Zn, Ni, and Pb. Some of these elements are actually necessary for humans in trace amounts (Fe, Zn, Co, Cu, Cr, Mn, Ni), while more than necessary amounts of trace elements and some others are carcinogenic or toxic, affecting, among others, the central nervous system (Hg, Pb, As, Mn), the kidneys or liver (Hg, Pb, Cd, Cu) or skin, bones, or teeth (Ni, Cd, Cu, Cr), Rai and Pal (2002), Chen et al. (2008), Lavery et al. (2009), Zevenhoven and Kilpinen (2001), Stankovic et al. (2011a), Jovic et al. (2012), Stankovic and Jovic (2012), Markovic et al. (2012).

Metals are significant to human because some of them are most important trace elements in various metabolic enzymes and constituents of cells. Zn, Cu and Fe form important component of cell and co-factor in several metalloenzymes, while organically chelated $Cr^{3+}$-ion act as a co-factor in insulin hormone response controlling carbohydrate metabolism in humans, Rai and Pal (2002). Some non-essential elements are present in all tissue and organs of man. Under normal conditions, more than 90 % of Pb is retained in the skeleton, Stankovic et al. (2011a).

The importance of Cr as trace nutrient in humans and animals has been known for about 40 years. Cr is necessary for an optimal metabolism in humans and some animals. The daily need for Cr in humans should be around 50 μg. Most frequently occurring damages at increased uptake of Cr are damages to skin and mucus membranes. International Agency for Research on Cancer (IARC) has assessed that Cr (VI) compounds are carcinogenic to humans, Rai and Pal (2002). The most common effect of exposure to Ni is allergic contact eczema. Acute poisonings are very rare, as for Cr. Observed effects of Ni compounds are various damages to the respiratory system. IARC has assessed that Ni compounds are carcinogenic to humans, Jovic et al. (2012).

In the 1956 an unusual and painful disease of rheumatic nature was recorded in the humans from a village in Japan. During subsequent years it became known as 'itai-itai' disease, chronic Cd poisoning, Stankovic et al. (2011a). Cd induced disturbances in Ca metabolism accompanied by softening of bones, fractures and skeletal deformations. The main source of Cd exposure in the general population is food, representing >90 % of the total intake in non-smokers, Stankovic et al. (2011a).

Hg is considered to be highly toxic metal for living organisms. Even at very low concentration, Hg and its compounds present potential hazards due to enrichment in food chain. Poisoning by MeHg presents a bizarre neurological picture as observed in large-scale outbreaks in Japan, China, Iraq and various parts of the world, Rai and Pal (2002). In 1959 the humans who had consumed fish and shellfish around Minamata bay in Japan progressively suffered from the disease known as Minamata disease. The humans' deaths were caused by the consumption of the fish and other foodstuffs contaminated with MeHg, Stankovic et al. (2011a), Stankovic and Jovic (2012). Human exposure to MeHg occurs mainly through the diet, more specifically from the consumption of fish. Humans can also be exposed to elemental Hg vapors and other inorganic Hg species, but the contributions from the air and consuming drinking-water are insignificant, Rai and Pal (2002).

In 1960 fatal incidents of lung cancer were reported from the Kiryama factory of Nippon-Denki concern. The Nippon chemical industry has deposited approximately 530,000 t of wastes containing Cr(VI) around Tokyo in Japan. Due to its hardening properties the material has found extensive use for construction purposes. Medical warnings were issued that inhalation of dust containing Cr(VI) was associated with malignant growth in the respiratory tract, Rai and Pal (2002).

The importance of As as a health hazard, which is also known as 'slow killer' is now well recognised. As is the most common cause of acute heavy metal poisoning in humans, and does not leave the body once it enters. The most obvious signs are the blisters on the palms of the hands and soles of the feet, which can eventually turn gangrenous and cancerous, Rai and Pal (2002). Meanwhile, the poison also attacks internal organs, notably the lungs and kidneys, which can result in illnesses including cancer, Stankovic et al. (2011a), Stankovic and Jovic (2012), Markovic et al. (2012), Jovic et al. (2012).

From several places As poisoning has been reported: in the vicinity of a coal power plant in Czechoslovakia where coal that was used contained about 1,000–
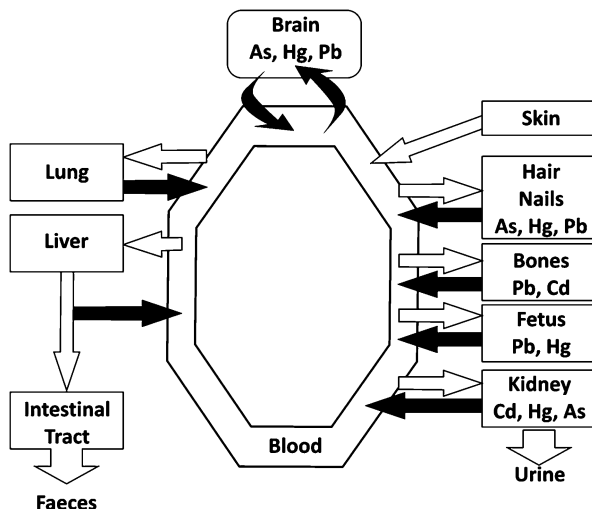
**Fig. 5.3** Deposition of metals in human, Kakkar and Jaffery (2005)

1,500 g of As per tonne, children showed respiratory symptoms and hearing loss, Rai and Pal (2002). Similar cases of As poisoning have been reported from West Bengal (India) and Bangladesh in recent years. There is no medicine available for chronic As toxicity. The only treatment is safe water, nutritious food and time, Rai and Pal (2002). Long term ingestion of As contaminated drinking water produced gastrointestinal, skin, liver and nerve tissue injuries, Rai and Pal (2002) and cancer Stankovic et al. (2011a), Stankovic and Jovic (2012), Markovic et al. (2012), Jovic et al. (2012).

Even the Romans were aware that Pb could cause serious health problems. Pb is the number one environmental poison amongst the toxic heavy metals all over the world, Rai and Pal (2002), causing serious health hazards to humans, especially to young children, affecting the membrane permeability of kidney, liver and brain cells, resulting in either reduced functioning or a complete breakdown of these tissues since Pb is a cumulative poison, Stankovic et al. (2011a), Stankovic and Jovic (2012). The full impact of Pb poisoning on the health of children and adults is becoming clearer to most countries, and many governments have begun to take action.

Figure 5.3 depicts the deposition of metals in humans, Kakkar and Jaffery (2005). Different levels of exposure to heavy metals can lead to three different effects: acute effects in which symptoms appear immediately after exposure of heavy metals during a short exposure period; chronic effects are a result of low-level exposure over a long period. Lastly, lethal effects can be defined as responses that occur when physical or chemical agents interfere with cellular and subcellular processes in the organism at the high level thus causing death, Kakkar and Jaffery (2005).

Problems related to pollutants include the emission of certain substances, even in small quantities, which disturb natural processes and threaten the health of humans

and ecosystems due to their toxicity, carcinogenity, mutagenicity or hormone mimicking properties, der Voet et al. (2000). Metal toxicity causes irregular metal-lothioneins (MT) protein synthesis, renal damage, and disruption of bone structure in humans. Metal-binding proteins have special functions in the detoxification of toxic metals and also play a role in the metabolism of essential metals. MTs are involved in the regulation of the essential metals Cu and Zn and in the detoxification of Cd and Hg, Zhou et al. (2008), Freisinger (2010), Sevcikova et al. (2011).

Pulmonary absorption of inhaled Cd ranges from 10 % to 50 % and the average normal gastrointestinal absorption of ingested Cd in humans' ranges from 3 % to 7 %, while in the tissues is mainly bound to metallothionein. The synthesis of this protein probably represents the body's defence mechanism against the toxic Cd-ion. Liver and kidney tissues are the two main sites of Cd storage and these organs accumulate considerable amounts of Cd, about 40–80 % of the body burden, WHO (2007). Important health endpoints include kidney and bone damage and cancer, Lavery et al. (2009), Stankovic et al. (2011a), Stankovic and Jovic (2012).

Heavy metals have been utilized by humans for thousands of years. Exposure to heavy metals continues although several adverse health effects of heavy metals have been known for a long time, Rai and Pal (2002), Schwartz et al. (2011), Stankovic et al. (2011a), Stankovic and Jovic (2012), Markovic et al. (2012). The main threats to human health from heavy metals are related with exposure to Pb, Cd, Hg and As. Pb is a toxic metal and can accumulate in the blood, bones, and soft tissues, Markovic et al. (2012); even low exposure to Pb can cause mental retardation in children. More toxic is Hg. Once Hg from the air reaches water, microorganisms can change it into MeHg, a highly toxic form that builds up in fishes. People are primarily exposed to Hg by eating contaminated fish and seafood, Stankovic et al. (2011a), Stankovic and Jovic (2012), Markovic et al. (2012), Jovic et al. (2012). MeHg exposure is a particular concern for women of childbearing age, unborn babies, and young children, because studies have linked that high level of MeHg damaging a developing nervous system. Other toxic metals As, Cr and Ni emitted from power plants and burning coal, can cause cancer in humans, Stankovic et al. (2011a), Stankovic and Jovic (2012). For example, Hg is still used in gold mining in many parts of Latin America. Pb remains a common additive to petrol, although this use has decreased in the developed countries, and As is still common in wood preservatives, fungicides and pesticides. Coal fuels and waste especially contain heavy metals which should be a central concern in the consideration of their use. Since the mid-nineteenth century the production of heavy metals has risen abruptly and their emissions into the environment, especially in less developed countries.

## 5.4 Regulations

Experience gained in the1960s and early the 1970s has led to the conclusion that the geographical marine toxic metal contamination and the associated biological impact were largely unknown and undocumented. The first monitoring program of

that kind was established by Goldberg (1975), 'Mussel Watch' monitoring program, and proposed to assess the spatial and temporal trends in chemical contamination in estuarine and coastal areas. Nowadays Agenda 21 is an action plan of the United Nations (UN) related to world sustainable development and their environment protection.

On the basis of the Agenda 21 the action should be taken globally, nationally, and locally by organizations of the UN, governments, and major groups in every area in which humans directly affect the environment. This meeting, known in UN circles as simply "Rio," was the first time that the international community agreed that the environment was something worth protecting and that long term economic development cannot be achieved without attention to principles of sustainability.

Today, compelled by the growing environmental and health awareness of the public, assessments of an array of trace metals in soils, sediments, water, air, as well as foodstuffs are demanded by regulatory guidelines, Wong et al. (2006), Smolders et al. (2009), Stankovic et al. (2011a), Joksimovic et al. (2011a), Jovic et al. (2011), Joksimovic and Stankovic (2012), Jovic et al. (2012). Those routinely regulated trace metals include As, Ba, Cd, Co, Cr, Cu, Hg, Pb, Mo, Ni, V, and Zn.

The significance of heavy metals for the environment quality has been reported in several European Union Directives 2004/107/EC (As, Cd, Hg, Pb, Ni, for instance) and Directive 2008/50/EC; Directive 2004/35/EC; Directive 2008/1/EC (for bioindicators), where it was stressed on the serious threat for human health evoked by the presence of these metals in the food chain, as well as in the environment. The aim of a directive is to protect the environment by monitoring water, air, sediment, soil, biota, and to establish common quality rules for their chemical analysis.

Within the European Union the first directive in the field of nature protection was the Bird Directive in 1978 (79/409/EEC), EEC (1979). Within the EU countries free access to environmental information is guaranteed according to the information directive 90/313/EEC, EEC (1990). Intensive Monitoring of Forest ecosystems were established in 1994 within the framework of the UN-ECE ICP Forest, UN/ECE and EC (2000). At gaining a better understanding of the effects of air pollution and other stress factors on forest ecosystems in 2001 was done by implementation of the multi-disciplinary Integrated Monitoring Programme ICP IM, UN/ECE Convention on Long-range Transboundary Air Pollution (2001).

Air pollution and the problems it causes are not confined by any geopolitical boundaries. In the last decade there has been significant improvement in air quality in urban areas as a result of strict legislation, the introduction of "cleaner" technologies and the migration industry in uninhabited areas. Monitoring of heavy metals (Pb, As, Cd, Hg, and Ni) and other air toxicants are planned according to the directives of the CAFE (Clean Air for Europe) program and those of the European Union (Directive 2004/107/EC 2004). EuroBionet – "European Network for Assessment of Air Quality by the Use of Plants Bioindicator" established and financially supported by the European Commission Environment Programme and EU obliges its member states to establish national networks for monitoring air quality.

The increasing importance of bioindicators is also encouraged within the European Union's Water Framework Directive (WFD), EC (2000). The directive aims to achieve a good ecological status in all European water bodies, such as rivers, lakes and coastal waters, and requires that the assessment of the ecological status of a system be accomplished primarily utilizing biological indicators. Among the wide range of bioindicators, five biological elements are listed within the WFD: phytoplankton, macroalgae, angiosperms, benthic invertebrates and fish, Frontalini and Coccioni (2011). One major goal of the WFD is to achieve the "good ecological status" and "good chemical status" in all surface water bodies of the EU. The term ecological status corresponds to the general philosophy of an integrated approach for evaluating the ecological integrity of water bodies.

The Marine Strategy Framework Directive 2008/56/EC (MSFD) are formulated as "concentrations of contaminants are at levels not giving rise to pollution effects". Contaminants are defined as chemical elements and compounds or groups of substances that are toxic, persistent and liable to bioaccumulate, and other substances or groups of substances which give rise to an equivalent level of concern. This definition is in line with the definition of hazardous substances used in the Water Framework Directive 2000/60/EC (WFD), EC 2000. The Water Framework Directive (WFD) establishes requirements for good surface water chemical status.

The recent adoption of human biomonitoring (HBM) as Action 3 in the Environment and Health Action Plan 2004–2010 of the European Commission COM (2004) has motivated the implementation and application of HBM in European environment and health research.

### 5.4.1 Toxic Metals Regulations for Human's Intake

Humans can be exposed to metals through inhalation or ingestion through food and drink. Once a metal is incorporated in a human, it tends to distributed to human's tissues and organs and persists in sites like the liver, bones, and kidneys for years or decades, Hu (2002), although some its excretion can occur through the kidneys and digestive tract. With respect to essential and non-essential elements, the Joint Food and Agricultural Organization and World Health Organization (FAO/WHO) have established the Provisional Tolerable Weekly Intake (PTWI) level, which is defined as an upper intake limit above which adverse effects might be expected, FAO/WHO (2004, 2007, 2010). The term 'provisional' is used to emphasize the lack of safety data on contaminants; consequently, the levels of PTWIs are continually re-evaluated.

According to WHO (2007) in most foodstuffs the Hg concentration is below 0.02 mg/kg. To safeguard public health, limits on total Hg levels in seafood have been established in various countries. Fish and fish products are the predominant sources of human exposure to MeHg. The United States Food and Drug Administration have set a maximum total mercury limit of 1 mg/kg wet weight for fish. In

**Table 5.2** Tolerable intake levels for trace elements appointed by the FAO/WHO (From http://www.inchem.org/ 6 November 2009)

| Element | Provisional tolerable weekly intake (PTWI) (mg kg$^{-1}$ bodyweight) | Provisional maximum tolerable daily intake (PMTDI) (mg kg$^{-1}$ bodyweight) |
|---|---|---|
| Arsenic | 0.015 | – |
| Cadmium | 0.007 | – |
| Copper | – | 0.50 |
| Iron | – | 0.80 |
| Lead | 0.025 | – |
| Mercury | 0.005 | – |
| Methylmercury | 0.0016 | – |
| Zinc | – | 1.00 |

Europe, a value of 0.5 mg/kg was set by the European Commission for seafood, EC (2006). In Japan, fish with total Hg concentrations higher than 0.4 mg/kg are considered unfit for human consumption, WHO (2007).

The provisional tolerable weekly intake (PTWI) set by WHO and the Food and Agriculture Organization of the United Nations (FAO) for Cd is 500 µg (a weekly intake of 7 µg/kg body weight (kgbw)), corresponding to a daily intake of 70 µg or 1 µg/kgbw/day, FAO/WHO (2010). The USEPA Reference Dose amounts to 1 µg/kgbw/day in food and 0.5 g/kgbw/day in drinking-water, USEPA (1999). These values are based on the chronic effects of Cd on kidney function. However, it has been suggested that the PTWI should be lowered, WHO (2007). Data on the toxicology of tolerable intake of the most important elements are given in Table 5.2.

## 5.5 Bioindicators

There is a continual influx of toxic metals into the biosphere from both natural and anthropogenic sources. The concentrations of contaminants may, however, be too small to be detected by using chemical methods. So, biological indicators, also called bioindicators, can be used to provide an indication of environmental conditions including the presence or absence of contaminants in trace. The term 'bioindicators' was first used by Clements in 1920 in order to signify the organisms, which by their presence in a particular habitat, clearly indicated the ecological conditions of that habitat.

The loss of ecosystem services (e.g., clean air, drinking water, plant pollinators) has increasingly focused world's attention on the health of natural ecosystems. All species tolerate a limited range of chemical, physical, and biological conditions, which we can use to evaluate environmental quality. Various species of biota can be potentially used or were used as bioindicator organisms for measuring diversified types of pollutants at different situations.

Considering the 1.7 million species currently documented on Earth, how do we chose just one as a bioindicator? The answer is simple: no single species

can adequately indicate every type of disturbance or stress in all environments. Depending upon the specific environment, the present species and their local disturbances, an appropriate bioindicator species or groups of species need to be selected. Ecologists have established a broad set of criteria that species must exhibit to be considered as good bioindicators, McBride et al. (2011). An organism, to be employed as a bioindicator, has to have the next characteristics: to be easily identified and sampled; to be widely spread in the studied area; to have a low mobility and a long-life cycle; to have a good genetic uniformity on all the considered area, and to be present all-year long, Zhou et al. (2008).

In practice, a bioindicator can be any animal, plant or microbial system that can be used to formulate conclusions about the environmental conditions they are continuously exposed to. Bioindicator is an organism that can be used to establish geographical and temporal variations in the bioavailability of contaminants by measuring the accumulated concentrations of chemicals in the whole body or in specific tissues, Hall et al. (2008). Previously used or suggested indicator organisms include plants, beetles, benthic invertebrates, butterflies, amphibians, fishes, birds, and mammals. As it was shown in the review of Burger (2006) over 40 % of the bioindicator papers were about metal pollution, wherein fish, plants, invertebrates, mammals were the dominant used bioindicator species. For aquatic metal pollution, the common used bioindicators mainly contained organisms including plankton, insects, mollusks, fishes, plants, and birds, Zhou et al. (2008).

Methods to measure and evaluate the effects of metals in the environment are complicated not only by bioavailability and susceptibility, but by measurement tools and detection limits, environmental conditions, and the interrelatedness of the metals themselves. The numerous benefits of bioindicators have spurred legislative mandates for their use in countries around the world. Examples of environmental, ecological, and biodiversity indicators can be found in many different organisms inhabiting many different environments. For example, lichens (a symbiosis among fungi, algae, and/or cyanobacteria) and bryophytes (mosses and liverworts) are often used to assess air pollution. Lichens and mosses serve as effective bioindicators of air quality because they have no roots, no cuticle, and acquire all their nutrients from direct exposure to the atmosphere.

A bioindicator is an organism, or part of an organism or a community of organisms, that contains information on the quantitative aspects of the quality of the environment. Furthermore, exposure can be measured by either levels or effects. The importance of metals to ecosystems and their component parts was initially evaluated by measuring metal levels in air, water, and soil, and inferring potential effects on organisms. Specific physiological and behavioural changes in bioindicators are used to detect changes in environmental health.

According to Hodkinson and Jackson (2005) bioindicator is a species or group of species that reflects biotic and abiotic levels of contamination of an environment. However, to the organism used as a bioindicator of pollution it must meet certain criterions. First of all, the body must constantly accumulate and tolerate large amounts of pollutants, must be tied to one place to make it real "representative" for the environmental area: soil, air, water; must be readily available for the collection,

identification and handling; must be sufficiently long lifetime to ensure sampling over a longer period of time; to have enough tissue for chemical analysis and must tolerate the physical and chemical changes of the environmental parameters. Bioindicators are species that are particularly sensitive to the environment, and provide information about ecosystem health, respond well both to the presence or absence of other species, as well as the presence or absence of contaminants.

### 5.5.1   Animals as Bioindicators

Ideally, metal concentrations in the animal's body reflect environmental pollution levels quantitatively. Such biological accumulators have often been used as accumulation indicators of environmental metal pollution. In reality, however, many factors like the nutritional, physiological and reproductive status, the sex and age of the animals influence such quantitative relationships. Generally, metal accumulation by animals is favoured by their limited ability to excrete these contaminants directly after their uptake because of metal inactivation by binding to metallothioneins, Zhou et al. (2008). The animal's kinds mostly used as bioindicators are zooplankton, invertebrate and vertebrate. Animal species have been commonly used as indicators of aquatic ecosystems.

### Zooplankton

Plankton is composed of phyto- and zooplankton microscopic organisms that float freely with oceanic currents and in other bodies of water. As primary source of food in the aquatic food chain, phytoplankton use chlorophyll to convert energy (from sunlight), inorganic chemicals (like nitrogen), and dissolved carbon dioxide gas into carbohydrates. A phyto- and other plankton are zooplankton food.

The freshwater cladoceran *Daphnia magna* is one of the oldest and most widely used zooplankton as test organism in aquatic toxicology, Ratte et al. (2003). The genus *Daphnia* is an important link in freshwater trophic chain, as it is both a phytoplankton consumer, as well as a food source to invertebrate and vertebrate predators.

The species of the genus *Daphnia* differ in their world distribution. *Daphnia magna* is a pond species with limited geographical range (Eurasia), whereas *Daphnia pulex* is a more widespread species (North and South America, Greenland and Europe), Ratte et al. (2003).

The choice of *Daphnia magna* as standard test species was influenced by several advantageous characteristics. It is of small size (compared to mollusks, fish, or macrocrustaceans) and is relatively sensitive to chemicals, compared to other freshwater organisms. The ecology of *Daphnia* has long been studied so there are a lot of background information on its biology and ecology. Thus, *Daphnia magna* is the most commonly tested freshwater species, in acute and also in chronic tests, on toxic metals such as Cu, Cd, Zn and Se, Lam and Wang (2008).

**Invertebrate**

The earthworm *Eisenia fetida* is one of the mostly used test organism for the terrestrial toxic metals bioindicators. This terrestrial species representing the soil fauna is used in acute tests as well as in reproduction tests. The earthworm *Eisenia fetida/E. andrei* is recommended in various guidelines as standard test organism for the terrestrial environment, Ratte et al. (2003). The use of *Enchytraeids* as standard test organisms is discussed in the recent years. Soil-inhabiting enchytraeids are more ecologically relevant than *E. fetida/E.andrei* as important members of the soil biocoenosis in many different habitats, especially where earthworms are rare occurring, Ratte et al. (2003).

Due to their ubiquitous distribution and enormous species number, mollusks play important ecological roles in the different aquatic and terrestrial ecosystems of the world. Although mollusks are basically a marine group of animals, gastropods and bivalves have also expanded their distribution to various freshwater systems, Moloukhia and Sleem (2011). Gastropods have additionally penetrated into a huge variety of terrestrial habitats so that mollusks can be found today from the abysses of the sea to mudflats, from lakes and rivers and their banks to forests, alpine mountains, but also in steppes and desserts; they occur on nearly all latitudes of the planet from polar to tropical temperatures. Thus, mollusks have been successfully used to obtain information on the quality of terrestrial, marine and freshwater ecosystems and to quantify contaminants in their environment, Oehlmann and Schulte-Oehlmann (2002). This is particularly the case for the two most diverse classes of mollusks, gastropods and bivalves, Moloukhia and Sleem (2011).

Oehlmann and Schulte-Oehlmann (2002) listed the following reasons why mollusks are well suited as bioindicators:

– Gastropods and bivalves are widespread and abundant in all marine and freshwater ecosystems worldwide; additionally, gastropods can also be found in almost all terrestrial environments;
– Most mollusks, especially those living in the aquatic environment, exhibit a broad distribution within and even between continents, facilitating their use in geographical large scale surveys;
– A number of species and genera are even cosmopolitans: mussels of the genus *Mytilus* with the two species *M. edulis* and *M. galloprovincialis* being the most widespread, Stankovic et al. (2011a).

The majority of gastropod and bivalve species exhibits an extremely limited mobility or is completely sessile as adults. Mollusks are in direct contact with the ambient medium, water or soil/sediment. Therefore, these mollusks represent the contamination of their habitat ideally. Toxic metals can be taken up not only from the diet, via the gastro-intestinal tract, but also additionally from ambient water or soil/sediment, resulting in greater accumulation potency for contaminants.

Compared with vertebrates, mollusks exhibit only a limited ability to excrete pollutants directly via their kidneys or other excretory organs and tissues. As a consequence, mollusks attain higher bioaccumulation or bioconcentration factors

for many toxicants than other groups. Most gastropod and bivalve species used for biomonitoring and bioindication purposes are relatively large and therefore easy to handle. Consequently, they can be used both under laboratory and field conditions, for active and passive biomonitoring.

Gastropods represent the only molluskan class in terrestrial ecosystems and consequently, snails are the only mollusks which can be used for bioindication and biomonitoring purposes in these environments. Biomonitoring attempts with snails have found an increasing interest during the last decade and surrounding pollution effects. Particularly terrestrial snails can be utilised as accumulation indicator for metal pollution. There are numerous reports on the use of snails as accumulation bioindicators so that only a limited number of examples can be presented here.

Fritsch et al. (2011) compared the concentrations of Cd, Cu, Pb and Zn in the grove snail *Cepaea sp* and the glass snail *Oxychilus draparnaudi*, an herbivorous and carnivorous species, respectively. Grove snails were abundant in both agricultural and woodland landscapes but rare in urban areas. The glass snail was often present in urban areas but less often in woodlands, and it was rarely found in agricultural areas. Toxic metal concentrations increased with total soil concentrations at the sampling points in the glass snail. Based on their results, toxic metals accumulation in snails and small mammals is governed by ecological (diet, habitat preferences and mobility) and physiological (assimilation and excretion of toxic metals) characteristics of animals, Fritsch et al. (2011).

The land snail *Eobania vermiculata* was used to monitor the Hg exposure in Italy in an area affected by a chlor-alkali complex. Additionally, Hg residues were also assessed in plants, soils and air from the same region. The results indicate a low bioavailability of Hg for the snails. The concentrations were in the same range as measured in plants at the same sampling sites indicating a low bioaccumulation of Hg in this snail species, Oehlmann and Schulte-Oehlmann (2002).

The so-called mussel watch, initiated in the United States of America in 1976, was one of the first environmental surveillance programmes, which made use of living organisms in an extended geographical area, Stankovic et al. (2011a), Stankovic and Jovic (2012). The mussel watch comprised a coordinated, standardised sampling and measurement of heavy metals and radionuclides in four marine bivalve species (*Mytilus edulis*, *Mytilus californicus*, *Crassostrea virginica* and *Ostrea equestris*) at more than 100 sample sites on the coast of North America. At the same time, the potential of freshwater bivalves was also analysed in first investigations of heavy metals accumulation in the shell and soft tissues of six clam species, Oehlmann and Schulte-Oehlmann (2002).

Since 1976, bivalves have been used to assess the levels of contamination in marine ecosystems, and certain systematic groups, notably mussels and oysters, have been extensively studied worldwide, Stankovic et al. (2011a). Bioavailability and thus uptake of metals and other pollutants are highly dependent on chemical and biological factors. Among biological factors, there are major differences in bioaccumulation between bivalve species. Within a single species, accumulation

can be a function of age, size, sex, and genotype, nutritional and reproductive status. Factors that influence metal bioaccumulation are physico-chemical water properties: temperature, pH, dissolved oxygen, salinity, sediment grain size, and hydrologic features of the system, Stankovic et al. (2011a).

Mussels *Mytilus* spp. (*M. edulis, M. galloprovincialis,* and *M. californicus*), oysters (*Crassostrea virginica* and *Ostrea edulis*) and clams (*Mercenaria mercenaria*, *Venerupis* sp., and *Macoma balthica*) are the most commonly used bivalve groups for toxic metals bioindicators, Oehlmann and Schulte-Oehlmann (2002). Bellotto and Miekeley (2007) confirmed that the mussel *Perna perna* is efficient toxic metals bioaccumulator in Asia-Pacific. The same species was also employed as an indicator of heavy metal pollution in Brazil and in tropical areas.

The two bivalve species *Crassostrea cucullata* and *Anadara granosa* as suitable bioindicators for heavy metal pollution in India and other Asian countries and the mussel *Mytella strigata* for the same purpose proposed in central and south America. In many areas, marine gastropods have been considered as additional accumulation monitors and the highest concentrations of Cu, Zn, Cd, Pb and Cr were measured in gastropod species, while bivalves were better accumulators of Ni, As and Sn, Oehlmann and Schulte-Oehlmann (2002).

Freshwater mollusks have also been frequently used as bioaccumulator organisms, Moloukhia and Sleem (2011). Biomonitoring studies with freshwater mollusks have covered a wide diversity of species, contaminants, and water ecosystems. In a freshwater ecosystem, *Filopaludina (Siamopaludina) martensi* may be used as bioindicator of heavy metal contaminants. The results of Piyatiratitivorakul and Boonchamoi (2008) indicate that the chosen snail species may be used as a bioindicator for acute and subacute exposures to Hg and Cd.

Metal accumulation by the bivalve *Caelatura (Caelatura) companyoi* and the snail *Cleopatra bulimoides* was determined and results showed that both mollusks could accumulate Cr and Cd to a large extent and could tolerate their toxicity to high limits, Moloukhia and Sleem (2011). Two important advantages of snails and bivalves, over most other freshwater organisms for biomonitoring research, are their large size and limited mobility.

One of the most frequently employed freshwater bivalve accumulator species is the zebra mussel *Dreissena polymorpha*. The zebra mussel *Dreissena polymorpha*, a widespread invasive species in Europe and North America, is an important bioindicator of freshwater environments reflecting the site specific pollution, Minguez et al. (2012). Zebra mussel accumulates high amounts of potentially toxic metals and is therefore widely considered as a bioindicator, Rahnama et al. (2011).

Mollusks have been successfully used as bioindicators in monitoring waters programmes in the past. Terrestrial ecosystems were much less considered than the aquatic environment. It can be stated that terrestrial bivalves, gastropods and especially the other molluskan classes have not yet received the attention they probably deserve, speaking in terms of their ecological importance. A number of invertebrate species are known to be efficient accumulators of trace elements.

**Vertebrate**

The diet is appear to be the most important factor affecting the levels of metals in the tissues of a given animal. Usually, the higher levels of metals in plants and in animals from lower trophic levels, the greater the concentrations of metals in the tissues of higher-level of animals, such as fish, birds and mammals.

**Fish**

Heavy metals play an important role as substances affecting aquatic organisms. Their impact, particularly on fish, is receiving considerable attention. Fish are well recognized bioindicators of environmental changes, including metals pollution, and are adequate for waters monitoring programs, Hauser-Davis et al. (2012). In fishes some trace metals, such as Mn, Co, Fe, V, Cu, Zn and Se, are necessary in small amounts for metabolic processes being naturally assimilated by fish, but Ni, Pb, Cr, Cd, Hg as non-essential elements perform no biological role for fish and become toxic above certain concentrations, Hauser-Davis et al. (2012).

Fish are used as test organisms in aquatic toxicology because of their top-position in the trophic chain and their role as food for humans. The acute and chronic fish tests are used to assess effects of chemicals. Different test species are used as test organisms representing the aquatic vertebrates. Commonly used test species are the rainbow trout *Oncorrhynchus mykiss*, the Zebrafish *Danio rerio*, the Common carp *Cyprinus carpio*, the fathead minnow *Pimephales promelas*, and the guppy *Poecilia reticulate,* Chovanec et al. (2003). Up to now the main database of fish toxicity data exist for freshwater species. Bioindication using fish represents a good monitoring tool especially with regard to river engineering, e.g. river restoration and management. The mobility of many fish species makes difficult to identify not only the exact source of pollution, but also the time and duration of pollution exposure.

Comparative studies of toxicity data of freshwater fish and marine species show that marine species were more sensitive than freshwater species for the majority of substances tested, but for 91 % of all substances the sensitivity ratios between freshwater and saltwater fish were within a factor of 10, Chovanec et al. (2003). Gills, liver, kidney and muscle fish are the organs mostly used for toxic metals investigations.

Metallothioneins (MTs) and other selectively metal binding proteins have found a comparable attention and application for aquatic studies like in terrestrial surveys. It appears that in fish MTs should be considered as a kind of general stress protein, which is particularly responsive to heavy metals, Hauser-Davis et al. (2012). In aquatic mollusks and especially snails and mussels, Dallinger et al. (2000), Chovanec et al. (2003), Stankovic et al. (2011a), MTs seem to be more specifically involved in responses to heavy metals and can thus be considered as a biomarker of exposure to metal contamination. Increased expression of MT in response to harmful levels of toxic metals has been demonstrated for several aquatic species such as gastropods, insect, crustaceans, mussels, fishes, Zhou et al. (2008), Hauser-Davis et al. (2012).

MTs are low molecular weight proteins with many sulfhydryl groups binding a variety of metals, showing a strong affinity toward certain essential and nonessential trace elements, such as Cd, Cu, and Zn. So far, MTs have been identified in a large number of tissues and species throughout the animal kingdom, including a number of terrestrial snails, Dallinger et al. (2000), and mussels, Stankovic et al. (2011a). Although a variety of biochemical data prove MTs to be structurally well defined, but their biological function is still under discussion, Freisinger (2010).

A number of studies demonstrated that the synthesis of MTs can be induced by certain trace elements, but also by organic chemicals and other non-chemical stress factors, like infections, starvation and injuries. Nevertheless, it has been shown that Zn, Cu, Cd, Hg, and other trace elements, are the most potent inducers of MT synthesis and it has therefore been speculated that the detoxification of metals is the primary biological function of these proteins. The involvement of MTs in Cd detoxification of terrestrial gastropods has been proven in detail for a number of species, for example the Roman snail *Helix pomatia*, Dallinger et al. (2000). Compared to other invertebrate taxa such as earthworms, land snails have the advantage that their MTs are by far less unstable, facilitating their use in biomonitoring surveys, Dallinger et al. (2000).

MTs are found in all tissues of fish, particularly in the liver and kidney, and play an important role in the intracellular regulation of the essential metals Zn and Cu. MT concentrations in tissues increase by exposure of fish to Cu, Zn, Cd, or Hg, and the affinity of Cd and Hg to MTs is even higher, so that they may displace essential metals, Zhou et al. (2008). Increased levels of stress proteins reflect the gradual impact on the fish metabolism ranging from adaptive to degenerative responses with severe consequences for fish survival.

The number of quantitative studies of heavy metals in both fish and their parasites has increased recently, Sures (2008). Most investigations focus on either comparative studies of parasite community changes subsequent to environmental stress, or on single investigations of heavy metal concentrations in fish and their parasites where especially acanthocephalans accumulate high burdens of Pb and Cd and therefore may be used as sensitive indicators for monitoring heavy metals contaminations in aquatic ecosystems, Sures (2008). The use of parasites as indicators of heavy metals contamination is of increasing in environmental control.


**Birds**

A few groups of birds dominate the contaminant literature, particularly raptors, waterfowl, and seabirds, Burger and Gochfeld (2004). Birds can play an important role as bioindicators. The general biology and ecology of birds are well known and birds are easy to identify. However, despite their undoubted advantages as bioindicators, birds are not being used as often or as effectively as they could be. One disadvantage is that many species are migratory, making it difficult to determine where exposure occurred, Burger and Gochfeld (2004).

Birds have been successfully used to indicate temporal and spatial trends in toxic metal pollutions in terrestrial and aquatic ecosystems, Burger and Gochfeld (2004),

Deng et al. (2007), Hargreaves et al. (2011), Berglund et al. (2011), Zhang and Ma (2011), Kitowski et al. (2012). Top predators, like raptors, water birds and seabirds accumulate toxic metals, which affect physiology, reproduction and even cause birds death, all of which lead to population declines and which have often in the past been early warning of environmental pollution. If birds are chronically exposed to heavy metals it could result in detrimental effects on their growth, behavior, reproduction, and resistance to diseases, Deng et al. (2007).

Birds have been proposed as useful biomonitoring species of pollutants from the year 1993. Until than focus have been on waterfowl or raptors, terrestrial passerines, such as great tit (*Parus major*), coal tit (*Periparus ater*), blue tit (*Cyanistes caeruleus*), pied flycatcher (*Ficedula hypoleuca*) and collared flycatcher (*Ficedula albicollis*), Berglund et al. (2011). They have successfully been used to monitor the environment close to a variety of different metal industries.

Birds have the advantage of being large, wide-ranging, abundant, long-lived, easily observed, and important to people. Many species are at the top of the food chain where they bioaccumulate contaminants with age, Burger and Gochfeld (2004). The metal concentration in birds can be assessed in a variety of samples, though reports of metal content in egg, blood, feathers, kidney and liver dominate the literature. Samples such as feathers or eggs are easy to collect which reduce the costs of sampling and is the non-destructive and noninvasive sampling technique, although they might not represent the body burden of metals, Berglund et al. (2011).

Birds' contaminations with Cd, Hg, and Se have been shown to adversely affect the condition of birds by reducing their growth or body weight, Zhang and Ma (2011). Since birds usually head the food web, they can provide valuable monitoring of environmental Hg. In many European areas, and also on other continents, birds have proven to be very useful bioindicators of Hg, especially sea and waterbirds, Burger and Gochfeld (2004), Kitowski et al. (2012).

Burger and Gochfeld (2004) analyzed methyl and inorganic Hg separately, MeHg makes up more than 90 % of the total mercury in liver, kidney, muscle, and feathers of birds. Feathers are good indicators of metal pollution, Zhang and Ma (2011), especially for organic bound metals like Hg or Sn, which accumulate in the plumage, and for which feathers are the main elimination routes. There is usually a significant correlation between concentrations of Pb in feathers and those in internal tissues, including blood; therefore the concentration of Pb in feathers is a good predictor of internal bird dose, Burger and Gochfeld (2004). The quantities of a metal incorporated into the feather represent the body level at the time of feather growth, during the development of the young or during moult.

As feathers reflect a bird's body burden during feather growth, body feathers from adult birds or chicks can be used to show long term and spatial trends in Hg contamination. The most frequently used matrix is the bird egg too. Waterbirds' eggs were used a lot as biomonitor to detect heavy metals' concentration or its temporal-spatial trends, as they are easier to obtain and can be saved for a long period compared with soft tissues and nestlings, Zhang and Ma (2011).

Analysis of Hg concentration in bird kidneys has been recognized for years as a valuable tool in monitoring environmental contamination levels. After Hg gains

entrance to a bird, it can be either stored in internal tissues such as kidneys and liver or it can be excreted in feathers and eggs, Kitowski et al. (2012). The level of Hg concentration was found to be significantly lower in young birds than in adults. Increased Hg concentration was also registered in the kidneys of birds, Kitowski et al. (2012).

Deng et al. (2007) analyzed the concentrations of 11 trace metals in tissues from 10 body parts of Great Tits and Greenfinches collected in the Western Mountains, China. The highest concentrations of Hg, Ni, Zn, and Mn were found in the feather; Pb and Co in the bone; Cd, Cr, and Se in the kidney, and Cu in the liver and heart of birds. In general, the concentrations of the non-essential elements (Hg, As, Pb, and Cd) were lower than that of essential elements (Zn, Se, Cu, Cr, and Mn). In both species, Hg had the highest concentration in the feather.

Kitowski et al. (2012) were determined Hg concentration levels in the kidneys of 131 specimens of 42 species of birds with different food preferences. The highest concentrations were found in piscivorous species such as the goosander, great white egret, white-tailed eagle and black stork. The following sequence was identified for concentration levels in kidneys of birds in different trophic guilds: piscivorous > small mammal eating > bird eating > aquatic invertebrate eating > terrestrial invertebrate eating > herbivorous > omnivorous.

Hargreaves et al. (2011) investigated As, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, and Hg concentrations in the tissues, food and abiotic environment of Arctic shorebirds: Hg, Se, Cd, Cu, and Zn bioconcentrated from soil to invertebrates, and Hg, Se, and Fe biomagnified from invertebrates to shorebird blood; As, Ni, Pb, Co, and Mn showed significant biodilution from soil to invertebrates to shorebirds. They tested elements in blood, feathers and eggs of six shorebird species (*Pluvialis squatarola, Calidris alpina, C. fuscicollis*, *Phalaropus fulicarius, Charadrius semipalmatus,* and *Arenaria interpres*). Levels of most elements in shorebird tissues were well below those associated with known pollution sources or toxicological thresholds. The exception was Hg being in blood and eggs, Hargreaves et al. (2011). The highest levels of metals were recorded in tissues of carnivore seabirds, Jakimska et al. (2011).

Berglund et al. (2011) measured the concentration of several elements, As, Ca, Cd, Cu, Ni, Pb, Se, and Zn, in adult and nestling pied flycatchers (*Ficedula hypoleuca)* and great tits (*Parus major*) at different distances to a Cu-Ni smelter. The uptake of Cu and Ni were regulated, but As, Cd, Pb, and Se accumulated in liver tissue. Pied flycatchers had generally higher element concentrations than great tits. The higher accumulation of As and Pb in pied flycatcher livers was explained by a more efficient absorption, whereas the higher Cd concentration was primarily due to different intake of food items. Age-related differences occurred between the two species, though both Cd and Se accumulated with age.

Birds are indeed useful indicators of the state of the environment in which they live, and have proved effective bioindicators as well as biomonitors of various kinds of environmental change. Consequently, avian bioindicators are already important components in current monitoring programmes in many countries and in a variety of ecosystems. Over the last decade the International Council for the Exploration

of the Sea (ICES) has established a working group on "Seabird Ecology", dealing with the question of the usefulness of seabirds as indicators of the state of marine environments.

Birds are abundant, widely distributed and, in some cases, long-lived. Birds occupy various positions in the food chain, especially the higher trophic levels, indicating toxic metals contamination in various compartments of ecosystems by biomagnification of persistent metals. Many birds are widespread species allowing comparisons between different ecosystems, countries or even continents. The widespread distribution of some bird species allows comparisons of contamination on large scales, even between oceans or continents. Not only can birds monitor local food webs, but also, if they are migratory they can be used to compare exposure in different regions. The mobility of birds implies an integrative value in bioindication over broad spatial scales.

## Mammals

Mammals represent useful organisms for biomonitoring purposes and can be used when both temporal and spatial information is required. Among the numerous members of the class of mammals, free ranging animals or "wildlife" fit best the requirements for a biomonitor. This is because they depend exclusively on the quality of food, water and air in their habitat. They consume flora or fauna that reflect the local soil, water and air contamination. Any contamination present will influence the animal and can have an effect on its health. The metal toxic levels in mammals depend on their diet composition and often influenced by food chain effects: do they eat either other animals or/and plants.

An omnivore is a kind of animal that eats either other animals or plants (generally only the fruits and vegetables). Some omnivores will hunt and eat carnivores, eating herbivores and other omnivores. A carnivore is an animal that gets food from killing and eating other animals. The most widespread predator in the order Carnivora is the red fox *Vulpes vulpes*. It lives in almost the entire northern hemisphere, from the Arctic Circle to North Africa, Central America and the Asian steppes, with the exception of Iceland, the Arctic islands and parts of Siberia, Kalisinska et al. (2012). A herbivore is an animal that gets its energy from eating only plants, including grasses. Some others are scavengers and will eat dead matter. Many will eat eggs from other animals. Therefore a great number of environmental contaminants accumulate in mammals, potentially toxic trace of Pb, Cd, Hg, As and radionuclides, like the Cs and Sr isotopes, Pokorny (2006), Rudy (2010), and Kalisinska et al. (2012).

Domestic animals, like cattle, pigs or sheep, can also be used for assessing environmental quality, providing they feed only on foodstuffs originating from their immediate environment. Although grazing sheep still fall in this category, due to modern agricultural practice pigs and most cattle no longer do. Their feed is produced not only outside of the original living area, but possibly even in other continents, Rudy (2010). Studies on domestic animals have shown how diet can

influence Pb absorption. Low Ca and vitamin D levels as well as an insufficient Fe and protein supply increase the absorption rate of Pb, Markovic et al. (2012).

Although many wildlife species could be used for toxic metals biomonitoring, the available literature concentrates on only a few species: wild boar, red deer, brown hare, and red fox, Pokorny (2006), Rudy (2010), and Kalisinska et al. (2012). The suitability of a species for study is influenced by different factors: herbivores accumulate metals to a higher extent than carnivores, which in contrast are better monitors for organohalogen compounds. Additionally, the size of the home range is important. Home range size should be relatively constant for all seasons and over the animal's life span. This factor would therefore exclude wild boar (*Sus scrofa*) as a monitoring species since it shows a sex-dependent difference of home range size. Groups of female boar with their juveniles have a relatively constant home range, whereas males live solitary with no stable territory and migrate over many kilometers, Rudy (2010).

The populations of the European brown hare (*Lepus europaeus*) in Central Europe represents one of the best biomonitors for agriculturally used land, Rudy (2010). A very good bioindicator is the roe deer (*Capreolus capreolus*), the species abundant in nearly all parts of Europe in agricultural, as well as in forest areas, which is one of the most suitable species for bioindication of toxic metals pollution in terrestrial ecosystems (Pokorny 2006). Consequently, Pokorny (2006) suggested the roe deer as a bioindicator of habitat quality/toxic metals pollution for the European region.

Nowadays, especially in Europe, anthropogenic barriers like roads and human settlements inhibit animals' migration and they have to stay in the same habitat during the entire year. This makes them in fact more useful for monitoring studies. However, if the food shortage in winter is compensated for by additional feeding provided by hunters, this can alter the results, since the supplementary foodstuff is often imported from other areas.

Several taxa of small mammals, like shrews (*Soricidae*) and rodents, can also be used for toxic metals biomonitoring of terrestrial, Sanchez-Chardi et al. (2007) and mink (*Mustela vison*) for aquatic ecosystems, Basu et al. (2007). Shrews accumulate higher concentrations of more toxic elements, especially non-essential metals, while wood mice (*Apodemus sylvaticus*) are less tolerant to toxic metals. Wood mice showed lower bioaccumulation of Pb, Hg, Cd, Fe, Mg, Zn, Cu, Mn, Mo, and Cr, than shrews, Sanchez-Chardi et al. (2007).

Mink are monogastric animals with a digestive tract more similar to that of humans than that of rodents, Basu et al. (2007). They can be found in wooded areas and fields near streams and lakes, it is carniverous – a meat- eater that consume a range of prey items available in their local habitat, including small mammals, frogs, snakes, fish and birds. Mink can also provide data on spatial and temporal trends in environmental pollution because they are found over a wide geographical area. For example, temporal analyses of the data have shown that Hg content in mink tissues from Ontario have not changed over the past 20 years, Basu et al. (2007).

Forty percent of mammal species are rodents, and they are found in vast numbers on all continents other than Antarctica. Common rodents include mice,

rats, squirrels, porcupines, beavers, and hamsters. Different species of rodents in a small mammal community may feed on different food items, leading to differences in exposure potential among species. The advantages of these species are that they are very abundant, do not migrate over long distances, have a widespread distribution and have generalised food habits. The disadvantages of these animals to be bioindicators are their small body size, so that very often the whole carcasses have to be analysed and they are not easy to trap.

In the past decade marine mammals, seals and dolphins have been accepted as bioindicators due to their long lifespan and their position at the very top of the marine food chain, in particular for medium and long-term ecosystem changes, Agusa et al. (2011), Bellante et al. (2012), Kakuschke et al. (2012). Marine mammals are extremely susceptible to Hg contamination from both natural and anthropogenic inputs, Wintle et al. (2011), Kakuschke et al. (2012). They accumulate Hg throughout their lifespan, and other factors such as growth features, gender, reproductive and feeding preference are assumed to control Hg levels in these animals, Pompe-Gotal et al. (2009). Considering the high mobility of dolphins, the levels of Hg concentrations in their tissues reflect the general contamination of the broad and poorly defined area in which they live, Wintle et al. (2011).

Concentrations of Cd and As were determined in organs and tissues of different dolphin species during the period 2000–2009 in the Italian coastal area. Significant differences were found between Cd concentrations in the different analysed tissues, but there were no differences in As concentrations, Bellante et al. (2012). Considerable Cd and As concentration differences in samples from different geographical areas were found. Dolphins from the Mediterranean Sea generally show higher levels of toxic metals when compared to others from open oceans, possibly because of the anthropogenic pressure on the basin, Bellante et al. (2012).

Marine mammals such as harbor seals (*Phoca vitulina*) are also accepted indicators. Metals concentrations were measured in their liver, kidney and muscle tissue, as well as in blood and plasma in samples of harbor seals (*Phoca vitulina*) from the North Sea, Kakuschke et al. (2012). The concentrations of Cr, Fe, Mn, and Zn were significantly lower in the whole blood samples of free-living harbor seals of the Elbe estuary than the range measured in free-living seals of the German and Danish Wadden Sea, Kakuschke et al. (2012). In the harp seal (*Phoca groenlandica*) from the coastal waters of Canada, concentrations of V, Mn, Fe, Cu, Mo, Ag, and Hg in the liver, Co, Cd, and Tl in the kidney, and Ba and Pb in the hair were significantly higher than those in other tissues, Agusa et al. (2011).

## Absorption and Distribution of Toxic Metals Among Mammalian Organs

In addition to uptake by feeding and drinking, animals can also absorb and accumulate Pb by breathing. Absorption via the gastro-intestinal tract is relatively low (in the range of 5–10 %) in comparison to absorption via the lungs which can reach up to 50 %. However, the amount of resorption depends on the size of the

Pb containing particles. In general, the concentrations of Pb in soils, vegetation, invertebrates and selected tissues of mammals correlated with traffic densities. There was also a gradient effect on tissue levels with distance from the road. In all species, specimens from the direct vicinity of heavily used highways exhibited the highest Pb concentrations.

Usually liver and kidney samples are analysed in order to monitor the Pb, As, Hg and Cd exposure of an animal, Pokorny (2000), Jakimska et al. (2011), Kakuschke et al. (2012), Bellante et al. (2012). The distribution pattern of Pb and As within these organs is not as uniform as it is found with Cd for example, Bellante et al. (2012). This in turn also varies depending on the species. For red and roe deer, many authors report variable ratios of Pb between the liver and kidneys, whereas for brown hares, rabbits and wild boar higher levels are always found in the liver, Pokorny (2000), Kolesarova et al. (2008), Rudy (2010). The Pb uptake of these animals with their diet is higher in autumn than in spring. One of the most suitable mammalian indicator species for Pb is the brown hare, which accumulates Pb very strongly. The Pb contamination of the strictly herbivorous brown hare is not only influenced by the Pb content of its feeding plants, but also by the fact that this animal is much more exposed to soil particles and dust, Kolesarova et al. (2008).

Cd is a non-essential element for mammals. Under normal conditions, absorption is minimal at around 5–7 % of the total oral uptake. The retention rate of Cd increases in mammals when proteins, Cu, Zn, Fe, Cu or vitamin D are low in their diet. In addition to dietary uptake, another exposure pathway for Cd is the inhalation of contaminated air. Because Cd is better absorbed from inhaled air (10–40 %, depending on particle size) than from ingested food, inhalation of Cd containing particles is likely to be the most important exposure pathway in areas with elevated atmospheric Cd levels, Tataruch and Kierdorf (2003).

After absorption in the lung and gut, Cd is transported via the bloodstream to body stores, particularly the liver. In blood, more than 95 % of Cd is bound to protein in the blood cells. In the liver Cd is bound to metallothionein, Zhou et al. (2008) and the formed complex is transported to the kidneys, where it is almost completely absorbed. Interestingly, approximately 50 % of the total Cd burden of the body is found in the liver and kidneys, Jakimska et al. (2011). In muscles Cd concentrations are low.

As with Pb, Cd concentrations in the blood are not useful as an indicator of the general body burden. In the kidneys, Cd has a very long retention time. For cattle, it was estimated a Cd half life time of up to 12 years in the kidneys compared to only about 2 years in the liver, Tataruch and Kierdorf (2003). These long retention times in the kidneys result in a highly significant, positive correlation between the kidney concentrations of Cd and the age of the animal. Since the kidneys are the main target organ for Cd, they are the best tissue samples for analysis of Cd in mammals, Tataruch and Kierdorf (2003), Bellante et al. (2012).

A significant influence on Cd burden of animals is exerted by the geological properties of the habitat, in particular the soil. Several authors found higher Cd concentrations in roe deer than in other species from the same habitat, Tataruch

and Kierdorf (2003). Chemical analysis of the preferred feeding plants of different ruminant species showed that the difference is due to the selective feeding habit of the roe deer. Its diet consists of a high percentage of Cd accumulating plants like herbs, weeds, fungi and ferns, Tataruch and Kierdorf (2003). Together with its narrow home range; its feeding habits make the roe deer a very sensitive bioindicator, especially in forest areas, Pokorny (2006).

For small mammals, a positive correlation between the Cd content in soils of their habitat and the Cd concentrations in soft tissues was found. As for other mammals, the highest values were found in the kidneys with average Cd levels in this organ being tenfold higher than in the liver. Other tissues displayed much lower concentrations, Tataruch and Kierdorf (2003).

The level of gastrointestinal absorption of Hg in animals depends on its chemical form. Hg in its inorganic form is absorbed up to about 7 % from food, which is similar to other heavy metals. On the other hand, the absorption of MeHg can be as high as 95 %. Elemental Hg cannot be absorbed orally, however, if its vapours are inhaled, around 80 % will be absorbed. The organ distribution of Hg in mammals follows the sequence of kidneys > liver > spleen > brain, in descending order. Concentrations in blood and muscle are low, Tataruch and Kierdorf (2003).

Hg concentrations in the organs of terrestrial mammals have declined considerably in recent years. Only those species that feed on fungi, such as roe deer and wild boar, showed higher levels. Fungi are known to accumulate metals, like Hg and Cd. Roe deer feed intensively on growing fungi, Tataruch and Kierdorf (2003). Other species do not show such an extreme preference for fungi, resulting in a lower Hg level.

The literature on Hg in small mammals indicates that concentration levels are influenced by feeding patterns, Basu et al. (2007). Small mammals, which feed on grasses, always showed comparably low Hg level in their organs than mammals whose food includes earthworms, insects, snails, fish and showed a remarkably higher Hg uptake compared to herbivorous species, Rimmer et al. (2009). Among terrestrial mammals, Hg concentrations increased from herbivores to omnivores and carnivores. For example, herbivores such as mule deer and various species of rabbits usually contained less than 1.0 mg Hg/kg fresh weight in liver and kidney, but carnivores such as red fox (*Vulpes vulpes*) contained 10 mg/kg ww, Kalisinska et al. (2012).

Animals as accumulative monitors of pollution by heavy metals have some advantages over plants such as area related results and comparability to man, Pokorny (2006). Many animal groups have been hailed as valuable sentinels, including birds, marine mammals, aquatic organisms, and domestic animals. Mammalian wildlife has physiological systems that are similar to those of humans in mediating the uptake, distribution, metabolism, and elimination of toxicants, Basu et al. (2007). Humans and many mammalian wildlife species inhabit similar ecosystems and are exposed to common climates, food sources, and pollutants.

## 5.5.2  *Plants as Bioindicators*

Accumulation and distribution of heavy metals in algae and plant depend on the species, the levels of the metals in the soil, water and air, the element species and bioavailiability, pH, cation exchange capacity, vegetation period and multiple other factors. Algae and plants absorb heavy metals through the water and root from the soil/sediment and through over ground vegetative organs from the water and atmosphere. Which plant species will be used as bioindicators also depends on how widely they are distributed throughout the environment.

The main sources of trace elements in algae and plants are their growth media. Biomaterials such as micro and macro algae, fungi, lichens, mosses, tree bark and leaves of higher plants have been used to detect the deposition, accumulation and distribution of metal pollution in water and air. Mosses and lichens, due to lack of root, are probably the most frequently used organisms for monitoring metal pollution in an air, Serbula et al. (2012).

Algae, fungi (micro and macro), and other plant communities play a fundamental role for nutrition and life on earth. As non-mobile organisms they are always exposed to the environmental conditions: to air, soil and water pollutants at their sites of growth. Algae and plants growing in metal-polluted sites exhibit altered metabolism, growth reduction, lower biomass production and metal accumulation. Various physiological and biochemical processes in them are affected by metals.

Chemical analyses of plant materials have been used for biomonitoring and biogeochemical mapping for many years. Trace element input to plants is governed by a wide variety of factors, Suchara et al. (2011). Accumulation and distribution of heavy metals in the plant depend on the plant species, the levels of the metals in the soil/sediment, water and air, the element species and bioavailiability, pH, vegetation period and multiple other factors, Nagajyoti et al. (2010). The quantity or level of heavy metals absorption in plant depends not only on the concentration levels of the metals in the physical and chemical composition of the soil/sediment and water, but also varies in different parts of the plant. Plants also have the ability to accumulate heavy metals which have no known biological function, Filipovic-Trajkovic et al. (2012). Plants are able to minimize the adverse effects of excess heavy metals by regulating the distribution and translocation of them within their organs or cells, Hossain et al. (2012).

Elements are often classified as being macronutrients or micronutrients, and as either essential or non-essential for the plant, whereas their concentrations are generally indicated as deficient, sufficient or toxic. Plant nutrients yet identified and best known as essential are C, H, O, N, P, K, S, Ca, Mg (as macronutrients) and B, Cl, Co, Cu, Fe, Mn, Mo, Ni, Si, Na and Zn (as micronutrients). When these metals are present in bioavailable forms and at excessive levels, they have the potential to become toxic to plants, Nagajyoti et al. (2010).

### 5.5.3 Lower Plants

**Algae, Fungi, Lichine and Moss**

Many toxic and bioaccumulative pollutants are found in only trace amounts in water, and often at elevated levels in sediments. Data from sediments may not be representative of pollutant concentrations in the overlying water column and cannot give information on patterns of contamination at the higher levels of the food chain. For example, the uptake of toxic metals by phytoplankton is the first step in the bioaccumulation in aquatic food webs. Macro- and microalgae also play an important role in the removal of toxic metals, Torres et al. (2008), Azizi et al. (2012), Rybak et al. (2012).

In terrestrial environments, bacteria, fungi, algae and other lower plants play the predominant role in the biochemical cycling of heavy metals. In aquatic environments algae play a key role in biogeochemical cycling of metals and their accumulation in sediments. Metals sequestered by microalgae are a major contributor to the metal load of the water column as well as to the metal content of sediments, Torres et al. (2008).

Some green algae or phytoplankton like *Scenedesmus subspicatus*, *Chlorella vulgaris* or *Pseudokirchneriella subcapitata* are in use as standard bioindicators representing primary producers, Ratte et al. (2003). For example, dominated algal mats of the green algae genera *Klebsormidium* are good indicators of high Fe concentration in water, whereas the presence of *Fucus vesiculosus* suggesting heavy metal pollution in marine environment, Das et al. (2009). At low metal concentration, algae, accumulating metals, pass them to other trophic levels.

The adsorption of heavy metals by algae is highly variable, depending on the metal, the taxon, and other conditions. Algal and fungal biomasses are reported to show efficient metal removal from wastewater, Das et al. (2009). Many heavy metals are necessary micronutrients of algae and fungi in low concentrations, however at high concentrations those can be fatal. Metal toxicity to both, algae and fungi, occurs by affecting their essential metabolic cell processes. Significantly, many nonessential metals compete with essential for uptake into cells. Several of those nonessential metals are toxic at very low concentrations (0.1–1.0 ppm).

Mosses and lichines are particularly used as bioindicators of aerial heavy metals contamination because of their bioaccumulative properties, Backor and Loppi (2009), Blagnytė and Paliulis (2010). Analysis of indigenous mosses is currently used in international and national monitoring programmes, particularly in Europe, Harmens et al. (2008). Mosses have been applied to measure heavy metal levels and trends within and around urban and industrial areas, Suchara et al. (2011). The use of fungi in the monitoring of heavy metal pollution is limited but some fungal groups are better bioaccumulators than others. Little published literature exists with regard to fungi distribution patterns in response to aerial metal contamination.

The use of marine organisms as bioindicators for trace metal pollution is currently very common. Macroalgae and mollusks are among the organisms most

used for this purpose, Jakimska et al. (2011), Markovic et al. (2012), Joksimovic and Stankovic (2012). Freshwater and sea macroalgae are able to accumulate trace metals, reaching concentration values that are thousands of times higher than the corresponding concentrations in water, Vardanyan et al. (2008), Akcali and Kucuksezgin (2011), Joksimovic (2011b), Wolff et al. (2012).

Macroalgea is an important component of aquatic communities due to their role in oxygen production, nutrient cycling, sediment stabilization and providing habitat for aquatic life, Vardanyan et al. (2008). Fresh and sea water macrophites are probably the main source of metals for many animals like invertebrate and fish feeding on them. Invertebrate and fish are commonly consumed as human food. Therefore, the investigation of heavy metal concentrations in the macroalgae species may provide useful information on the transfer of potentially toxic elements from abiotic compartments, water and sediment, to higher consumers including man.

**Higher Plants**

Plants have been used as bioindicators in areas with significant air pollution in the absence of mosses. Some responses of higher plants to heavy metal contamination in the air have potential. The use of plants in the bioindication of heavy metal contamination is not routinely practised.

The most often used parts of higher plants are leaves/needles of: spruce, birch, pine, oak, olive, poplar; also a bark of: oak, pine, ash tree, Serbula et al. (2012). Different plant organs (leaves, flowers, bark or roots) from naturally occurring wild plants and trees, and cultivated plants (vegetables and fruits) were evaluated as possible bioindicators of heavy-metal pollution, Filipovic-Trajkovic et al. (2012). The bioavailability of trace elements from aerial sources through leaves also has a significant impact on plant contamination. Trace elements taken up by leaves can be translocated to other plant organs according to Filipovic-Trajkovic et al. (2012) and Serbula et al. (2012). Unlike Pb, Cd contamination cannot be removed from plants by washing them; it is distributed throughout the organism. It is often difficult to be certain of the cause of a Cd content found in fruit or vegetables, as the substance in its natural form exists everywhere in the soil and is absorbed by the roots.

However, excessive accumulation of metals can be toxic to most plant species. Generally, it is accepted that the normal Cd concentrations in plants are between 0.2 and 0.8 mg kg$^{-1}$ and toxic Cd concentrations are defined as 5–30 mg kg$^{-1}$ and Zn is not considered to be highly phytotoxic and the toxicity limit for Zn (300–400 mg kg$^{-1}$) depends on the plant species as well as on the growth stage. According to Kabata-Pendias and Pendias (1992) normal concentrations of Pb in plants are 0.1–10 mg kg$^{-1}$ d.w. and also expressed that uptake and release of elements depend on plant species, growth stage and composition of the soil/sediment, especially Ca. Generally, toxic Pb concentrations are defined as 30–300 mg kg$^{-1}$, Filipovic-Trajkovic et al. (2012).

The results of the Filipovic-Trajkovic et al. (2012) indicate the existence of differences in accumulation of Pb, Zn and Cd in different plant organs, tissues and different plant species. Distribution of heavy metals is unequal and the largest is in the tree's bark, which is explained by the time of its exposure to the environmental conditions and structure (roughness), enabling a larger absorption of heavy metals from the air. After the tree bark, heavy metals are mostly accumulated in the roots, then in the leaves and as well as in the plant fruits. In over ground organs the highest amounts of Pb were found in the leaves and then in the fruits and vegetables, Filipovic-Trajkovic et al. (2012).

Hg present in soil has a very low availability to plants as the roots function as a barrier. Therefore, in general Hg concentrations in the environment are comparably low and are limited mostly to specific and/or localised aerogenous emissions. Significant accumulation of Hg has been observed in fungi, carrots and potatoes. When organomercuric compounds were still in use as fungicides it was shown that in wheat, barley, oats and corn, Hg was transferred from the seed dressing into the new seedling. During growth, dilution of the mercury occurred and the concentration in the grain was reduced, Tataruch and Kierdorf (2003).

The terrestrial plants are promising indicators for metal pollution in the soil too. It is important during metal biomonitoring programmes that the background of examined elements concentrations in the soil should be established. The design of a monitoring programme should involve the selection of appropriate species, sampling locations, sample collection, sampling frequency, metals to be analysed, chemical technique and data analysis.

It was well known that chemicals themselves can interact significantly with each other, showing either antagonistic (less than additive effects) or synergistic (more than additive effects) behaviour, Joksimovic et al. (2011a), Joksimovic and Stankovic (2012), but combined effects of natural environmental conditions and toxic metals are more important on the biota than interactions among chemicals. It has been shown in a number of studies that natural environmental conditions can significantly modify responses of organisms to toxicants, Laskowski et al. (2010), Holmstrup et al. (2010), Avgın and Luff (2010), Dondero et al. (2011).

The knowledge about these interactions and how they affect organisms is scarce. For example, already over 20 years ago it was noticed that salinity and temperature may significantly affect results of aquatic toxicity tests, Laskowski et al. (2010). Most studies on interactions between toxicants and natural factors showed that effects of toxic chemicals on organisms can differ vastly depending purely on external conditions: in 62.3 % cases Laskowski et al. (2010) were found significant interactions between natural factors and chemicals.

### 5.5.4   Biomarkers of Metal Toxicity in Living Organisms

A conventional monitoring system of environmental metal pollution includes measuring the level of selected metals in the whole organism or in respective

organs. However, measuring only the metal content in particular organs does not give information about its effect at the subcellular level. Therefore, the evaluation of biochemical biomarkers metallothioneins (MTs), phytochelatins (PCs) and antioxidant enzymes (catalase – CAT, superoxide dismutase – SOD, glutathione S-transferases – GST and glutathione peroxidases – GPXs, lipid peroxidation – LP) may be useful in assessing metal exposure and the prediction of potential detrimental effects induced by environmental metal contaminations, Valavanidis et al. (2006), Vlahogianni and Valavanidis (2007), Vlahogianni et al. (2007), Torres et al. (2008), Nordberg and Nordberg (2009), Shariati and Shariati (2011), Semedo et al. (2012).

After having crossed the plasma membrane, the incoming metal is bound immediately by ligands and distributed between sites of storage, efflux, or toxic action. Toxicity does not depend on total accumulated metal concentration. Accumulated metal concentrations should be interpreted in terms of different trace metal accumulation patterns dividing accumulated metals into two components: metabolically available metal and stored detoxified metal. The relationship between metal accumulation and toxicity is influenced by physiological activity of living organisms, Shariati and Shariati (2011).

A number of trace metals are used by living organisms to stabilize protein structures, facilitate electron transfer reactions and catalyze enzymatic reactions, Nagajyoti et al. (2010). For example, Cu, Zn and Fe are essential as constituents of the catalytic sites of several enzymes. Although some heavy metals are essential micronutrients for animals, plants and many microorganisms, depending on the route and dose, all heavy metals demonstrate toxic effects on living organisms via metabolic interference and mutagenesis. The mechanisms by which metals exert their toxicity in living organisms is very diverse, especially their involvement in oxidative biochemical reactions through the formation of reactive oxygen species (ROS), Torres et al. (2008). Heavy metals are involved in toxic redox mechanisms through the generation reactive oxygen species, associated with oxidative damage to important biomolecules, and molecular mechanisms of metal toxicity and carcinogenicity, Vlahogianni and Valavanidis (2007).

Metal ions can penetrate inside the cell, interrupting cellular metabolism and in some cases can enter the nucleus. The entrance of the metal in the cell can mobilize several metabolic pathways and genetic processes to neutralize the source of toxicity, Azevedo and Rodriguez (2012). The entrance of certain metals into the nucleus can enhance the synthesis of RNA that codes from metallothioneins. Metallothieneins (MTs) are peptides found mainly in the cytosol, lysosomes and in the nucleus, low molecular weight peptides, high in the amino acid cysteine which contains a thiol group (-SH). The thiol group enables MTs to bind heavy metals, Nordberg and Nordberg (2009).

MT in the physiological system has several roles, especially in the metabolism and kinetics of metals: transport and detoxification of metal ions and protection from metal toxicity, free radical scavenging, storage of metal ions, metabolism of essential metal ions, immune response, genotoxicity and carcinogenicity, chemo-resistance, and radiotherapy resistance, Shariati and Shariati (2011). Considering

the heavy metal detoxification significance of MTs, these proteins can serve as biomarkers of heavy metal pollution of the environment, Valavanidis et al. (2006), Nordberg and Nordberg (2009), Shariati and Shariati (2011).

MT can be used as an indicator in both environmental and biological monitoring reflecting exposure to metals, and as a good biomarker of renal dysfunction. MT in urine can be used as a sensitive biomarker for metal induced nephrotoxicity. MT is an established biomarker in biomontoring of human Cd exposure and may also be useful in the risk assessment of other metal exposures. MT mRNA in lymphocytes in humans has been suggested as an indicator of susceptible groups in relation to metal exposure, Nordberg and Nordberg (2009).

Oxygen and nitrogen free radicals are essential in the physiological control of cell function in biological systems and are continuously produced in living cells. Basic cellular metabolism in aerobic organisms involves the production of oxygen free radicals and nonradical reactive species, referred to as reactive oxygen species (ROS). The potential of oxygen free radicals and other reactive oxygen species, able to damage tissues and cellular components in biological systems, called oxidative stress, have become a topic of significant interest for environmental toxicology studies, Valavanidis et al. (2006).

Both animal and plant cells are capable of generating – via multiple sources – a number of different reactive oxygen species (ROS), including the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$) and the hydroxyl radical ($\cdot OH$), Torres et al. (2008). These species occur transiently and are regular products of oxidative metabolism. Although some ROS may function as important signaling molecules that alter gene expression and modulate the activity of specific defense proteins, but all ROS are harmful to organisms at high concentrations, Torres et al. (2008).

Metal ions possess the ability to produce reactive radicals, resulting in DNA damage, lipid peroxidation and depletion of protein sulphydryls. When ROS levels exceed antioxidant defences, the cells go into oxidative stress which causes membrane lipid peroxidation and changes in the activity of the antioxidant defence enzymes, Vlahogianni et al. (2007). This system includes antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), and nonenzymatic antioxidants, such as glutathione, vitamin E, ascorbate, bcarotene, and urate, whose activities are modified in response to cellular oxidative stress, Valavanidis et al. (2006).

Antioxidant defence enzymes play an important role in cellular antioxidant defence systems and protection from oxidative damage by ROS. Heavy metals are involved in toxic redox mechanisms through the generation of ROS, associated with oxidative damage to important biomolecules and molecular mechanisms of metal toxicity and carcinogenicity. Although there are considerable gaps in the knowledge of cellular damage, response mechanisms, repair processes, and disease etiology in biological systems, but free radical reactions and the production of toxic ROS are known to be responsible for a variety of oxidative damages leading to adverse health effects and diseases, Valavanidis et al. (2006).

Many enzymes need cofactors to work properly, such as $Fe^{2+}$, $Mg^{2+}$, $Cu^{2+}$ $Ca^{2+}$. The substitution of one heavy metal ion by another leads to the inhibition

or loss of enzymatic activity. Antioxidant enzyme activities, oxidative damages (lipid peroxidation – LP) and metal content in the marine species have been studied, Vlahogianni et al. (2007), Duarte et al. (2011), Giarratano et al. (2011), Semedo et al. (2012). Lipid peroxidation (LP) by ROS is considered to be a major mechanism by which heavy metals can cause tissue damage. The negative correlations found for LP with SOD and mainly GST activity highlighted the importance of these enzymes in preventing oxidative damage in the digestive mussel gland. CAT activity was also positively correlated with SOD and GST activities, which emphasizes that the three enzymes respond in a coordinated way to metal induced oxidative stress, Semedo et al. (2012). Correlations between metal accumulation and biomarkers of oxidative stress (LP, CAT and SOD) in marine mollusks were found, Vlahogianni and Valavanidis (2007), Duarte et al. (2011), Giarratano et al. (2011). The study of Vlahogianni et al. (2007) showed that seasonal variations of the activity of antioxidant defence enzymes and LP concentrations in mussels can be used as potential biomarkers of toxicity for long-term monitoring in marine coastal ecosystems.

Heavy metal toxicity is one of the major abiotic stresses also leading to hazardous effects in plants. A common consequence of heavy metal toxicity is the excessive accumulation of reactive oxygen species (ROS) and methylglyoxal (MG). Both can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, DNA damage and/or interact with other vital constituents of plant cells. Higher plants have evolved a sophisticated antioxidant defense system to scavenge ROS and MG, Hossain et al. (2012).

Heavy metal toxicity results in the accumulation of excessive ROS inside the plant cell. For example, Cu can directly generate ROS, whereas Cd is a redox-inactive heavy metal and can only generate ROS indirectly by enzyme inactivation. Potentially a very important mechanism of heavy metal detoxification and tolerance in plants under heavy metal stress is chelation of heavy metals in the cytosol or intracellular fluid by high affinity ligands. Plants make two types of peptide metal binding ligands: phytochelatins (PCs) and metallothioneins (MTs), Hossain et al. (2012).

MTs and PCs have been identified in a wide variety of plant species and in some microorganisms, Hegelund et al. (2012), Hossain et al. (2012). PCs and MTs are different classes of cysteine-rich heavy metal-binding protein molecules. MTs are cysteine-rich polypeptides encoded by a family of genes. In contrast, PCs are a family of enzymatically synthesized cysteine-rich peptides, Hossain et al. (2012). PCs are a family of Cys-rich polypeptides, although the most common PC forms have 2–4 peptides, Hossain et al. (2012).

PCs are structurally related to glutathione (GSH) and numerous physiological, biochemical, and genetic studies have confirmed that GSH is the substrate for PC biosynthesis. GSH is a tripeptide with thiol group (-SH) of cysteine. It can be synthesized in the human body from the amino acids. While all cells in the human body are capable of synthesizing glutathione, liver glutathione synthesis has been shown to be essential. In animal cells GSH is catalyzed by glutathione S-transferase enzymes. It is an antioxidant, preventing damage to important cellular components

caused by reactive oxygen species such as free radicals and peroxides, Pompella et al. (2003). GHS has a vital function in Fe metabolism, Kumar et al. (2011) and it is the major free radical scavenger in the brain, Gawryluk et al. (2011).

Recent plant molecular studies have shown that GSH by itself and its metabolizing enzymes act additively and coordinately for efficient protection against heavy metals damage in plants. PC synthase is primarily regulated by activation of the enzyme in the presence of heavy metals, Hossain et al. (2012). The biosynthesis of PCs is induced by many heavy metals, including Cd, Hg, Ag, Cu, Ni, Au, Pb, As, and Zn; however, Cd is by far the strongest inducer.

Plants are not able to metabolize or eliminate Cd, Hossain et al. (2012). They adopt the strategy of making Cd-GSH and Cd-PCs complexes. Zhang and Ge (2008) found a close relationship between Cd level and GSH content as well as enzyme glutathione $S$-transferase (GST) activity in rice, suggesting that these two parameters of antioxidant defense system may be used as biomarkers of Cd-induced stress in plants. Glutathione peroxidases (GPXs) are key enzymes of the antioxidant network in plants. Cuypers et al. (2002) suggested that peroxidase activity can be used as a potential biomarker for heavy metal toxicity in plants. Similarly, a significant increase in GPX activity was also observed in red onion exposed to a variety of Hg, Pb, Cr, Cu, Zn, or Cd concentrations suggesting that the elevated activity of GPX was a result of heavy metal-induced free radical generation, Fatima and Ahmad (2005).

Research on plant MTs lags behind what is known about the vertebrate forms, Freisinger (2010). The large diversity in the metalbinding regions of plant MTs suggests that they have the ability to bind a greater range of metals than their animal counterparts and, consequently, a greater range of function, Cobbett and Goldsbrough (2002). In plants MTs are extremely diverse, Hossain et al. (2012) and their role in the detoxification process has not been conclusively shown, Freisinger (2010). The high metal ion binding capacity of MTs suggests a role in metal ion storage, metabolism and trafficking of essential $Cu^+$ and $Zn^{2+}$ ions, as well as the detoxification of nonessential metal ions such as $Cd^{2+}$ and $Hg^{2+}$ in living organisms. The precise function of the MT role in living organisms remains elusive, Freisinger (2010).

PCs have been shown to play an important role in the detoxification of certain heavy metals in both plants and animals. PCs play a wider role in heavy metal detoxification in biology than previously expected, but it appears that some organisms probably do not express a PCs synthesis. There is, for example, no evidence for PC synthase in mouse and human genomes. Organisms with an aquatic or soil habitat are more likely to express PCs, Cobbett and Goldsbrough (2002).

## 5.6 Bioindicators of Toxic Metals

Different species are used worldwide in scientific studies evaluating the risk of toxic chemicals, but only a few of this species have been established as standard organisms as bioindicators of worldwide use. The standardized bioindicators of

**Table 5.3** The most analyzed heavy metals in the biota of environmental compartments from reviewed literature

| Environmental compartment | Taxa studied | Heavy metals |
| --- | --- | --- |
| Air | Mosses, lichens, high plants | Pb, Zn, Cd, Cu |
| Water | Bacteria, plankton, macro algae | Pb, Cd, Cu, Zn, Hg, Cr, As |
|  | Mollusks, crustaceans, fish, birds, mammals |  |
| Soil/sediment | Bacteria, protozoa, nematodes, oligochaeta (earthworms), amphibians, birds, mammals | Pb, Cd, Cu, Zn, Hg, Cr, As |

the different trophic levels for the most commonly studied toxic metals in the environment compartments (air, soil and water) is given in Table 5.3. This part will mainly focus on organisms as a standard and some potential bioindicators of toxic metals in air, soil and aquatic ecosystems.

The oldest and widely used species for toxic metal bioindications are fish, bird and mosses species, while species representing soil organisms are currently becoming more important in toxic metals bioindications. Representative species traditionally used for risk assessment of chemicals are various freshwater species representing bacteria, microalgae, invertebrates and fishes. Up until now, among the terrestrial species mainly used in risk assessment of chemicals are the earthworm *Eisenia fetida* and some higher plants. Generally speaking, toxic metals distributions were considered more homogeneously in aquatic systems as compared to terrestrial ones.

### 5.6.1 Bioindicators for Air Metal Pollution

Biological monitors and vegetations are used to measure the levels of atmospheric trace metal concentration as the cheapest and simplest indicators for monitoring the trace metal concentrations in the air, Kord et al. (2010). Numerous different bio-indicators are used in monitoring air pollution: bacteria, fungi, mosses, lichens, grasses, agricultural crops, ornamental plants, vascular plants, and woody plants, Joshi (2008), Baslar et al. (2009), Kord et al. (2010), Suchara et al. (2011), Filipovic-Trajkovic et al. (2012). Samples were collected from industry, roadside, suburban and rural areas.

Which plant species will be used as bioindicators also depends on how widely they are distributed throughout the region. The most often used parts of higher plants are leaves/needles of: spruce, birch, pine, oak, olive, and poplar; also a bark of oak, pine, ash tree, Serbula et al. (2012). The usage of air pollution biondicators usually covers metal toxicity in traffics, smelters, mining industries, industrial pollution, coal-burning power plants and agriculture, Conti and Cecchetti (2001), Wolterbeek (2002), Harmens et al. (2008), Blagnytė and Paliulis (2010), Suchara et al. (2011), Stankovic et al. (2011c), Paoli et al. (2012).

Lichens and mosses may be considered as the most commonly applied organisms as bioindicators. Typical examples of a biological indicator of air pollution are lichens and mosses, Conti and Cecchetti (2001), Wolterbeek (2002), Blagnytė and Paliulis (2010). Today, lichens and mosses are mostly used as lower plants for atmospheric trace elements bioindicators due to their capacity to accumulate and store heavy metals and other toxins and, because they do not have roots, they are thus able to provide a direct indication of the air pollution, Suchara et al. (2011).

The idea of using mosses to measure atmospheric heavy metal deposition was developed already in the late 1960s, well established in the Scandinavian countries in the early 1980s, Sabovljevic et al. (2009) and in Europe a moss monitoring programme has been repeated every 5 years since then, Suchara et al. (2011). Naturally growing mosses have been widely used as effective bioindicators of heavy-metal air pollution, Harmens et al. (2008). Mosses properties as good bioindicator are: large surface to weight ratio, their slow growth rate and a habit of growing in groups, minimal morphological changes during the mosses lifetime, ease sampling, an ability to survive in highly polluted environment and the possibility to determine concentrations in the annual growth segments, Dragovič and Mihailovič (2009). Moss appears to be an excellent deposition bioindicator for monitoring elements such as Pb, Cd, Cu, V, and partially Zn. Secondary processes seriously affect monitoring of Ni, Cr, Mn, Fe, such as rain washing down of entrapped particles from mosses, which could result in loses reaching up to 20 %.

A high proportion of the pollutant load accumulates in mosses through wet deposition. The amount, duration and intensity of precipitation affect accumulation and leaching by mosses, depending on the meteorological conditions and seasonal growth. The metal uptake by mosses decrease in the order: Cu > Pb > Ni > Co > Cd > Zn, Mn. The most commonly used lichen species for toxic metals biomonitoring were *Parmelia sulcata*, *Hypnum cupressiforme*, *Hylocomium splendens*, and *Pleurozium schreberi*, Blagnytė and Paliulis (2010).

Many European countries have used mosses in national and multinational surveys of atmospheric-metal deposition. According to Harmens et al. (2008) metals deposition trends in some European countries during 1990–2005/6 measured on the basis of mosses were next: the lowest toxic metals concentrations in air were generally found in (north) Scandinavia, the Baltic States and northern parts of the United Kingdom in 2005/6. The concentration in European mosses since 1990 has declined the most for As, Cd, Fe, Pb and V (45–72 %) followed by Cu, Ni and Zn (20–30 %) with no significant reduction being observed for Cr (2 %) and Hg (12 % since 1995).

Moss (*Pleurozium schreberi*), grass (*Avenella flexuosa*), and 1- and 2-year old spruce (*Picea abies*) needles were collected over the territory of the Czech Republic. The samples were analysed for 36 elements: Ag, Al, As, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, S, Sb, Se, Sn, Sr, Th, Tl, U, V, Y, and Zn, Suchara et al. (2011). Most elements showed the highest concentrations in moss, considerably lower concentrations in grass, even lower concentrations in the 1-year old spruce needles and again somewhat higher concentrations in the 2-year old spruce needles: As, Bi, Ce, Cr, Fe, La, Nd, Pb, Pr,

Sb, Se, Sn, Th, U, V, and Y. Mo is the element that was especially enriched in grass. Grass appeared to be a good model crop and allowed estimation of the level of Hg contamination in leafy vegetables.

Different moss types are currently widely used as bioindicators since they obtain most of their nutrients directly from the air and by dry deposition. The procedures for the moss as a bioindicator, the methods of collection, processing and analyses of moss samples, are outlined in the international moss monitoring manual, Markert et al. (2011). But it is important to note that a unique species that can be a suitable bioindicator for biomonitoring of toxic metal pollution all over the world has not been found yet. For this reason, different species of mosses are useful as bioindicators in different parts of the world, Blagnytė and Paliulis (2010).

Moss still appears to be one of the best air pollution bioindicator, but on the basis of literature search of Aboal et al. (2010) it could be concluded that mosses are possible to be bioindicators for certain metals such as Pb or Cd, probably because these elements are of almost exclusively atmospheric origin. According to Suchara et al. (2011) mosses can be identified as the most effective collectors of fine atmospheric dust than of wet depositions and can be used as bioindicator of atmospheric pollution in megacities, because this plant is able to absorb water and nutrients directly from the air, Markert et al. (2011).

Lichens have been widely used as trace elements air biomonitors as they are widespread and capable of absorbing elements directly from the atmosphere and accumulating them in their tissues. Many countries have performed biomonitoring research on metal levels in industrial areas and in clean areas based on lichens as air toxic metal bioindicators. But, lichens of similar composition are not easy to find, because of the differences caused by the tree on which lichens are growing. As different lichen species in the same location contain varying amounts of metal, it is obvious that the amount of metal contained by lichen is species-dependent.

Lichens are symbiotic organisms of fungus and algae, and have been widely used in biomonitoring of air pollution by trace elements. It is well known that the chemical composition of lichens reflects the availability of trace elements in the air environment, Backor and Loppi (2009) and can be used to monitor air quality changes in urban areas over intervals of a few years, Paoli et al. (2012).

Paoli et al. (2012) used the lichen *Flavoparmelia caperata* as indicators of pollution around a landfill in central Italy along 14 years of waste management. Lichens revealed an increased deposition for some elements (Cd, Cr, Fe, and Ni) and a decrease of the lichen diversity was subjected to high airborne dust concentrations, Paoli et al. (2012).

Numbers of phylloplane fungi and bacteria were used as a bioindicator for toxic metals air pollution, Joshi (2008). Analysis of phylloplane microorganisms, Pb, Zn, Cu, and Cd from plant leaves indicated that the bacterial and fungal population was higher at the unpolluted site. Numbers of phylloplane fungi and bacteria were significantly reduced in the toxic metals polluted sites. Bacterial population showed a significant negative correlation with Pb, Zn, Cu, and Cd. Microfungal species composition and their diversity in the phylloplane were directly correlated to the roadside toxic metals pollution, as Joshi (2008) suggested for bacteria.

In the last decades of the twentieth century a rapid increase in bioindication studies for pollutant loads in higher plants can be observed. Both the broadleaves and coniferous trees are used in toxic metals air pollution studies. Air pollutants can be taken up via stomata, cuticle or indirectly by uptake via roots after deposition of the air pollutants to the soil. The surface wax layer of leaves and needles works as an accumulator of particle bound air pollutants, Weiss et al. (2003). This observation clearly demonstrates the effective accumulation of particle bound elements on plant surfaces and supports the principal suitability of higher plants to monitor the corresponding atmospheric load.

Particularly for the following elements a significant contribution of the plant cuticle layer to the overall needle or leaf concentration was shown: Al, As, Br, Co, Cr, Cs, Cu, Fe, Hg, La, Mo, Na, Ni, Pb, Sc, Sb, Sn, and Th, Weiss et al. (2003). On the contrary, cuticular deposits usually do not contribute significantly to the overall needle or leaf concentration of Mg, K, Ca, Ba, Cd, Mn, Tl, and Zn, Weiss et al. (2003). These general statements, however, may be not valid for all plants.

Other plant materials are occasionally used as bioindicators of air toxic metal deposition includes pine and spruce bark, and grass. Tree bark is appropriate in indicating longer term of air pollution. In literature, there are many studies focusing on using barks of different tree species as bioindicator. Results of such studies identified that the barks of the pine trees are good adsorbents of airborne pollutants, including anthropogenic heavy metals. Among the studied barks of pine tree are Turkish red pine (*Pinus brutia Ten*.), Italian stone pine (*Pinus pinea L.*), Austrian pine (*Pinus nigra Arnold*.) Masson pine (*Pinus massoniana Lamb.*) and Scots pine (*Pinus sylvestris L.*), Baslar et al. (2009). Coniferous trees indicate pollution over a longer time period, and since bark is exposed to air pollutants directly from the atmosphere, tree bark is appropriate in indicating longer term air pollution.

Depend upon the type of the tree, the deployment of heavy metal content and ways of accumulation show a great variety: in some trees heavy metals are filtered out by the leaves from the air, while in others they are taken up by their crown or by their roots. According to Baslar et al. (2009) Mn or Zn are mainly taken up by the root system and are then transported from there to the crowns, since they are highly mobile elements. Therefore, it is better to investigate its deposition in the crowns.

Different plant organs (leaves, flowers, stems or roots) from naturally occurring wild plants and trees, and cultivated plants (vegetables and fruits) were evaluated as possible bioindicators of heavy-metal pollution too, Filipovic-Trajkovic et al. (2012). Filipovic-Trajkovic et al. (2012) were found the highest amounts of heavy metals in the leaves, especially Pb, and then in the fruits and vegetables. They concluded that fruits and vegetables were metal avoiders. Naveed et al. (2010) used leaves of roadside plants (*Dalbergia sissoo* Roxb., *Prosopis juliflora* L. and *Eucalyptus* spp.) as bioindicator of traffic related to Pb pollution. They conclude that all the three selected plants proved to be good indicators of Pb air pollution and, due to their diverse distribution in different parts of the world, their leaves can be used as bioindicators of Pb pollution.

General problems of air bioindicators that measure toxic metals concentrations do not only depend on anthropogenic elements inputs via the atmosphere, but also

depend on different uptake mechanisms and element preference/rejection by certain plant species, Suchara et al. (2011). Lower plants, especially mosses and lichens, are probably the organisms most frequently used for monitoring metal pollutions in urban environments. They have an important advantage because they do not need soil substrate, but there are limitations in using them as in situ bioindicators, because of difficulties to find a single species spanning a large area, Dmuchowski and Bytnerowicz (2009), Backor and Loppi (2009). Tree bark and higher plants leaves started to be used to detect the deposition, accumulation and distribution of air metal pollution in large-scale of air pollution, Baslar et al. (2009), Kord et al. (2010), Stankovic et al. (2011c), Serbula et al. (2012).

## 5.6.2   Bioindicators for Aquatic Metal Pollution

In this part, principles of algal bio-indication and bio-monitoring in the environment are outlined for streams and rivers, lakes and reservoirs, as well as for marine ecosystems. Aspects of sediment testing are included. Since environmental contamination and pollution has severely expanded in the recent years, using bioindicators became increasingly important.

Monitoring of trace toxic metals in the aquatic environment using biological indicators has been well established since chemical analysis of the environment matrix such as water and sediment is the most direct approach to reveal the heavy metal pollution status in the environment, while it cannot afford the powerful evidence on the integrated influence and possible toxicity of such pollution on the water organisms. Speciation of metals, their solubility and complexation, are important factors that influence the toxicity of metals in the aquatic environment. The amount of dissolved metal strongly depends on water pH. The interaction of metals can alter their toxic effects on aquatic organisms both positively and negatively, Sevcikova et al. (2011).

Aquatic heavy metal pollution usually represents high levels of Hg, Cr, Pb, Cd, Cu, Zn, Ni etc. and introduced into environmental water system may pose high toxicities on the aquatic organisms, Zhou et al. (2008). The most important application of biomonitoring is for the evaluation of metal pollution in aquatic ecosystem including harbors and continental waters. Many pollutants are associated with sediments in aquatic systems. Hazard assessment to establish sediment quality criteria require rapid, inexpensive examination too. Direct bioassay with algae has proven to be very sensitive indicators of contaminant stress.

Since the 1950s, numerous studies have demonstrated the value of benthic foraminifera in detecting ecosystem contamination. They are among the most common marine plankton species. Foraminifera have typically been included in the group *Protozoa* as unicellular protists with animal-like behavior and movement. Living benthic foraminiferal taxa were studied in surface sediment samples collected from the Italian sea, marsh and likes sediments over the last three decades, Frontalini and Coccioni (2011) and foraminifera have been proven to be successful

candidates as part of an integrated monitoring program of pollution. The statistical analysis reveals a strong relationship between trace elements, in particular Hg, Mn, Ni, Pb, and Zn, and the occurrence of abnormalities in foraminiferal taxa, Frontalini and Coccioni (2011).

The value of algae as biomonitors for fresh waters has already been recognised in the mid nineteenth century. Species which might be used as indicators are *Aulacoseira granulata, Actinocyclus normanii, Stephanodiscus neoastrea, Cyclotella meneghiniana* among many others, the usefulness of river plankton for bioindication the toxicity of chemicals, mixtures of chemicals or polluted waters is measured as their inhibitory effect on the photosynthesis of natural algal assemblages. Several algal species accumulate considerable amounts of metals and can thus be used as monitors for elements such as Cd, Cu, or Pb. Most metals are slightly to highly toxic to algae, As, Cu, Hg, and Zn having the greatest effect. Impacts on algae in natural waters are highly variable. Cyanobacteria and green algae, both species concentrate various metals proportionally to ambient concentrations, Ratte et al. (2003), Azizi et al. (2012).

The most effective way of studying algal populations with respect to lake water quality is to analyse the microfossils present in surface sediments from lakes with known water chemistry, since, lake water quality changes in the last 5–10 years and several toxic elements can be monitored using sedimentary algal remains, Ratte et al. (2003).

Most what has been used for freshwater indicators, equally applies to bioindicators in the marine environment, such as bacteria and phytoplankton, but macroalgae in coastal marine waters are far more important as bioindicators than macrophytic algae in fresh-waters. Macroalgae have several intrinsic advantages as organisms for monitoring environmental impacts. Because of their sessile nature they can be easily collected in abundance and used to characterize locations over time. Accumulation of heavy metals from the surrounding water makes them ideal as bioindicators.

Red algae (*Glaciralia* sp.), often dominating macroalgae of sea communities are frequently employed for Cd, Cu, and Zn coastal monitoring, Jakimska et al. (2011). Members of the Fucales (*Fucus, Ascophyllum*) and Laminariales (*Laminaria, Macrocystis*) received most attention. Pollution assessment studies have also used red algae (*Rodophyceae*). Among the green macroalgae, the genera *Ulva, Enteromorpha,* and *Posidonia,* have attracted considerable attention as toxic metal bioindicators, Richir et al. (2010), Akcali and Kucuksezgin (2011), Wolff et al. (2012), Rybak et al. (2012), Luy et al. (2012).

Specific programs for monitoring toxic metals in aquatic systems were undertaken as early as in 1960s using various animals like invertebrate, mollusks, and vertebrate, like mammals and birds. Various aquatic organisms occur in rivers, lakes, seas and marines, and they are potentially useful as metal pollution bioindicators of sediments and waters, Zhou et al. (2008), Markovic et al. (2012), Joksimovic and Stankovic (2012), including fish, Yilmaz (2009), shellfish, oyster, mussels, Özden et al. (2010), Tsangaris et al. (2010), Valavanidis and Vlachogianni (2007),

Jovic et al. (2011), Jakimska et al. (2011), Stankovic et al. (2011a), Hauser-Davis et al. (2012), clams, Singh et al. (2012), aquatic plankton, Das et al. (2009) and macroalgae, Joksimovic et al. (2011b), Rybak et al. (2012).

## Microorganisms as Bioindicators of Water Quality

Plankton (phytoplankton, zooplankton and bacteriaplankton) are any organisms that live in the water column, on the surface and on the bottom. Phytoplankton live near the water surface with sufficient light to support photosynthesis. Bacterioplankton play an important role in the remineralising organic material down the water column. This scheme divides the plankton community into broad producer, consumer and recycler groups. They provide a crucial source of food to many large aquatic organisms, such as fish and marine mammals.

Many bacteria live in surface water, ground water, and other natural environmental water, which offers the possibility for water quality assessment especially for hygiene using bacteria test, Zhou et al. (2008). Trace elements in aquatic ecosystem accumulate on organic, inorganic sediment particles and their associated microorganism community. Fungi, bacteria, protists, and exudates of microorganisms form the biofilm on sediments. Microorganisms of the biofilm like bacteria and fungi are known to accumulate high amounts of metals and metalloids. Many species associate with metals and metalloids polluted sediments in aquatic systems, Schaller et al. (2011).

Various zooplankton species show their special advantages in the actual biomonitoring researches. Zooplankton containing protozoa are the important component of aquatic ecosystem. Many zooplankton species can accumulate and metabolize heavy metals which offer the possibility for its use in biomonitoring of water quality, Zhou et al. (2008). For example protozoa communities can be used for the monitoring of Hg and Cu levels in the water, Zhou et al. (2008). Most of protozoa species are worldwide distributed and not restricted by seasonal variations and regional discrepancies making them good bioindicators.

Zooplankton species *Daphniidae* as the representative of crustacean in biomonitoring of aquatic metal can sensitive response to heavy metals and pesticides. Thus, *Daphnia magna* is the most commonly tested freshwater species in acute, as well as in chronic tests, Zhou et al. (2008). Daphniidae toxicity test is the essential assay for worldwide water quality assessment.

Phytoplankton as the important elementary producers in marine and inland water plays key role to the whole ecosystem. Some phytoplanktons are bacteria, some are protists, and most are single-celled plants. Among the common kinds are cyanobacteria, green algae and fungi, Das et al. (2009). The aquatic algae species and amounts can directly reflect the water quality, Zhou et al. (2008). As an example, the green algae *Chlorella ellipsoidea* was reported to exhibit growth inhibition due to Cu, Zn, Ni, and Cd exposure. *C. ellipsoidea* also showed different toxicity

response to different metal speciation exposure. The accumulation rate of some metals like Zn, Cu, and Ni in different plant organs shows significant accumulation difference, like roots than others, Zhou et al. (2008).

Microorganisms, such as protozoa, green algae or bacteria, reflect the water quality of only 1 or 2 weeks prior to their sampling and analysis, whereas insect larvae, worms, snails, and other macroinvertebrate organisms reflect more than a month, and possibly several years. Microorganisms have proven capability to take up heavy metals from aqueous solutions especially, when the metal concentrations range from less than 1 to about 20 mg/L, Ahmad (2006). Microbes such as bacteria also exist at the lowest trophic level, so bacteria have the ability to detect toxic compound before others do. Thus, bioindicators using bacteria have been commercialized such as the *Lux-Fluoro*, the Polytox™, the Deltatox™ and the Microtox™, Ahmad (2006).

The environmental monitoring of toxic metals by bioindicators like bioluminescent bacteria (BLB) in marine environment is reported by Ahmad (2006). The test method is design as a rapid evaluation of the toxicity of wastewaters or aqueous extracts from contaminated soils and sediments using luminescent marine bacteria. The *Photobacteria phosoreum* was used to the test wastewater or aqueous soil/sediment extract and the inhibition of light output measured over a specified time, Ahmad (2006).

Microorganisms also have evolved various measures to respond to heavy metal stress in aquatic systems via processes such as transport across the cell membrane, biosorption to cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation reduction reactions, Ahmad (2006), Burnat et al. (2010), Schaller et al. (2011), Jackson et al. (2012), and Murthy et al. (2012).

## Animals as Bioindicators of Water Quality

Since the mid-1970s, scientists of several countries have used bivalve-filter feeding mollusks to monitor selected contaminants in coastal marine waters. This then led to the establishment of similar local or regional 'Mussel Watch' programs in many countries of the world, OSPAR (1992). The mussel watch program was initially used for analyzing trace metals in marine coastal waters. Mollusks (mainly mussels, *Mytilus* sp.) and fish (*Mullus* sp., *Platichthys flesus* L., *Zoarces viviparus*, *Perca* sp.) have both been employed as sentinel organisms in routine biomonitoring programs, at a national and an international level (Med Pol, UNEP Mediterranean Biomonitoring Program and OSPAR Convention).

Mussels are recognized as pollution bioindicator organisms because they accumulate pollutants in their tissues at elevated levels in relation to pollutant availability in the marine environment. Moreover, this ability has led to the adoption of the international "Mussel Watch Project" and several national programs on Mussel Watch in the marine environment have been carried out. As a result, much work on invertebrates has focussed in the aquatic environment, where mollusks have been

used as effective bioindicators of toxic Hg, As, Cd, Pb, and trace elements: Fe, Mn, Ni, Co, Zn, Cu, Özden et al. (2010), Tsangaris et al. (2010), Jovic et al. (2011), Stankovic et al. (2011a), Joksimovic et al. (2011a), Jakimska et al. (2011).

Freshwater mussels are used as bioindicator of Pb, Cd, Hg, and Zn too, Mosher (2008), Faria et al. (2010), Rahnama et al. (2011), Minguez et al. (2012). It appears that freshwater gastropods and freshwater species in general, may be less sensitive than their marine relatives, although there are insufficient freshwater data to be sure if there is a real difference in sensitivity. Freshwater invertebrate toxicity tests are in current use and after many years of experimenting have reached a satisfactory level of precision. Most of these tests are described in the book Standard Method for the Examination of Water and Wastewater, APHA (1989).

Reptiles, particularly water snakes (*Nerodia* spp.), could serve as bioindicators of toxic metals contamination too, Burger et al. (2005), because some are comparatively long-lived, exhibit different trophic levels, and are at the top of their food chains. Water snakes can be useful bioindicators of As, Se, Cd, Cr, Pb, and Hg accumulation for multiple spatial and temporal scales, Burger et al. (2005).

Studies with fish species were introduced in the 1930s for the toxic effects determination in the field of toxicology investigation, especially for the screening of toxic chemicals, agricultural and industrial effluents and for pollution studies in rivers, lakes and the marine environment, Valavanidis and Vlachogianni (2010). Fish are used as test organisms in aquatic toxicology because of their top-position in the trophic chain and their role as food for humans. During their life-cycle, fish feed on algae, rotifers, microcrustaceans, macroinvertebrates, higher plants and other small fish. The use of fish in ecotoxicological studies has been advanced by various international organizations, Valavanidis and Vlachogianni (2010).

Fish are one of the most frequently used groups of bioindicators in ecotoxicological field studies. Various metal ions are involved in an oxidative stress in fishes. In the review Sevcikova et al. (2011) concentrated on the most important and most studied metals (Fe, Cu, Cr, Hg, and Pb) and metalloids (As, Se) in a fish. Apart from the mentioned ones, other metals are also connected with oxidative stress in fish (Ni, V, and Co), and can be detected in aquatic environments, Kandemir et al. (2010).

Due to quality assurance issues and to achieve adequate geographical and temporal representation, mussels (blue mussel – *Mytilus edulis*, Mediterranean mussel – *Mytilus galloprovincialis*) and fish (Atlantic cod – *Gadus morhua*, herring – *Clupea harengus* and flounder – *Platichthys flesus*) were selected as state indicators, EEA (2003). They are widely monitored in a comparable way. Mussels are attached to shallow-water surfaces, thus reflecting exposure at a fixed point, i.e., local pollution. Mussels are, however, restricted to the coastal zone. Atlantic cod is a widely distributed and commercially important fish species in the North Atlantic. It is a predator and as such will also to some extent reflect contamination levels in its prey. Herring is a commercially and ecologically important species both in the Baltic and the North-East Atlantic. Flounder is found in some of the most highly contaminated estuaries and is a common species along most European coasts, EEA (2003).

Other marine species, like marine mammals (seal, sea lion) and seabirds can also be used for the marine pollution biomonitoring due to their strong toxic metals

bioaccumulation ability, Jakimska et al. (2011). Levels of accumulated toxic metals like Hg and Cd in marine mammals can be high, but without showing any signs of having been poisoned, Jakimska et al. (2011). Many studies have reported that Hg, Cd, and Se are accumulated in high levels in several seabirds, Horai et al. (2007), Hargreaves et al. (2011), and seabirds also have higher Hg levels than land birds, Jakimska et al. (2011). The high migration, however, limits their wide use in pollution biomonitoring in the investigated areas.

**Plants as Bioindicators of Water Quality**

Aquatic macrophytes have been used for monitoring the contamination level by various pollutants in aquatic environments, including heavy metals, such as Zn, Pb, Cd, and Hg and more others, as these plants have the ability to accumulate metallic ions, Vardanyan et al. (2008), Joksimovic et al. (2011b), Richir et al. (2010), Akcali and Kucuksezgin (2011), Rybak et al. (2011), Copat et al. (2012), Luy et al. (2012).

The results suggest that freshwater macroalgae *S. auriculata* and *Ulva* show good potential for use as a bioindicator and it can be used in the biomonitoring of aquatic ecosystems contaminated by Cd, Ni, and Pb, Rybak et al. (2011), Wolff et al. (2012). The studies of Richir et al. (2010), Joksimovic et al. (2011b), Copat et al. (2012), and Luy et al. (2012) confirm the relevance of the use of sea macrophyte *P. oceanica* as a biological indicator of metal contamination in coastal ecosystems and as a good tool for seawater quality evaluation. By biomonitoring study of heavy metals in macroalgae (the brown algae *Cystoseira* sp., the green algae *Ulva* sp. and *Enteromorpha* sp.) from eastern Mediterranean coastal areas, Akcali and Kucuksezgin (2011) concluded that *Cystoseira* and *Entromorpha* were the strongest accumulators of Cd, Cr, Fe, and Hg, Pb, Zn, respectively. *Ulva* turned out to be the highest Cu accumulator whereas this species was seen to accumulate Pb, Zn, and Fe with the minimum aptitude, Akcali and Kucuksezgin (2011). Freshwater populations of *Ulva* exhibited a greater efficiency to bioaccumulate Ni as compared to species derived from marine ecosystems, Rybak et al. (2012).

The use of these plants as bioindicators of contamination by heavy metals has advantages: (a) high tolerance to the pollutant, (b) easy sampling, (c) selective absorption ability, and (d) individuals are relatively big and easy to handle in laboratories. Data for better quantification of contaminants fluxes and inputs into water bodies and their water/air and water/sediments interfaces exchanges is lacking. These data are also essential for predictive and mass balance modelling of contaminants fates in the aquatic systems, Law et al. (2010).

## 5.6.3 Bioindicators for Soil Metal Pollution

Population growth combined with the increasing industrialization is responsible for generating tonnes of waste per day, which, many times, are accumulated in the

environment without any previous treatment. Soil becomes a cheaper and practice alternative for the final disposal of these residues, but not without consequences: contaminated soil is a broad problem, since the contaminants can be leached into groundwater, rivers, lakes, and seas and adsorbed into plants and animals, and finally, into a soil food web.

Various abiotic and biotic soil characteristics can be used as indicators for evaluating soil health. With increasing heavy metal concentrations, the activities of microbes, soil enzymes, and nitrogen fixation are inhibited, and growth of microfloral communities such as fungi, algae, and photosynthetic bacteria is reduced. In this context, some taxonomic groups of soil invertebrates and higher plants have been proposed as bioindicator organisms. Plants and soil-inhabiting organisms such as soil microflora, and microfauna (protozoa), fungi, nematodes, earthworms, mites, and insects have been used as biotic indicators of soil toxicity, Park et al. (2011).

The microbial community concept is based on the interactions among in all of the various species of bacteria, fungi, protozoa, and microfauna that carry out the various broad level functions of the soil, ranging from nutrient cycling to organic matter formation, and plant disease protection, Crowley (2008). Acute responses of microbial populations to metal toxicities include reduction in species diversity and in biomass of microorganisms, and can result in long term, in reduction of soil quality and primary productivity. Some microbial communities can adapt to metal pollution, but loss of species diversity results in potential loss of biological functions and reduced soil resilience. The extent to which this occurs depends on soil physicochemical properties that affect the bioavailability of metals, Crowley (2008).

There are many ways in which bacteria and microfungi and algae can take up toxic metal ions from soil, Ahmad (2006). Heavy metal ions can be entrapped in the cellular structure and subsequently biosorbed onto the binding sites present in the cellular structure. There is also reported that a cell may develop metal resistance systems in an attempt to protect sensitive cellular components, Fontanetti et al. (2011).

The environmental monitoring of toxic pollutants by bioluminescent bacteria (BLB) in terrestrial environment is reported, Ahmad (2006). The presence of toxicants in the sample reduced light emission of the bioluminescent microorganisms. General reductions in microfungal number have often been noted in soils polluted with Cu, Cd, Pb, As, and Zn, Gadd (1993). The soil protozoa *Tetrahymena pyriformanis* is used for Cu and Zn determination in soil, Ahmad (2006). Other soil protozoa that were used as bioindicators for toxic metals were naked amoebae, testate amoebae, flagellates and sporozoans.

Mosses and also higher fungi have developed accumulation mechanisms with regard to heavy metals from the soil. Their heavy metal uptake is distinctly higher than from atmospheric deposition. Some higher fungi are known to have the ability to accumulate toxic elements such As, Cd or Pb from the environment. According to Kalac (2010), content of many trace elements, especially of Cd and Hg, increases in wild growing edible fungi from polluted areas as compared with those from unpolluted rural sites, but the relationship between substrate contamination with a trace element and its content in wild edible fungi is not tight enough to enable usage of a mushroom species as a reliable bioindicator, Kalac (2010).

## Fauna Soil Bioindicators

Much work on invertebrates has focussed on the aquatic environment, where mollusks have been used as effective bioindicators of toxic metals and sea water quality, Markovic et al. (2012), Jovic et al. (2011), Joksimovic and Stankovic (2012). The types of invertebrate soil fauna used in monitoring pollutant effects include nematodes, oligochaetes (ertworms), gastropods, springtails, isopods, arachnids, Park et al. (2011), Sanchez-Hernandez (2006), Nahmani et al. (2006), Hirano and Tamae (2010), Olayinka et al. (2011).

Isopods are more abundant in grasslands and woodlands and accumulate tolerate high levels of Cu, Zn, Pb, and Cd, Nahmani et al. (2006). They can also potentially be used as bioindicators in assessing levels of heavy metals in soil. The soil nematode community has been suggested to be a useful indicator of the status of soils pollutants and ecological disturbance, because of their influence in soil foodwebs and plant–soil interactions, Sochova et al. (2006).

The concentrations of heavy metals such as Cr, Cd, Pb, Zn, and Ni influence on the soil nematode community structure, Park et al. (2011). Single nematode candidates have been investigated far more widely, Boyle and Kakouli-Duarte (2008). The nematode *Caenorhabditis elegans* is a widely investigated in many studies demonstrating its usefulness as a test species for toxicity testing. In the United States a guide was accepted describing the use of *C. elegans* in soil toxicity tests (American Society for Testing and Materials 2002) and updated and also published in 2008, Boyle and Kakouli-Duarte (2008).

By far, the most common invertebrate soil bioindicator used to assess soil and contaminant toxicity are members of the Family *Lubricidae* and *Eisenia* spp. earthworms (Anellida, Oligochaeta), Sanchez-Hernandez (2006), Hirano and Tamae (2010). They are important members of the soil fauna. In soils, earthworms play a critical ecological role. For example, it is estimated that under favourable conditions, earthworms can move up to 18 t of soil per acre per year, Olayinka et al. (2011). Earthworms (*E. foetida*) are capable of accumulating Hg, Cd, Cu, Pb, and Zn and a positive correlation exists between Hg levels in worm tissues, the substrate they consume and the length of exposure. Earthworms have been mainly studied in response to Cd, Cu, Pb, and Zn exposure because significant positive correlations have been found between Cd, Cu, Pb, and Zn concentrations in the earthworm and the metals concentrations in soil, Hirano and Tamae (2010), Olayinka et al. (2011).

Earthworms may be available alternative to traditionally applied organisms in aquatic ecosystems (e.g. fish), as they are simple and they can provide indications of bioavailability in a short time at relatively low costs. Earthworms accumulate heavy metals from polluted soil; they ingest large quantities of soil and are in full contact with the substrate they consume. As well, they constitute up to 92 % of the invertebrate biomass of soils and participate in many food chains, acting as a food source for a wide variety of organisms including birds, fish, insects, amphibians, various mammals, and reptiles, and they can be used to assess bioavailability and to estimate food web transfer and impacts. In addition, they are easily bred, have

been extensively studied, and are approved for use in toxicity testing by the US EPA and the European Economic Community and the Organization for Economic Cooperation and Development.

The effectiveness of invertebrates as bioindicators has been demonstrated using a range of soil invertebrates, Nahmani et al. (2006). These include families and ecological groups of soil macrofauna chilopoda, diplopoda, dermaptera, isopods, earthworms, gastropoda, but most work has focussed on earthworms or insects, Hall et al. (2008). In the terrestrial trophic systems, invertebrates are of great ecological importance in terms of pollution transfer. Beside aquatic and marine gastropods, terrestrial gastropods (snails) are recognized as adequate bioindicators, because of their ability to accumulate Pb, Zn, Cu, and Cd, Madoz-Escande and Simon (2006). They also exhibit a very wide distribution of a limited number of species, which would allow the accurate comparison of data from a variety of habitats. For example *Deroceras reticulatum* species is known to be found across much of North America, Europe, North Africa and Atlantic islands, Hall et al. (2008). Snails are known to play an important role in the diet of many species, including snakes, toads, beetles and birds.

In the case of vertebrates, in general only a few vertebrate species spend the majority of their time throughout the year in close contact with the soil ecosystem. Examples would include some small mammals, Hubbart (2012), such as ground squirrels, and some larger mammals, such as fox, and several species of snakes and amphibians, Alleva et al. (2006), Kalisinska et al. (2012). California ground squirrels (*Spermophilus beecheyi*) are ideal mammals for such studies, Hubbart (2012). Red fox is a species with a wide geographical range, occurring for the entire year in a small home range. It has a high position in the trophic pyramid and accumulates various toxic metals, including Hg. Taking into account Kalisinska et al. (2012) results and findings of other authors, Alleva et al. (2006), it may be argued that the fox exhibits a measurable response to environmental Hg pollution and meets the requirements for bioindicators not only between European countries but entire continents, e.g. Europe and North America.

Exposure among terrestrial vertebrates occurs through ingestion of contaminated biotic or abiotic matter, contaminant absorption through skin, or via inhalation of volatile, aerosolized, or particle bound contaminants. Terrestrial organisms consume a wide array of vegetative and animal matter, and in many cases free-standing water. In most cases, exposure of wild mammals to contaminants is likely through oral consumption, either by ingesting contaminants incorporated into dietary food and water. Dietary exposure in mammals is a function of age, sex, and season, with mammalian dietary range from pure herbivory to an exclusive carnivorous diet, with virtually all gradients in between, also influence contaminant exposure, Smith et al. (2007).

Nahmani et al. (2006) were investigated grasslands, forests, and cultivated lands using soil macrofauna at different taxonomic levels: species, families and ecological groups. They come to conclusion that the species level is the most accurate taxonomic level in bioindication studies. But it is difficult to use animals in environmental studies, because of their mobile lifestyle, especially when analyzing the effects of chronic exposure.

**Flora Soil Bioindicators**

As a result of their sedentary nature, plants have evolved diverse abilities for dealing with toxic compounds in their environment. Plants act as solar-driven pumping and filtering systems as they take up contaminants mainly water soluble, through their roots and transport/translocate them through various plant tissues where they can be metabolized, sequestered, or volatilized, Abhilash et al. (2009). Plants utilize different types of mechanisms for dealing with environmental pollutants in soils. Variety of contaminant-degrading enzymes can be found in plants, Singer et al. (2004). Long-term exposure to soil contaminants has a strong impact on the physiological composition of plant populations with many evidences of toxic metals pollutions.

Investigations of the content of heavy metals in soil are mainly focused on heavily urbanized areas such as industrial regions and city agglomerations, as well as on the areas of constant and linear emitters, which include industrial plants, waste landfills and roads. The investigations also concern the soils used for agricultural purposes, Szyczewski et al. (2009) and the results confirm degradation of urban and industrial areas where the concentrations of the studied elements are usually high, Szyczewski et al. (2009).

Most heavy metals accumulate in the top soil and in the long term their contaminations increase in the soil as a result of an increased absorption and accumulation in plants. There is a potential of using plants in the monitoring of heavy metals in the environment on the basis of a significant correlation between heavy metal content of foliage and soil, Wisłocka et al. (2006). Chemical composition of foliage varies with season and rainfall.

Plants require for their development not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients such as Fe, Zn, Mn, Ni, Cu, and Mo. Most of these micronutrients accumulate in the plant tissues for their metabolic needs, but they never exceed $10 \text{ mg kg}^{-1}$. Yildiz et al. (2010) have reported the normal natural concentration intervals for toxic metals in terrestrial plants as Cd: 0.2–2.4 µg/g, Ni: 1–5 µg/g, Zn: 20–400 µg/g, Fe: 70–700 µg/g, Pb: 1–13 µg/g, Mn: 20–700 µg/g. They found that the level of accumulation in the high plant sample was soil-oriented. Higher levels of these metals and other heavy metals in plant tissue might have phytotoxic effects, sometimes resulting in death, Winkelmann (2005).

Grass has been used as a bioindicator of both: atmospheric metal deposition and metal levels in soils, Baslar et al. (2009), Suchara et al. (2011). They assume that grass will for Pb predominantly reflect atmospheric Pb input because the uptake of other toxic metals is strongly regulate via the root system. According to Brun et al. (2010) the relative importance of atmospheric input versus root uptake differs widely.

Depend upon the type of the tree, the deployment of heavy metal content and ways of accumulation show a great variety. For example, in some trees heavy metals are filtered out by the leaves from the air, while in others they are taken up by their crown or by their roots. According to Baslar et al. (2009) Mn or Zn are taken mainly up by the root system and are then transported from there to the crowns, since they are highly mobile elements. Therefore, it is better to investigate its deposition in the crowns.

The observed elevated levels of heavy metals in the soils are reflected by the high content of Fe, Zn, Pb, and Cd in the leaves of *B. pendula*, Zn, Pb, Cd, and Ni in *S. caprea* and Pb and Cd in *R. idaeus*, Wisłocka et al. (2006). The highest amounts of heavy metals were found in the root of the sensitive *Plantago major* and less in the resistant *Rumex acetosella*, Filipovic-Trajkovic et al. (2012). Thus, high levels of heavy metals in the soil do not always indicate similar high concentrations in plants. Brej (1998) showed that in a polluted soil most seeds are not sensitive to heavy metals occurring in the study area (Cu, Zn, Pb, Cd, and Ni). Also the root/shoot ratio is determinant in the plant tolerance to soil pollutants.

Metallophytes (chalcophytes) are characteristic of sites with heavy metal concentrations in soil above the normal background values of the micronutrients Fe, Mn, Zn, Cu, Co, Mo, Ni, V, and the trace metals Cd, As, U, Pb, Tl, Cr, and Hg. A classic Zn indicator is *Viola calaminaria* which occurs in association with the heavy metal ecotypes of *M. verna* and *S. cucubalus*. *B. pendula* and *S. caprea* have proved to be good bioindicators of Pb and Cd in contaminated soil, Wisłocka et al. (2006) and *Lolium* ssp. and *Sinapis* ssp. are considered to be suitable Pt bioindicators, especially because of their ability to grow along roads, Babula et al. (2008).

In an investigation of Cd, Cu, Ni, and Pb uptake from air and soil by *Achillea millefolium* (milfoil) and *Hordeum vulgare* (barley) in Denmark, Cu and Pb plant concentrations correlated with aerial deposition but not with soil concentrations; in contrast, Ni and Cd content in the plants correlated with aerial deposition and soil content. Contrary to Pb, Zn is a micro plant nutrient and Zn concentrations will thus be much more influenced by soil uptake into the higher plants than Pb values, Suchara et al. (2011).

Vascular plants have many species-specific mechanisms to restrict the cellular uptake of heavy metals and to detoxify them internally. The symptoms of reduced root growth, reduced seed sprouting and seedling stunting, necrosis, and chlorosis appear in susceptible plants grown in soils contaminated with heavy metals, Park et al. (2011). The use of physiological and biochemical plant parameters in the bioindication of heavy metal soil contamination is not routinely practiced yet. Some responses of higher plants to soil heavy metal contamination have potential.

### 5.6.4 Metal Bioindicators for Humans

For human contaminants, the approach involve first examining contaminants in all the media which form pathways for human exposure – food, drinking water, air and soil, and then setting out data on contaminants in different human tissues: adipose, blood, urine, breast milk, hair, nails, and so on. There has been a huge interest in developing alternative analytical methods to monitor the presence and levels of trace elements in children, apart from invasive blood method, especially in developing countries, Cole et al. (1998). Low cost and easy sample collection methods can be done through hair, nails, exhaled air, serum, placenta, breast milk, urine and saliva, Mehra and Juneja (2004), Khuder et al. (2007), Hussein et al. (2008), Dastych et al.

(2008), Esteban and Castano (2009), Priya and Geetha (2010), Ayodele and Bayero (2010), Ogboko (2011), Hubbart (2012), Gundacker and Hengstschläger (2012).

The use of hair as an indicator is not new. Over 200 years ago, hair was analyzed to measure As levels in the body, Hubbart (2012). Hair is a complex matrix, formed over a period of time, consisting of high levels of both organic and inorganic materials. Hair can provide a more permanent record of trace element associated with normal and abnormal metabolism, as well as trace element assimilated from the environment, Hashem and Abed (2007). Further, hair is easily collected, and may better reflect the total body pool of some elements, than either blood or urine as short-term indicators, Hubbart (2012). Hair retains an incorporated substance for long periods, since there is no active metabolism/excretion to remove the substance once deposited, Hubbart (2012). For example, hair is a long-term exposure bioidicator to MeHg: once Hg is incorporated into the hair, it remains unchanged, WHO (2007). One approach to assessing the status of environmental health is to compare the levels of trace element concentrations of hair in populations living in areas with toxic metal pollution of soil, water or air, Hubbart (2012).

The placenta can be used to detect the presence of As, Hg or Pb, and also urine, finger/toenails, and human milk have been repeatedly used as indicators of toxic metals exposure, Smolders et al. (2009). The heavy metals Hg, Pb, and Cd are toxicants that are well-known to cross the placenta and to accumulate in fetal tissues, Gundacker and Hengstschläger (2012). Prenatal exposure to Hg and Pb poses a health threat particularly to the developing brain. Fetal exposures to Pb and Cd correlate with reduced birth weight and birth size. The placental passage of Cd is limited suggesting a partial barrier for this metal. It is very likely that metallothionein is responsible for placental storage of the metals, especially of Cd, Gundacker and Hengstschläger (2012).

Unlike the urine, blood, placenta, and human milk, hair and fingernails can record the level and changes of elements in the body over a long period of time, Ayodele and Bayero (2010). Mehra and Juneja (2004, 2005) suggested that hair and nail samples provide reliable indications of exposure to many toxic metals over periods of a year or more, and can be used in epidemiological studies related to environmental pollution.

Humans can be directly affected by air, water and soil metal pollutants, as well as indirectly, through contaminated food supplies. In recent past, a large number of silent epidemics have been reported due to metal contamination, which is escalating day by day. Even metals that are biologically essential have the potential to be harmful to humans and other living organisms at high levels of exposure, Valavanidis et al. (2006), Freisinger (2010), Jovic et al. (2012). An increased concentration of heavy metals in biotic and abiotic environment can affect mineral and enzyme status of human beings, Nordberg and Nordberg (2009). The metals irreversibly bind to active sites of enzymes, thereby destroying normal metabolism by producing high-level toxicity, Valavanidis et al. (2006). The excessive content of heavy metals in humans' body may affect the body and psychophysical development, Szyczewski et al. (2009).

## 5.7   Conclusion

Toxic metals contamination is a great concern at global, regional and local level and influencing the functional and structural integrity of an ecosystem. Toxic metals or "heavy metals" are individual metals and metal compounds that negatively affect living organisms. In very small amounts, many of metals are necessary to support life. However, in larger amounts, they become toxic. They may build up in biological systems and become a significant health hazard.

There are hundreds of sources of metal pollution, including natural and much more anthropogenic sources: industries, mines, agricultural, domestic, and urban pollution. They are emitted to atmosphere, soil, fresh and marine waters via a number of pathways and can have detrimental effects on biota and man. Humans can be exposed to heavy metals through the ingestion of contaminated drinking water, of contaminated food and throughout a food web. Therefore, the content of heavy metals needs to be known not only in water, air, soil/sediment, but also in biological samples: plants, animals, and on the end, in the human population.

Toxic metals introduced into the environment can penetrate ecosystems and can be found in the whole biosphere causing changes in the functions of organisms. Adverse effects of toxic metals on living organisms can be observed. In the last few decades investigations have focused on searching for bioindicators like microorganisms, plants and animals that accumulate toxic metals, even a man.

Biondicators represent organisms that may indicate the levels of environmental pollution. Bioindication is the use of an organism, a part of an organism or a society of organisms, to obtain information on the quality of its environment. It is important to highlight that the choice of the bioindicator organism is essential to the success of environmental monitoring. Thus, the use of bioindicators should help to describe the natural environment, to detect and assess human impacts. Considering all the results presented here, it can be concluded that Zn, Cu, Pb, Cd, and Hg are the most intensively investigated elements in presented bioidicators of air, water and soil. Their concentrations were depending on the investigated species and environmental compartment.

The largest number of bacteria, microorganisms, plant and animal species are used as bioindicators of heavy metals in the aquatic ecosystems. The lowest number is used for the soil contamination. Mosses are particularly effective bioindicators of aerial heavy metal contamination. Analysis of mosses is currently used in international and national monitoring programmes, particularly in Europe. Mosses mainly applied to measure heavy metal levels and trends within and around urban and industrial areas. Higher plants have appeal as indicators in air pollution monitoring in highly polluted areas where mosses are often absent. The use of plants in the bioindication of heavy metal contamination is not routinely practiced. Some responses of higher plants as bioindicators of soil contaminations to heavy metals have potential.

Without question, economic growth and social development are critical for improving environment, human health and well-being. The toxic metals are widely used and consequently distributed in the environment and their early identification

is fundamental to prevent or to control possible damages to the environment and humans. Because of that, a wide range of legislations exists in Europe and other countries related to the metal release and impact on the environment, including air, water and soil, and legislations related to toxic metals levels in a food.

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# Chapter 6
# Natural Dyes and Antimicrobials for Textiles

**Masoud B. Kasiri and Siyamak Safapour**

**Abstract**  Many studies on extraction, purification, and modification processes of natural dyes and antimicrobials, and their subsequent application on textiles in recent years, demonstrates the revival of natural dyeing and finishing. Natural dyes have been widely used in textile coloration since ancient times. However, with advent of man-made synthetic dyes in the mid-nineteenth century, the dye market has been captured due to variety of competitive properties of synthetic dyes. Such properties are lower cost, color variety, ability to dye synthetic fibers, and availability in large industrial scale. Subsequently the use of natural dyes fainted. However, most synthetic dyes raise serious problems for human health and cause environmental risks. As a consequence there is now a worldwide interest for the production of dyes from natural sources such as plants and microorganisms.

The use of natural dyes in textile processing is increasing because of higher dyestuff quality, environmental compatibility and lower costs. In addition many natural dyes have inherently antimicrobial properties. Natural dyes are extracted from microorganisms and plant organs such as bark, leaf, root, fruit, seed, and flowers. Natural dyes are not only a rich and varied source of dyestuff, but also could be considered as safe, environmentally friendly and low cost treatments with additional benefit of coloring in a single stage. In this article, we review extraction and application of natural dyes on textiles as effective coloring and antibacterial agents. Extraction and treatment methods are discussed

**Keywords**  Textile dyes • Antimicrobials • Dye extraction • Bark • Leaf • Flower • Root • Chitosan • Berberine

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## Contents

## 6.1   Introduction

For centuries, natural colorants have been used for numerous purposes varying from tribal or funeral ceremonies, body painting, decorative art and clothing or domestic decoration. At first, colorants were mainly extracted from minerals, insects and plants (Guinot et al. 2006). But since 1856, the manufacture of synthetic colorants became more important as a result of: (I) moderate washing and light fastness of the natural colorants; (II) high cost of the production of natural dye; (III) the advancements in chemical synthesis and characterization techniques and; (IV) the greater production efficiency of synthetic dyes in terms of quality, quantity and the potential to produce low-cost raw materials (Samanta and Agarwal 2009). Consequently, natural colorants were increasingly replaced by synthetic ones, produced in cheaper ways, with moderate to excellent fastness. It is estimated that some 700,000 t of dyes are consumed annually and world production of pigments is also about five million tons, with inorganic pigments accounting for approximately 96 % (McCarthy 1997). Almost all these synthetic colorants being synthesized from petrochemical sources through hazardous chemical processes pose threat towards the environment and human body health (Kasiri et al. 2008; Safapour et al. 2010; Kasiri and Khataee 2011; Samanta and Konar 2011).

Global concern for environmental pollution has put stringent rules forward for high level pollutant industries. Textile industry with huge amount of hazardous wastewater production is one of such industries. Numerous researches in recent years have focused on development of totally new technologies and/or modification of conventional technologies for cleaner and environmentally friendlier production processes.

Hence, in the last few years the use of non toxic, antimicrobial and eco-friendly natural dyes on textiles, preferably natural fiber products, has become a matter of increasing importance due to the increased awareness of synthetic dyes environmental hazards (Hill 1997; Dawson 2009). Some of the advantages of natural dyes are

**Fig. 6.1** Natural dyes are extracted from different parts of plants such as (**a**) bark (Purple bark; http://www.wickedsmileys.com/GothicGreetingCards/index2.html), (**b**) leaf (Eucalyptus; http://herbs.ygoy.com/what-is-eucalyptus/), (**c**) flower (Marigold; http://flowerwallpapers.co/marigold/download-yellow-marigolds-flower-images-inpretty-orange-marigolds-wallpaper.html), (**d**) fruits (Pomegranate rind; http://www.anniesremedy.com/herb_detail518.php?gc=518b) and (**e**) root (Madder; http://microsites.merton.gov.uk/riverandcloth/image/madder-roots.html)

eco-friendliness, i.e., they do not create any environmental problems at the stage of production or use and maintains the ecological balance (Sivakumar et al. 2011).

On the other hands, textiles, by virtue of their characteristics and proximity to human body, provide an excellent medium for the growth and multiplication of microorganisms, where they could find the required nutrition including; water, carbon, nitrogen and some inorganic salts. This could help microorganisms to transfer, propagation of infection and causing microbial diseases (Gupta and Bhaumik 2007).

Most of natural dyes have inherently antimicrobial properties and could consequently possess high medicinal activity. Natural dyes are extracted from different parts of plants including bark (e.g. Purple bark, Sappan wood, Shillicorai, Khair, Red and Sandalwood), leaf (e.g. Indigo, Henna, Eucalyptus, Tea, Cardamon, Coral Jasmine, Lemon Grass), root (e.g. Turmeric, Madder, Onions, Beet-root), fruits or seeds (e.g. Latkan, Pomegranate rind, Beetle nut, Myrobolan) and flower (e.g. Marigold, Dahlia, Tesu, Kusum) that contain coloring materials like tannin, flavonoids, quinonoids, etc (Fig. 6.1).

The natural dyes also come from some types of microorganisms such as fungi, algae and bacteria. These dyes can offer not only a rich and varied source of dyestuff, but also could be considered as safe, environmentally friendly and low cost treatments with additional benefit of coloring in a single stage (Samanta and Agarwal 2009; Samanta and Konar 2011).

Application of natural dyes has, of course, some limitations. It is difficult to reproduce shades by using natural dyes. Natural dyeing requires skilled workmanship and is therefore expensive. They have poor fastness and the colors produced are of poor quality. Moreover, these dyes require time-consuming extractions, are difficult to use and the dyed textile may change color when exposed to the sun, sweat and air (Hill 1997).

For using natural dyes in an industrial scale, the appropriate and standardized extraction methods and dyeing techniques must be developed without sacrificing the required quality and quantity of dyed textiles. Therefore, to obtain stable shades with acceptable color fastness behavior and reproducible color yield, appropriate extraction, identification, dyeing and finishing techniques need to be derived from the current scientific studies.

In this review, an attempt has been made to give the latest scientific overview on extraction and application of natural dyes/antimicrobials on textiles as effective coloring and antibacterial agents. Different methods of extraction of natural dyes/antimicrobials will be discussed and examples of early applications of these dyes on textile processing, properties, advantages and disadvantages will be reviewed.

## 6.2 Natural Dyes/Antimicrobials

Natural dyes/antimicrobials are dyes, colorants, and agents derived from natural resources. The resurgence of natural dyes in the early twenty-first century is due to some their peculiar benefits. However, there are some drawbacks restricting the application of these beneficial natural materials (Dawson 2008). In this section, some main benefits and restrictions of natural dyes/antimicrobials have been summarized.

### 6.2.1 Benefits

According to the results of previous works, growing global interest toward dyes/antimicrobials from natural resources is due to some main following benefits:

- Some natural dyes have intrinsic additional properties such as antibacterial, moth proof, anti-allergy, anti-ultra violet, etc.

- Natural dyes produce different uncommon, eye-catching, and soothing shades on textiles.
- Wide range of shades can be produced with an individual natural dye either in mixture with mordants or by change in dyeing condition.
- Different natural constituents along with natural dye may enhance dyeing/finishing process efficiency as auxiliary, mordant, fixing component.
- Natural dyes/antimicrobials are obtained from inexpensive renewable resources with huge potential.
- From two aspects, natural dyes/antimicrobials are environmentally friendlier than synthetic ones: first, in natural materials all synthesis processes are accomplished by nature with no pollution of environment. Second, these materials are readily biodegradable and do not produce hazardous wastewater upon degradation in environment, and so, there is no need for treatment of wastewater before discharging into the environment.
- Majority of natural dyes/antimicrobials are extracted from wild and self growing plants with no additional cost for their cultivation.
- Color characteristics of some natural dyes mellow with time. This characteristic imparts unique properties and appearance to the stuff dyed, e.g., old carpet piles dyed with natural dyes.
- Most of natural antimicrobial components do not have negative effects on beneficial non-target microorganisms.
- Most of natural antimicrobials do not have side effects on human body.
- Extraction, purification, and application of natural dyes/antimicrobials have become easier and more possible with different modern characterization techniques developed.

## 6.2.2 Restrictions

Despite various benefits of natural dyes/antimicrobials, they have some drawbacks limiting their application on textiles. Some important restrictions can be summarized as follows:

- Generally, natural dyes/antimicrobials have low wash, rub, light, sweat, and gas fastness on textiles probably due to weak bonding, weak interaction, etc. with textiles.
- To improve fastness of natural dyes/antimicrobials, some additional chemicals are used such as metallic mordants, crosslinking chemicals, etc. Discharge of wastewater of dyeing and finishing processes containing these hazardous materials poses some serious environmental risks.
- Only little information is available about extraction, purification, chemical structure, and application methods of natural dyes/antimicrobials onto textiles.

- Color matching and reproduction of color is another disadvantage of natural dyes since the quantity and quality of natural materials steadily change with climate, plant genus, etc.
- There is no standard method of natural dyeing.
- The efficiency of the natural dyes/antimicrobials extraction process is generally low, only few grams of natural component per kg of raw materials are normally obtained.

## 6.3 Extraction of Natural Dyes

Extraction of dyes/antimicrobials from the natural sources could be one of the most important steps for treatment of textiles to achieve the desired dyeing properties and/or antimicrobial activities. Moreover, obtaining a standard extraction process and optimizing the extraction variables for a particular natural source, are economically important and consequently, affect the end-products price.

Natural dyes/antimicrobials could be extracted from different types of microorganisms as well as various parts of the plants such as bark, leaf, root, fruit, seed, flower, etc. There are different methods to extract these materials from above-mentioned natural sources including; aqueous extraction i.e. using water for the extraction with or without addition of salt/acid/alkali/alcohol in the extraction bath, chelating with alum and precipitating with acid, enzyme assisted extraction, alcoholic/organic solvent extraction by using relevant extracting equipment, such as soxhlet apparatus, with use of alcohol, hexane or benzene solvents, adsorption of the pigments on silica gel and finally, ultrasonic extraction method. In the following paragraphs, four main important techniques, i.e. aqueous extraction methods, alcoholic/organic solvent extraction, ultrasonic extraction and enzyme assisted extraction will be reviewed.

## 6.3.1 Aqueous Extraction Methods

Due to the chemical structure of the most natural dyes/antimicrobials, simple set-up and cost-effective aqueous extraction method is the most widely used extraction technique. Routinely, dried and finely cut materials of natural source are grinded in powdered form and then the dye/antimicrobial is extracted in water employing different standard processes.

For instance, the aqueous solution could be filtered and concentrated under reduced pressure (rotary evaporator) to give a crude extract (Farizadeh et al. 2009).

Extraction time, pH of the solution, and temperature are the most important parameters influencing the yield of extraction. In an interesting work, the natural dye was extracted from petals under different operating conditions such as extraction

**Fig. 6.2** A schematic representation of a soxhlet extractor; (**1**) stirrer bar (**2**) still pot (**3**) distillation path (**4**) thimble (**5**) solid (**6**) siphon top (**7**) siphon exit (**8**) expansion adapter (**9**) condenser (**10**) cooling water in (**11**) cooling water out (http://en.wikipedia.org/wiki/Soxhlet_extractor)
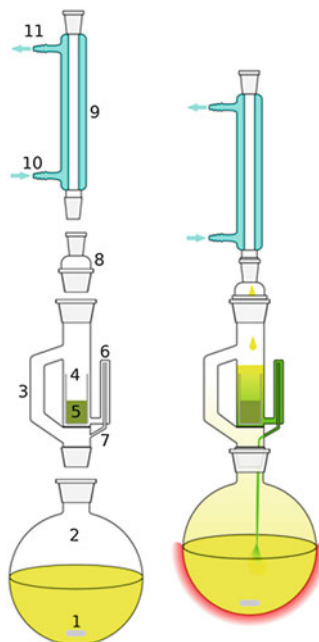


time (45–120 min), temperature (60–90 °C) and mass of the petals (0.5–2 g) by conventional extraction technique, where response surface methodology (RSM) was used for optimization of the extraction process (Sinha et al. 2012).

## 6.3.2 Alcoholic/Organic Solvent Extraction Methods

The organic nature of some dyes/antimicrobials from natural sources implies the application of non-aqueous extraction methods. During this method, a mixture of organic solvents, usually alcohols with hexane, is used for extraction of natural agents.

As an example, the roots of the authentic sample of *Ratanjot* were air-dried and ground to coarse powder. The powdered roots were extracted with *n*-hexane in soxhlet apparatus (Fig. 6.2) till the color of the decoction became very light. Solvent was removed and the percentage yield of the hexane extraction from roots was estimated (Arora et al. 2009).

Similarly, for extraction of carminic acid, a natural colorant that can be obtained from the dried bodies of females of the *Dactylopius coccus* Costa insect species, Borges et al. (2012) have tried the following method. Three different mixtures of solvents i.e. methanol:water, ethanol:water and pure ethanol were employed to achieve extracts with different compositions. Moreover, extractions were performed

at three different extraction temperatures (100 °C, 150 °C, and 200 °C) and 30 min as extraction time. All extractions were performed in 11 ml extraction cells, containing 2.0 g of sample.

Another example has been explained at flavonoid dye extraction from marigold flower (Guinot et al. 2008). Dried ground flowers were soaked by decoction for 10 min in distilled water (1:100 w/v). Extracts of the dried ground flowers were obtained by maceration (1:100 w/v) at 60 °C for 30 min and 60 min under mechanical stirring (180 rpm). The solvents used were ethanol:water mixtures of various compositions, i.e. 2:8, 3:7, 5:5 v/v. The resulting suspensions were filtered through gauze and the filtrates were made up to their initial volume with the appropriate solvent. Finally, 2 ml of filtrate were centrifuged for 10 min (7,176 g) before analysis.

### 6.3.3   Ultrasonic Extraction Method

State of arts process like ultrasonic, enzymatic and enzyme mediated ultrasound extraction techniques are nowadays widely being studied for their high efficiency over the conventional methods. In this method, the energy of the ultrasonic waves enhances the yield of extraction.

An example of application of this extraction technique has been explained by Mishra et al. (2012). The petals of the flowers were picked and dried at 50 °C in tray drier. Methanol (15 ml/g of petals) was added in dried petals and then pH was adjusted to the desired value. The beakers was then placed in ultrasonic bath and sonicated for 5–30 min at 27–30 MHz and 160 V. After sonication, the contents of beakers were filtered through standard test sieve to remove solid materials. The filtrated dark red liquid was vacuum evaporated in a rotary vacuum evaporator to about half of the original volume and then, the concentrated red liquid was spray-dried.

In this method, extraction bath pH, salt concentration, ultrasonic power, extraction time and temperature are the most influencing parameters. Kamel et al. (2005) have studied the extracting of lac as a natural dye with ultrasonic assisted method varying amounts of the dye materials (2–12 %) at different temperatures (50–80 °C) using different ultrasonic powers (100–500 W) and for different time intervals (20–120 min), yielding different amount of dyeing agent.

Similarly, plant extracts (1:100 w/v) were obtained from dried leaves and stems in ethanol/water (3:7 v/v) with ultrasonication (15 min, 24 kHz, R.E.U.S-GEX 180). After passing through filter paper, the filtrate was centrifuged for 10 min (7,176 g) (Guinot et al. 2009).

As another example of ultrasonic extraction described by Sivakumar et al. (2011), 1 g of the plant samples was taken and added to 50 ml water taken in the beaker. The beaker was covered using aluminum foil to prevent loss of solvent by evaporation. The ultrasonic probe was positioned in the beaker and the ultrasound power set at 80 W, while the temperature was maintained at around 45 °C.

### 6.3.4 Enzyme Assisted Extraction Method

As a recipe for enzymatic extraction technique, a 2 % solution of pectinase:cellulase (2:1) was sprayed on pomegranate rind (25 g) for better soaking and contact and then, left overnight. The enzyme treated material was washed with little amount of distilled water and pH of the solution was adjusted to 10. The sample was shaken at 150 rpm for 20–80 min at desired temperature. The contents of beakers were filtered through standard test sieve to remove solid materials and the concentrated dye extract was vacuum evaporated in a rotary vacuum evaporator to about half of the original volume and finally, the concentrated liquid was spray-dried (Tiwari et al. 2010).

## 6.4 Application of Natural Dyes/Antimicrobials in Textiles

### 6.4.1 Treatment Methods

In application of materials from natural sources onto textiles, fastness properties such as wash, light, perspiration, etc. are often in low and undesired level. This can be due to heterogeneity, diversity and complexity of natural matters as well as their weak or negligible interactions with textiles. Low fastness properties limit the application area of such useful, eco-safe, low cost natural materials in the treatments of textiles in spite of their various beneficial properties. Hence, to surpass this drawback, additional treatments are often needed to enhance the durability and value of textile products. Treatment processes can be performed on textiles (Das et al. 2007; Pruthi et al. 2008; Ammayappan and Moses 2009; Çalış et al. 2009; Giri Dev et al. 2009; Kiumarsi et al. 2009; Mongkholrattanasit et al. 2011) or on natural matters (Iqbal et al. 2008; Syamili et al. 2012) or on both (Iqbal et al. 2008), where the first one is more common and frequently used. In literature survey to prepare the present review, we have found some interesting treatment methods to enhance the properties and values of final products. Although addressing all available treatment methods is not possible here, some important examples will be briefly reviewed in the following paragraphs.

**Structure Modification of Textiles**

In the dyeing process with indicaxanthin natural dye, Guesmi et al. (2013) used modified acrylic fibers with amino and hydroxyl functional groups. They reported that the affinity of the dye and dyeability of fibers were strongly dependent on the pH value of dyeing bath with the highest amount of dye up-take at pH 3. It was attributed to the action of functional groups available in both fiber and dye structures controlling ionic interaction of carboxyl groups of dye with the protonated amino groups of the modified acrylic fibers.

**Fig. 6.3** Chemical structure of berberine extracted from a Chinese herb, *Rhizoma coptidis*
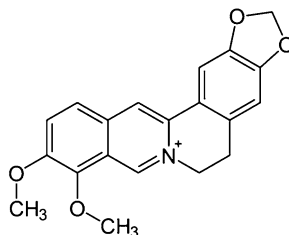


## Treatment with Chemicals

In a series of studies, cotton was dyed with Berberine as natural colorant (Kim et al. 2004; Kim and Son 2005; Son et al. 2008). Berberine is a quaternary ammonium salt and strong yellow colored natural dye with both dyeing and antimicrobial properties (Fig. 6.3). Berberine has high affinity for protein fibers such as wool and silk whereas it shows low affinity for cellulosic fibers because of lack of ionic charges on cotton surface compared to protein fibers. Accordingly, cotton was reacted with an anionic bridging agent to produce negative charges on the surface, being responsible for electrostatic interactions with cationic natural dye, in order to enhance the affinity of Berberine to cotton. Dyeing tests demonstrated significantly enhanced properties in both Berberine exhaustion and fastness properties.

In another work, Glyoxal as crosslinking chemical were employed to enhance the application of *Azadirachta indica*, a natural antibacterial agent, on polyester/cotton blend fabrics (Joshi et al. 2007). Antibacterial properties retained after different wash cycles demonstrated the efficiency of chemicals in crosslinking yield of bioactive matter on the substrate. Similarly, citric acid was used as crosslinking agent in antibacterial finishing of cotton with green tea leaves extracts to increase the durability against wash (Syamili et al. 2012).

Another example of treatment with chemicals is the work of Ammayappan and Moses (2009). They reported that pre-treatment of cotton with hydrogen peroxide and wool fibers with formic acid increase antibacterial activity of fibers treated with aloevera, chitosan, and curcumin as natural antimicrobials. It was attributed to the increase in the accessible areas in the case of cotton, and disulphide linkage modification and change in wettability in the case of wool/hair fibers. Moreover, additional reasons stated were the increase in the quantity of antimicrobials absorbed and decrease of impurities for all fibers after treatment with selective chemicals.

## Treatment with Chitosan

Chitosan is a linear polysaccharide produced commercially by deacetylation of chitin (Fig. 6.4), the structural element in the crustaceans such as crabs and shrimp

**Fig. 6.4** Chemical structure of chitosan, a linear polysaccharide
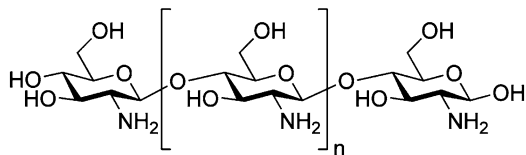
and cell walls of fungi. It has been widely used in various industries because of its multifunctional properties. Chitosan is also extensively used in treatment of textiles such as dyeing, finishing, printing, etc.

For instance, chitosan has been used in pre-treatment of cotton fibers prior to dyeing with lac dye (Rattanaphani et al. 2007). Due to lack of cationic sites in cotton fibers, lac dye has low affinity for cotton. It was shown that treatment with chitosan produced cationic sites on cotton and subsequently the affinity of lac dye toward fiber, dye up-take, and fatness properties have been enhanced. It was discussed that chitosan could be a suitable eco-friend alternative in treatment of cotton compared to use other toxic chemicals.

In another work, cotton has been pre-treated with chitosan and then dyed with turmeric (Kavitha et al. 2007). Treatment with chitosan enhances antibacterial activity and improves some mechanical properties of fibers. Similar attempt has been made to pre-treat wool fibers with this biopolymer prior dyeing with henna dye (Giri Dev et al. 2009). Increasing in henna dye up-take and antimicrobial finish were properties imparted to wool fibers after treatment with chitosan.

## Treatment with Enzyme

Protease is an enzyme, biological molecules that catalyze chemical reactions, that conducts proteolysis. In the study of antimicrobial characteristics of some natural dyes on wool fibers, effects of protease enzyme treatment on dyeing and antimicrobial yield were studied (Raja and Thilagavathi 2011). It was demonstrated that alkaline protease enzyme process enhances the quantity of natural dye exhausted. It was ascribed to partial or complete damage of cuticle facilitating easier penetration of natural dye molecules into fibers.

## Treatment with Ultraviolet (UV) Radiation

Iqbal et al. (2008) have studied the effect of UV irradiation on the henna dyed cotton fabrics. Henna and cotton were separately UV irradiated and then after coloration, the results were compared with non-irradiated samples. At first step, untreated fabrics were dyed with both virgin and irradiated henna. It was observed that both color strength and shade of fabrics dyed with irradiated henna are higher compared to non-irradiated henna. It was concluded that lawsone, the coloring matter in henna, upon irradiation is hydrolytically degraded and thereby considerable increase in the

sorption of degraded dye occurs. In the next step, effect of radiation treatment of cotton substrate on its dyeability with henna was investigated and similarly higher color strength values were obtained for treated samples. It was inferred that increase in the interaction between dye molecules and cellulose macromolecules, resulted from developed spaces within fibers after irradiation, was responsible for higher dye up-take values. Finally, Iqbal et al. (2008) concluded that to obtain the highest color strength both henna dye and cotton must be UV irradiated.

## Treatment with Ultrasound

Nowadays, ultrasound energy is exploited in dyeing of textiles so-called sonicator dyeing. Sonicator dyeing has some significant advantages over the conventional dyeing, e.g., decrease in temperature and time of dyeing, and decrease in quantity of dye and electrolyte required. Wool fibers were dyed with lac as a natural dye using both conventional and ultrasonic techniques (Kamel et al. 2005). About 47 % increase in lac dye up-take was reported using ultrasonic dyeing. Also, the amount of dye extracted by ultrasonic technique was 41 % higher than conventional extraction methods. Similar study was conducted on the dyeing of cationized cotton fabric with same dye using both conventional and ultrasonic methods, exhibiting 66.5 % improvement in lac dye up-take using ultrasonic dyeing technique (Kamel et al. 2007).

In another study, cotton was dyed with natural dye Eclipta using both conventional and sonicator dyeing. The efficiency of sonicator dyeing was reported to be 7–9 % higher than conventional method (Vankar et al. 2007).

Guesmi et al. (2013) also compared dyeing properties of modified acrylic fibers with indicaxanthin natural dye using both conventional and sonicator dyeing and reported 49.62 % higher natural dye up-take, better wash and light fastness with less process time and energy consumption for sonicator dyeing technique.

Similarly, improved natural dye exhaustion, fastness properties as well as waste reduction of dyeing were reported in the ultrasonic dyeing study of cotton with natural dye from plant species *Symplocos spicata* (Vankar et al. 2008).

## Treatment with Plasma

Plasma treatment is a modern method of textiles treatment. Plasma is the fourth state of the matter in the form of partly ionized gases. When a gas under controlled pressure interacts with electromagnetic field, plasma is produced. Plasma technology is gradually being replaced by chemical methods in treatment of textiles. Low-temperature plasma is widely used in non-destructive surface modification of textiles where a wide range of properties can be obtained. Ghoranneviss et al. (2010) have studied dyeing properties of plasma treated wool fibers with two natural dyes, namely, madder and weld. It was proved that the treatment of wool fibers with plasma enhances natural coloration process and properties. Moreover, plasma

treatment was introduced as a suitable replacement for metallic mordant treatment with some additional benefits. Chen and Chang (2007) used low temperature microwave plasma to treat cotton fabric prior to grafting with onion skin and onion pulp extracts in order to impart antimicrobial properties to cotton fabrics. It was discussed that functional groups produced on the fiber surface after low-temperature oxygen plasma treatment significantly increase the hydrophilic nature of cotton and thereby the grafting yield of both onion skin and pulp extracts enhances.

### 6.4.2  Examples of Early Applications

Numerous reports have been published on natural dyes/antimicrobials application, including variability in the quantity and quality in the natural source, their use in combinations, their dyeing and antimicrobial properties, the effect of mordants and auxiliaries, their fastness, improvements in extraction methods and the development of alternative sources. Natural dyes/antimicrobials are extracted mainly from two different resources: plants and microorganisms. In the following parts, examples of early applications of dyes/antimicrobials from these two resources will be reviewed.

**Dyes/Antimicrobials from Plants**

Plants are the major source of natural dyes/antimicrobials in the nature. These materials are extracted from different parts of the plants such as bark, leaf, root and flower containing commonly coloring materials like tannin, flavonoids, quinonoids, etc. During the following paragraphs, the examples of applications are classified according to the different parts of plants used as the natural source.

Root

Root is one of the common parts of the plants that has been extensively used for dyes/antimicrobials extraction (Table 6.1). **Madder**, the ground root of the *Rubia* species with anthraquinone substituted chemical structure, has traditionally been an important natural source of red and brown color on wool and cotton along with different mordants (Gupta et al. 2001a, 2004a). Chemical structure of some colorants presented in madder root is shown in Fig. 6.5.

Gupta et al. (2001a) have tried the dyeing of nylon with the pigment extracted from indian madder. Nordamncanthal, a bright red pigment with a hydroxyanthraquinone chemical structure, was extracted from madder by chelating with alum and precipitating with acid. This pigment was used for dyeing of nylon and polyester, and the results showed good affinity for the fibers studied.

The authors have also studied the dyeing process of nylon with another extract of madder (Gupta et al. 2001b). Purpurin with 1,2,4-trihydroxyanthraquinone structure

**Table 6.1** Characteristics of dyes/antimicrobials extracted from roots of the plants and their application properties

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Indian madder | *Rubia* | *Hydroxyanthra-quinones (nordamncanthal)* | Bright red | Nylon and polyester | Good affinity for the fibers | – | Chelating with alum and precipitating with acid | *Gupta et al.* (2001a) |
| 2 | Indian madder | *Rubia* | *1,2,4-trihydroxy-anthraquinone (purpurin)* | Red | Nylon and polyester | Good affinity for the fibers | – | Chelating with alum and precipitating with acid | *Gupta et al.* (2001b) |
| 3 | Indian madder | *Rubia* | *1,2,4-trihydroxy-anthraquinone (purpurin), 1,3-dihydroxy-2-carboxy anthraquinone (munjistin)* | – | Nylon with mordant | Color | Purpurin($G$) Munjistin($P$) | Extraction with alum | *Gupta et al.* (2004a) |
| 4 | Madder | – | Alizarin, purpurin and 1,4-dihydroxy-anthraquinone | – | Wool | Adsorption dependent on the pH and temperature | – | Extraction with distilled water | *Farizadeh et al.* (2009) |
| 5 | Madder | – | Alizarin, rubierythric acid, rubiadin, munjistin purpuroxanthin or xanthopurpurin | – | Wool | Accelerate dye diffusion | – | Extraction with distilled water | *Montazer et al.* (2007) |
| 6 | Madder | – | *Hydroxyanthra-quinones* | Red | Wool with mordant | Fading of the madder-dyed yarns | – | – | *Montazer and Parvinzadeh* (2004) |

| No. | Source | Botanical name | Chemical class | Color | Substrate | Property | Fastness | Extraction | References |
|---|---|---|---|---|---|---|---|---|---|
| 7 | Madder, cochineal, walnut, weld, white onion, red onion, pomegranate | *Rubia tinctoria, Dactylopius coccus, Juglens regia, Reseda luteola, Allium cepa, Allium cepa, Punica granatum* | *Anthraquinone, naphthaquinone, flavone and tannin* | – | Wool with mordant | – | – | – | Montazer et al. (2004) |
| 8 | Ratanjot | *Arnebia nobilis* Rech.f | Naphthoquinonoid, hydroxynaphtho-quinone, isohex-enylnaphthazarin | Pink, blue, purple | Polyester, nylon, cotton, wool, silk, acrylic | Color | Wash(*E*) Light(*P*) Rub(*E*) | Soxhlet extraction with n-hexane | Arora et al. (2009); Arora et al. (2012a); Arora et al. (2012b) |
| 9 | Turmeric | *Curcuma longa L.* | Natural phenols | Yellow | Wool | Color, anti-microbial | Wash(*G*) Light(*P*) | Aqueous ethanol extraction | Han and Yang (2005) |
| 10 | Turmeric | *Curcuma longa L.* | Natural phenols | Yellow | Cotton coated with chitosan | Color, anti-bacterial | – | Aqueous ethanol extraction | Kavitha et al. (2007) |
| 11 | Aloevera, chitosan, curcumin | – | – | – | Cotton, wool, rabbit hair | Antimicrobial | Wash(*G*) | – | Ammayappan and Moses (2009) |

(continued)

**Table 6.1** (continued)

| Source No. (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] method | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|
| 12 Turmeric, sapanwood, phellodendron, chinese yellow berries, gardenia, safflower, madder, gromwell, indigo | Curcuma longa L., Caesalpinia sappon L., Phellodendron amurense Ruprecht, Sophora japonica L., Gardenia jasmoides Ellis, Carthamus tinctorius L., Rubia cordifolia, L., Lithospermum erythrorhizon, Indigo | Curcumin, berberine, rutin, crocin, safflower yellow, safflower red, purpurin, munjistin, brazilin, shikonin, indigo | – | – | – | – | – | Choo and Lee (2002) |
| 13 Cochineal, madder, alkanna, henna, brazilwood, red sandalwood, safflower, indigo, logwood | Porphyrophora polonica L., Rubia tinctorum L., Alkanna tinctoria Tausch., Lawsonia inermis L., Caesalpinia trees, Pterocarpus santalinus L., Carthamus tincorius L., Indigofera tinctoria, Hematoxylon compechianum L. | – | Red, red, red, orange, red, red, red, blue, black | Cotton and wool with mordants | Significant improvement of the dye adsorption on the cotton fabrics | Wool: Wash(G) | Extraction with boiling water | Zarkogianni et al. (2010) |

| No. | Common names | Botanical names | Chemical/dye | Color | Substrate | Property | Fastness | Notes | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 14 | Acacia, indigo, lac, kamala, pomegranate, gall nuts, cutch, myrobolan, himalayan rhubarb, indian madder, golden dock | *Acacia nilotica, Indigofera tinctoria, Kerria lacca, Mallotus philippinensis, Punica granatum, Quercus infectoria, Acacia catechu, Terminalia chebula, Rheum emodi, Rubia cordifolia, Rumex maritimus* | Bark, indigo, laccaic acid, flavonoid, tanin, tanin, tanin, tanin, hydroxyan-thraquinones, hydroxyan-thraquinones, hydroxyan-thraquinones | – | Cotton | Antibacterial | Wash(*G*) | – | Gupta et al. (2004b) |
| 15 | Chinese herb | *Rhizoma coptidis* | Berberine | Brownish yellow | Wool with mordant | Deeper shades and darker colors, good antibacterial property | Wash(*P*) | Extraction with boiling water | Ke et al. (2006) |
| 16 | Amur cork tree, goldenseal coptis, oregon grape, barberry, tree turmeric, yerba mansa | *Coptis chinensis, Berberis aquifolium, Berberis vulgaris, Berberis aristata, Anemopsis californica* | Isoquinoline alkaloid | Yellow | Nylon 66 | Color, anti-microbial | – | – | Son et al. (2007) |
| 17 | Dolu | *Rheum emodi* | Anthraquinone | Yellow, olive green | Wool, silk with metallic, mordants | Color | Wash(*G*) Light(*M*) | – | Das et al. (2008) |

[a]Excellent (E), Very good (VG), Good (G), Medium (M), Poor (P)
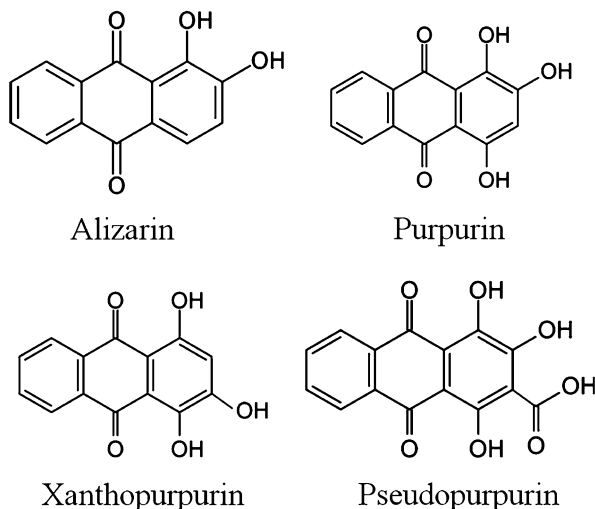
**Fig. 6.5** Chemical structure of alizarin, purpurin, xanthopurpurin and pseudopurpurin extracted from madder root

was extracted from madder in a similar way and was used for dyeing of nylon fabrics. The authors have reported that this red pigment has also good affinity, like nordamncanthal, for the nylon fibers.

The light fastness of madder extracts applied on nylon has been reported in a related work (Gupta et al. 2004a). The authors have extracted two pigments, purpurin and munjistin, from the indian madder by soaking and extraction with alum and applied it for dyeing of nylon with ferrous sulphate, copper sulphate, alum and stannous chloride as mordants. The authors have reported a good light fastness for the fabrics while the dye fastness of fabrics with munjistin was poor.

Extraction of dyes from madder at different conditions has been studied by Farizadeh and his colleagues (2009), too. The extracted pigments with distilled water were analyzed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) techniques and identified as alizarin, purpurin and 1,4-dihydroxyanthraquinone. Extracted pigments were used for dyeing of wool fabrics at different temperatures and dyebath pHs and the results proved that the affinity of the dye on the fabrics dependent mainly on the pH and temperature of the bath, where increasing of temperature led to decrease the values of partition ratio and affinity.

In an attempt, dyeing process of wool with madder and liposomes as an auxiliary in dyeing has also been optimized (Montazer et al. 2007). The pigment were extracted with steeping the madder in water, heated and passed through a filter. The dyeing process was optimized by response surface methodology, where the bath temperature, time, and liposome percentage were chosen as input variables and the fabrics color strength as the response. The dyeing process was optimized, but it was also found that wash, light, wet and dry rub fastness properties of samples dyed with madder including liposomes have not changed significantly.

**Fig. 6.6** Chemical structure
of alkannin extracted from
Ratanjot
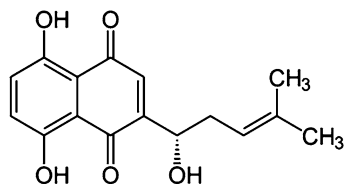


On the other hand, wool yarns were dyed with madder along with aluminum potassium sulfate as the mordant and then treated with different ammonia solutions by Montazer and Parvinzadeh (2004). It has been reported that a change of color is observed on the dyed samples when wash-fastness tests were carried out, while the light-fastness tests showed more fading of the madder-dyed yarns after ammonia treatment.

They have also studied the effect of ammonia on the coloristic properties of wool dyed with madder and other natural dyes containing anthraquinone, naphthaquinone, flavone and tannin structures (Montazer et al. 2004). Wool was first mordanted with aluminium potassium sulphate and then dyed using madder, cochineal, walnut husks, weld, red and white onion skins, and pomegranate shells. The results showed that the color changes could be due to the extension of dye molecule conjugated system by one lone pair electron in ammonia solution.

**Ratanjot**, *Arnebia nobilis* Rech.f is the root of herbal plant that has been widely used as a potent resource for obtaining both herbal drugs and color matter (Arora et al. 2009). It contains a variety of naturally occurring chemicals, mainly a naphthoquinone derivative called alkannin (Fig. 6.6). Alkannin has a deep red color in a greasy or oily environment and a violet color in an alkaline environment (Papageorgiou et al. 1999).

Arora et al. (2009) have tried to extract coloring matters from Ratanjot. Due to variety of plant species, they used different identification methods such as macroscopic, microscopic, chemical and TLC analysis of the roots. The extraction was performed using soxhlet system with n-hexane and the TLC analysis showed the presence of five naphthoquinone derivatives with red and pink colors.

In another work, Arora et al. (2012a) have used ratanjot extract for coloration of variety of textiles, i.e., cotton, wool, silk, nylon, polyester, and acrylic. Interestingly, the strong sensitivity of extracted natural dye solution to pH and temperature changes was observed. With gradual increasing of pH in the range 3–12, continuous changes in color from red to purple and then to dark blue was observed. It was attributed to the conversion of dye structure from quinonoid in acidic solution to benzenoid in alkaline solution. Dyeing results showed that as the dyeing pH increases, the dye up-take decreases which was ascribed to decomposition of dye structure in alkaline solution.

Wool, cotton, silk, nylon, polyester, and acrylic were dyed with ratanjot extract in similar condition and different shades were obtained such as pink on polyester, blue on nylon, and purple on the rest fibers. Different results for affinity and dye up-take were observed for fibers. Very low dye up-take on cotton fabric was attributed to non-polar character of dye and low affinity to cotton fibers. Wool had higher

**Fig. 6.7** Chemical structure
of curcuminoids extracted
from turmeric



Curcumin

Demethoxycurcumin

Bis-demethoxycurcumin
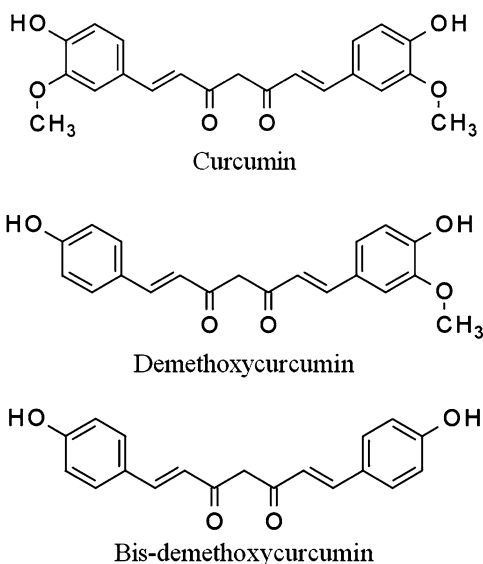
dye up-take compared to silk possibly because of lower crystallinity of wool fibers. Polyester showed lower dye up-take at 130 °C compared to 100 °C, not due to lower affinity of the dye to polyester but owing to more decomposition of dye at enhanced temperatures decreasing effective dye concentration in the solution (Arora et al. 2012a).

Interestingly, by means of oxidation/reduction test, the reason of completely different observed shades was addressed to be the structure transformation of dye from normal state (quinonoid) to the reduced reversible state (benzenoid) on all substrates. Fastness tests data demonstrated poor light fastness whereas wash, rub and perspiration fastnesses were excellent. Finally, the findings of the study showed that *Arnebia nobilis* Rech.f can be used as a good natural dye resource for dyeing and production of variety of eco-friend textiles where light fastness is not key factor in dye selection (Arora et al. 2012a).

Using *Arnebia nobilis* Rech.f dye, kinetics and thermodynamics of dyeing on wool has also been studied by Aora et al. (2012b) and the results were compared with those of juglone, lawsone, and Rheum emodi from literature. Lower diffusion coefficient than other three natural dyes with exothermic dyeing process was reported for this dye.

**Turmeric**, *Curcuma longa*, is a rhizomatous herbaceous perennial plant of the ginger family native to tropical south Asia. The dried rhizomes are ground into a deep orange-yellow powder commonly used as spice in cuisine and for dyeing. Turmeric's active ingredient is curcumin which is the principal curcuminoids, natural phenols that are responsible for the yellow color of turmeric. Other two curcuminoids present in turmeric are desmethoxycurcumin and bis-desmethoxycurcumin, known as curcumin I-III (Chan et al. 2009). Chemical structures of curcuminoids are shown in Fig. 6.7.

Turmeric has been used to impart either color or antibacterial property on textiles (Popoola 2000; Han and Yang 2005; Kavitha et al. 2007; Ammayappan and Moses 2009). Han and Yang (2005) used curcumin as antimicrobial finish for wool against two bacteria *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) with no additional treatment through conventional dyeing process. Very low concentration of curcumin was effective in bacterial growth inhibition on wool, e.g., only 0.05 % and 0.2 % curcumin were able to impart inhibition rate of 70 % and 95 % against *E. coli*, respectively. An equation was also established with good accuracy to predict the inhibition rate of bacteria growth using color strength values (K/S). The advantage of the developed equation was in the measuring of antibacterial property indirectly with no need to perform antimicrobial tests.

Using chitosan and turmeric, an attempt has also been made to develop green textiles for medical application. Kavitha et al. (2007) have studied the effects of chitosan pretreatment and subsequent dyeing with turmeric on the properties of cotton yarns. Improvements in mechanical properties such as tensile strength, flexural rigidity, and shear strength as well as natural dye up-take were achieved through chitosan treatment. Excellent antibacterial activity against two pathogenic bacteria, *E. coli* and *S. Aureus*, was another beneficial result of the treatment of cotton yarns with chitosan and turmeric.

In an interesting attempt to develop an eco-friend antibacterial finish using materials from green resources, curcumin along with two other natural bioactive agents, aloevera and chitosan, have been applied lonely or in binary or in ternary combinations on cotton, wool and rabbit hair (Ammayappan and Moses 2009). Then, their bactericidal properties in both pretreated and untreated substrates were examined. It was found that antibacterial activity of substrates finished after pretreatment with selective chemicals is higher than directly finished fabrics. Such behaviors were explained through enhanced wettability, critical surface tension change, and available area in the fiber responsible for better diffusion, adhesion and up-take of antibacterial agent.

When natural antibacterials applied individually on substrates, the antibacterial activity was in descending order: aloevera > curcumin > chitosan (Ammayappan and Moses 2009). The higher antibacterial activity of aloevera was concluded to be related to diverse ingredients, such as acemannon, anthraquinone, salicylic acid, amino acids, zinc, saponins, and many others compared to curcumin with individual benzenoid structure (only phenolic groups) and chitosan with polysaccharide structure (only hydroxyl and amino groups). Antimicrobial performance of binary or ternary combined agents was much better than corresponding individuals. It was explained through some determining factors, e.g., (I) the number and concentration of components present in natural material; (II) the number and diversity of functional groups and their determining role on affinity/binding to fiber; (III) Their mutual interaction with other chemical components and/or fibers and; (IV) synergistic, antagonistic or neutral effects of ingredients on each other in combined applications. Wash fastness tests proved that antibacterial finish durability on substrates are quite high where up to 25 wash cycles completely retained.

Choo and Lee (2002) have also tried to recreate traditional korean dyeing methods using colorants extracts from nine plants, used either singly or in combination, to produce a wide range of colors. In addition to madder, the extracts of indigo, turmeric, sapanwood, phellodendron, chinese yellow berries, gardenia, safflower and gromwell were used as dyeing agents and the fibers dyed were studied by microchemical tests, visible spectroscopy, thin layer chromatography and high performance liquid chromatography.

In a similar study, reconstruction of traditional textile dyeing techniques was the subject of the scientific research done by Zarkogianni et al. (2010). In this work, extractions of cochineal, madder, alkanna, henna, brazilwood, red sandalwood, safflower, indigo and logwood were obtained by aqueous extraction of the powdered mixture of plant/insect. These nine pigments were used for dyeing of wool and cotton fabrics after mordanting of the textile material with a variety of metallic salts. The results demonstrated significant improvement of the dye adsorption on the cotton fabrics and very good washing fastness of the wool samples.

The antimicrobial properties of 11 natural dyes from madder and other plants against three types of gram-negative bacteria were studied experimentally in an interesting work (Gupta et al. 2004b). The pigments were extracted from different parts of acacia, indigo, lac, kamala, pomegranate, gall nuts, cutch, myrobolan, himalayan rhubarb, indian madder and golden dock and were used for dyeing of wool fibers. The authors have reported that certain dyes are able to reduce microbial growth almost completely in the case of *Escherichia coli* and *Proteus vulgaris*, and since the selected dyes showed reasonably good wash fastness it could be assumed that the antimicrobial effect will be durable in practice.

The roots of a chinese herb, *Rhizoma coptidis,* is a source of natural cationic yellow colorant called berberine (Fig. 6.7). In a research work by Ke et al. (2006), the plant root was minced and dipped in distilled water for 30 min, then boiled in distilled water to extract the brownish yellow pigment and used for dyeing of wool fibers with mordanting.

Wool fabrics dyed with extraction of *R. coptidis* under different conditions showed various yellow colors with deep shades and good antibacterial properties, but the washing fastness of dyed fibers was poor.

Berberine has been similarly used as colorant and antimicrobial finish on nylon 66 fibers (Son et al. 2007). It imparted excellent bactericidal property to nylon fibers. It was concluded that finishing with berberine could be practical, eco-friendly, very simple and effective finish.

Das et al. (2008) carried out an interesting research on the dyeing of protein fibers using a natural dye. Wool and silk fibers were dyed with *Rheum emodi* extracted dye with/without Mn, Al, Fe metallic mordants and various shades from yellow to olive green were obtained. Chrysophanic acid, an anthraquinone derivative, is the main coloring matter of *Rheum emodi* whose structure is shown in Fig. 6.8. Interestingly, dyeing results exhibited no changes in the dye up-take of this dye in the pH range 4–8. Another finding was the unusual higher dye up-take, dye affinity, and dyeing rate of silk than wool fibers. The dyeing mechanism was shown corresponded to partition mechanism similar to those of disperse dyes.

**Fig. 6.8** Chemical structure of Chrysophanic acid the main color matter of *Rheum emodi*
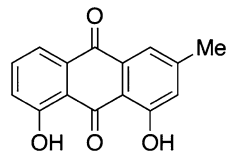
It was concluded that those observations were related to typical structure of dye component behaving like disperse dyes in the dyeing process. Wash and light fastness of dyed fibers were moderate where wash fastness was further enhanced with iron mordant.

Seed

Seed of the plants could be a rich source of natural dyes/antimicrobuals (Table 6.2). **Bixin** is a natural dye which has been exploited in yellow to orange coloration of textiles. It is an apocarotenoid found in annatto, a natural food coloring obtained from the seeds of the achiote tree, *Bixa orellana*. Annatto seeds contain about 5 % pigments, which consist of 70–80 % bixin. The chemical structure of bixin is shown in Fig. 6.9. Bixin is chemically unstable when isolated and converts through isomerization into trans-bixin, double-bond isomer. This extract is soluble in fats but insoluble in water. Upon exposure to alkali, the methyl ester is hydrolyzed to produce the dicarboxylic acid norbixin, a water-soluble derivative.

Wool and silk have been dyed using bixin natural dye with/without metallic mordants (Das et al. 2007). Bixin colorant was extracted from the seeds of annato in alkali solution. Wool and silk in the fiber and fabric form, respectively, were first pre-treated, then pre-, post- and simultaneous mordanted, and finally dyed with (10 % on weight of fiber) annatto solution at 90 °C at wide range of pH 2–10. The shades obtained were from yellow to brown depending on the mordants employed.

Annato dye up-take was increased when dyeing bath pH decreased and maximum dye up-take and color strength was obtained at pH 4.5. Bixin with carboxylate groups can be considered as an acid dye (Fig. 6.9). It was discussed that with decreasing pH, the amino groups in protein fibers were protonated, ionic interaction of bixin carboxylate anions with amino cations increased, and as a result the affinity and dye up-take increased (Das et al. 2007). Interestingly, the color imparting ability of mordants for both wool and silk fibers was found to be in the descending order with bixin dye: ferrous sulphate > aluminium sulphate > magnesium sulphate > no salt. It was attributed to the ease and rapid salt formation of iron and aluminium with bixin compared to magnesium.

Generally low light fastness was reported for bixin on both wool and silk fibers. After mordants incorporation through pre- and post mordanting, light fastness was enhanced by one point scale. It was ascribed to the good complex formation ability of iron with tannin, associated with bixin, and subsequent deposition on the fibers. However, such improvement was not that much marked in simultaneous mordanting due to early combination of iron with dye in the solution before sorption with fibers.

**Table 6.2** Characteristics of dyes/antimicrobials extracted from seed of the plants and their application properties

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Annato | *Bixa orellana* | Carotenoids (bixin, norbixin) | Yellow, orange, brown | Wool, silk with metallic mordants | Color | Wash(*M*) Light(*P*) | – | Das et al. (2007) |
| 2 | Bixin | *Bixa orellana* | Carotenoids | Yellow, orange, brown | Cotton | Color | Wash(*P*) Light(*M*) | Soxhlet extraction with n-hexane | Popoola (2000) |
| | Curcumin | *Curcuma longa* | Natural phenols | Yellow | Cotton | Wash(*M*) Light(*G*) | Soxhlet extraction with methanol | |
| 3 | Ban-ajwain | *Thymus serpyllum* | – | – | Cotton | Antibacterial | Wash(*VG*) | Soxhlet extraction with methanol | Sathianarayanan et al. (2011) |

[a]Excellent (E), Very good (VG), Good (G), Medium (M), Poor (P)
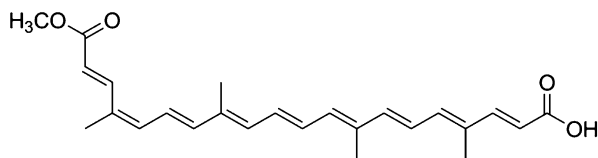
**Fig. 6.9** Chemical structure of bixin found in annatto seed

Magnesium did not improve the light fastness. Wash fastness data were in the range 2–3 without mordants. Wash fastness was improved by one point scale only in pre-mordanting condition (Das et al. 2007).

Popoola (2000) have studied bixin and curcumin in the coloration of cotton and compared their fastness properties. Bixin seeds and turmeric roots were used for extraction of coloring matter using soxhlet apparatus reflux system with n-hexane and methanol as extracting solvents, respectively. Afterwards, dyeing was performed without any mordant. The produced shades on cotton were yellow, orange and brown for bixin and yellow for curcumin. Both natural dyes had moderate light and alkaline wash fastness on cotton but, overall curcumin had higher light and alkaline wash fastness than bixin in identical conditions. It was attributed to the difference in chemical structure of coloring components, bixin with extended open-chain conjugated structure making it less stable against light and related photo-reactions compared to curcumin with two benzenoid rings, giving rise resonance stability, in its structure. Alkaline wash fatness data of both dyes were slightly higher than medium. But, similar to light fastness, curcumin dyed cotton had slightly higher wash fastness probably due to less solubility in equal alkaline condition.

In another report, Sathianarayanan et al. (2011) have tried to impart antibacterial finish on cotton fabrics using the bio-active ingredients extracted from ban-ajwain seeds, *Thymus serpyllum*. The extract was applied using different methods such as direct application, micro-encapsulation with acacia gum, crosslinking with resin, and the mixture of them. In direct application method, durability of finish against wash was very low. Wash fastness was enhanced with crosslinking and micro-encapsulation, however, some physical/mechanical properties were lost. Combined method gave the best fastness results so that the finish was durable up to 15 wash cycles.

Leaf

In some researches, leaves of different plants have been used as color resource (Iqbal et al. 2008; Ali et al. 2009; Giri Dev et al. 2009; Mongkholrattanasit et al. 2010, 2011) (Table 6.3). **Henna**, *Lawsonia inermis*, is a shrub or small tree frequently cultivated in India, Pakistan, Egypt, Yemen, Iran and Afghanistan. Henna has been used since ancient times to dye skin, hair, fingernails, leather, silk and wool. Henna's coloring and antimicrobial properties are due to lawsone (2-hydroxy-1,4-naphthoquinone), an organic compound also known as hennotannic

**Table 6.3** Characteristics of dyes/antimicrobials extracted from leaves of the plants and their application properties

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Henna | Lawsonia inermis L. | Lawsone: 2-hydroxy-1,4-naphthaquinone | – | Cotton with mordants | Much higher color strength with alkaline extract method | Light(M to G) | Alkaline extract method | Ali et al. (2009) |
| 2 | Henna | Lawsonia inermis | Naphthoquinone | – | Wool treated with chitosan | Color, antimicrobial | Wash(VG) Rub(M) Perspiration(M) | Aqueous ethanol extraction | Giri Dev et al. (2009) |
| 3 | Eucalyptus | Eucalyptus camaldulensis | Polyphenol (quercetin, rutin, ellagic acid, gallic acid) | Yellow to brown, grey | Wool, silk with metallic mordants | Color, UV protection | Wash(G to VG) Light(M to G) Rub(M to G) | Aqueous extraction | Mongkholrattanasit et al. (2011) |
| 4 | Tea | Camellia sinensis | Tannin | Black | Cotton, Jute with mordants | Deep shad on jute | Wash(G to E) Light(G to E) | Extracted with boiling water | Deo and Desai (1999) |
| 5 | Green tea | Camellia sinensis | – | – | Cotton | Antibacterial | Wash(M) | Methanolic extraction | Syamili et al. (2012) |
| 6 | Deciduous | Silver oak, Flame of the forest, Tanner's senna, Wattle, Serviceberry | Tannin | Yellow, brown, yellow, orange, dark grey | Wool with mordants | Color | Light(M to G) | Extraction with boiled water | Raja and Thilagavathi (2008) |
| 7 | Dom Sheng | Symplocos spicata | Flavonol | Yellowish brown | Cotton, wool, silk with metallic mordants | Color | Wash(VG) Light(VG) Rub(VG) | Aqueous extraction | Vankar et al. (2008) |

[a]Excellent (E), Very good (VG), Good (G), Medium (M), Poor (P)

**Fig. 6.10** Chemical structure of lawsone, a red orange dye extracted from henna leaves
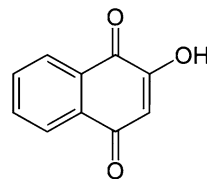


acid (Fig. 6.10). Lawsone is a red-orange dye primarily concentrated in the leaves, especially in the petioles of the leaf. Lawsone reacts chemically with the protein known as keratin in skin and hair, in a process known as Michael addition, resulting in a strong permanent stain that lasts until the skin or hair is shed. Lawsone strongly absorbs UV light, and aqueous extracts can be effective sunless tanning and sunscreens.

As an example of application, the extract of henna leaves has been used for dyeing of cotton fibers (Ali et al. 2009). The results revealed that extracts produced with alkaline extraction of henna leaves have moderate to good fastness properties and better color strength than the dye extracts obtained in distilled water.

In a similar study to develop an eco-friend, nontoxic and multifunctional finish on wool, Giri Dev et al. (2009) studied the role of chitosan treatment, individually and in combination with natural henna dye, on the antimicrobial property and dyeability of wool fabrics. They found that chitosan simultaneously showed bi-functional effects, i.e., imparted antimicrobial property against two common pathogenic bacteria, *S. aureus* and *E. coli*, and enhanced dye up-take. Fastness tests demonstrated good wash, light, and perspiration durability of finish on fabrics.

In another attempt to extend eco-friendly textiles processing techniques, Iqbal et al. (2008) have tried to improve the color strength and color fastness properties of henna dye on cotton fabric through UV irradiation treatment. They irradiated cotton and henna dye separately, extracted coloring matter using water, alum, and methanol, dyed irradiated cotton with the dye extract and subsequently compared the results with non-irradiated samples. They found that, generally, irradiated dyed samples demonstrate more color strength and deeper shades, and color fastness enhanced from medium to good level.

The observed behaviors were explained from one hand via enhanced affinity and facile sorption of hydrolytically degraded lawsone, decrease of insoluble impurities in the case of irradiated henna, and on the other hand via an increase of number and size of irradiated fiber pores and carboxylic acid groups produced upon UV photo-oxidation of cotton fiber (Iqbal et al. 2008). The industrial aspects of the process were discussed as well and the optimum dyeing condition were introduced as following: 65 °C dye bath temperature, 120 min dyeing time, 3 % NaCl, and 4 % copper sulphate (pre-mordanting).

**Eucalyptus**, *Eucalyptus camaldulensis*, is another example of natural dye resource. It is a rich source of natural polyphenols and tannins. Leaves contain 11 % quercetin as major component. Other components are rutin, gallic acid, ellagic acid, etc. that are very useful in dyeing yield and durability of substrate (Mongkholrattanasit et al. 2010, 2011). Chemical structures of these coloring matters are shown in Fig. 6.11.
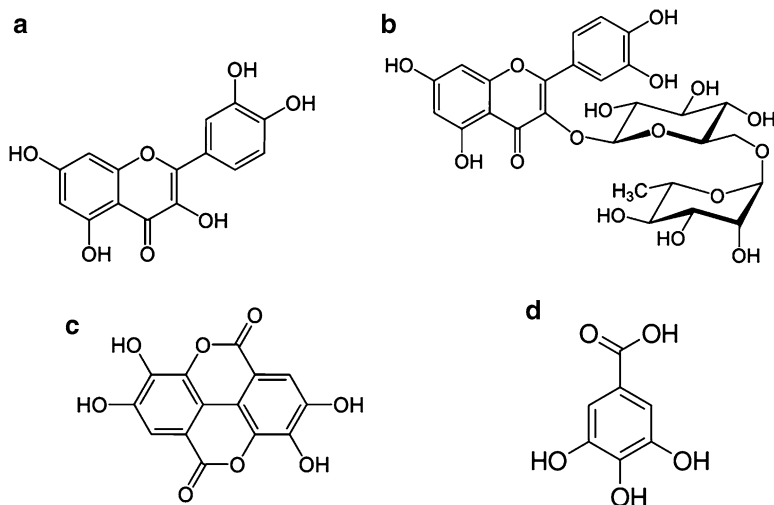
**Fig. 6.11** Chemical structures of (**a**) quercetin, (**b**) rutin, (**c**) ellagic acid and (**d**) gallic acid, extracted from eucalyptus leaf

Mongkholrattanasit et al. (2011) demonstrated that water extract of eucalyptus leaves could be effectively used in exhaustion coloration process of wool and silk. They used aluminium, iron, copper, and tin as mordants. The optimum dyeing condition was determined to be at pH 4, time 40 min, and temperature 90 °C. Wide variety of shades was achieved where wool fibers had higher color strength than silk fibers. Shades obtained were yellowish brown with aluminium and copper, dark grayish brown with iron and bright yellow with tin on both wool and silk substrates. Protection against UV was additional property achieved for both substrates. Both fibers showed good to very good wash fastness and medium to good light and rub fastness.

**Tea** plant, *Camellia sinensis*, is the rich resource of different beneficial natural components such as antioxidants, flavanols, flavonoids, polyphenols, and catechins. It is processed using various methods to produce variety kinds of teas: green, yellow, dark, white, oolong, and black. Aqueous extract of tea could also be used for textiles. Deo and Desai (1999) have studied the dyeing of cotton and jute with an aqueous extraction of black tea where the tannins were the main colorant. The mixture of black tea was stirred, heated, held at the boil for 30 min, allowed to stand for 15 min and then filtered and used as black pigment for dyeing of fabrics, where ferrous sulphate heptahydrate, alum and copper sulphate pentahydrate were used as mordants. The results showed deep color shad on jute fabrics, while good to excellent washing and light fastnesses were observed.

In a related study and with the aim to production of eco-friendly medical textiles, Syamili et al. (2012) have tried to apply extract of green tea leaves onto cotton to impart antibacterial properties. Various tests were performed to identify the effective ingredients of green tea. The ingredients were extracted using ethanol, chelated with

copper to exploit intrinsic bactericidal property of copper, applied on cotton with and without citric acid as crosslinker agent. Significant inhibition against bacteria, *S. aureus* and *E. coli,* growth was obtained for finished fabrics.

The wash durability of antibacterial finish was tested and the results showed that after 10 wash cycles the bacterial inhibition rate was less than 25 % for fabrics finished directly with no crosslinker agent and more than 50 % for crosslinked finished fabrics. It was concluded that to increase the durability of antibacterial finish, extracted components should be chemically fixed onto substrate surface using proper crosslinker. This finish process was also introduced as an eco-safe, facile and economic process (Syamili et al. 2012).

Similarly, the leaves of deciduous plant with high content of tannin have been used as a natural dye sources for dyeing of wool (Raja and Thilagavathi 2008). The colorant agent of five different deciduous plants, namely, silver oak, flame of the forest, tanner's senna, wattle and serviceberry, was extracted by boiling 250 g of the leaf material with 5 l of water for 1 h and, were used with mordants to dye wool. The authors have introduced the use of plant leaves more eco-friendly and economical than using their barks or other parts, showing a moderate to good fastness of the fibers dyed.

*Symplocos spicata* plant leaves extract have also been used in ultrasound coloration of cotton, wool, and silk with Al, Cu, Fe, Cr, Sn(*II*), Sn(*IV*) mordants (Vankar et al. 2008). An additional pretreatment with tannic acid was also done for cotton to provide carboxylic acid groups on the fiber surface for better mordanting. Dye up-take was markedly improved and became almost double in ultrasonic dyeing compared to common exhaustion dyeing process. Yellow to brown shades with high color fastness properties were obtained for all fibers. Lower required time, energy, and dye amount to obtain the same results of conventional dyeing process proved the superiority of ultrasonic natural dyeing technique.

Fruit

Fruits of the plants are another source of natural dyes and have been extensively used for textile dyeing (Table 6.4). **Pomegranate** is the fruit of deciduous shrub or small tree, *Punica granatun*. Fruit rind has been widely used in traditional coloration of textiles since ancient times. However, in open literature there are rare reports addressing the application of pomegranate rind on textiles scientifically except some recently published papers (Çalıs et al. 2009; Sathianarayanan et al. 2010; Kulkarni et al. 2011a; Rajendran et al. 2011). Rind of the pomegranate is a rich resource of tannins, phenolic contents, alkaloids, etc. Granatonine is the main coloring component found in the fruit rind in the form N-methyl granatonine (Fig. 6.12).

The extract of pomegranate rind has been used in environmentally friendly dyeing (Kulkarni et al. 2011a) and bactericidal treatment (Sathianarayanan et al. 2010; Rajendran et al. 2011) of cotton fabric. Wide range of shades such as yellow, brown, and black with good fastness properties were achieved using copper and iron mordants with different ratios (Kulkarni et al. 2011a).

**Table 6.4** Characteristics of dyes/antimicrobials extracted from fruits of the plants and their application properties

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Pomegranate (*peel*) | *Punica granatum* | Alkaloid (granatonine) | Yellow, brown, black | Cotton with metallic mordants | Color | Wash(*G*) Light(*G*) Rub(*G*) | Soxhlet extraction with aqueous ethanol | Kulkarni et al. (2011a) |
| 2 | Tulsi(*leaf*) Pomegranate (*rind*) | *Ocimum sanctum Punica granatum* | Eugenol, germacrene, phytol, tannin | – | Cotton | Antibacterial | Wash(*G*) | Soxhlet extraction with methanol | Sathianarayanan et al. (2010) |
| 3 | Pomegranate (*peel*) | *Punica granatum* | Alkaloid | – | Cotton with metallic mordants | Color, anti-microbial | Wash(*G*) Light(*G*) Rub(*G*) Per-spiration(*G*) | Aqueous extraction, Ethanolic extraction | Rajendran et al. (2011) |
| 4 | Pomegranate (*rind*) | *Punica granatum* | Tannin, alkaloid | Yellow, grey | Wool, silk with metallic mordants | Color | Wash(*G*) Light(*G*) Rub(*G*) | Aqueous extraction, Ethanolic extraction | Das et al. (2006) |
| 5 | Madder(*root*) Onion(*skin, pulp*) Pomegranate (*rind*) Peppermint(*leaf*) | *Rubia tinctorum, Allium cepa, Punia granatum L., Mentha SP* | Anthraquinone, flavoniod, tannin, terpinene menthol | – | Wool with metallic mordants | Color, anti-microbial | – | Aqueous extraction | Çalıs et al. (2009) |
| 6 | Fruit | *Opuntia ficus-indica* | Indicaxanthin, betanin | – | Wool with mordant | Color | Wash(*G*) | Extraction with ethanol | Guesmi et al. (2012) |

| 7 | Fruit | *Opuntia ficus-indica* | Indicaxanthin | Orange-yellow | Modified acrylic fibers | Improvement of dye uptake by sonicator dyeing | Light(*M*) | Extraction with ethanol | Guesmi et al. (2013) |
|---|---|---|---|---|---|---|---|---|---|
| 8 | Prickly pear(*fruit*) | *Opuntia Lasia-cantha* Pfeiffer | Betalain | Red | Wool with mordants | Showing high bacteria inhibition activity | Light(*G*) | Extraction with water | Ali and El-Mohamedy (2011) |
| 9 | Mangosteen (*hull*) | *Garcinia mangostana Linn* | Anthocyanin | Red | Cotton, Silk with metallic mordants | Color | Wash(*G*) Light(*G*) Rub(*G*) | With aqueous citric acid solution | Chairat et al. (2007) |
| 10 | Amla(*fruit*) | *Emblica officinalis G.* | Tannin | – | Cotton, Silk with natural and metallic mordants | Antibacterial | Wash(*G*) | Ethanolic extraction | Prabhu et al. (2011) |
| 11 | Green chili | *Capsicum annum* | Oleoresin | Yellow | Cotton with modrants | – | Wash(*M*) Light(*G*) Rub(*G*) | Solvent extraction method | Kulkarni et al. (2011b) |

[a]Excellent (E), Very good (VG), Good (G), Medium (M), Poor (P)

**Fig. 6.12** Chemical structure of N-methyl granatonine found in the fruit rind of pomegranate
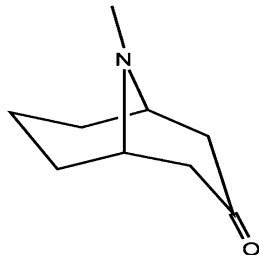
As an example, pomegranate rind extract was used for bi-functional treatment of cotton, i.e., color and bactericidal finish (Rajendran et al. 2011). The dye was extracted using water and ethanol, separately. The dye extract showed good affinity for cotton, and was applied with alum and copper. Good wash, light, rub, perspiration fastnesses and excellent antibacterial property against common bacteria, *S. Aureus* and *E. coli*, was resulted. However, antibacterial property steadily decreased by successive wash cycles.

Pomegranate rind has also been utilized for dyeing of wool and silk with/without mordants (Das et al. 2006; Çalış et al. 2009). The best dyeing pH was found to be four. Iron and aluminium were resulted in grey and yellow shades on fibers, respectively. Dye up-take, and light fastness were improved with mordants, however, wash fastness was not markedly changed (Das et al. 2006).

In a similar study, four common natural dyes were applied on wool and resulting antimicrobial effect was examined (Çalış et al. 2009). Bacterial growth reduction of 4–80 % for pomegranate rind, *Punica granatum*, 53–86 % for onion, *Allium cepa L.*, 32–52 % for madder, *Rubia tinctorium L.*, and 28–91 % for peppermint, *Mentha piperita L.*, was obtained against variety of common pathogenic bacteria used.

Guesmi et al. (2012) have extracted indicaxanthin from *Opuntia ficus-indica* fruit and used for dyeing of textile fabrics. Extraction method was to mix 50 g of juice from cactus pears with 100 ml of 80 % aqueous ethanol as solvent. This plant contains mainly two dyes, indicaxanthin and betanin (Fig. 6.13).

Extracted indicaxanthin was used for dyeing of wool fabrics with different mordants and the results showed that the fabrics dyed have good washing fastness, where $KAl(SO_4)_2$, $MnSO_4$ and $CoSO_4$ gave the best results.

In a similar work, indicaxanthin has been used for dyeing of modified acrylic fibers by both conventional and ultrasonic techniques (Guesmi et al. 2013). The authors have reported that sonicator dyeing with this orange-yellow natural dye showed marked improvement in dye uptake and fair to good fastness properties of the dyed fabrics.

Relevantly, Ali and El-Mohamedy (2011) have tried to extract the dyeing agent of red prickly pear plant, *Opuntia lasiacantha* Pfeiffer, and used it for dying of wool with different types of mordants. Betalain (Fig. 6.14), the red dye extracted from this plant, was used for dyeing of wool fabrics with ferrous sulfate, copper sulfate, potassium dichromate and tannic acid as mordants.
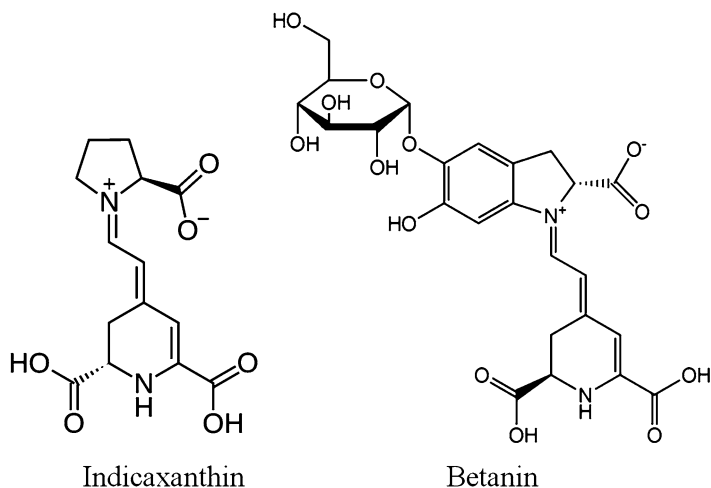
**Fig. 6.13** Chemical structures of indicaxanthin and betanin extracted from *Opuntia ficus-indica* fruit

**Fig. 6.14** Betalain pigment structure extracted from *Opuntia lasiacantha* Pfeiffer fruits
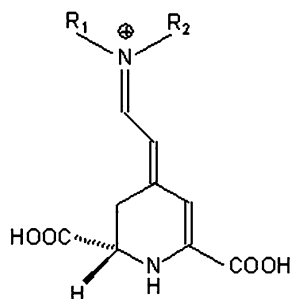
The fabrics dyed with betalain showed high bacteria inhibition activity and good fastness property.

*Garcinia mangostana Linn*, is a tropical evergreen tree which grows especially in Thailand, Indonesia, Malaysia, and Philippines. It is known for its best tasting fruits. Fruit shells contain various useful natural components such as mangosteen that is a red pigment. Chairat et al. (2007) have extracted mangosteen using an aqueous acetic acid solution where extract solution had dark red color. The extract solution was used in dyeing of silk and cotton with Al, Ca, Fe, Zn, and a natural mordant. Generally, color depth, wash and light fastnesses of dye were markedly improved using mordants and the best results obtained in post-mordanting method with Fe and Ca.

*Emlica officialis G.* fruit is a rich resource of natural tannins used as natural mordants. It contains mainly two hydrolysable tannins that on hydrolysis give gallic acid and ellagic acid. Prabhu et al. (2011) have used the ethanol isolated tannins
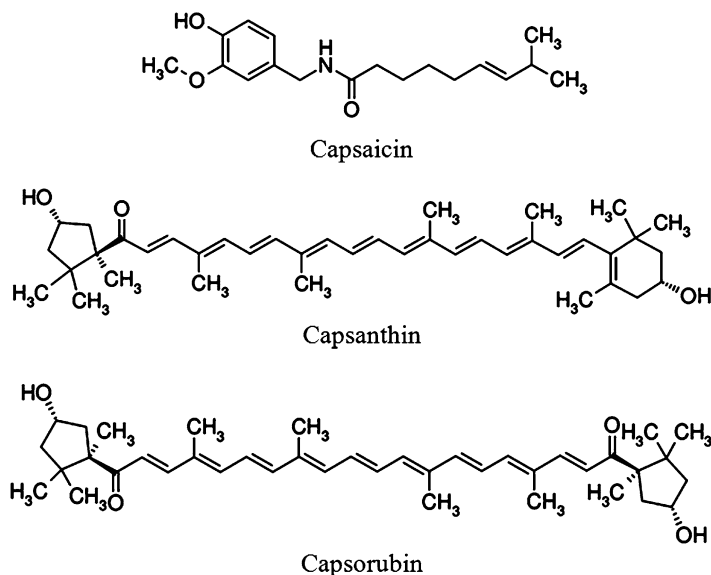
**Fig. 6.15** Chemical structure of oleoresin ingredients; capsaicin, capsanthin and capsorubin

individually and/or in mixture with copper sulphate for enhancement of color yield and color fastness properties in coloration of cotton and silk using natural dyes. Four natural dyes used were turmeric, *Curcuma longa L.*, pomegranate rind, *Punica granatum L.*, henna, *Lawsonia inermis L.*, and indian madder, *Rubia cordifolia L.* Different shades were obtained using these dyes together with mordants showing good color fastness and color strength. Antibacterial test was also carried out to evaluate the bactericidal property of *Emlica officialis G.* on cotton and silk and it was found that cotton and silk treated with this natural mordant have good antibacterial activity which was durable up to 20 washes.

In a similar work, Kulkarni et al. (2011b) have tried to produce different shades of yellow color using the dye extracted from chili skin. The yellow pigment of *Capsicum annum*, that mainly contains oleoresin, was extracted by solvent extraction method and was used for dyeing of cotton fibers by mordants. Oleoresin is an oil soluble extract from the fruits of *Capsicum annum Linn*, and is primarily used as a coloring and/or flavouring in food products. It is composed of capsaicin, the main flavouring compound giving pungency in higher concentrations, and capsanthin and capsorubin, the main coloring compounds (Pérez-Gálvez et al. 2003) (Fig. 6.15).

Based on the results obtained, the authors have reported good light and rub fastness, and moderate wash fastness for the fabrics dyed with the green chili extract.

Bark

Some studies have reported the use of bark of various plants as natural dye resource (Table 6.5). Reuse of ash-tree bark for extraction of a natural dye for textile dyeing

**Table 6.5** Characteristics of dyes/antimicrobials extracted from bark of the plants and their application properties

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Ash-tree | *Fraxinus excelsior* L. | *Quercetin* | Beige olive | Wool with mordant | Good shade re-producibility and levelness of dyed fibers | Light(*G*) | Extracted with distilled water | Bechtold et al. (2007) |
| 2 | Chandada(*bark*) | *Macaranga Peltata* | – | Yellow, red | Silk | Color | – | Soxhlet extraction with methanol | Vinod et al. (2010) |
| 3 | Barberry(*bark*) | *Berberis aristata* DC. | – | Wide range of shades | Silk with metallic mordants | Color | Wash(*P*) Light(*VG*) Rub(*VG*) | Aqueous extraction | Pruthi et al. (2008) |
| 4 | – | *Sticta coronate lichen* | – | Lilac (pale purple) | Silk with metallic, natural mordants | Color | – | Ultrasonic extraction with aqueous acetone | Mansour and Heffernan (2011) |
| 5 | Ficus(*bark*) | *Ficus Religiosa L.* | – | Orange, red, brown | Silk with metallic, natural mordants | Color | Wash(*G*) Light(*G*) Rub(*G*) | Aqueous extraction | Saravanan and Chandramohan (2011) |
| 6 | Jackfruit(*wood*) | *Artocarpus heterophyllus Lam* | Flavanol (Hydroxy-flavone) (Morol) | Yellow to golden brown | Cotton, jute with metallic, natural mordants | Color | Wash(*VG*) Light(*M*) Rub(*G*) | Aqueous extraction | Samanta et al. (2007) |

(continued)

**Table 6.5** (continued)

| Source No. (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|
| 7 Jackfruit wood Manjistha/ Madder (root) Red sandalwood Marigold (flower) Babool(bark, wood) | Artocarpus heterophyllus L., Rubia cordifolia, Pterocarpus santalinius, Tagetes patula/Tagetes erecta, Acacia arabica | Morol putpurin, manjistin, pupuroxanthin, pseudo-purpurin, nordamncanthal, santalin A, santalin B, deoxyxantalin, flavonoid, cretonoid, polyphenol | Variety of shades | Jute with natural, metallic mordants | Color | Wash(VG) Light(M) Rub(VG) | Aqueous extraction | Samanta et al. (2008) |

[a]Excellent (E), Very good (VG), Good (G), Medium (M), Poor (P)

was the subject of an interesting research (Bechtold et al. 2007). A quercetin-rich beige olive pigment extracted with distilled water from ash-tree bark, *Fraxinus excelsior* L., was used for dyeing wool fabrics with different mordants. Good shade reproducibility and levelness of the dyed material along with the acceptable fastness to light and wash were obtained.

Again, the colorant extracted from the tree bark has been used by Vinod et al. (2010) for dyeing of textile fabrics. In this work, pigment from the bark of *Macaranga peltata* was extracted by methanol and, using spectral techniques, ellagic acid was identified as the main coloring agent. Then, this yellow pigment was used for dyeing silk with tannic acid and potash alum $[Al(NH_4)(SO_4)_2.12H_2O]$ as mordant, and the results demonstrated good fastness of dyed fabrics.

Barberry plant, *Berberis aristata DC.*, is another plant that its bark has been used in textile coloration (Pruthi et al. 2008). Silk was dyed with aqueous extract of barberry bark and post-mordanted with different combination of various mordants. Wide range of shades was obtained with high dye fastness properties. Based on the results several conclusions were drawn: best dyeing method for silk is simultaneous mordanting because of the highest dye up-take in this method; alum must be used in combination with other mordants due to its dye-up take improvement ability; the use of combination of mordants produce wide range of shades.

Similar attempt has been made to make use of *Sticta coronata* lichen as an abundant resource of natural dye in New Zealand in ultrasonic dyeing of silk (Mansour and Heffernan 2011). Catechu, *Acacia catechu*, a natural mordant and alum were used in dyeing process. Ultrasound energy improved dye up-take markedly. The dye showed good affinity and produced brilliant lilac color with good fastness on silk fibers.

In similar study, bark of Peepal tree, *Ficus religiosa* L., were used in natural dyeing of silk (Saravanan and Chandramohan 2011). The color component was extracted with water and then was used for dyeing of silk with metallic mordants such as Al, Cu, Cr, Ni, and Sn and natural mordants like myrobolan, *Terminalia chebula*, and cow dung. Myrobolan or harda is an important natural mordant with chebulinic acid as main ingredient whose chemical structure is shown in Fig. 6.16. The color fastness properties on fibers were overall acceptable.

Similarly, Samanta et al. (2007) have used the extraction of Jackfruit wood, *Artocarpus heterophyllus* Lam, in coloration of cotton and jut fibers with myrobolan, aluminium, iron, tin and EDTA mordants. The main coloring component of jackfruit is morol, a typical flavanol that imparted yellow to golden brown shade with various mordants on cotton and jut fibers. The optimum dyeing condition was, time 90 min, temperature 70–90 °C, 20–30 % mordant, 30–40 % dye with pre-mordanting. Good color fastness properties were reported for both dyed fibers.

After that, Samanta et al. (2008) in another study have used binary mixture of jackfruit extract with other natural dyes, manjistha, red sandal wood, marigold, sappan wood, and babool in dyeing of jute fibers with myrobolan and aluminium mordants. They also examined the compatibility of mixed dyes. After dyeing, additional treatment with various chemicals was performed to enhance wash and light fastness.

**Fig. 6.16** Chemical structure of chebulinic acid, the main component of myrobolan natural mordant
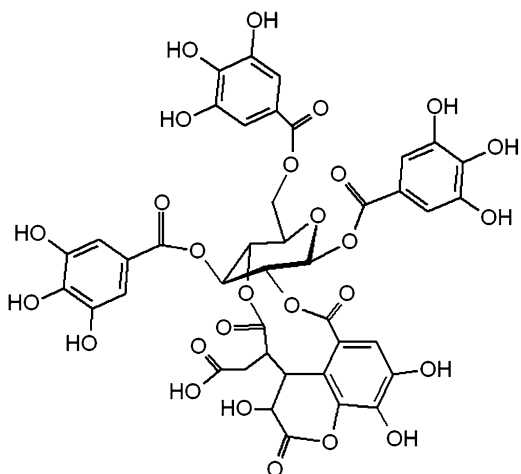
Flower

Natural dyes/antimicrobials can also be extracted from flower part of various plants (Table 6.6). Pigments extracted from marigold flowers, *Tagetes,* with a quite dark and saturated yellow to orange red color on textiles is another example of natural dyes/antimicrobials. The aqueous extraction of african marigold, *Tagetes erecta* L., that contains mainly lutein has been used for dyeing of cotton and silk fabrics with mordants (Jothi 2008). The author has reported good light fastness and good to excellent wash fastness for the fabrics dyed with this natural yellow pigment.

In a similar work, Guinot and co-workers (2008) have evaluated the dyeing potential of pigments from marigold flowers, *Tagetes patula* L. Flavonoids were obtained by water-ethanol mixture extraction technique, giving the highest extraction efficiency, and were used for dyeing of woven wool with mordants. Based on the results observed, the authors have introduced marigold flower as a potential dyeing plant.

A greenish yellow natural dye called luteolin was also extracted by methanol from leaves, stems and flowers of weld (*Reseda luteola*) (Cerrato et al. 2002). Then, it was used for dyeing of cotton and wool fibers with alum as mordant. A composite design experiment allowed the effects of particle size and liquid/solid ratio to be determined and the results demonstrated that luteolin concentrated extract could be used for home dyeing of fabrics of kits specially designed textiles.

Also, Vankar and Shanker (2009) have tried the pretreatment of silk fiber in an eco-friendly dyeing with flower extract. A bright reddish brown pigment, containing quercetin, was extracted by soaking the dried and ground flower sample, *Delonix regia,* in distilled water and then, was used for fiber dyeing with mordants.

Wool fabrics have also been dyed with catechu (*Acacia catechu*) extract by Bhattacharya and Shah (2000). The light brown pigment, containing mainly tannin,

**Table 6.6** Characteristics of dyes/antimicrobials extracted from flower of the plants and their application properties

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | African marigold | *Tagetes erecta* L. | Lutin | Yellow to orange red | Cotton and silk fabrics with mordants | – | Wash(*G to E*) Light(*G*) | Extraction with water | Jothi (2008) |
| 2 | Marigold flower | *Tagetes patula* L. | Flavonoid | – | Woven wool with mordant | Development of new natural coloring matters | – | Extraction with water-ethanol mixture | Guinot et al. (2008) |
| 3 | Weld | *Reseda luteola* | Luteolin | Greenish yellow | Cotton and wool with mordants | Color | – | Extraction with methanol | Cerrato et al. (2002) |
| 4 | Flower | *Delonix regia* | Quercetin | Bright reddish brown | Silk with mordant | Eco-friendly textile pretreatment that does not utilize metal mordanting | Light(*M*) | Extraction with water | Vankar and Shanker (2009) |
| 5 | Catechu | *Acacia catechu* | Tannin | Light brown | Wool with mordant | Production of different colors | Light(*G*) | – | Bhattacharya and Shah (2000) |
| 6 | African tulip tree | *Spathodea campanulata* | – | Various shades | Cotton with metallic natural mordants | Color | Wash(*E*) Light(*M*) Rub(*E*) Perspiration (*G-E*) | Aqueous extraction | Kumaresan et al. (2012) |

(continued)

**Table 6.6** (continued)

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 7 | Plants | *Acacia catechu, Kerria lacca, Quercus infectoria, Rubia cordifolia, Rumex maritinus* | – | – | Wool | Antimicrobial activity | – | – | Singh et al. (2005) |
| 8 | Oak | *Quercus infectoria* | – | – | Cotton with mordants | Antimicrobial | – | – | Gupta and Laha (2007) |
| 9 | Herb leaves, stem and flower | *Ricinus communis, Senna auriculata, Euphorbia hirta* | – | – | Denim fabric | Antibacterial | – | Methanol extraction | Sumithra and Raaja (2012) |
| 10 | Herb | *Sappanwood* | – | – | Wool with mordant | Anti-ultraviolet effect | Rub(*G*) | Dissolution and extraction | Zhang and Cai (2011) |

[a]Excellent (E), Very good (VG), Good (G), Medium (M), Poor (P)

was used for dyeing of fabrics with different mordants. Different colors produced on wool fabric with catechu using various metal mordants. Moreover, the fastness properties were sufficiently good for practical dyeing.

*Spathodea campanulata* is a flowering tree that is planted extensively in tropical countries as an ornamental tree. It has very showy reddish-orange or crimson (rarely yellow), campanulate flowers. Kumaresan et al. (2012) have investigated the dyeing properties of cotton with dye extracted from *Spathodea campanulata* flowers without and with various binary mixtures of natural/metallic mordants. Extraction of dyed was carried out in boiling water and ethanol separately. Ethanol extract was good to obtain brown shades. Generally, in almost all mordant-dye-fiber cases, color fastness to wash, rub, and perspiration was quite high. It was inferred that this dye can be used as an eco-friendly natural dye in coloration of textiles.

On the other hands, antibacterial activity of fibers dyed with extracts from plants has been widely investigated by the scientists. The antimicrobial activity of five natural dyes, *Acacia catechu, Kerria lacca, Quercus infectoria, Rubia cordifolia, Rumex maritimus*, on wool fabrics has been shown by Singh et al. (2005). Moreover, tannin-rich extract of oak has been used as an antibacterial agent for textiles by Gupta and Laha (2007). In this work, the extract of *Quercus infectoria* plant in combination with alum, copper and ferrous mordants have been used as antibacterial agents for treatment of cotton fabrics. The results showed that the cotton textiles can be successfully treated with this extract to show antibacterial activities against both Gram-positive and Gram-negative bacteria.

In a similar work, the antibacterial activity of extracts from herb leaves, stem and flower has been studied (Sumithra and Raaja 2012). The methanol extracts of *Ricinus communis*, *Senna auriculata* and *Euphorbia hirta* were examined for their antimicrobial efficiency on the four variant of denim fabrics directly by using dip method and the findings demonstrated that the fabrics had good antibacterial activities. Similarly, the natural pigment from sappan was used for coloration of wool fabrics after treatment with the protease and transglutaminase (Zhang and Cai 2011). The pigment of sappan was extracted by dissolving the herb in distilled water and allowed to boil in a beaker kept over water bath for quick extraction. Then, this pigment was used for dyeing of wool fabrics with ferrous sulphate, potassium alum, and tannic acid as mordants. This process had a good anti-ultraviolet effect on the fibers while modification with protease led to some decrease in wet rub fastness, whereas transglutaminase had almost no influence on rub fastness. But, the treated fibers had good dry rub fastness.

Other Parts of Plants

Natural extracts of other parts of plants have also important dyeing/antimicrobial properties and their application on textile has been widely studied (Table 6.7).

A natural dye was extracted by ultrasonic method from a vegetable with botanic name *Sargentodoxa cuneata,* and its application on wool fabric was studied (Xinsheng et al. 2008). The authors have reported that the color of fabrics dyed with ultrasonic extraction solution is deeper.

**Table 6.7** Characteristics of dyes/antibacterial extracted from other parts of plants and their application properties

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Vegetable | *Sargentodoxa cuneata* | – | – | Wool with mordant | Color | – | Ultrasonic extraction technique | Xinsheng et al. (2008) |
| 2 | Weld, buckthorn, bastard hemp | *Rhamnus petiolaris* Boiss, *Datisca cannabina* L., *Reseda luteola* L. | Flavonoid and anthraquinone | Yellow | Wool with mordant | Color | – | Extraction of with hydrogen chloridemethanol-water mixture | Deveoglu et al. (2012) |
| 3 | Plant | *Dahlia variabilis* | Flavonoids – anthocyanins | – | Wool with mordant | Good potential to replace phenolphthalein as an indicator | Light(*G*) | Ultra sound assisted extraction | Mishra et al. (2012) |
| 4 | Madder, weld and woad | *Reseda luteola* L., *Isatis tinctoria*, *Rubia tinctoria* | – | Red Yellow Blue | Cotton with mordant | Color | Light(*P*) | – | Cristea and Vilarem (2006) |
| 5 | Tesu | *Buteamonosperma frondosa* | Butein | Yellow, Orange, Brown | Jute with metallic, natural mordants | Color | Wash(*G*) Light(*G*) Rub(*G*) | Aqueous extraction | Samanta et al. (2011) |
| 6 | Silver oak | *Grevillea robusta* | Tannin | – | Wool with enzyme and mordants | Color, antimicrobial | – | Aqueous extraction | Raja and Thilaghavati (2011) |
|  | Flame of forest | *Spathodea campanulata* | – |  |  |  |  |  |  |
|  | Tanner's senna | *Cassia auriculata* | – |  |  |  |  |  |  |
|  | Wattle | *Acacia decurrens* | Tannin |  |  |  |  |  |  |

[a]Excellent (E), Very good (VG), Good (G), Medium (M), Poor (P)

In a similar work, the mordanted wool fabrics with alum, were dyed in 50 % *Reseda luteola* L. (weld), 20 % *Rhamnus petiolaris* Boiss (buckthorn) and 50 % *Datisca cannabina* L. (bastard hemp) dye-baths (Deveoglu et al. 2012). Extraction of yellow pigments, containing mainly flavonoid and anthraquinone, was carried out with a hydrogen chloridemethanolwater mixture. The authors have reported as a conclusion that, it would be possible to analyze and to identify the natural dyes present in historical textiles using a reverse-phase high-performance liquid chromatography.

Natural dye from *Dahlia variabilis* have been similarly used for dyeing of textile (Mishra et al. 2012). The flavonoids – anthocyanins rich colorant was extracted by ultra sound assisted technique and was used for dyeing of wool fibers with alum and milk of tartar as mordants. The authors have reported good fastness properties of dyed fibers as well as intrinsic potential of this dye to replace phenolphthalein as a pH indicator.

Cristea and Vilarem (2006) have also studied the light fastness of selected natural dyes from madder, weld and woad. Red, yellow and blue extracts of *Rubia tinctoria*, *Reseda luteola L.* and *Isatis tinctoria*, respectively, were used for dyeing of cotton with mordants. The authors have concluded that, in spite of poor fastness of fiber dyed with these three natural dyes, using UV absorbers or antioxidants improves the light fastness of dyed fabrics.

Samanta et al. (2011) have used aqueous extract of flower petals of tesu flowering tree, *buteamonosperma frondosa*, in coloration of jute fabric in alkaline medium to obtain various shades like creamish orange, yellowish orange, ochre brown, and reddish brown. The main color component in the yellowish red/orange extract was butein (Fig. 6.17). Good color fastness and color yield was reported for proper selected dyeing condition (dye-fiber-mordant system) and further improvements in light fastness were obtained with proper post-chemical treatment.

In an interesting and recent research work, Raja and Thilaghavati (2011) have tried to make use the inherent antibacterial characteristic of some natural dyes in bi-functional environmentally friendly treatment of textiles. In their work, four natural dyes, with in vitro antimicrobial activity against both gram positive and gram negative bacteria, were chosen. The effect of mordant and protease enzyme treatment of wool on the bactericidal performance of natural dyes were examined and compared with untreated wool results. Various test results showed higher dye up-take and antibacterial property in enzyme treated wool that was ascribed to increased dye diffusion and affinity due to hydrolysis of outer layer of wool. Moreover, although the color fastness properties of dyed wool were increased upon mordant treatment, but none of dyed fibers mordant treated showed antibacterial activity. Such behavior was attributed to the blocking of bio-active ingredients in natural dye through possible complex formation of metal ion with those components (Raja and Thilaghavati 2011).

**Fig. 6.17** Chemical structure
of butein extracted from
flower petals of tesu
flowering tree
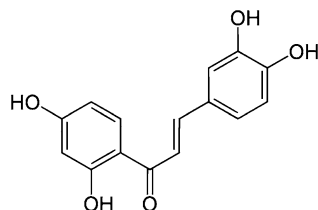


## Dyes/Antimicrobials from Microorganisms

The main disadvantage of natural dyes/antimicrobials from plants is the low
efficiency of the extraction process (a few grams of pigment per kg of dried raw
material). This makes their application limited to high-value-added natural-colored
garments only (De Santis et al. 2005). To overcome this drawback, it was suggested
to exploit the potentiality of microorganisms such as fungi, bacteria and algae that
are fast growing and have the potential of being standardized commercially (Sharma
et al. 2012).

There are several microorganisms which can produce pigments, which are
one of the important classes of secondary metabolites and are often referred to
as biopigments. Microorganisms produce various pigments including carotenoids,
melanins, flavins, quinones, prodigiosins and more specifically monascins, violacein
or indigo (Khanafari et al. 2006; Venil and Lakshmanaperumalsamy 2009). So,
researchers have recently focused on the production, identification and application
of these pigments on textiles and have studied the dyeing and antimicrobial
characteristics of the dyed fibers (Table 6.8).

**Chitosan** is a linear polysaccharide produced commercially by deacetylation of
chitin, the structural element in the crustaceans such as crabs and shrimp and cell
walls of fungi.

Chitosan, *N*-deacetylated derivative of chitin that is a linear polysaccharide, is
also an important natural antibacterial agent. It has been used in wool finishing, but
its weak binding to the wool constitutes the main problems in its application. For
instance, Ranjbar-Mohammadi and her co-workers (2010) have studied the grafting
of chitosan onto wool fabric using anhydride bridge and its antibacterial property.
The results showed that the wool fabrics treated by chitosan had a considerable anti-
microbial and antifelting activities as well as good wash fastness.

In a similar works, chitosan has been used to impart antibacterial properties to the
cotton yarns (Karolia and Mendapara 2007; Kavitha et al. 2007). It was observed
that the chitosan finish provides better functionality to the fabrics with increasing
tensile strength, flexural rigidity and shear strength as well as showing excellent
activity of the cotton fabrics against bacteria.

Prodigiosin is a bright red pigment with a pyrrolylpyrromethane chemical struc-
ture that possesses antibacterial activity, too. Venil and Lakshmanaperumalsamy
(2009) have studied the prodigiosin potential of *Serratia* and the effect of different
factors influencing the production of this pigment. They have reported the optimum

**Table 6.8** Characteristics of dyes/antibacterial extracted from microorganisms and their application properties

| No. | Source | Scientific name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | – | *Chitosan* | Polysaccharide | – | Wool | Anti-microbial, antifelting | Wash(*G*) | – | Ranjbar-Mohammadi et al. (2010) |
| 2 | – | *Chitosan* | Polysaccharide | – | Cotton | Antimicrobial | Wash(*G*) | – | Karolia and Mendapara (2007) |
| 3 | – | *Chitosan* | Polysaccharide | – | Cotton | Increasing tensile strength, flexural rigidity and shear strength. Excellent activity against bacteria | – | – | Kavitha et al. (2007) |
| 4 | Bacteria | *Serratia* | Tri-pyrrylmethene | Bright red | – | Antibacterial | – | – | Venil and Lakshmanaperumalsamy (2009) |
| 5 | Bacteria | *Serratia marcescens* | Pyrrolylpyrromethane | Bright red | wool, silk, nylon, cotton and polyester | Antibacterial, antifungal, antioxidant | Resistant to acid, alkali and detergents | Centrifugation | Gulani et al. (2012) |
| 6 | Bacteria, marine sediments | *Vibrio gazogenes* | – | Bright red | wool, nylon, acrylics, and silk | Antibacterial | – | Fermentations | Alihosseini et al. (2008) |

(continued)

**Table 6.8** (continued)

| No. | Source | Scientific name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 7 | Fungi | *Monascus purpureus, Isaria farinosa, Emericella nidulans, Fusarium verticillioides, Penicillium purpurogenum* | – | Red, pink, red, yellow, yellow | Cotton fabric and leather, with mordant | Anti-microbial | Light(*G*) | Absorption of the pigments on silica gel | Velmurugan et al. (2009) |
| 8 | Fungi | *Monascus purpureus* | – | Red or orange | Wool with mordant | Cost-effective production | Light(*G*) | Extraction by ethanol | De Santis et al. (2005) |
| 9 | Bacteria: bacillus | *Protease* | – | – | Wool and Silk | Increasing the degree of dye uptake | – | Dilution and incubation | Kim et al. (2005) |
| 10 | Bacteria | *Serratia sakuensis* | – | Red | Silk, wood and cotton | High color strength values | Color(*G*) | Solid-state cultivation | Vaidyanathan et al. (2012) |
| 11 | Fungi | *Trichoderma virens, Alternaria alternate, Curvularia lunata* | – | – | Wool and silk with mordants | Antifungal | Wash(*G*) Rub(*G*) | – | Sharma et al. (2012) |
| 12 | Fungi | *Monascus purpureus, Isaria farinosa, Emericella nidulans, Fusariumverti-cillioides, Penicillium purpurogenum* | – | Red pink, reddish brown, and yellow | Cotton with mordant | Considerable shade | Wash(*G*) | Extraction with ethanol | Velmurugan et al. (2010) |

[a]Excellent (E), Very good (VG), Good (G), Medium (M), Poor (P)

conditions for prodigiosin biosynthesis as well as the different techniques used for its extraction. It has been concluded that prodigiosin is a remarkable species for its diverse range of biological and dyeing effects, although much deeper insight into the mode of action of these compounds is required.

Gulani et al. (2012) have studied the effect of different parameters on the production of prodigiosin form *Serratia marcescens*. The effects of various media components and process parameters like carbon and nitrogen sources, temperature, pH, incubation period were investigated. The pigment was extracted by centrifugation and applied on wool, silk, nylon, cotton and polyester fibers. They have reported that the dyed fibers show good antibacterial, antifungal, and antioxidant activity where a good resistant to acid, alkali and detergents was observed.

This natural pigment has also been isolated from marine sediments by Alihosseini and her co-workers (2008). They have used this bright red pigment for dyeing wool, nylon, acrylics, and silk fibers and have reported that fabrics dyed with the microbial prodiginines demonstrate antibacterial activity.

Velmurugan et al. (2009) have studied the anti-bacterial activity and dyeing property of the pigments obtained from five fungal species. The fungi species were *Monascus purpureus, Isaria farinosa, Emericella nidulans, Fusarium verticillioides,* and *Penicillium purpurogenum,* producing red, pink, red, yellow and yellow pigments, respectively. The pigments were extracted by absorption on silica gel and used for dyeing of cotton fabric and processed leather with mordanting. The results showed that the dyed fibers have anti-microbial activity and good color fastness property.

In a similar work, dyeing properties of pigment from fungi *Monascus purpureus* has been investigated by De Santis and co-workers (2005). Red to orange pigment has been extracted by ethanol and along with alum or stannic chloride as mordants applied for dyeing wool fibers. The authors have reported good dye fastness for the dyed fibers and introduced this process as a cost-effective production.

Kim et al. (2005) have used the protease extracted from bacteria "bacillus" for improvement of dyeing quality in wool and silk. The enzyme was extracted by dilution and incubation of the soil sample containing the bacteria and was used for dyeing the fibers. Their results showed that by increasing the protease treatment, the dyeing characteristics of the fibers are enhanced, and as a result of soil removal, the fibers become smoother.

Extraction, characterization and use of a pigment produced by bacteria, *Serratia sakuensis* has been tried by Vaidyanathan et al. (2012). This novel red pigment was produced by solid-state cultivation of a bacterial isolate obtained from garden soil. It was a prodigiosin-like pigment that shows properties similar to prodigiosin. Then, the pigment was used for dyeing of silk, wool and cotton fabrics and the results showed that the dyed fabrics have good color fastness, high color strength values and dye uptake as well as antibacterial activity.

Similarly an attempt has also been made to apply the dyes obtained from fungus, *Trichoderma virens, Alternaria alternate,* and *Curvularia lunata* to the fibers by Sharma et al. (2012). Wool and silk fibers were dyed unmordanted and the results obtained show very good wash and rub fastnesses as well as antifungal activity.

Velmurugan et al. (2010) have also studied the dyeing of fibers with soluble pigments of selected fungi. They have used water soluble fungal pigments obtained from *Monascus purpureus, Isaria farinose, Emericella nidulans, Fusariumverti-cillioides,* and *Penicillium purpurogenum* species. These red pink, reddish brown, and yellow pigments extracted by ethanol were used for dyeing of cotton fibers accompanying mordanting. The authors have reported that a strong variation in shade and color depth could be achieved by using alum and ferrous sulfate that gives good wash fastness properties to the fabrics.

**Reusing and Recycling of Natural Dyes**

Some researchers have focused on the economical aspects of the application of natural dyes for textile such as reusing or recycling (Table 6.9). Mirjalili et al. (2011) have studied the dyeing of wool fibers with the extract of weld and concluded that in the case of dyeing with this extract, and keeping the good light fastness, the exhaustion rate for the extracted dye increased by 49 % compared to the raw dye.

In a similar work, Meksi and co-workers (2012) have used the olive mill wastewater as a potential source of natural dyes for wool dyeing. This wastewater was filtered and the coloring agents such as pectines, mucilages and tannins were extracted with hexane. Along with the darker shades and with generally good fastnesses, the results proved that mordanting give deeper shades and enhanced fastness properties.

In another work, plant waste materials including *Terminalia catappa, Artocarpus heterophyllus, Tectona grandis* and *Morinda citrofolia* were extracted by methanol and used for dyeing of silk fibers (Prusty et al. 2010). The fibers dyed with the extracts showed excellent antimicrobial activity and good durable fastness properties.

Another example for recycling of useless by-products of natural materials in sustainable treatment of textiles is the study that has been accomplished by Ahmad et al. (2011). They extracted color component from *Gluta aptera* wood (Rengas) saw dust and used it in environmentally friendly dyeing of cotton and silk. Water at boil and methanol at room temperature were used as extracting solvents. Rice husk ash and Al were used as natural and metallic mordants, respectively. Different brownish shades with good color fastness properties were obtained for both substrates.

In another similar work, Lee (2007) has tried to use the coffee sludge extract for dyeing of textile. The natural colorant was extracted from *Coffea arabica* L., using water as extractant and was applied for dyeing of cotton, silk, and wool with a variety of metallic salts as mordants. The author has reported that the fabrics dyed show good deodorization performance as well as excellent light fastness with mordanting.

Interestingly, Shams-Nateri (2011) has tried to reuse the dyebath containing madder extract for dyeing of wool fibers. The dyebath contained mainly alizarin, purpurin, and water, was reconstructed by adding madder. The author has reported

**Table 6.9** Characteristics of dyes/antimicrobial studied for economical aspects of their application

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Weld (flower) | – | – | – | Wool | Good potential of weld extracts for commercial dyeing | Light(M) | Extraction with soxhlet apparatus | Mirjalili et al. (2011) |
| 2 | Olive mill (wastewater) | – | Pectines, mucilages and tannins | – | Wool with mordants | Deeper shades and enhanced fastness properties | Light(M) | Filtration of wastewater and extraction with hexane | Meksi et al. (2012) |
| 3 | Plant (waste materials) | Terminalia catappa, Artocarpus heterophyllus, Tectona grandis, Morinda citrofolia | – | – | Silk with mordants | Excellent antimicrobial activity | Light(M) | Solvent extraction | Prusty et al. (2010) |
| 4 | Rengas (wood) | Gluta Aptera | Flavonoid (phenolic compounds) | Brown | Cotton, Silk with metallic mordants | Color | Wash(VG) Light(M) Rub(VG) | Aqueous extraction, Methanolic extraction | Ahmad et al. (2011) |
| 5 | Coffee (sludge) | Coffea arabica L. | – | – | Cotton, silk, and wool with mordants | Good deodorization performance | Light(E) with mordanting | Extraction with water | Lee (2007) |

**Table 6.9** (continued)

| No. | Source (used part) | Botanic name | Chemical base | Shade on substrate | Substrate applied | Properties achieved | Dye fastness[a] | Extraction method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 6 | Madder dyebath (wastewater) | Rubia tinctorum L. | Alizarin: 1,2-dihydroxy-anthraquinone Purpurin: 1,2,4-trihydroxyanthra-quinone | – | Wool | Reusing successfully the dyebath, 19.91% cost saving in wool dyeing | Light(VG) | Reconstruction of dyebath by adding madder and water | Shams-Nateri (2011) |
| 7 | Banana (peel) | Musa cavendish | Flavonoid | – | Cotton with metallic mordants | Color, antibacterial, UV protection | – | Alkaline extraction | Salah (2011) |
| 8 | Neem(seed) Mexican daisy(flower) | – | Alkaloids | – | Cotton | Antimicrobial | Wash(VG) | Methanolic extraction | Thilagavathi and Krishna Bala (2007) |
| 9 | Tulsi(leaf) Pomegranate (rind) | Ocimum sanctum, Punica granatum | Eugenol, germacrene, phytol, tannin | – | Cotton | Antibacterial | Wash(G) | Soxhlet extraction with methanol | Sathianarayanan et al. (2010) |
| 10 | Neem(seed) | Azadirachta indica | Azadirachtin, nimbin, salannin | – | Polyester and cotton blend fabric | Antimicrobial | Wash(M) | Methanolic extraction | Joshi et al. (2007) |
| 11 | Yarrow(flower) | Achillea millefolium | Flavonoids, luteolin and apigenin | – | Wool with metallic mordants | Color | Wash(G) Light(G) | Aqueous extraction | Kiumarsi et al. (2009) |
| 12 | Saffron(flower petal) | Crocus sativus L. | Anthocyanidin | Yellow to brown | Wool with metallic mordants | Color | Wash(G) Light(G) | Aqueous extraction | Mortazavi et al. (In press) |
| 13 | Indeciduous | Caesalpinia sappan L. | – | – | Poly(ethylene terphthalate) | Higher fastness, shortening of the dyeing time | – | – | Park et al. (2008) |

[a]Excellent (E), Very good (VG), Good (G), Medium (M), Poor (P)
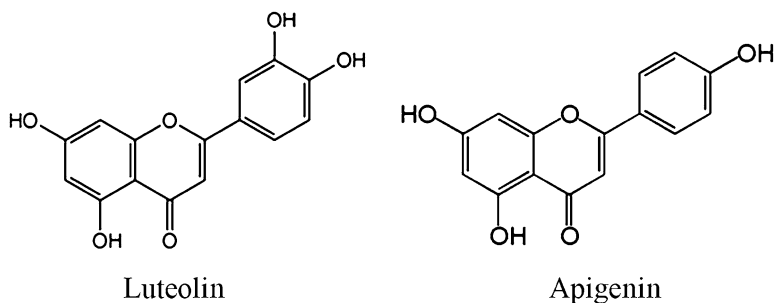
**Fig. 6.18** Chemical structure of luteolin and apigenin extracted from *Achillea millefolium* plant

that dye fastness of the samples dyed in reconstructed dyebath is the same as initial wool dyeing while the economic analysis showed that the reusing wastewater causes 19.91 % cost saving in wool dyeing with madder.

In exploring to find an alternative to decrease environmental pollution from burning of useless by product of banana fields, the studies led to find out the coloring components present in banana peel (Salah 2011). Natural dye extracted from banana peel wastes, *Musa cavendish*, by alkaline extraction was used for coloration of cotton with iron mordant. In addition to good color imparted, dyed cotton showed excellent antibacterial activity and high UV protection properties.

To develop an eco-friendly and economical alternative for production of medical cotton and polyester/cotton, the extracts of pomegranate rind, *Punica granatum*, and leaves of tulsi, *Ocimum sanctum* (Sathianarayanan et al. 2010), neem, *Azadirachta indica*, and mexican daisy (Thilagavathi and Krishna Bala 2007) have been applied on cotton through variety of methods such as direct application, pad-dry-cure, micro-encapsulation, resin crosslinking and their combinations. Excellent antibacterial property was obtained in all methods with good wash durability except for direct application of extracts where wash fastness was poor (Joshi et al. 2007).

Interestingly, Kiumarsi et al. (2009) have recently optimized the coloration process of yarrow, *Achillea millefolium*, on wool using Taguchi statistical method. *Achillea millefolium* is a pharmaceutical plant known as a powerful healing herb containing flavoniods such as luteolin and apigenin as natural dyes (Fig. 6.18).

The dye aqueous extract was used for dyeing of wool with aluminium, tin, and copper mordants. Interesting variety of shades from pale yellow to dark green was obtained with very good wash and light fastness for all dyed samples. The process was claimed to be economical due to saving time and energy that could also be commercialized.

In a related and most recent work, Mortazavi et al. (2012) introduced saffron, *Crocus sativus L.*, as a potent natural dye resource. They used saffron flower petals and sepals extract, a rich resource of anthocyanidin natural dye previously discarded as useless wastes, in coloration of wool. They used different metallic mordants such as tin, copper, aluminium, iron, and chrome together with oxalic and tartaric acid to obtain a variety of shades as well as to enhance fastness properties of natural dye on

wool. Although the color of fresh petals and sepals was light purple, but the shade range obtained on wool varied from yellow to brown with different mordants. Wash and light fastnesses of wool fibers were excellent with iron and chrome, moderate with copper and aluminium, and poor with tin mordants.

Dyeing of poly(ethylene terphthalate) fabrics with an aqueous extract of an indeciduous tree, *Caesalpinia sappan* L., was also investigated by Park and co-workers (2008). The results showed that the pretreatment with chitosan and/or plasma is better than a metal mordant in terms of the dye uptake and shortening of the dyeing time.

## 6.5   Conclusion

Due to the increasing awareness of the high risk impact associated with the synthetic colorants used for textiles, the application of natural or green extracts as colorants or antimicrobials has been these days increasingly considered. Review of the related published papers revealed that the non-reproducibility, time-consuming extraction methods and poor fastness properties are the main problems ahead the dyers and finishers to use these products in textile industry and consequently, must be the cores of scientific researches in this field.

The papers published in this field show that most of natural dyes have inherently antimicrobial properties and could consequently possess high medicinal activity. They are extracted from the different types of microorganisms as well as various parts of the plants such as bark, leaf, root, seed, fruit, and flower containing coloring materials like tannin, flavonoids, quinonoids, etc. Natural sources can offer not only a rich and varied source of dyestuffs, but also could be considered as safe, environmentally friendly and low cost treatments with additional benefit of coloring in a single stage. There have also been undeniable developments in extraction techniques of natural dyes/antimicrobials such as the invention of ultrasonic or enzyme assisted ultrasonic extraction methods where they prove high extraction efficiency over the conventional methods. But, there are still some important issues to solve. Most particularly, the application of metallic mordants for better fastness purpose is one of the problems concerning the environmental impact of natural dyes. The result of this survey showed that the shade and colorfastness of the fiber dyed depend not only on the natural colorant but also on the mordant and mordant assistants used. While dyes from natural sources are eco-friendlier than synthetic ones, the level of mordants used is normally higher than the permitted official concentration and poses environmental problem. It is expected that mordants will be replaced by modern environmentally friendlier technologies like plasma treatment. Moreover, studying and enlightening different processes involving natural dyes will be helpful in identification and analyzing color characteristics of historic/old textiles.

Real effort has been put to include all the relevant referred publications on the exploiting natural dyes/antimicrobials in green treatments of textiles in this review.

However, the limitation of our resources and the sheer number of publications in this field may have prevented the comprehensiveness of this report. Our sincere apologies are extended to any and all authors whose works are not included in this report.

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# Chapter 7
# Surfactants in Agriculture

**Mariano J.L. Castro, Carlos Ojeda, and Alicia Fernández Cirelli**

**Abstract** Adjuvants such as surfactants improve pesticide efficiency by multiple mechanisms. In particular surfactants increase the foliar uptake of herbicides, growth regulators, and defoliants. Therefore, the choice of the adjuvant in an agrochemical formulation is crucial. Surfactants include anionic, nonionic, amphoteric and cationic surfactants. This review describes the role and properties of new adjuvants for agriculture. In particular adjuvants such as glyphosate formulations are modified to decrease ecotoxicity.

**Keywords** Surfactant formulation • Pesticide • Agriculture • Adjuvant • Plant cell • Glyphosate • Cuticular • Alkyl polyglycosides • Saccharose esters • Chitosan

## Contents

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## 7.1   Introduction

The world population is projected to continue increasing into the next century. Population growth is assumed to follow the United Nation medium projection leading to about 10 billion people by 2,050. A central question is how global food production may be increased to provide for the coming population expansion. It would be necessary to increase current levels of food production more than proportional to population growth so as to provide most humans with an adequate diet (Kindall and Pimentel 1994).

Agronomic sciences and their different disciplines should be able to supply solution in order to increase food production. Agricultural science includes knowledge of the land, conducted by the geology, geochemistry and soil science, environmental analysis by the ecology, biogeography, bioclimatology, biometeorology and crops sown, with its potential for improvement through the creation of new seed varieties designed for hybridization and genetic modification. Moreover, there are a lot of factors with influence in the crops yields, such as irrigation, drainage, tillage and fertilizer applied to soil. The type of treatment above described improves the physical qualities of soil, while the use of fertilizers improves their chemical qualities. Finally, the fight against pests and weeds through pesticides is of paramount importance within the modern agricultural activity. The agricultural breakthrough occurred during recent decades by the incorporation of new laboratory techniques, statistics, computer science, satellite information. On the other hand, crop modified

by genetic engineering produced a huge increase in yields per hectare of different crops. All the disciplines above described are modifying year by year through the incorporation of new technologies.

Top-10 drivers for the changes of the agricultural industry are (Heinemann 2010):

1. Rising number and age of human beings and their nutritional behaviour
2. Increasing competition of food, feed and energy for agricultural outputs
3. Scarcity of water and arable land relative to the rising demand
4. Constantly increasing regulations & requirements for healthiness and sustainability
5. Genetically modified organism (GMO) and precision-farming enabled increase of productivity and quality
6. Higher volatility of demand vs. supply due to political and financials interventions
7. Ongoing commoditization of essential inputs like agrochemicals and fertilizer
8. Beyond consolidation: business model deconstruction and recomposition
9. More and quicker information due to novel media platforms
10. Personalization-driven convergence of health and nutrition

Agrochemical formulations cover a wide range of systems forms for crop protection that are prepared to suit a specific application. All these formulations require the use of a surface active agent or surfactant, which is not only essential for its preparation and maintenance of long-term physical stability, but also to enhance biological performance of the agrochemical. Most active ingredient used in agrochemical formulation are water-insoluble compounds. The obvious reason for adding surfactants is to enable the spray solution to adhere to the target surface, and spread over it to cover a large area. In addition, the surface active agent plays a very important role in the optimization of the biological efficacy. The surfactants commonly used in adjuvants illustrate the wide array of surfactant chemistry available which includes anionic, nonionic, amphoteric and cationic surfactants; all can be found in today's adjuvant market. Generally some solvent or glycol can also present to hold the composition in a homogeneous state or control viscosity. Increasingly, if a solvent is essential it will have a high flash point and low vapour pressure since the practice of using isopropanol, acetone, or other flammable materials in adjuvant formulations is quickly disappearing due to the dangers of low flash point products. Other components common to adjuvants depending on type include oils (based on natural raw materials and petrochemical), gums and/or polymers, and oleochemicals like fatty acids or glycerin.

Adjuvants usually have multiple functions in relation to pesticide efficacy (Green and Beestman 2007). Increasing the foliar uptake of active ingredients is of particular importance for herbicides, growth regulators, and defoliants (Fig. 7.1). Therefore, the choice of the adjuvant in an agrochemical formulation is crucial (Tadros 2005).

Formulation/Spray Tank
- Compatibility
- Solubility
- Stability
- pH
- Antifoam

Atomization
- Drift
- Evaporation

Deposition
- Reflection
- Adhesion

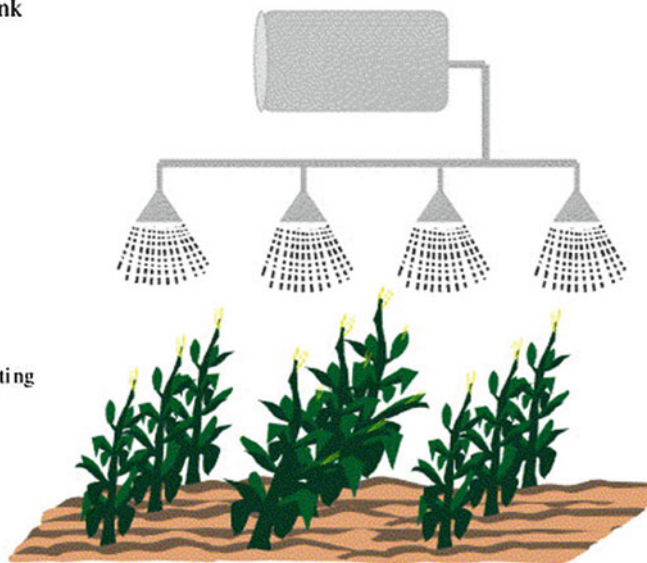Spray Deposit
- Spreading/Wetting
- Physical Form

Penetration/Uptake
- Hydrophilic
- Lipophilic
- Stomata

Translocation

**Fig. 7.1** Influence of formulation and adjuvants on herbicide performance (Reprint from Green and Beestman (2007))

It is actually difficult to ignore adjuvants when dealing with foliar uptake of pesticides. Indeed, the use of adjuvants dated back as early as about 200 years ago, although most of the synthetic adjuvant products started to emerge only a few decades ago.

Adjuvants are fascinating compounds. Typically showing no biological activity at all on their own, they help to exploit the full biological potential of many active ingredients. They can be absolutely necessary for some actives or to preserve activity, for example, under unfavorable environmental conditions or adverse water quality. Sometimes their mode of action appears obscure, since for a single active many different adjuvants with completely different modes of action, and used at differing rates per hectare, increase performance similarly. The other quite common extremes are situations where a particular adjuvant fits best to a particular active at a particular ratio in a particular formulation type, or conversely, where adjuvants are antagonistic to the product performance. In the case of adjuvants that somehow improve coverage, the visible mode of action correlates often with the achieved gain in performance.

However, most adjuvant effects are not visible at all since they occur at the molecular level, are time dependent and are often variable, since the adjuvant effects are based on interactions with the biological target and environmental factors. There are several good reasons to predict that adjuvant innovations will become even more important in the future.

The variety of products and technologies in the agricultural adjuvant industry has grown with the need to maximize the performance of pesticide products. These

products have been shown to improve the performance of pesticides through aiding on-target delivery, maximizing efficacy of the active ingredient and improving spray water quality just to mention a few general areas of use. Development of pesticide formulations can be a rigorous science with the competing demands of maximizing performance of the active ingredient, maximizing the physical stability of the formulation, ensuring the human health and environmental effects of the formulation are minimized. Meanwhile, the costs of the product have to keep low enough so it makes business sense to bring it to market and for the customer to have value from its use. All of these factors must be balanced and can only be balanced by a team approach to developing the product.

Climate changes are likely to occur and possibly become more pronounced in the next decades. Weather is becoming more variable and drought spells will increase in many regions. Challenges for crop production are thus to increase crop productivity further considering that drought is only but one of the adverse conditions which crop production is already facing. Others include e.g. soil compaction, salinity, acidity or alkaline conditions, nutrient fixation or depletion. All those conditions interfere as well with the nutrient uptake of crops from soil. The development of new surfactant based-system as bio-activator for actives is a key factor to improve the cost-effective performance increasing process efficiency, energy and raw material savings. On the other hand, sustainability should take into account the use of renewable resources and the improvement of eco-friendly product profile.

## 7.2   Generic Pesticides of Growing Interest

Agrochemicals are an aging industry. While over 800 pesticide active ingredients remain on the market, the number of new substances being developed has fallen considerably over the last 10 years. In some industrialized country markets older products are losing their registrations. For example, the European Union review has removed over 360 active ingredients to date. But these and many other chemicals are still available elsewhere.

The overall global agrochemical pesticide market was valued at US$32 billion in 2004 with generic products making up an increasing percentage of this market. Latest estimates indicate that generic products account for US$18 billion, or around 66 % of overall sales and by volume, generic active ingredients may account for approximately 95 % of all product sales worldwide.

The types of generic most sold are herbicides with 57 % of the market, followed by insecticides with 34 % and then fungicides with 11 % of sales. The biggest selling generic is glyphosate with annual sales approximating US$5 billion followed by the insecticide imidacloprid with annual sales of around US$1 billion. The biggest selling fungicide is mancozeb, seventeenth in the list with sales of $220 million (see Table 7.1). On the other hand, generic active ingredients glyphosate, chlorpyrifos and cypermethrin, are produced by between 31 and 39 manufacturers.

**Table 7.1** Market profile of the top 24 generic active ingredients (Adapted from Agrow's Complete guide to generic pesticides. Volume 2, Products and Markets (DS250), August 2005, pp 22–23)

| Rank | Active ingredient | Activity | Value sales Sales ($m) | Prospects |
|---|---|---|---|---|
| 1 | Glyphosate | Herbicide | 5000 | Rising |
| 2 | Imidacloprid | Insecticide | 1000 | Rising |
| 3 | Malathion | Insecticide | 400 | Stable |
| 4 | Paraquat | Herbicide | 400 | Falling |
| 5 | Acephate | Insecticide | 350 | Rising |
| 6 | Pendimethalin | Herbicide | 350 | Stable |
| 7 | 2,4-D | Herbicide | 335 | Stable |
| 8 | Acetochlor | Herbicide | 300 | Rising |
| 9 | Chlorpyrifos | Insecticide | 300 | Falling |
| 10 | Trifluralin | Herbicide | 300 | Stable |
| 11 | Atrazine | Herbicide | 280 | Stable |
| 12 | Imazethapyr | Herbicide | 280 | Falling |
| 13 | Lambda-cyhalothrin | Insecticide | 275 | Rising |
| 14 | Permethrin | Insecticide | 270 | Falling |
| 15 | Carbofuran | Insecticide | 250 | Falling |
| 16 | Deltamethrin | Insecticide | 250 | Stable |
| 17 | Mancozeb | Fungicide | 220 | Rising |
| 18 | Delta-cypermethrin | Insecticide | 200 | Rising |
| 19 | Carbendazim | Fungicide | 200 | Stable |
| 20 | Chlorothalonil | Fungicide | 200 | Stable |
| 21 | Cypermethrin | Insecticide | 200 | Rising |
| 22 | Dichlorvos | Insecticide | 200 | Falling |
| 23 | Linuron | Herbicide | 200 | Falling |
| 24 | Methomyl | Insecticide | 200 | Stable |

## 7.3 Adjuvant Classification, Functionality and Chemistry

Although the first recorded use of adjuvants to increase herbicide performance was in the late 1890s, it was not until after the discovery of effective organic herbicides like 2,4-D in the 1940s that significant adjuvant research began (Foy 1989).

Adjuvants are defined as "an ingredient in the pesticide prescription, which aids or modifies the action of the principal ingredient" (Foy 1987). Adjuvants can be divided into two general types: (1) formulation adjuvants and (2) spray adjuvants. The first type consists of adjuvants, which are part of the formulation, while the second type of adjuvants is added along with the formulated product to the water in the tank of the spray equipment before application on the fields. Spray adjuvants are sometimes called tank mixing additives or just adjuvants, whereas the formulation adjuvants are called additives or inerts (Hochberg 1996).

The term adjuvant has developed several meanings in the agrochemical industry. In some parts of the world, adjuvant can refer to anything other than the pesticide formulation which is added to the spray tank. In other parts of the world, the term adjuvant is used interchangeably with the term formulant and refers to
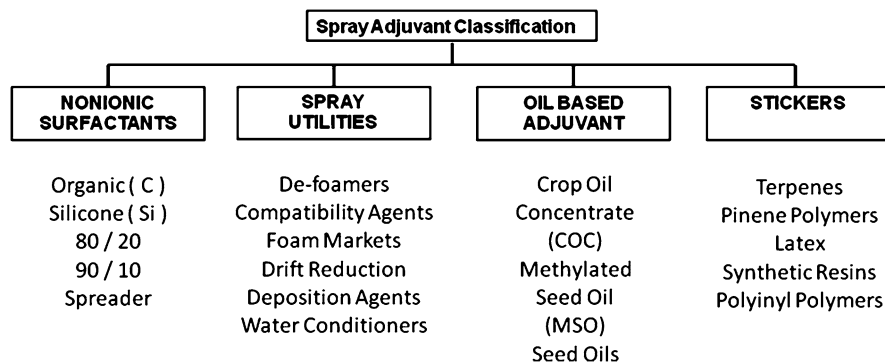
**Fig. 7.2** Classification of adjuvants by tank mix function (Adapted from Roberts 2010)

any nonpesticidal ingredient in the formulated pest control product. Adjuvant terminology globally became such a large issue in the 1990s that several trade organizations decided to attempt standardizing both, the terminology and functionality of adjuvants.

Although several authors attempted to write materials for adjuvant terminology, only ASTM International (formerly American Society of Testing and Material) began to work on definitions for standard terms. ASTM E 1519 defines an adjuvant as a material added to a tank mix to aid or modify the action of an agrochemical, or the physical characteristics of the mixture (ASTM E 1519).

Although ASTM definitions are widely used, in some cases different terminology is adapted for unique circumstances. In 2009 the US Environmental Protection Agency (U.S. EPA) announced it was considering requiring the submission of residue data for active ingredients including an adjuvant as part of spray treatment when a spray tank adjuvant was included on the labelling. In order to facilitate EPA's investigation, industry proposed a set of classifications for tank mix adjuvants to minimize the testing necessary to provide the residue data EPA suggested it needed to establish a tolerance on food. The Spray Classification system presented to EPA utilized the ASTM definitions but aggregated specific adjuvants by broad type as a more general level of classification. Figure 7.2 illustrates the proposed classification of tank mix adjuvants (Roberts 2010).

The functions of adjuvants selected for use in tank mixtures vary widely depending on the needs of the active ingredient with which the product is applied. For example, certain active ingredients show enhanced performance from improved wetting while others may show enhanced performance from water conditioning or drift reduction. In some cases, the presence of an oil adjuvant may improve penetration and uptake of the active ingredient. A list of common crop protection products which respond to the use of a spray tank adjuvant along with the possible function provided by the adjuvant appear in Table 7.2 (Roberts 2010).

We have considered in the present review the term adjuvants to refer to any non-active ingredient in the formulated pest control product added to aid or modify the action of an agrochemical.

**Table 7.2** Crop protection product combinations with spray tank adjuvants (Adapted from Roberts 2010)

| Crop protection product | Desired adjuvant functionality |
|---|---|
| Glyphosate | Wetting, water conditioning |
| Sethoxydim | Cuticular penetration |
| Atrazine (post use) | Wetting, compatibility |
| Paraquat | Wetting, sticker |
| Glufosinate | Wetting, penetration, water conditioning |
| Imazapyr | Cuticular penetration |
| Acifluorfen | Wetting, cuticular penetration |
| Sulfonyl urea | Wetting, pH buffering |
| Fluazifop | Wetting, cuticular penetration |
| Pyraclostrobin | Cuticular penetration |

## 7.4 Types of Surfactants

Surface-active agents have a characteristic molecular structure consisting on a structural group that has a very little attraction for water, known as a hydrophobic group, together with a group that has strong attraction for water, called hydrophilic group. This is known as an amphiphilic structure. The hydrophobic group is usually a long-chain hydrocarbon, and the hydrophilic group is an ionic or highly polar group. According to the nature of the hydrophilic group, surfactants are classified as: anionic, cationic, nonionic and amphoteric (Fernández Cirelli et al. 2008).
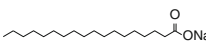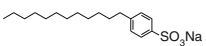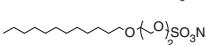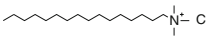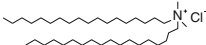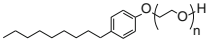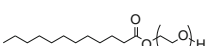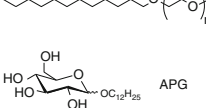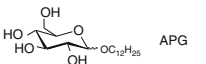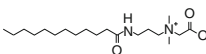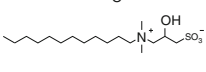
### 7.4.1 Anionic Surfactants

The hydrophilic groups of anionic surfactants consist in most cases of sulfonate, sulfate, or carboxylate groups with either a sodium or a calcium a counterion (Table 7.3). Amongst them, linear alkylbenzene sulfonate (LAS) are produced in the largest quantities worldwide. These are mainly used in powdery and liquid laundry detergents and household cleaners. It is important to point out that calcium linear alkylbenzenesulfonate is employed as adjuvant in many agrochemicals formulations.

### 7.4.2 Nonionic Surfactants

The hydrophilic behavior of nonionic surfactants is caused by polymerized glycol ether or glucose units (Table 7.3) (Fernández Cirelli et al. 2008). They are almost

**Table 7.3** General classification and characteristic features of surfactants

| | Alkyl tail | Polar head | Example |
|---|---|---|---|
| Anionic | $C_8$-$C_{20}$ linear or branched-chain | –COOH |  Soap |
| | $C_8$-$C_{15}$ alkylbenzene residues | –$SO_3Na$ |  LAS |
| | $C_8$-$C_{20}$ linear-chain ethoxylated | –$OSO_3Na$ |  LES |
| Cationic | $C_8$-$C_{18}$ linear-chain | –$N(CH_3)_3Cl$ |  CTAC |
| | $C_8$-$C_{18}$ linear-chain | –$N(CH_3)_2Cl$ |  DODAC |
| Non-ionic | $C_8$-$C_9$ alkylphenol residues | –$(CH_2CH_2O)_n$–OH n: 4–22 |  APEO |
| | $C_8$-$C_{20}$ linear or branched-chain | –$COO(CH_2CH_2O)_n$–OH n: 4–22 |  FAEO |
| | $C_8$-$C_{20}$ linear or branched-chain | –$(CH_2CH_2O)_n$–OH n: 2–22 |  AEO |
| | $C_8$-$C_{20}$ linear or branched-chain | –$NH(CH_2CH_2O)_n$–OH n: 2–22 |  APG |
| | $C_8$-$C_{20}$ linear or branched-chain | Glucose | |
| Amphoteric | $C_{10}$-$C_{16}$ amido-propylamine residue | –$N^+(CH_2)_2CH_2COO^-$ |  (CAPB) |
| | $C_8$-$C_{18}$ linear-chain | –$N^+(CH_2)_2CH_2CH(OH)CH_2SO_3^-$ |  (CAHS) |

exclusively synthesized by addition of ethylene oxide or propylene oxide to alkylphenols, fatty alcohols, fatty acids, fatty amines or fatty acid amides. Nonionic surfactants found major applications as detergents, emulsifiers, wetting agents, and dispersing agents. They are used in many sectors, including household, industrial and institutional cleaning products, textile processing, pulp and paper processing, emulsion polymerization, paints, coatings, and agrochemicals. A large amount of them are employed as adjuvant in many agrochemicals formulations.

## 7.4.3  Cationic Surfactants

Cationic surfactants contain quaternary ammonium ions as their hydrophilic parts (Table 7.3). This class of surfactants has gained importance because of its bacteriostatic properties. Therefore, cationic surfactants are applied as disinfectants and antiseptic components in personal care products and medicine. Because of their high adsorptivity to a wide variety of surfaces, they are used as antistatic agents, textile softeners, corrosion inhibitors, and flotation agents.

### 7.4.4   Amphoteric Surfactants

Amphoteric surfactants containing both cationic and anionic group in their structure and sometimes are referred to as zwitterionic molecules (Table 7.3). They are soluble in water and show excellent compatibility with other surfactants, forming mixed micelles. The change in charge with pH of amphoteric surfactants affects wetting, detergency, foaming, etc. The properties of amphoteric surfactants resemble those of non-ionics very closely. Zwitterionic surfactants have excellent dermatological properties, they also exhibit low eye irritation and are frequently used in shampoos and other personal care products. Amphoteric surfactants are now starting to be used in agrochemicals formulation.

## 7.5   Mode of Action of Adjuvants

Despite the negative perception of the public, pesticides are still going to be used for many decades to ensure the food supply for the ever growing world population. The simple reason for this is that products more costly for farmers mean more expensive foods for the population, alternative methods for plant protection are either inefficient or too costly for farmers.

The global pesticide production in 2000 amounted to over 3 million tons of active ingredients (Tilman et al. 2002). It is estimated that of the total amount of pesticides applied for weed and pest control, only a very small part (<0.1 %) actually reaches the sites of action, with the larger proportion being lost *via* spray drift, off-target deposition, run-off, photodegradation and so on (Pimentel 1995). Agrochemical industries are continuously seeking for new products and formulations of expanding area of use and market share. It typically takes at least 10 years of intense research and development from the discovery of an active ingredient until its market introduction as an agrochemical product (Cordiner 2004). In addition, it has become increasingly more and more difficult to find materials of high activity that are environmentally friendly, safe and cheaper to manufacture. Most government and regulatory authorities are now demanding formulations that are cleaner and safer for the users, have minimal impact on the environment, and can be applied at the lowest dose rate (Holden 1992).

The transporting-limiting barrier restricts the performance of foliar-applied agrochemicals molecules such as pesticides, fruit chemical thinning, and growth regulators (Schreiber 2006; Petracek et al. 1998). An agrochemical active ingredient is often ineffective and of little use to the end user if applied to the target surface alone (Tominack 2000). In most cases the active ingredient is formulated into complex, carefully designed multi-agent preparations that facilitate mixing, dilution, application and stability. To enhance the efficacy of foliar-applied agrochemicals, surfactants are widely used in spray solution to increase active ingredient solubility, to improve wetting of the plant cuticle, and to increase cuticular penetration.

Around 230,000 t of surfactants is used annually in agrochemical products, with a formulation typically containing 1–10 % of one or more surfactants (Edser 2007). Surfactant, as a plasticizer, softens the crystalline waxes in cuticle and thus increases the mobility of the agrochemicals across the cuticular membrane (Schönherr et al. 2000).

With the presence of surfactant, the sorption behavior of plant cuticle becomes very complex. Derived from similar studies, surfactant demonstrates two opposite effects on the cuticle sorption behavior: (i) surfactant solutions decrease the distribution of organic contaminant onto the cuticle by increasing the solute aqueous solubility, that is, a negative effect; (ii) surfactants increase the sorption capability by softening of cuticular wax (i.e., plasticizing effect) or by forming a new partition phase for the cuticle-sorbed surfactant, that is, a positive effect (Zhu et al. 2004; Lee et al. 2000, 2004). The apparent effects of surfactants on sorption depend on the balance of the two opposite effects, which are dominated by the compositional characteristics of plant cuticles, surfactant type and concentration, and the solute's properties (Tamura 2001).

On the other hand, it is well known that droplets of between 50 and 250 mm diameter will adhere to plant surfaces, but larger droplets may rebound or shatter (Hartley and Brunskill 1958). However, smaller droplets are more prone to drift, so we have the classic conundrum of how to achieve maximum on-target adhesion/retention, with minimum off-site drift. The exact mechanism by which these molecules elicit this acceleration of movement is not yet fully understood.

In the case of systemic pesticides, if there is little or no uptake, then regardless of the efficiency of deposition and retention, this will have been to no avail. Obviously environmental factors, such as rain (causing wash-off), wind and relative humidity (affecting droplet drying), will materially affect pesticide uptake. However, a major factor in all cases is the plant leaf itself, including its surface and cuticle. One of the main functions of spray adjuvants is to overcome or minimise the effect of leaf waxes and the cuticular barrier. Appropriate adjuvants will substantially aid droplet spread, redistribution and leaf coverage, but can also enhance uptake of the active ingredient. Penetration and absorption of a compound by leaves is determined by the ability of the compound to move through the cuticle, a process known as transcuticular penetration or movement (Solel and Edgington 1973).

Foliar uptake of pesticides is a diffusion process across the epicuticular wax, the cuticle, and the plasma membrane of epidermal cells. The diffusion rate of both lipophilic and hydrophilic compounds across isolated leaf cuticles was negatively correlated with their molecular weight (MW), or molecular size (Bauer and Schönherr 1992; Schönherr and Schreiber 2004). In fact, the plasmodesmata of plants can only allow free circulation of molecules smaller than 1 kDa (Oparka and Roberts 2001). It is thus not a coincidence that most foliar-applied herbicides have a molecular weight between 100 and 500, as any out-ranged molecules would be eliminated at the screening stage if they cannot enter plant leaves or move between cells (Tice 2001).

Lipophilicity is probably the single most important property of pesticides related to foliar uptake. Generally, an octanol/water partition coefficient (log $Ko/w$ or log$P$)

is used to describe the lipophilicity of a compound. Chemicals with log $P < 0$ are considered as hydrophilic and those with log $P > 0$ lipophilic. Due to the lipoidal nature of the epicuticular wax and the cuticle, foliar uptake tends to increase with increasing lipophilicity of the chemicals (Baker et al. 1992; Stevens et al. 1988). A best example showing this is the faster uptake of 2,4-D ester (lipophilic) compared to 2,4-D salt (hydrophilic) (De Ruiter et al. 1993).

However, like molecular size, log$P$ is not the only property that determines the foliar penetration. Indeed, based on the uptake data obtained with 26 chemicals and four plant species, only a moderate correlation ($R2$: 24–45 %) was found between log$P$ and the foliar uptake of chemicals. In fact, some hydrophilic compounds, such as paraquat, are actually able to penetrate into plant leaves very fast.

## 7.6 Plants Cells Morphology

Plant cells, unlike animal cells, are surrounded by thick walls that form rigid tissues. They do not need the junctions found in animal tissues. But some higher plant cells are interconnected by plasmodesmata, tubelike structures that penetrate through cell walls and form fluid connections between adjacent cells. Like gap junctions in animal cells, plasmodesmata allow the free exchange of small molecules and help coordinate the activities of neighboring cells.

On the surface of the leaves of higher plants is a layer of waxy material known as the cuticle or cuticular membrane (Kirkwood 1999; Riederer and Schreiber 2001). It is theorized that after plants migrated from the sea onto land 400 million years ago, in the Silurian period of the Paleozoic era, cuticles evolved as a means of reducing water loss to the surrounding air (Stewart et al. 1993; Thomas and Spicer 1987). The plant cuticle is a thin continuous layer of predominantly lipid material synthesized by the epidermal cells and deposited on their outer. Cuticle composition and structure of modern higher plants vary greatly from species to species but are generally 0.1–10 μm thick, being composed of two major classes of lipids: (i) solid waxes, usually mixes of long chain aliphatic compounds and (ii) insoluble, high molecular weight polyester cutins. Plant cuticle contains too terpenoids and phenolic compounds, some of which serve as anti-feedants to discourage grazing by herbivores.

There are five basic types of cuticles: smooth, ridged, papillose, glaucous (having an additional covering of microcrystalline wax), and glandular where trichomes are present in high number and comprise the main surface of the leaf (Hess and Foy 2000).

Generally, the plant cuticle is composed of two regions. The lower region consists of cutin, long chain alkyl ketones, alcohols, and closest to the cells, polysaccharides; the upper region consists of epicuticular wax, mostly very long chain alkanes (Li and Chen 2009; Chen et al. 2008).

The epicuticular wax present in the outer surface of the membrane has two main forms, crystalline and amorphous (Jeffree 1996; Chen and Li 2007). Whereas there

are some important exceptions, the dominant epicuticular wax on leaves of grass species is crystalline and on broadleaf species is amorphous. The crystalline wax is undoubtedly an obstacle for spray retention and an intimate contact between pesticide droplets and leaf surface, but it does not seem to be a formidable barrier for pesticide uptake. All aerial surfaces of plants are covered by the cuticle. It is widely known that the waxy sheet of cuticle not only prevents water loss, but also functions in defense by forming a barrier that resists physical damage and microbial invasion. The cuticle is incontestably the most important barrier for the penetration of pesticides. The microscopic structure and chemical composition of plant cuticles were reviewed by Holloway (Holloway 1993).

The cuticle permeability for pesticide molecules varies greatly with plant species and growth stage, but is not correlated to the thickness (Becker et al. 1986; Buchholz et al. 1998). It is clear that both lipophilic and hydrophilic compounds can diffuse through the cuticle. It was postulated that lipophilic and hydrophilic chemicals follow distinct pathways, i.e., polar (aqueous pores) and apolar (cutin matrix and wax) routes (Crafts 1960). Until now the existence of aqueous pores inside the cuticle is only supported by indirect evidence obtained through studying the diffusion characters of calcium salts and glyphosate across isolated leaf cuticles (Schönherr 2000, 2002).

Their high sorption capability may be seriously suppressed and even inhibited by the cuticular waxes deposited within and on the surface of the polyester matrix because of their partially cristaline structure. More importantly, it is the arrangement of the wax into amorphous and crystalline domains that is believed to be the transport limiting barrier (Reynhardt and Riederer 1994; Santier and Chamel 1998). Removal of waxes from bulk plant cuticles promotes their sorption capability and leads to an increase in the permeability by several orders of magnitude (Chefetz 2003; Popp et al. 2005).

## 7.7  Mechanism of Action of Adjuvants

It is generally recognized that not any surfactant can increase the uptake of any herbicide. At present, there is no practical theory or comprehensive model that can predict quantitatively the effect of a particular surfactant on the uptake of a specific herbicide. Applying the right concentration and type of surfactant is not an easy task for the user. Systematic studies will be needed to elucidate the mechanisms involved in the complex interactions between herbicides, surfactants and plants. In order to improve the uptake of post-emergence herbicides, numerous surfactants have been tested over the last 30 years. However, until the early 1990s, most experiments involved only a few randomly selected surfactants and it was not possible to know which parameter of surfactants was most important for enhancing herbicide uptake.

A first review of the mechanisms of action is listing various contributions like wetting and spreading, dissolution or disruption of epicuticular waxes, solubilisation

of agrochemicals in deposits, prevention or delay of crystal formation in deposits, retention of moisture in deposits by humectant action, and promotion of uptake of solutions via stomatal infiltration (Stock and Holloway 1993).

A first mechanistic approach of the adjuvant/wax interactions was reported by Schreiber (Schreiber 1995). From the observation that the surfactant-induced acceleration of the diffusion of pentachlorophenol is completely reversible if the surfactant is removed, it was tentatively concluded that the effect of the surfactant on wax is an unspecific plasticising interaction. It was not before a decade later that again a plasticising effect was revealed from macro and micro-thermal analysis of plant/surfactant interactions (Perkins et al. 2005). Finally, a series of results from different experiments with reconstituted cuticular wax, like the already mentioned reversibility of diffusion rates when adjuvants are removed, like the decrease in size selectivity in the presence of adjuvants, and like the fluidity increase in the presence of adjuvants as determined by electron spin resonance (ESR) and by nuclear magnetic resonance (NMR), clearly allow the conclusion that the adjuvants act by a non-specific, plasticizing mechanism (Schreiber 2006; Petracek et al. 1998).

An even deeper insight into the uptake-enhancing mechanism of adjuvants is reported by Burghardt et al. (Burghardt et al. 1998). In this paper, a free volume model was applied to predict diffusion coefficients of active ingredients on the basis of the transport properties of the wax, the molar volume of the diffusing compound and the adjuvant concentration in the wax. Increase in the mean free volume by the plasticising action of adjuvants raises the probability for a diffusing compound to find a fitting void. Despite these experimental and theoretical advances, it was stated by Wang et al. that a more multidisciplinary approach is needed to elucidate the transcuticular diffusion behaviour of active ingredients and the mode of action of adjuvants (Wang and Liu 2007).

## 7.8 Efficiency Parameters to Increase the Agrochemicals Performance

### 7.8.1 Cuticular Uptake

One of the most important ways to improve the efficacy of pesticides and minimize their impact on off-target organisms is through increasing the penetration of active ingredients into plant foliage. If foliar uptake is important for the efficacy of systemic fungicides and insecticides, then diffusion into plant tissues is a prerequisite for the activity of foliar-applied herbicides, growth regulators, and defoliants. Foliar uptake of pesticides is a complex process, depending on leaf surface characters of plants, physicochemical properties of the chemicals, types and concentration of the additives, and environmental conditions. How each of these factors influences the uptake is only partially understood, despite an urgent demand for efficient use of pesticides.
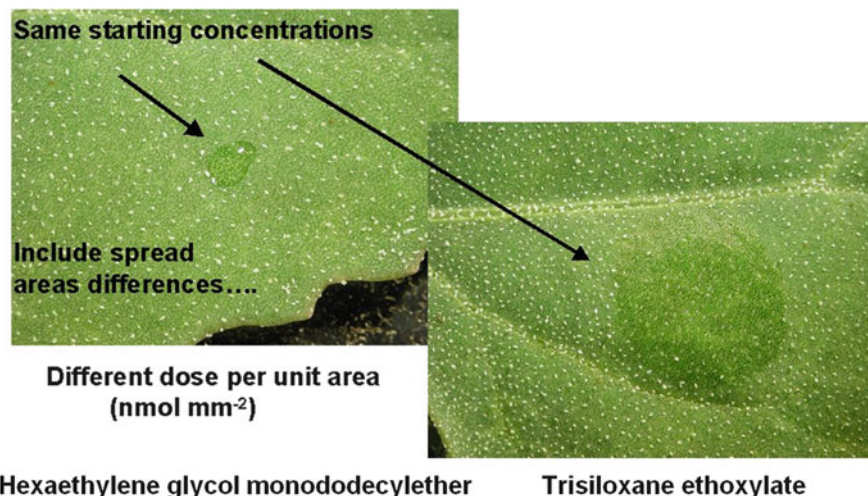
**Fig. 7.3** Illustration of droplets spread effects on *Chenopodium album* with different spray formulation (Reprint from Zabkiewicz (2007))

Cuticular uptake (diffusion of the chemical directly through the cuticle) of pesticides has been studied for a longer time, but progress in understanding the factors controlling this mechanism has been much slower. Attention was focussed on relating the physical properties of the formulation to percentage uptake, with less focus on the interactions of adjuvant(s) in a spray formulation, or the importance of plant cuticle and epidermal layers (Stevens and Bukovac 1987; Stock et al. 1993). Just as it is essential to consider application and formulation together in the case of adhesion and retention by plants, so the interactions of plant, active and adjuvant must all be considered in the uptake process.

The fundamental mechanism of uptake has been considered, with most attention given to the epicuticular lipids and their role in modifying active ingredient diffusion through cuticles (Kirkwood 1999; Riederer and Markstädter 1996; Schönherr et al. 1999). However, there is a much simpler effect on the leaf surface that needs to be considered first. If a spray formulation contains adjuvants that cause droplet spread on a leaf surface (Fig. 7.3), this will in effect lower the mass of active per unit area, and without any change in concentration until the spray solution begins to evaporate. In any case, there will be a "solution residue", where the concentration of the active is many times more than in the starting spray solution (Zabkiewicz 2003).

## 7.8.2  Translocation

Absorption and translocation of herbicides can be significantly increased when adjuvants are added to foliar applied spray mixtures level of increased control from
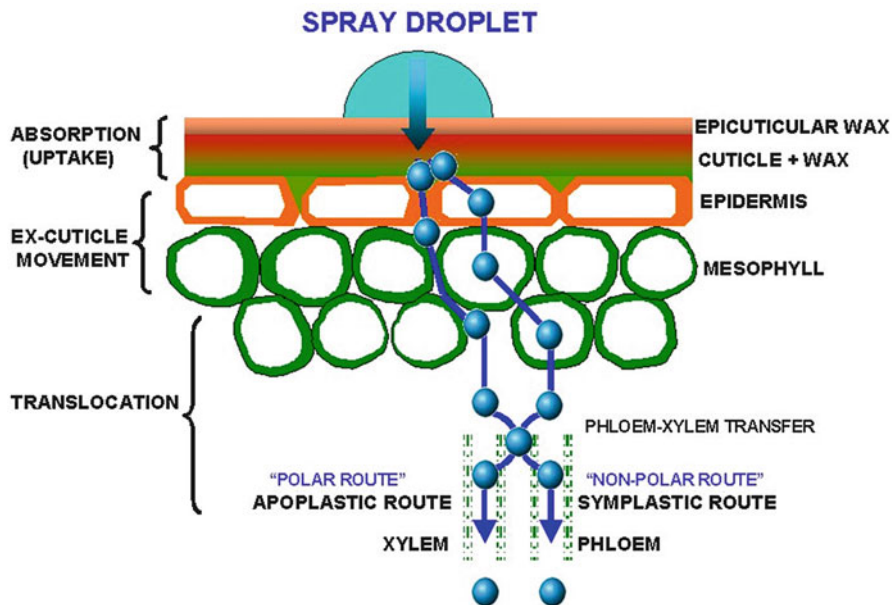
**Fig. 7.4** Representation of different trans-cuticular pathways and subsequent apoplastic (*polar*) and symplastic (*non-polar*) pathways (Reprint from Zabkiewicz (2007))

adjuvants is usually affected by the environmental conditions at the time of herbicide application. In most cases, herbicide absorption and translocation are greatest under environmental conditions which are most favorable for growth of the treated plant. Adjuvants added to treatment solutions often increase absorption and translocation of foliar applied herbicides, especially when plants are under stress at time of treatment (Wills and McWhorter 1988).

Translocation has received the least attention, though long distance transport has been well studied and reviewed (Price 1976; Coupland 1988). In the case of foliar applied pesticides, it is known that lipophilicity is important, as these compounds have to cross hydrophobic membranes or structures other than the cuticle proper. Hydrophilic molecules are readily transported in either the phloem (downwards) or the xylem (upwards), though their initial movements through the cuticle, epidermal cells and into the mesophyll are not well understood (Devine and Hall 1990). The presence of separate "hydrophilic" and "lipophilic" pathways as part of the uptake process, may in turn determine the efficiency of the subsequent translocation pathway, but it is also difficult to define when uptake becomes translocation (Fig. 7.4).

Practically all herbicides and all systemic pesticides are weak electrolytes. Bromilow et al. (1990) represented polarity or lipophilicity and acid strength (pKa), as two main herbicide characteristics that help in understanding the transport pattern in plants. As the chemicals become more polar and more lipophilic, the permeation rate decreases. The pH in the exterior of the cell is approximately 5.5, herbicides
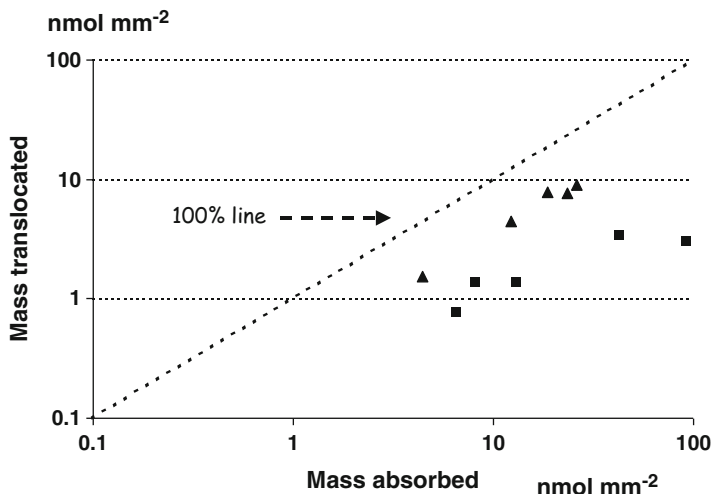
**Fig. 7.5** Glyphosate mass uptake into *Chenopodium album* versus mass absorbed from Roundup®
Ultra (▲) and Touchdown (■) commercial formulations; (- - -) maximum uptake line, representing
100 % uptake over the initial dose range (Reprint from Zabkiewicz (2007))

with pKa below 5.5 are in a non-dissociated form and are thus able to penetrate the
cell more easily. Once at the cytoplasm, where the medium is more alkaline (pH
approximately 7.5), these herbicides disassociate and turn into the most active, and
less capable, translocating form of the molecule. Because the dissociated form is less
capable of spreading to the exterior of the cell, the molecules of these herbicides get
"stuck" in the cytoplasm; this behavior receives the name "ionic trap". In addition,
plasmodesmata may have significant participation in translocation of molecules that
have pKa (dissociation constant) below the pH of the xylem, which is approximately
5.5; it may also assist symplastic movement of these molecules by other routes
besides phloem (Trapp 2004).

It is clear that "long-term" translocation will be affected by plant growth stage
and environmental conditions, but "short-term" translocation (over hours rather than
days) may have other rate limiting or modifying features.

Adjuvants are known to facilitate cuticular "transport" (foliar uptake) but are not
thought to play any significant part in further short or long-distance translocation
processes. However, in theory, if adjuvants could reach the cellular plasmalemma,
then they could affect the initial stage of the sub-cuticular transport process
(Fig. 7.4). The recent use of mass or molar relationships, instead of percentages,
for xenobiotic uptake into plants from differing formulations, may be a means of
elucidating some of the interactions among actives, adjuvants and plants (Forster
et al. 2004).

It has been demonstrated that a relationship similar to that developed for mass
uptake, for translocation vs. mass absorbed (which is related to the initial dose
applied) can also apply to the translocation process in model systems but less well
with complex formulations (Fig. 7.5) (Zabkiewicz 2007). For example, the commer-

cial glyphosate formulations show considerable interaction, if the reduction in both mass uptake and translocation at higher initial dosages, is a correct indication. This manner of presenting mass uptake and translocation has the potential to identify anomalous behaviour or complex interactions.

The advantage of the mass relationship is that it can provide information similar to that used for drug delivery dose prescriptions. Knowing the mass uptake, an estimation can be made of the mass translocated in specific systems; with subsequent studies on the influence of physiological and environmental influences, appropriate "dosages" could be applied at specific growth stages or conditions. It can also be used to estimate the change in overall efficacy of spray formulations, as to date no generic quantitative relationship has been identified.

The principal factors controlling foliar uptake appear to be solute mobility in the cuticle, cuticle tortuosity and solute-driving force.

### 7.8.3 Environmental Conditions

It is well known that environmental conditions have an important influence on herbicide efficacy. In particular, the effect of humidity on herbicide uptake has been attributed to changes in cuticle hydration and droplet drying. As early as the 1950s, it was hypothesized that humectants such as glycerol would enhance herbicide uptake by not letting droplets dry, thus maintaining the herbicide in solution, and hence making it available for uptake. Shortly thereafter, evidence was found to support this hypothesis and humectants were used successfully in warm, dry areas to increase herbicide efficacy. Furthermore, current evidence suggests that highly water-soluble, ionic herbicides may be more sensitive to low humidity and rapid drop drying than lipophilic herbicides. The interaction of water-soluble herbicides with surfactants, the cuticle, and humidity, with particular emphasis on the impact of low humidity and humectants on herbicide uptake is presented by Ramsey et al. 2005. It was found that when one focuses on research performed at low humidity the importance of humectants emerges, which is not in keeping with what is now commonly accepted (Ramsey et al. 2005).

It is well known that the activity of foliar-herbicide sprays is influenced by the environmental conditions at the time of spraying. Current evidence suggests that water-soluble herbicides may be more sensitive to variations in spray conditions than lipophilic herbicides.

Among the many environmental factors that can affect herbicide uptake, two of the most important are temperature and humidity, with optimal uptake being favoured by warm, humid conditions (Muzik 1976).

Temperature can affect herbicide uptake by changing the viscosity of cuticle waxes, the rate of diffusion, and in conjunction with humidity, cuticle hydration (Price 1983). Increased diffusion of solutes into cuticles with increasing temperature has been explained as a result of lower partition coefficients (log P) as temperature increases (Schönherr et al. 1999).

By the mid-1980s, it appeared as though research on humectants gave way to investigations on the effect of ethylene oxide (EO) content on surfactant performance. Ethoxylated surfactants have been shown to be effective at increasing the uptake of both lipophilic and hydrophilic herbicides.

However, an important observation from research on ethoxylated surfactants is that for water-soluble herbicides, surfactants that increase droplet spreading often result in poor uptake compared to surfactants that are poor spreaders and have higher surface tension. The reason for this is unclear, but it may be related to the fact that droplets with high surface tension have smaller surface areas for evaporation than droplets with low surface tension, which disperse across the leaf surface (Zhu et al. 2008).

## 7.9  Agro-adjuvants Models

The traditional method of study and development of different formulations has been to conduct experimental trials (both field trials and controlled environment trials) which are time consuming, expensive, and product specific. More recently, models are being developed that take a more mechanistic approach to the different aspects of spray application with the hope of gaining insight into the underlying physical processes (Zabkiewicz 2000). Often these models consist of sub-models of individual aspects of interest. The challenge in the future is to bring these modeling approaches together in a coherent and systematic way so that an integrated formulation efficacy support system can be developed.

By their nature comprehensive detailed models have large numbers of parameters that tend to be plant and a.i. specific and may be difficult to determine. An example of such a comprehensive model is given by the series of papers by Satchivi et al. (2000a, b, 2001, 2006). The results from these types of models are usually excellent but care must be taken that they are not used outside of their limitations (Trapp 2004). By their very nature these comprehensive models include many competing mechanisms for active ingredient uptake. Due to this the effects of varying one parameter (say droplet size) is not always obvious. The benefit of simplified models, such as that presented here, is that results are not obscured by surfactant adjuvants (Tu et al. 2003). The precise nature of this increased foliar uptake is not well understood but hydrophilic compounds generally show larger increases in uptake than lipophilic ones (Stock et al. 1993). Initial dose per unit area has been shown to be a strong determinant of uptake for surfactants of various spread characteristics (Forster et al. 2004). The addition of a surfactant can lead to greater droplet spreading and so the dose per unit area is reduced but it is applied over a larger area. These phenomena were investigated to determine what effect an increased spread area has on uptake. To be able to model the effect of droplet size and spread area a model with both horizontal (along the leaf surface) and vertical (into the leaf) spatial dimensions are needed as well as time dependence. To simplify the analysis circular symmetry of the droplet is assumed so the along leaf dimension is simply
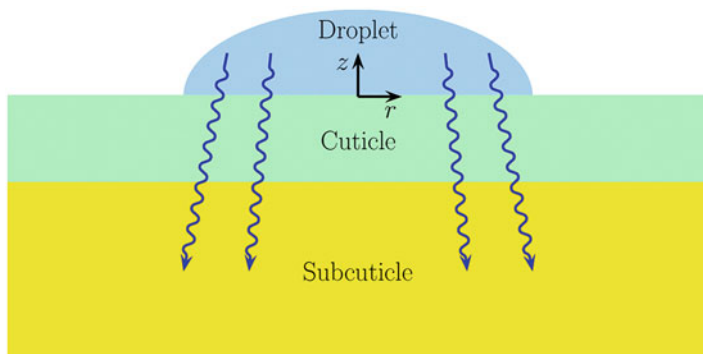
**Fig. 7.6** The schematic of the layers used in the numerical solution (Reprint from Mercer (2007))
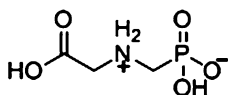


**Fig. 7.7** Glyphosate structural formula

the radius from the centre of a circular droplet. A three layer model is used as shown in Fig. 7.6. The upper layer is the droplet, the middle layer the cuticle and the lower layer the subcuticle. The governing process is assumed to be a simple diffusion process with removal of active ingredient in the subcuticle region (Mercer 2007).

The effect spray droplet size and spread area have on the diffusion of a hydrophilic active ingredient through a leaf cuticle and therefore on its eventual uptake by the plant. The model presented here is not designed to be a comprehensive detailed model of all the physical processes involved but rather a simplified model so that specific size and area related factors can be investigated.

## 7.10  Toxicity of Adjuvants for Glyphosate

Toxicity of wetting agents employed in the formulations of glyphosate will be analyzed in this section, because glyphosate is the most important pesticide worldwide and the amount of wetting agent present in glyphosate formulation becomes as high as 150 g/lt.

Glyphosate, N-(phosphonomethyl)glycine, the active ingredient of very well-known herbicide preparations, such as Roundup®, is a systemic and non-selective herbicide utilized for weed control, i.e. in agriculture, forestry, urban areas and even aquaculture (Woodburn 2000; Williams et al. 2000).

The use of this non-selective and broad-spectrum herbicide increased dramatically after the introduction of genetically modified glyphosate-resistant crops in

1997 (Giesy et al. 2000). Although glyphosate is already one of the most used xenobiotics in modern agriculture, we should expect an increasing utilization of glyphosate largely due to the number of transgenic plants developed to be tolerant to this herbicide (May et al. 2002; Nadler-Hassar et al. 2004; Stephenson et al. 2004).

The main formulation of glyphosate is Roundup®, where glyphosate is present as an isopropylamine (IPA) salt and its efficiency is enhanced by addition of the surfactant polyoxyethylene amine (POEA) (Tsui and Chu 2003).

Virtually, every pesticide product contains ingredients other than those identified as the "active" ingredient(s), i.e. the one designed to provide the killing action. These ingredients are misleadingly called "inert". Commercial glyphosate formulations are more acutely toxic than pure glyphosate, since the amount of Roundup® required to kill rats is about 1/3 of the amount of glyphosate alone (Martinez and Brown 1991). Similar results have been obtained in cell division, thus indicating a synergy between glyphosate and Roundup® formulation products (Marc et al. 2002). There are in the literature important studies regarding the possible impact on environment and human health toxicity of glyphosate, particularly since there is a paucity of data regarding chronic exposure to sublethal doses during embryonic developments (Paganelli et al. 2010) but they have not included due to it is out of the scope of this review.

Moreover, regarding the herbicide commercial formulation, the negative impact of polyoxyethylene tallow amine (POEA) surfactant towards examined amphibians was emphasized as correlated with the pH value of water environment. This latter finding is well harmonized either with the literature data (Krogh et al. 2003). POEA represents the group of nonionic alkylamine ethoxylates (ANEOs), which have been employed in agrochemical formulations and as spray adjuvants since the 1970s of twentieth century. For many years these chemicals were treated as non-toxic biodegradable additives; however, over the past two decades the knowledge on their adverse impact, especially on aquatic biota, increased dramatically (Krogh et al. 2003).

The traditional glyphosate surfactant system (called MON 0818) is a blend of components that contains a complex polyethoxylated tallow-amine (POEA) surfactant mixture. The primary component of MON0818 is POEA surfactant (about 75 %) (Wan et al. 1989). The other 25 % of ingredients have been determined to be relatively non-toxic (LC50 > 100 mg $l^{-1}$ for various ingredients to a variety of freshwater species; US Environmental Protection Agency ECOTOX Database), and therefore are unlikely to substantially contribute to the toxicity of MON 0818. The POEA surfactant component consists of a complex mixture of polyethoxylated long-chain aliphatic amines, which can be considered non-ionic in the neat form. In the aqueous environment of the microcosms used in the study, it is at least partially protonated and can be considered cationic.

Wade et al. reported that Mon 0818, a tallow amine surfactant, increased the diffusion of glyphosate into isolated plasma membrane vesicles, suggesting that surfactants could increase the membrane permeability (Wade et al. 1993).

On the other hand, the application of herbicides to control emergent aquatic vegetation or to treat the banks of waterways can produce localized concentrations

of glyphosate and surfactant in water that are greater than those from runoff associated with terrestrial uses (e.g., associated with agriculture). Glyphosate has been shown to be slightly toxic or non-toxic to aquatic organisms, whereas the MON 0818 surfactant containing POEA is considered to be moderately toxic (Giesy et al. 2000). Some studies have noted that the concentrations of MON 0818 in shallow water could reach levels that present an elevated risk to aquatic organisms (Wan et al. 1989; Folmar et al. 1979; Servizi et al. 1987). The risk potential of MON 0818 in shallow water is a function of both toxicity and environmental fate. Although numerous studies have been conducted to evaluate the fate and toxicity of glyphosate, limited information is available on the fate and toxicity of the surfactant alone (Giesy et al. 2000; Wan et al. 1989; Folmar et al. 1979; Goldsborough and Brown 1993; Beyers 1995; Gardner and Grue 1996; Franz et al. 1997; Henry et al. 1994; Paveglio et al. 1996). The majority of information available on the environmental fate of non-ionic ethoxylated surfactants pertains to linear alcohol ethoxylates and alkylphenol ethoxylates, which dissipate in the aquatic environment through adsorption to sediment and biodegradation by microbial action (Krogh et al. 2003; Urano et al. 1984; Cano and Dorn 1996).

Since aquatic weed control can occur in relatively shallow water bodies containing various types of sediments, Wang et al. assessed changes in aqueous concentrations of MON 0818 and changes in its toxicity to the cladoceran *Daphnia magna* over time in microcosms with and without natural sediments (Wan et al. 1989). The toxicity of the POEA surfactant, MON 0818, decreased rapidly in water from microcosms containing sediment, and this decrease in toxicity was correlated with the decline of MON 0818 concentrations in the overlying water. These results indicate that toxicity and concentration of the surfactant can be rapidly reduced in shallow water due to interactions with sediments. Although the present study did not directly address the potential for chronic exposure of aquatic organisms to the POEA surfactant, the strong tendency for this surfactant to adsorb rapidly to sediment, together with biodegradation, suggest that risks of chronic toxicity would be low. Furthermore, it also appears that dissipation is increased in the presence of sediment containing higher percent clay and total organic carbon, and higher microbial mass. The information obtained from the chemical analyses and toxicity tests illustrates the utility of applying different approaches to understanding complex surfactant-related issues. The mechanism of dissipation is likely to be related to either sorption or microbial degradation of the POEA surfactant (Giesy et al. 2000).

On the other hand, the organic carbon partition coefficients ($K_{oc}$) of $^{14}$C-labeled POEA surfactant in three different soil types (silt loam, silt clay loam, and sandy loam) ranged from 2,500 to 9,600, suggesting that the surfactant would strongly adsorb to soil (Marvel et al. 1974). In the present study, the POEA surfactant could rapidly adsorb to the surface of the sediment layer and to suspended sediment (a small amount of sediment was suspended into the water column when adding the MON 0818 solution into microcosms and when removing the sheets of aluminum foil from the surface of sediment at the beginning of the study). Microbial degradation can also contribute to the dissipation of POEA from natural

water bodies. However, the rate and extent of degradation will depend on the types and amounts of microbes present in the water and sediment. While no study on the microbial degradation of POEA surfactant is available in a water–sediment system, a study using $^{14}$C-labeled POEA surfactant in natural water containing suspended sediment reported a half-life of less than 3–4 weeks (Banduhn and Frazier 1978).

The half-lives of POEA surfactant reported by Banduhn and Frazier were obtained in a system of natural water and suspended sediment that likely had a relatively low microbial population (Banduhn and Frazier 1978). Using limited data, Giesy et al. estimated that the aquatic half-life for POEA surfactant would range from 21 to 42 d, which is substantially longer than the half-lives of 13 and 18 h that we estimated for shallow (12 cm) water columns in contact with sediments (Giesy et al. 2000). Sorption and degradation could be expected to occur in the presence of sediment.

Contardo-Jara et al. showed that glyphosate accumulates in L. variegatus, despite the hydrophilic character of the herbicide (Contardo-Jara et al. 2009). The accumulated amounts of glyphosate and the added surfactants in Roundup® Ultra cause an elevation of the biotransformation enzyme sGST at non-toxic concentrations and antioxidant enzyme superoxide dismutase was significantly increased by glyphosate but in particular by Roundup® exposure indicating oxidative stress. The accumulation and the enzymatic response of the worms were clearly higher in the animals exposed to Roundup®, indicating that the formulation Roundup® is of more ecotoxicological relevance than the glyphosate itself.

The alterations demonstrated by Peixoto[102] in mitochondrial bioenergetics caused by Roundup® cannot be exclusively attributed to the active ingredient, but may as well be the result of other chemicals e.g. POEA, or due the possible synergy between glyphosate and Roundup® formulation products (Peixoto 2005). This synergy between glyphosate and Roundup® inert ingredients has also been reported in cell division, where the delay observed in the cell cycle could be the result of alterations on the mitochondrial bioenergetic reactivity's, which have drastic consequences on cellular function through the perturbation of the bioenergetic charge and balance of the cell (Marc et al. 2002). Therefore, the reduced energetic efficiency of mitochondria may account for some toxic effects resulting from the impairment of the energy requirements of the cell and from the crucial importance of energy metabolism in active tissues, e.g. liver. Marc and co-workers found that the so called "inert ingredients" used in Roundup® are greatly responsible for the observed toxicity at the bioenergetic level. Data obtained in this study clearly demonstrate the ability of Roundup® to impair mitochondrial bioenergetic reactions. Alteration of basic mitochondrial functions was monitored by the detection of changes induced in mitochondrial respiration and membrane energization ($\Delta_\Psi$). Bearing in mind that mitochondria is provided with a variety of bioenergetic functions mandatory for the regulation of intracellular aerobic energy production and electrolyte homeostasis, these results question the safety of Roundup® on animal health.

## 7.11 Screening and Efficacy of New Surfactants for Glyphosate

Developing new plant protection formulations is a challenging task. The formulation of the active ingredient must assure a long shelf life stability including very high and low temperatures, a high loading and at the same time, the active ingredient needs to be optimal bioavailable. Greenhouse tests to evaluate the biological performance are regularly done with small plants and spray chambers using nozzles and water amounts which are not in accordance to practical field conditions.

Glyphosate, the most important generic active ingredient produced of the world is the most active patent area by far in order to improve glyphosate performance. Many companies sell glyphosate and the type and amount of surfactant in glyphosate formulations varies greatly. Green and Beestman focused on new formulation and adjuvant technologies available to maximize performance and minimize safety and environmental impact of herbicides (Green and Beestman 2007). Even though governments have not approved many new chemicals for use with agrochemicals during the past decade, there is a rich record of patent applications and product introductions. Formulation technology is the principal mechanism agrochemical companies use to renew products when the initial patents covering the active expire. The adjuvant and formulation industry has done an impressive job finding useful combinations and new utilities of currently approved chemicals.

Current data suggest that surfactant efficacy may be the result of charged surfactants ability to diffuse away from the cuticle into the subtending apoplastic space, where they act directly on the plasma membrane to increase glyphosate uptake. Cationic polyoxyethylene tertiary amine surfactants are more effective than nonionic surfactants at promoting glyphosate phytotoxicity (Wyrill and Burnside 1976; Riechers 1992). Surfactants promoting greatest glyphosate phytotoxicity are required at higher concentrations than necessary for maximum reduction of the spray solution surface tension, indicating that their mode of action is not limited to increasing the spreading characteristics of the spray droplet (Sherrick et al. 1986). This observation suggests that effective surfactants are also involved in increasing the permeability of the cuticle, plasma membrane, or both in increasing foliar uptake of glyphosate and promoting phytotoxicity. Richard and Slife determined that cellular absorption may offer greater resistance to foliar glyphosate uptake than cuticular penetration (Richard and Slife 1979). They suggested that the negative charges associated with the cell wall and the negative membrane potential of the plasma membrane repel the anionic glyphosate molecule. Sherrick et al. hypothesized that the surfactant MON 0818, is able to penetrate the cuticle and act at the plasma membrane (Sherrick et al. 1986). These authors reported that less than 20 % of [$^{14}$C]MON 0818 applied to field bindweed leaves was recovered in combined surface and chloroform washes, indicating surfactant penetration past the cuticle into underlying tissue.

Riechers et al. suggested that surfactant efficacy involves, at least partially, its ability to diffuse out of the cuticle into the apoplast where it alters membrane permeability to glyphosate (Riechers et al. 1994).

On the other hand, systematic studies conducted by and Nalewaja and co-workers indicate that surfactants of low ethylene oxide (EO) content may be optimal for promoting the uptake of lipophilic pesticides ($\log P > 3$), while those of high EO content were more beneficial for the uptake of hydrophilic compounds ($\log P < 0$) (Stock et al. 1993, 1992; Nalewaja et al. 1996). For compounds of intermediate lipophilicity, uptake is independent on the EO content of the added surfactant. The results were later confirmed by other studies on leaves and also on isolated cuticles (Nalewaja et al. 1996; Sharma et al. 1996; Coret and Chamel 1995).

One of the best way to evaluate the efficacy of glyphosate is measuring the efficiency of EPSPS (5-enolpyruvylshikimate 3-phosphate synthase) inhibition. Starting with a dose that is sprayed from a nozzle, only a fraction is foliarly retained, absorbed, translocated, and ultimately delivered to the plastids where EPSPS is localized. Each of these processes is in turn impacted by many physical, environmental, and physiological variables. For example, foliar retention can be affected by spray interception and droplet rebound which can be affected by plant morphology (e.g., leaf orientation and cuticle structure), environmental conditions (e.g., temperature and humidity), and surfactant concentration and droplet size (De Ruiter et al. 1990; Feng et al. 2003a; Buhler and Burnside 1987; Liu et al. 1996; Casely and Coupland 1985). Absorption is a major barrier for glyphosate efficacy and is affected by both surfactant class and concentration (De Ruiter et al. 1996; Denis and Delrot 1997). The efficacy of the tallow amine based cationic surfactants has been attributed to effective cuticle disruption (Feng et al. 1998; Ryerse 2001; Feng et al. 1999). Absorption is also impacted by the concentration gradient between the droplet and leaf, and efficacy enhancement has been reported at low spray volumes (Cranmer and Linscott 1990; Duncan Yerkes and Weller 1996; Feng et al. 2003a).

Glyphosate is translocated in the phloem from source to sink tissues following the sucrose gradient (McAllister and Haderlie 1985; Gougler and Geiger 1984). The excellent systemicity of glyphosate is derived from its unique combination of three acidic and one basic groups, and loss of any one of its functional groups resulted in decreased translocation (Bromilow and Chamberlain 2000). How glyphosate is loaded into the phloem is not well understood, but may involve phosphate transporters (Morin et al. 1997; Denis and Delrot 1993). Reductions in photoassimilate production and translocation ultimately reduce glyphosate translocation (Geiger et al. 1999). This self-limitation effect reduces the time window in which glyphosate can be efficiently translocated.

In the end, a plant that has been treated with glyphosate will die only if all the tissues have been killed. Any surviving tissue provides the opportunity for the plant to re-grow under favorable environmental conditions. The fate of individual tissues is decided based on whether a sufficient concentration of glyphosate was
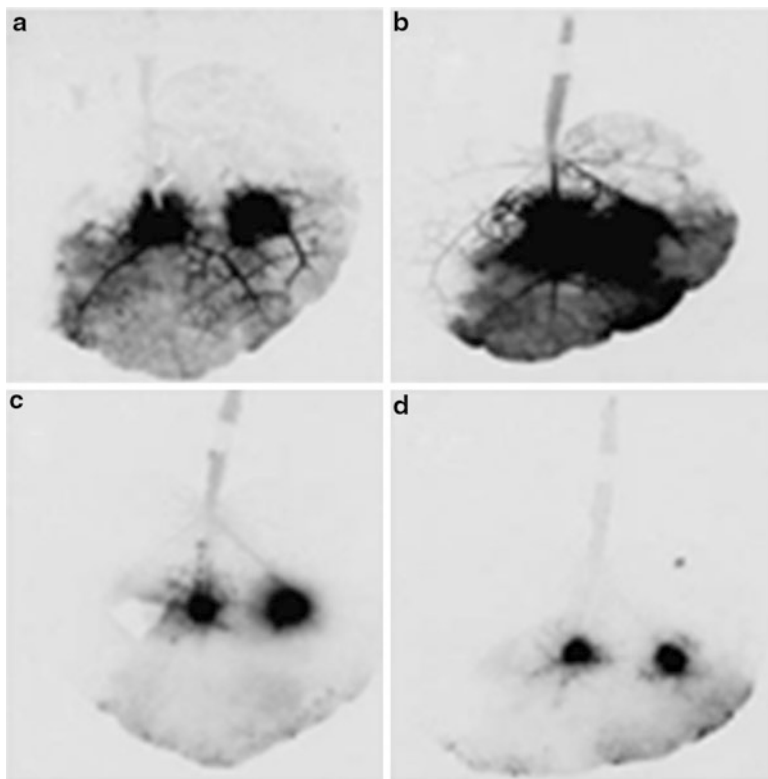
**Fig. 7.8** Analysis by autoradiography of leaves with [$^{14}$C]glyphosate in MON0818 (0.1 %) as a function of harvest time: 2.5 (**a**), 5.0 (**b**), 24 (**c**) and 48 HAT (**d**) (Reprint from Feng et al. (2003b))

attained to inhibit EPSPS. Feng et al. examined the role of glyphosate distribution and tissue sensitivity in overall efficacy (Feng et al. 2003b). The process by which glyphosate is exported out of the treated leaf was examined by autoradiography. A sub-lethal dose of [$^{14}$C]glyphosate was formulated in 0.1 % MON0818 (tallow amine ethoxylated an nonylohenol ethoxylated mixture) and applied as two 1 μl droplets to the first leaf of 3.5 leaf velvetleaf plants. At various times (2.5, 5.0, 24, and 48 h after treatment, HAT) the treated leaf was excised and washed with water to remove surface residues (Fig. 7.8). Exposure of tissues to X-ray film allowed visualization of glyphosate that was in the leaf and provided a time course of glyphosate leaf export.

The studies used velvetleaf as a model and [$^{14}$C]glyphosate as the marker, and showed that plant response to glyphosate was impacted by both differential tissue distribution as well as sensitivity. The second conclusion is that the amount of

**Fig. 7.9** Accumulation on shikimic acid in the third leaf of 3-week-old rape 48 h (◇), 24 h (•) and 6 h (○) after spraying with glyphosate (Reprint from Harring et al. (1998))
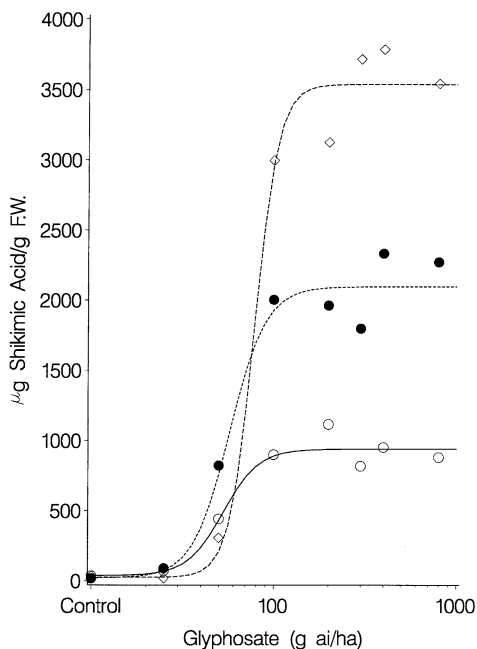
glyphosate delivered to tissues is linearly proportional to dose. This means that the dose does not change how glyphosate is distributed only how much is distributed. The current understanding is that whole plant efficacy is dependent on application of a dose of glyphosate that results in sufficient uptake and distribution to attain the lethal threshold in all tissues quickly and before the onset of self-limitation.

On the other hand, accumulation of shikimic acid was related to the dose of glyphosate by a nonlinear logistic dose response models as early as 5 h after spraying (Fig. 7.9). Within the same period shikimic acid accumulation made it possible to distinguish between a formulation of the pure isopropylamine salt of glyphosate and formulations containing different surfactants. ED50 (effective dose 50) estimates based on accumulation of shikimic acid gave a good indication of the relative strength of the evaluated glyphosate formulations. Ranking of ED50 based on accumulation of shikimic acid was the same as achieved by visual assessment of plant death 14 days after spraying.

Wan Kim and Amrhein found glyphosate induced accumulation of shikimic acid within 24 h after treatment of tomato (Wan and Amrhein 1995). Harring et al. studied the glyphosate action on phenylalanine ammonia-lyase activity and shikimic acid accumulation (Harring et al. 1998). Plants sense glyphosate toxicity within hours after application resulting in changes in carbon allocation and photosynthesis (Geiger and Bestman 1990).

## 7.12 Replacement of Fatty Amines and Nonylphenol Ethoxylated by New Renewable Resources-Based Surfactants

The selection of the type of formulation, active ingredient concentration, selection of the proper adjuvant and amount to maximize performance, maintaining product stability, all while insuring the toxicological properties of the product should meet customer, market and regulatory requirements. There is increasing regulatory and public pressure to decrease the amount of pesticides released into the environment. The challenge is to maintain and safeguard their efficiency with effective adjuvants. The use of petrochemical based surfactants like nonylphenol ethoxylates and fatty amine ethoxylates have declined significantly in the past decade, due to the ecological and toxicological reasons. Hence, there is continuing demand for alternatives based on a renewable source.

Run-off, leaching, adsorption and biodegradation are some of the possible exposure routes and main fate processes. Potential transport routes via rainwater may be either horizontal as run-off ending up in surface waters or vertical leaching to groundwater. Another fate route is adsorption to soil or biological material such as plants, plant roots and plant debris or micro-organisms in the soil. Additionally, the adjuvants may be biodegraded either aerobically or anaerobically.

The effects of nonylphenol ethoxylates and nonylphenol in the environment have been extremely controversial. Concerns first emerged in 1983–1984 when Giger and coworkers from Switzerland established that nonylphenol ethoxylates and products of degradation were more toxic to aquatic life than their precursors (Giger et al. 1984).

The first evidence that alkylphenols could be oestrogenic was published in 1938 by Dodds and Lawson but it was Soto and co-workers who accidentally observed that nonylphenol, which was employed in the manufacture of the test tubes used in their experiments, was capable of initiating proliferation in breast tumour cells as if oestrogens were present (Dodds and Lawson 1938; Soto et al. 1991). Endocrine disrupters can disturb the hormonal system by mimicking the occurrence of natural hormones, blocking their production or by inhibiting or stimulating the endocrine system. Nonylphenol was found to mimic the natural hormone 17β-oestradiol by competing for the binding site of the oestrogen receptor (Fig. 7.10a), due to their structural similarity (Fig. 7.10b) (Lee and Lee 1996; White et al. 1994).

For nonylphenol to exhibit endocrine activity the para-position of the phenolic OH-group and the branched aliphatic side chain appear to be determinant, meaning that not all nonylphenol isomers are capable of inducing oestrogenic activity (Kim et al. 2004; Odum et al. 1997; Tabira et al. 1999). 17β-oestradiol is a natural hormone that influences the development and maintenance of the female sex characteristics, and the maturation and function of accessory sex organs and it is also involved in the neuroendocrine and skeletal systems and is capable of promoting carcinogenicity in target tissues (Lee and Lee 1996; Alberts et al. 1983). Hence, nonylphenol is expected to initiate a variety of responses in organisms. Recently
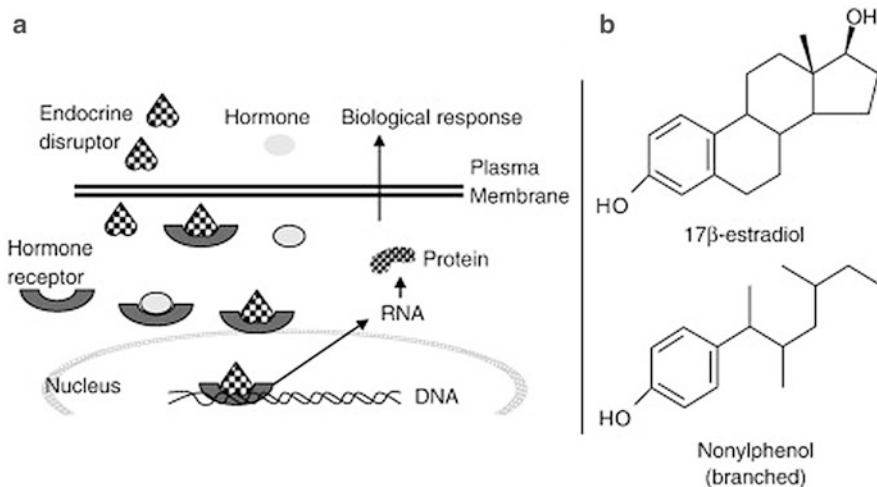
**Fig. 7.10** Competition for the oestrogen binding receptor between nonylphenol and 17b-oestradiol in the cell (Reprint from Soares et al. (2005))

it has also been established that nonylphenol has antiandrogenic activity, i.e., is capable of interfering with the proper functioning of androgens that are essential for the normal development of males and their reproductive systems (Lee et al. 2003).

The responses of the endocrine system to nonylphenol have been thoroughly studied in different organisms (White et al. 1994; Laws et al. 2000). For instance, nonylphenol was capable of inducing the production of female proteins in rainbow trout (Oncorhynchus mykiss) at 20.3 mg/l, medaka fish (Oryzias latipes) exposed to 0.1 mg/l nonylphenol, and the platyfish (Xiphophorus maculates) testis morphology and male fertility were negatively affected at nonylphenol concentrations ≥0.96 mg/l after 4 weeks of exposure (Jobling et al. 1996; Tabata et al. 2001; Kinnberg et al. 2000).

According to the data above showed, nonylphenol ethoxylates compounds have therefore been added to Annex I to Regulation (EC) No 689/2008 of the European Parliament and of the Council concerning the export and import of dangerous chemicals (OJ L 204, 31.7.2008, p. 1–35). The pesticides agreed as 'priorities for action' are listed here (updated in 2004), as well as a list of substances of 'possible concern' including 98 pesticides (updated in September 2005).

However, many other countries, including China, India and several South American countries use and produce nonylphenolic compounds in large amounts and no action has been taken by any of these countries to reduce or eliminate their usage. Nonylphenol ethoxylates are being replaced by other surfactants in most European, Canadian and Japanese markets, mainly by alcohol ethoxylates (Krogh et al. 1997). These surfactants are less efficient but considered to be environmentally safer as they degrade more rapidly (Campbell 2002). However, few studies have been performed on the metabolites that are produced as a result of degradation of

alcohol ethoxylates and it has been recently pointed out that their degradation gives rise to compounds with low water solubility and these adsorb to the sludge solids (Soares et al. 2005).

Meanwhile, tallow amine ethoxylates are the market reference of built-in adjuvants for glyphosate since many years, due to the excellent weed control performance they provide. However due to their toxicity profile there is a growing demand for alternatives with reduced labelling.

Traditionally, tallow amine ethoxylates (TAM-EO) were the built-in adjuvant of choice for standard isopropylamine (IPA) glyphosate 360 a.e./l formulations. These products are mediocre surfactants from a physico-chemical point of view, as indicated by fairly high surface tension and contact angle values. However, they are excellent adjuvants: it is believed that they act by facilitating the penetration of glyphosate, a relatively hydrophilic compound, through the rather hydrophobic cuticle which covers the external surface of higher plants, due to the disruption of cell walls allowing.

TAM-EOs and more especially TAM-15 EO and TAM-20 EO are harmful and hazardous for the environment. Quite unusual for a pesticide formulation is the co-formulant considered to be more toxic than the active ingredient. Glyphosate formulations with reduced labelling but with similar potency are therefore required in today's market.

The main disadvantages of these products is the high risk of damage to the eyes, the high toxicity to aquatic organisms, even in the concentration levels used in the formulation of glyphosate (8–15 %) and its high content of 1.4 dioxane (up to 25,000 ppm) due to the use of ethylene oxide during the process of manufacture of TAM-EO.

Several references were found in the literature about the toxicity of POEA (van Ginkel et al. 1993). TAM-EO has got more toxicity due to their high toxic by-products, without alkyl chain, resulting from the biodegradation process.

Finally in 2000, Bean and Cutler noted the low compatibility of alkoxylated alkylamine surfactants in general with high-strength glyphosate concentrates (Bean and Cutler 2000). This, in turn, has triggered the development of suitable formulants. Another driving force for new glyphosate additive developments is to save costs since the market has polarised into performance and low-cost brands since 2008. Many of the 700 glyphosate patent publications are directed at this issue, but still most of the improved adjuvant systems still contain amine-based surfactants. The current market situation could be called "the era of the small difference" where continually improved formulations are essential for success (Pimentel 1997).

## 7.13   Trends and Perspectives in Agrochemicals Formulations

The history of agricultural adjuvants dates back to eighteenth and nineteenth centuries when additives such as pitch, resins, flour, molasses, and sugar were used with lime, sulfur, copper, and arsenates to improve "sticking" and biological performance

by modifying the physical and chemical characteristics of the applied mixture. Fundamentally, the goal of using adjuvants has stayed the same. Using substances that are inactive when used alone to improve the performance and application of an active ingredient by modifying the physical and chemical characteristics of the spray mixture is a fundamental part of all agrochemical research.

Despite the significance of agrochemicals use for pest control, the environmental problems caused by overuse of agrochemicals have brought scientists and publics much concern in recent years (Dayan et al. 2009). Of the reasons for this are (1) the high toxicity and non-biodegradable of agrochemicals; (2) the lack of scientific formulations. Thus, formulation scientists now are facing the challenge to explore novel green or environmental friendly agrochemical formulations to improve the biologic efficacy and develop techniques that can be employed to reduce pesticide use while maintaining plant protection. The tremendous increase in crop yields associated with the 'green' revolution has been possible in part by the discovery and utilization of chemicals for pest control. However, concerns over the potential impact of pesticides on human health and the environment has led to the introduction of new pesticide registration procedures, such as the Food Quality Protection Act in the United States. These new regulations have reduced the number of synthetic pesticides available in agriculture. Therefore, the current paradigm of relying almost exclusively on chemicals for pest control may need to be reconsidered. New pesticides, including natural product-based pesticides are being discovered and developed to replace the compounds lost due to the new registration requirements. Dayan et al. covered the historical use of natural products in agricultural practices, the impact of natural products on the development of new pesticides, and the future prospects for natural products-based pest management (Dayan et al. 2009).

## 7.14 New Methodologies in Agrochemicals Formulation

### 7.14.1 Microemulsions and Nanoemulsions

Microemulsions are considered as thermodynamically stable colloidal dispersions that are optically transparent or translucent with drop size in the range of 100–200 nm (Prince 1977). Thus, microemulsion may be regarded as one phase system (Lindman and Danielson 1981). As indicated above, the cloud point is one of the specific characteristics of the microemulsions. When the temperature of the microemulsion system is increased, the solubility of non-ionic surfactant decreases. The cloud point of microemulsion can be defined as the temperature at which the transparent microemulsion solution becomes cloudy, i.e. from one phase to two phases or three phases (Strey 1996).

It is common knowledge that by hydrating of polyethylene oxide group (PEO) chain, non-ionic surfactant is dissolved in water medium, but it may dissociate with

water as a result of dehydration when the temperature exceeds its cloud point. The cloud point increases with increasing the amount of ethylene oxide groups in a chain, i.e. the higher hydrophilicity of non-ionic surfactant, the higher the cloud point. The cloud point is also affected by the concentration of the surfactant solution and the electrolytes in the aqueous solution. In the later case, usually lower the cloud point (Yoshihara et al. 1995; Saito and Shinoda 1967; Minanaperez et al. 1995; Tadros 1994). Chen et al. described, two new-types of pesticide microemulsions used for control *Liriomyza* spp., namely 16 wt % beta-cypermehtrin (inside microemulsion xylene oil droplets) plus monososultap (dissolved in water medium) and 20 % abamectin (inside microemulsion xylene oil droplets) plus monososultap (in the water medium) were prepared (Chen et al. 2000). The effects of agrochemical concentration, various surfactants at various concentrations on the cloud points of microemulsions have been studied. The stability of microemulsions containing 5 wt % abamectin and 1 wt % beta-cypermethrin is also discussed. Similar to the cloud point of surfactant aqueous solution, at constant surfactant concentration the cloud point of the agrochemical microemulsions increases as the hydrophilicity of the surfactant increases. The cloud point of the formulated microemulsions depends on the characteristics of the agrochemical, the kinds and the amounts of the added surfactant and co-surfactant.

The results above described shown that the values of the cloud point of microemulsions were depended on the nature of agrochemical, the surfactants used and the concentration of surfactants. Surfactants with higher HLB value at same concentration produce more stable agrochemical microemulsions, i.e. with higher cloud point. The results showed that the match between surfactant and oil phase or pesticide oil phase was the key to formulate stable microemulsions. However, the water quality was showed almost no effect on the cloud point of the agrochemical microemulsions in the studies.

Meanwhile microemulsions, which contain non-polar agrochemicals, usually have higher cloud points than those of containing polar ones, especially electrolytic agrochemicals. As discussed, higher cloud points can be obtained with an increase in surfactant concentration, but it is more reasonable to use more co-surfactant rather than surfactant, because the former is much cheaper.

On the other hand, Nanoemulsions have uniform and extremely small droplet sizes, typically in the range of 20–100 nm (Forgiarini et al. 2001). In addition, high kinetic stability, low viscosity and optical transparency make them very attractive systems for many industrial applications; for example, in agrochemicals for pesticide delivery (Lee and Tadros 1982). Wang et al. investigated potential applications of the system developed with water insoluble pesticide, $\beta$-cypermethrin ($\beta$-CP), incorporated into the precursor microemulsion concentrate (Wang et al. 2007). The effect of this active pesticide on stabilities of the concentrate, and the corresponding nanoemulsion, were also investigated. The incorporation of $\beta$-CP in the concentrate showed no effect on the phase behavior when present at less than 12 wt %. Compared with the commercial $\beta$-CP microemulsion, the excellent stability of sprayed solution diluted from the concentrate makes this system an ideal

candidate as a water-insoluble pesticide delivery system. Thus, the application of the new methodology designing by spray formulations of $\beta$-CP may enable a reduction in the applied amounts, relative to those formulated as O/W microemulsions. These characteristics make the new methodology promising from both environmental and economical points of view.

### 7.14.2    Liposomes

Liposomes are structures made of lipid bilayers forming one or more concentric spheres which entrap part of the solvent in which they freely float, into their interior. Their unique properties have triggered numerous applications in various fields of science and technology, from basic studies of membrane function to the use as carriers of very different substances. In agriculture, they can be used to improve the efficacy of different biocides and to deliver some essential nutrients (Lasic 1993). Herbicides, fungicides and pesticides are rapidly washed from the leaves of plants and encapsulation in liposomes may prolong the action of these agents on plants and reduce the damage in soil cultures. Pons and Estelrich described the optimization of a cheap and easy method of preparing liposomes (Pons and Estelrich 1996). Among the studied formulations there are some with a high stability and this property makes them very suitable to be used as an agrochemical product.

### 7.14.3    Nanomaterials

Materials with a particle size less than 100 nm in at least one dimension are generally classified as nanomaterials. The development of nanotechnology in conjunction with biotechnology has significantly expanded the application domain of nanomaterials in various fields. However in the field of agriculture, the use of nanomaterials is relatively new and needs further exploration.

Khot et al. summarized the developments and application of novel nanomaterials in agriculture, and described nanopesticides and its applications. Nanopesticides "involve either very small particles of pesticidal active ingredients or other small engineered structures with useful pesticidal properties" and can increase the dispersion and wettability of agricultural formulations (i.e., reduction in organic solvent runoff), and unwanted pesticide movement. Nanomaterials and biocomposites exhibit useful properties such as stiffness, permeability, crystallinity, thermal stability, solubility, and biodegradability needed for formulating nanopesticides. Nanopesticides also offer large specific surface area and hence increased affinity to the target. Nanoemulsions, nanoencapsulates, nanocontainers, and nanocages are some of the nanopesticide delivery techniques that have been discussed recently for plant protection (Khot et al. 2012).

## 7.15   New Surfactants and Additives Apply to Agrochemicals Formulation

There is increasing regulatory and public pressure to decrease the amount of pesticides released into the environment. The current trends in the development of pesticide formulations are increased enormously to meet the needs of environmental safety, eliminate organic solvents or to improve the activity and persistence of the active ingredient (Knowles 2008).

### 7.15.1   Alkyl Polyglycosides

Alkyl polyglycosides or APG's are non ionic surfactants with a hydrophilic saccharide instead of an ethylene oxide chain. The alkyl chain has 8–16 carbons. APGs are water soluble with excellent adjuvant properties. APGs are made from renewable raw materials and can be put into very concentrated liquid of dry formulations (Pompeo et al. 2005). They are called "green surfactants" because they are very safe to the environment.

Short chain APGs show decent properties as wetting and penetrating agents and offer a high tolerance to saline solutions though they are non-ionic, and do not exhibit a cloud point typical for alkoxylates (Nickel et al. 1996). The importance of nonionic APGs for glyphosate was first recognized by Syngenta as potentiators for glyphosate (Burval and Chan 1995).

Alkyl polyglycosides (APG) are superior surfactants with outstanding wetting properties and are used as adjuvants for pesticides as they improve the spreading and enhance the uptake of the pesticides (Garst 1997). Nonetheless, the effect of APGs on weed control of glyphosate cannot match industry standards. Advantageously however, as compared to TAM-EO, APGs are generally classified as non-toxic and ready biodegradable. Several options were explored to take advantage of the benefits of APGs as high performance wetting agents whilst optimising the weed control.

A new class of non-ionic surfactants resulting from the direct ethoxylation of alkyl and/or alkenyl polyglycosides was designed as possible alternatives to TAM-EO (Behler and Clasen 2006). Their performance as adjuvants for glyphosate was assessed in greenhouse trials on two model plant species. The new derivatives were compared to TAM-EO and to straight APG. According to the greenhouse data, alkoxylated alkyl polyglycosides showed good weed control almost reaching performance of TAM-EOs and surpassing efficiency of standard APG. Alkoxylated alkyl polyglycosides exhibit also a much better toxicological profile compared to TAM-EOs reducing the risk to end-users.

### 7.15.2  Ethoxylated Saccharose Esters

In 2007, ethoxylated APGs were introduced. Due to their hydrophilic properties, they turned out to be suitable glyphosate potentiators and adjuvants (Abribat et al. 2007). However, their low lipophilicity makes them not compatible in oil based formulations such as emulsifiable concentrate (EC) or concentrated oil-in-water emulsion (EW) and consequently, new lipophilic surfactants based on ethoxylated saccharose esters have been developed.

In a recent application, Mainx and Hofer described alkylene oxide adducts of oligosaccharides like saccharose (Mainx and Hofer 2009). Their esterification leads to a new chemical class of label-free nonionic surfactants. Selected representatives of this class of chemicals showed outstanding results in boosting the performance of crop protection products in greenhouse studies. Indeed, the protective property of azoxystrobin applied with these new ethoxylated saccharose esters was more effective than the industry standards, such as nonylphenol ethoxylated and isotridecyl alcohol 6 EO. In curative tests, the activity of the fungicide epoxiconazole was improved as well, although the effects were less pronounced.

Epoxiconazole was improved as well, although the effects were less pronounced and even the activity of glyphosate was enhanced. Ethoxylated saccharose esters show a good toxicological and ecotoxicological profile. Their behaviour in pesticide formulations and tank mix solutions is excellent, so in total, they can be considered as a novel suitable class of adjuvants and surfactants for pesticide formulations.

The studies above mentioned showed that ethoxylated saccharose esters have an excellent potential as new adjuvants and emulsifiers in agrochemical compositions. Especially with fungicides at low concentrations, they show extraordinary results in improving the activity of the active ingredients suggesting their suitability as both, tank mix adjuvants and inerts for incan formulations. Due to good compatibility with most new solvents, this new chemical class of surfactants can also be considered to become suitable components in all pesticide formulations either alone or in combination with other nonionic or anionic surfactants.

### 7.15.3  Dimethylethanolamine Based Esterquats

Esterquat chemistries are widely used in the commercial laundry market as fabric softeners. They have been used since the 1970s as biodegradable alternatives to dihydrogenated tallow dimethyl ammonium chloride (DHTDMAC) chemistries. However, the use of esterquats in agrochemical formulations has not been thoroughly explored and would represent a biodegradable alternative chemistry (Zoller 2009). In addition, esterquats may offer reduced eye irritation or improved ecotoxicity profiles to an industry that is becoming ever more scrutinized for such formulation improvements.

Malec et al. explored the use of esterquats based on dimethylethanolamine (DMEA) in agricultural formulations (Malec et al. 2009). It was determined that DMEA based esterquats can function as wetters or spreaders, but can also act as emulsifiers. The surface active properties and aquatic toxicity of esterquats as surfactants will be explored as well. The esterquats have lower critical micelle concentrations, lower equilibrium surface tensions, and faster wetting times than traditional agricultural surfactants.

Various formulations were explored including herbicides, insecticides, and fungicides. These formulations included delivery systems such as soluble concentrates (SL) and emulsifiable concentrates (EC). The formulations were evaluated and in many cases provided performance properties that were equal to current standards for commercial products. Greenhouse trials were conducted evaluating the effectiveness of esterquats in glyphosate (N-(phosphonomethyl) glycine) formulations. It was found that esterquats were successful glyphosate adjuvants, utilizing lower surfactant use rates while offering equal efficacy to commercial products. Additionally, glyphosate formulations using DMEA esterquats have lower eye irritation compared to other commercial standard glyphosate surfactants. The aquatic toxicity of DMEA esterquats was also studied and the DMEA esterquats were found to be less toxic than TAM-EO.

The studies showed that DMEA esterquats may serve a valuable function in agrochemical formulations. They offer improved biodegradability, lower eye toxicity, improved wetting, spreading, and penetration, and comparable aquatic toxicity to commercial surfactants already in use in agrochemicals.

### 7.15.4   Chitosan Derivatives

Chitosan based polymeric micelle due to its outstanding biologic properties and functions such as biodegradability, biocompatibility, insecticidal and antibacterial activity, has been widely researched or applied in the fields of agriculture, medicine, pharmaceuticals, functional food in the last decade (Chellat et al. 2000; Risbud and Bhonda 2000; Badawy et al. 2004; Hejazi and Amiji 2003; Kumar et al. 2004; Rabea et al. 2005). However, chitosan has no amphiphilicity, cannot form micelle and load drug directly. In recent years, chitosan-based micelle system has been developed by introducing hydrophobic and/or hydrophilic groups to the chitosan backbone (Zhang et al. 2008). Amphiphilic chitosan derivatives which grafted sulfuryl as hydrophilic moieties and octyl as hydrophobic moieties had been reported (Zhang et al. 2003, 2008).

Lao et al. described novel amphiphilic chitosan derivatives designed and synthesized by grafting octadecanol-1-glycidyl ether to amino groups, sulfate to hydroxyl groups (e.g. N-(octadecanol-1-glycidyl ether)-O-sulfate chitosan (NOSCS)) (Lao et al. 2010). Rotenone as a model drug was chosen and used to assess the potential loading capability of novel chitosan derivatives. The insecticide rotenone was loaded and formed about 116.4–216.0 nm nanoparticles, and its solubility in

NOSCS micelles aqueous solution was increased largely. The highest concentration of rotenone was up to 26.0 mg/mL (NOSCS- 1), which was about 13,000 times that of free rotenone in water (about 0.002 mg/mL).

### 7.15.5 Ammonium Sulphate and Sulphated Glycerine as Additive

The leaf cuticle and plasma membrane have been identified as barriers limiting glyphosate activity (Riechers et al. 1994; Denis and Delrot 1993). Neither glyphosate nor its different salts are effective in overcoming these barriers easily without appropriate surfactants (Buhler and Burnside 1983b; Jordan 1981). Cationic surfactants have been found to be more effective than nonionic surfactants in increasing efficacy (Wyrill and Burnside 1976; Riechers et al. 1995). The addition of ammonium sulphate (AMS), an inorganic salt, to the glyphosate spray solution improved the efficacy of the herbicide (Blair 1975). Additionally, it has been found that salts dissolved in water used as the carrier for glyphosate may reduce its effectiveness, particularly calcium and magnesium salts. These salts have a positive charge and may associate with the negatively charged glyphosate molecule, replacing the isopropylamine or diammonium salts found in the formulated glyphosate product. AMS reduces the formation of calcium–glyphosate complexes on these leaves and therefore improves performance (Hartzler 2001). The inverse relationships between weed growth parameters and increasing concentration levels of AMS suggest that the ability of AMS to enhance glyphosate activity is to a large extent concentration dependent (Aladesanwa and Oladimeji 2005).

Glycerine and ammonium sulphate have both been popular additives in the long-held practice of adding chemical agents (adjuvants) to improve biological efficacy in crop protection sprays (Gednalske and Herzfeld 1994). Glycerine, a polyol of natural origins (triglycerides), is one of the principle building blocks of plant tissues. This biocompatibility as well as glycerine's humectancy is strong contributors to its adjuvancy with additional benefit as a hydrotrope to homogenize water-based spray solutions (Heldt et al. 2000). Ammonium sulphate is a proven sequestrant of hard water cations as well as a nitrogen source benefiting herbicides of diverse modes of action (Buhler and Burnside 1983a). One difficulty with ammonium sulphate solutions are their ionic strength and the difficulty that presents in creating shelf-stable blends with surfactants (Gednalske and Herzfeld 1994).

Anderson presented the reaction product(s) resulting from sulphating glycerine show strong adjuvant activity with herbicides (Anderson 2010). As with ammonium sulphate, the adjuvant activity of sulphated glycerine includes both, sequestering cations commonly found in hard water as well as increased nitrogen for non-complexing herbicides. The field treatments including sulphated glycerine, demonstrated statistically superior weed control when compared to un-sequestered reference samples. These "hard water" treatments including sulphated glycerine

were statistically equivalent to control observed for glyphosate sprays prepared with de-ionized water. The increased efficacy of the herbicide glufosinate was demonstrated when using ammonium sulphate or sulphated glycerine as an adjuvant. Both of these biological observations were confirmed with rates of sulphated glycerine significantly lower than current label rates for ammonium sulphate.

## 7.16   Conclusion

Uptake of pesticides into plant foliage varies with plants and chemicals, and can be greatly influenced by adjuvants and environmental conditions. Adjuvants usually have multiple functions in relation to pesticide efficacy. Increasing the foliar uptake of active ingredients is of particular importance for herbicides, growth regulators, and defoliants. It is known that the penetration of pesticides into plant leaves is related to the physicochemical properties of the active ingredients, especially molecular size and lipophilicity. However, the uptake rate of a compound cannot be predicted by either of them or even combination of them. For a specific chemical, uptake varies greatly with plant species and there is no simple method at the moment to quickly evaluate the leaf surface permeability of a plant.

Environmental conditions have an important influence on herbicide efficacy. In particular, the effect of humidity on herbicide uptake has been attributed to changes in cuticle hydration and droplet drying. Herbicide uptaking slows or stops when herbicide droplets dry, it was suggested that humectants or wetting agents could enhance herbicide uptake by keeping droplets moist, maintaining the herbicide in solution, and keeping it available for uptake.

Various adjuvants are being used to increase the penetration of pesticides into target plant foliage, but their effects vary with chemicals and plant species. The mechanisms of action of adjuvants in enhancing pesticide uptake remain unclear despite the effort made during the last three decades. Modern analytical and microscopic techniques provide powerful tools to deepen our understanding in this issue. However, a more multidisciplinary approach is urgently needed to elucidate the transcuticular diffusion behaviour of pesticides and the mode of action of adjuvants. A better understanding of the foliar uptake process should lead to a more rational use of pesticides and minimize their negative impact on the environment. Therefore, a systematic study on the complex interaction between active ingredients, surfactants and plants should lead to a more rational and cost-effective use of surfactants in weed control.

On the other hand, commercial glyphosate formulations are more acutely toxic than glyphosate, since the amount of Roundup® required to kill rats is about 1/3 of the amount of glyphosate alone. Similar results have been obtained in cell division, thus indicating a synergy between glyphosate and Roundup® formulation products. Although glyphosate is already one of the most used xenobiotics in modern agriculture, we should expect an increasing utilization of glyphosate largely due to the number of transgenic plants developed to be tolerant to this herbicide. Fatty

amine ethoxylates or co-formulant called "inert ingredients" used in Roundup® are greatly responsible for the observed toxicity at the bioenergetic level. Quite unusual for a pesticide formulation is the co-formulant considered to be more toxic than the active ingredient. Glyphosate formulations with reduced labelling but with similar potency are therefore required in today's market.

The fact that the adjuvant could be more toxic than the active ingredient in one of the most active ingredient used pesticides together with the need that more effectiveness is required to reduce the environmental effect of xenobiotics has lead to the development of new adjuvants, e.g. more than 600 patent of glyphosate formulations have been published in the last 12 years.

It is important to point out that there is increasing regulatory and public pressure to decrease the amount of pesticides released into the environment. The challenge is to maintain and safeguard their efficiency with effective adjuvants. The use of petrochemical based surfactants like nonylphenol ethoxylates and fatty amine ethoxylates have declined significantly in the past decade, due to the ecological and toxicological reasons. Hence, there is continuing demand for alternatives based on a renewable source. Suppliers are developing new surfactants and new surfactants formulations based on natural products such as vegetable oils, lecithin, sugars, amino acid and others. Microemulsions, liposomes and nanoemulsions as emerging technologies in agrochemicals formulations have reduced the use of petrochemical solvents, e.g. xylene and improve the efficiency of biocides. The development of nanotechnology in conjunction with biotechnology has significantly expanded the application domain of nanomaterials in various fields. However in the field of agriculture, the use of nanomaterials is relatively new and needs further exploration.

These products will become more popular in the near future as a means of increasing product safety and enabling quicker product regulation.

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# Chapter 8
# Cheap Materials to Clean Heavy Metal Polluted Waters

**Pei-Sin Keng, Siew-Ling Lee, Sie-Tiong Ha, Yung-Tse Hung, and Siew-Teng Ong**

**Abstract** The rapid growth of the human population and industrialization in the world has increased the environmental problems such as water, air and land pollution. Heavy metals can be considered as one of the most problematic pollutants. This chapter reviews developments and technical applicability of treatments methods for the removal of heavy metals. We focus on the use of low-cost materials to remove heavy metals. Isotherm equilibrium models and kinetics are discussed. Designing adsorption columns under continuous flow conditions are evaluated.

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**Contents**

## 8.1 Heavy Metals

In recent years, water pollution by heavy metals poses one of the most serious environmental problems and of the most difficult to solve. Activities such as extensive use of fertilizers, municipal waste to land, electroplating, energy and fuel production, and wastewater from manufacturing industries including textile industry are among the major contributions to the contamination of environment by heavy metals. These metallic agents, once concentrated in the biosphere, generally persist and are not broken down. It is reported that cadmium, lead, cobalt, copper, mercury, chromium, nickel, selenium and zinc are the most important metals related to chain food contamination (McLaughlin et al. 1999). These metals are carcinogenic to human beings if consumed in higher quantity. Therefore, heavy metal pollution poses a great potential threat to the environment and human health.

Heavy metals may be defined as the elements having atomic weights between 63.5 and 200.6 and have a minimum specific gravity of 5 (Srivastava and Majumder 2008). Specific gravity is dimensionless property of matter and its value is obtained by dividing the density of that substance with the density of water. In addition, other experimental conditions, such as temperature, for determining the two densities must be the same. Heavy metals are "trace elements" with individual concentrations that do not exceed 1,000 mg/kg (0.1 %) of earth's crust. They are classified as

essential, beneficial, or detrimental. For instances, iron, zinc, copper, chromium, iodine, cobalt, molybdenum, and selenium are recognised as essential for human health, of course with specific recommended daily allowances (RDA) or reference dose (RfD) set by respective authorities. On the other hand, some heavy metals are regarded as purely toxic metals such as cadmium, lead, nickel and mercury, which do not provide any essential or potentially beneficial health effect at any level of exposure (Nordberg et al. 2002). As arsenic, nickel and lead are metals of widespread use and frequently found in industrial wastewater, these metals were discussed in the following section.

### 8.1.1  Arsenic

Arsenic (As) is a metalloid belonging to group 15 of the periodic table with atomic number of 33 and atomic weight of 74.92. It is found naturally in the environment with estimated crustal abundance of 1.8 mg/kg and estimated oceanic abundance of 3–3.7 mg/L. The common oxidation states for arsenic are 5, 3 and −3, which appear widely in a variety of minerals. Realgar ($As_4S_4$) and orpiment ($As_2S_3$) are the two common arsenic sulfides. Arsenic also occurs as arsenide and sulfaresenides of heavy metals such as iron, copper, lead, silver, and gold (Yan Chu 1994). Loellingite ($FeAs_2$), safforlite ($CoAs$), niccolite ($NiAs$), arsenopyrite ($FeAsS$) and enargite ($Cu_3AsS_4$) are some of the examples of minerals containing arsenide and sulfaresenides. Arsenic can also be found in oxidised form such as in the mineral arsenolite ($As_2O_3$) (Greenwood 1989)

As a result of its toxicity, arsenic compounds are used mainly in wood preservation and insecticides. It is also employed in the making of special glass and semi-conductors, gallium arsenide (GaAs). GaAs is a semiconductor used in laser diodes and light emitting diodes (LEDs). While arsine gas ($AsH_3$) has become an important dopant gas in the microchip industry under strict guidelines. The widely used of arsenic in aforementioned activities, mining as well as erosion and weathering of rocks have contributed significantly to the content of this element in the environment.

Ingestion and inhalation of arsenic and its compounds cause various health effects. Arsenic interferes with enzyme action, DNA transcription, and metabolism. It causes chronic weakness, general debility and lassitude, loss of appetite and energy and sometimes a degree of dementia (Hindmarsh and McCurdy 1986; Yamanaka et al. 1996). Uptake of significant amounts of inorganic arsenic is associated with cancer development, especially development of lung, liver and lymphatic cancer. Prolong exposure to arsenic compounds will also cause dermatological effect such as hyperpigmentation and hypopigmentation, progressing to palmar/plantar hyperkeratosis and cause skin cancer eventually (Hindmarsh and McCurdy 1986). In addition, a very high exposure to inorganic arsenic contributes to infertility and miscarriages in women and most importantly to note that the lethal dose of arsenic is reported as 100 mg (Lenntech 2012a).

## 8.1.2    Nickel

Nickel (Ni) is a silvery white, hard, malleable and ferromagnetic metallic element belonging to group 10 of the periodic table with atomic number of 28 and atomic weight of 58.69. Nickel is a fairly good heat and electric conductor. It is a commonly found constituent in most meteorites with iron meteorites (siderites). Besides, nickel is also found sparingly in nature, usually combined with arsenic or iron and sulfur such as niccolite (NiAs) and pentlandite (NiS·2FeS). The estimated crustal abundance of Ni is $8.4 \times 10^1$ mg/kg and its estimated oceanic abundance is $5.6 \times 10^{-4}$ mg/L.

Nickel is corrosion resistant and used primarily for making of stainless steel and many other corrosion resistant alloys. Typical composition of stainless steel is 8 % nickel, 18 % chromium and the remaining is iron. In fact, approximately 65 % of the nickel consumed in the western world is used to make stainless steel (Lenntech 2012b) .Other uses of nickel are in coinage, electroplating and battery production, foundry products as well as catalyst for hydrogenation process.

Nickel is exposed to human beings through inhalation, ingestion and skin contact. Workers in the industries related to nickel applications are the main group to suffer of nickel poisoning via inhalation of nickel-containing aerosols, dusts, fumes, and mists, especially those involved in production, processing, and use of nickel. Smokers are also at high risk to nickel inhalation as cigarettes, on average, contain 1–3 µg nickel (ATSDR 1993; Von Burg 1997) and mainstream smoke from one cigarette contains 0–0.51 µg nickel (Smith et al. 1997). Out of the total inhalation of nickel, about 20–35 % is deposited in the lungs causing lungs inflammation. On the other hand, nickel is also exposed to human population through ingestion of food and drinking water. Drinking water is the main contribution to nickel ingestion with 27 % nickel in drinking water is absorbed in humans compare to food which is approximately 1 %. This is because more soluble nickel compounds have higher absorption efficiencies.

Absorbed nickel is unable to be metabolised by human body. It is eliminated via urine with the elimination half-time of 28 days for the oral route. In fact, serum and urine nickel levels are the most useful monitoring of nickel exposure. According to the Agency for Toxic Substances and Disease Registry (ATSDR) of United States, the reference values for nickel in healthy adults are 0.2 µg/L in serum and 1–3 µg/L in urine (ATSDR 2005). For dermal contact of nickel, it causes dermatitis which is the most adverse health effect associated with nickel exposure. The common dermal contact of nickel is via nickel alloy and nickel-plated products such as stainless steel, coin and jewelry.

## 8.1.3    Lead

Lead (Pb) is a soft, malleable, ductile and dense metal. It has bluish-gray colour and belongs to group 14 with atomic number 82 and atomic weight of 207.2.

Though lead do not occur abundantly in nature but it is readily accessible in the earth's crust as the mineral galena (PbS), anglesite (PbSO$_4$) and cerussite (PbCO$_3$). The estimated crustal abundance is $1.4 \times 10^1$ mg/kg and the estimated oceanic abundance is $3 \times 10^{-5}$ mg/L.

Lead is corrosion resistant, thus historically it was used in plumbing. Nowadays it is used to line tanks that store corrosive materials such as sulfuric acid (H$_2$SO$_4$) and as covering for cables. Lead's high density makes it useful as a shield against X-ray and gamma-ray radiation and is used in X-ray machines as well as in the glass of computer and television screens, where it shields the viewer from radiation (Lenntech 2012c). The other major use of lead is in lead-acid batteries manufacturing and formation of lead alloys. For instances, solder, an alloy constituted of half lead and half tin, is a material with a relatively low melting point that is used to join electrical components, pipes and other metallic items.

Like arsenic, lead is toxic in nature. Lead poisoning in human population is due to inhalation, ingestion and dermal contact to lead compounds. The emission of lead particulate in the air is primarily due to anthropogenic activities including automobiles using leaded gasoline. Lead is also found in soil due to adsorption at mineral interfaces, weathering and chipping of lead-based paint from buildings, bridges and other structures. Lead can also leach into drinking water from the leaded pipes in water distribution systems (Madden et al. 2002).

It is reported that children are more vulnerable to the effects of lead than adults. Children have higher gastrointestinal absorption of lead (40–50 %) compare to adults (3–10 %) (ATSDR 2007). A study on the lead content was conducted in Saratov, a town in Russia which its environment is exposed to lead discharge due to automobiles and operations of battery and lead glass factories. Blood lead and environmental samples from 579 children and their homes for soil, water, dust and paint are collected for lead content analysis. The study included the first pediatric blood lead screening among Russian children and the result shows that nearly one-fourth of the children had blood lead levels above the United State level of concern, 10 μg/dL (Rubin et al. 1997). Absorbed and retained of high dose of lead in children will disturb their neurological development. A well-developed literature argues that these neurological impacts reduce cognitive ability and lead to behavioral problems and learning disabilities later in life. Exposure to lead also increases the risk of kidney failure, disruption of central nervous system, brain damage and even death in cases of extremely high exposure (Jones 2012).

## 8.2 Heavy Metal Removal: Conventional and Emerging Methods

Extensive research and financial resources have been spent on the treatment of heavy metals pollution especially in industrial wastewater which is contaminated by heavy metals. These technologies include conventional physico-chemical methods

and biological treatments, where the latter have gradually getting momentum because they are more eco-friendly and cost-effective compare to the conventional techniques. The physical-chemical methods include chemical precipitation, ion-exchange, adsorption, membrane filtration, coagulation-flocculation and electro-chemical methods. Biological treatments involve the use of plants and microbes in any of the above said methods.

### 8.2.1 Chemical Precipitation

Chemical precipitation is the most common and effective method used in the industry for treatment of wastewater because it is relatively easy and inexpensive to operate (Ku and Jung 2001). This technique is employed in almost 75 % of electroplating facilities to treat wastewater (Karthikeyan et al. 1996). Generally, the metals are precipitated as insoluble hydroxides, carbonates, or sulfides and separated from water by sedimentation and filtration (Table 8.1).

**Hydroxide Precipitation**

Of all the chemical treatments, hydroxide precipitation is the most preferable and being extensively used method in the industry, particularly where complex chemical compounds are not involved and economic recovery is not a consideration. Lime, $Ca(OH)_2$ and caustic soda, $NaOH$ are the preferred choice of bases used to precipitate heavy metals as their respective hydroxides. There are numerous factors govern the efficiency of metal hydroxides precipitation. For instances, the pH of the metals solution, the electrolyte composition of the effluent, the presence of soluble and/or insoluble products following reaction with complexing agents, ionic strength, electrical potential, temperature, and the time of reaction (Lin et al. 2005).

Dean et al. (1972) have found that there is almost complete precipitation and removal of metals such as cobalt, copper, iron, manganese, nickel, and zinc as the hydroxide with no special modification. However addition of soda ash (for lead) or sodium sulfide (for cadmium and mercury) is required to complete the precipitation of cadmium, lead and mercury. Chen et al. (2009) also reported that fly ash is used to enhance lime precipitation of metals. The fly ash-lime-carbonation treatment increased the particle size of the precipitate and significantly improved the efficiency of heavy metal removal. As a result, the removal efficiency of chromium, copper, lead and zinc in effluents had achieved 99 % removal. For strong acidic wastes, limestone ($CaCO_3$) is used as part of the neutralisation together with lime since limestone is less expensive and it reduces the operational cost significantly. However, limestone must be evaluated carefully for each acid waste since its efficiency depends upon particle coating and size, and pH limitation of calcium carbonate (Dean et al. 1972).

**Table 8.1** Removal of heavy metals using chemical precipitation

| Cation (heavy metal) | Anion (precipitant) | Optimum pH | Contact time (h) | Initial metal concentration (mg/L) | Removal efficiency (%) | Reference |
|---|---|---|---|---|---|---|
| $Cu^{2+}$, $Cr^{4+}$ | $Ca(OH)_2$, NaOH, $Na_2CO_3$ | 12.0, 8.7 | 2 | 48.51, 30 | 98.6, 99.97 | Mirbagheri and Hosseini (2005) |
| $Cd^{2+}$, $Cr^{3+}$, $Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$, $Zn^{2+}$ | $Ca(OH)_2$, NaOH | 8.1–8.3 | 24 | 0.1–29.7 | 50–100 | Meunier et al. (2006) |
| Soil, fly ash and sewage leachates ($Al^{3+}$, $Ba^{2+}$, $Ca^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Cr^{3+}$, $Cu^{2+}$, Fe, $Mg^{2+}$, Mn, $Ni^{2+}$, $Pb^{2+}$, $Zn^{2+}$, P, S) | NaOH, $Ca(OH)_2$, NaOH/$Na_2S$, NaOH/$Na_2CO_3$, NaOH/$Na_2HPO_4$ | 5.0 7.5 7.5 8.0 | 0.3 | 0.13–719[a] 1.38–11,100[b] 0.23–827[c] | 0.0–100[a] 23.9–100[b] 3.5–100[c] | Djedidi et al. (2009) |
| $Ni^{2+}$, $Co^{2+}$, $Cu^{2+}$ | $Na_2S$ | 5.81–11.6 | 0.5–2 | 50–2,051 | 66.9–99.9 | Lewis and van Hille (2006) |
| $Zn^{2+}$ | $Na_2S$ | 6.5 | NA | 7,500 | 99.9 (<0.01 mg/L) | Veeken et al. (2003) |
| Acid mine drainage ($Fe^{2+}$, $Mn^{2+}$) | 1,3-benzenediamidoethane-thiolate | 4.5 | 20 | 194 4.65 | 99.9 (<0.009 ppm), 97.3 | Matlock et al. (2002) |

*NA* not available

[a]Refers to the soil leachates

[b]Refers to the fly ash leachates

[c]Refers to the sewage sludge leachates

Another important parameter for precipitation of heavy metals is the adjustment of optimum pH. Heavy metals, like chromium, copper, lead, manganese and zinc do not precipitate at pH below 7.0, thus allow their separation from ferric ion ($Fe^{3+}$) and aluminium ion ($Al^{3+}$), which precipitate at pH below 6.5 (Djedidi et al. 2009). The investigation from Mirbagheri and Hosseini (2005) agreed well with Djedidi et al. (2009). In their study of Cu(II) and Cr(IV) removal from wastewater using $Ca(OH)_2$ and NaOH, the Cr(VI) which was converted to Cr(III) using ferrous sulfate showed the maximum precipitation at pH 8.7 with the addition of $Ca(OH)_2$. At this pH value, the concentration of chromate was successfully reduced from 30 to 0.01 mg/L. While the maximum precipitation of copper occurred at pH 12.0 for both $Ca(OH)_2$ and NaOH and the concentration of copper was successfully reduced from 48.51 to 0.694 mg/L.

Although hydroxide precipitation is reported as the most widely used method for heavy metals removal in industry effluent, it also has some limitations. Hydroxide precipitation requires a large amount of chemicals to reduce metals to an acceptable level prior discharge especially if the effluents have high acidity where large amounts of lime are required for neutralisation. In this case, excessive secondary sludge, such as gypsum ($CaSO_4 \cdot 2H_2O$) are produced that require further treatment. These secondary precipitates not only increase the costs of sludge handling but also give long-term environmental impacts (Kurniawan et al. 2006). Secondly, some metal hydroxides are amphoteric, and the mixed metals create a problem using hydroxide precipitation since the ideal pH for one metal may change another metal back into solution. On top of these, some complexing agents present in the wastewater will inhibit the efficacy of metal hydroxide precipitation (Fu and Wang 2011).

## Sulfide Precipitation

Sulfide precipitation is another effective method used for removal of heavy metals from wastewater. It has several advantages over hydroxide precipitation. For instances, sulfide precipitates are not amphoteric, hence, the sulfide precipitation process can achieve a high degree of metal removal over a broad pH range compared with hydroxide precipitation. Moreover, the solubilities of the metal sulfide precipitates are intensely lower than hydroxide precipitates. Metal sulfide sludges also exhibit better thickening and dewatering characteristics than the corresponding metal hydroxide sludges, thus reducing the amount and cost of sludges handling (Fu and Wang 2011). The sulfide precipitation process can be employed using various sulfide sources, such as solid FeS, CaS, aqueous $Na_2S$, NaHS, $NH_4S$ or gaseous $H_2S$. Some researchers use the degeneration reaction of sodium thiosulfate ($Na_2S_2O_3$) as a source of sulfide for metal precipitation (Lewis 2010).

In fact, metal sulfide precipitation is preferable than hydroxide precipitation in hydrometallurgical processes in recent decades (Bhattacharyya et al. 1979; Lewis and van Hille 2006). The reasons that support its preferential use are based on the high degree of metal removal at relatively low pH values; the low solubility

of sulfide precipitates; favourable dewatering characteristics and the stability of the metal sulfides formed. In the batch study of metal recovery conducted by Lewis and van Hille (2006), the maximum metal removal achieved for nickel and cobalt are 99.8 % and 99.9 %, respectively, when the metal: sodium sulfide molar ratio was 1:1. However, when excess sulfide is used, it compromises metal removal efficiency due to the formation of aqueous polysulfide species that will re-dissolve in the solution. The reaction is described as following equation:

$$MS_{(s)} + HS^-_{(aq)} \rightarrow MS(HS)^-_{(aq)} \tag{8.1}$$
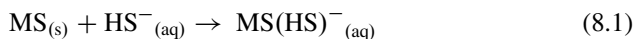
whereby MS: solid metal sulfide

$HS^-$: aqueous sulfide

$MS(HS^-)$: aqueous polysulphide complex

Hence, sulfide precipitation is not used as widely as it could due to this limitation. Moreover, it is rather difficult to control the dosing of sulfide due to the very low solubility of the metal sulfides and thus the sensitivity of the process to the dose. The colloidal precipitates that formed during metal sulfide precipitation also tend to cause some separation problems in either settling or filtration processes (Veeken et al. 2003).

In order to overcome the disadvantages between hydroxide and sulfide precipitation, nowadays, the combination of these two methods in treatment of heavy metals is being considered (Marchioretto et al. 2005; Djedidi et al. 2009). The set up for this treatment is usually a two-stage processes in which hydroxide precipitation is followed by sulfide precipitation. Djedidi et al. (2009) has reported that the combination of NaOH and $Na_2S$ led to better removal of $Pb^{2+}$, $Zn^{2+}$, $Cu^{2+}$ and $Mn^{2+}$ ions in soil, fly ash and sewage sludge leachates than with $NaOH/Na_2CO_3$ or $NaOH/Na_2HPO_4$. The metal sulfide precipitations occurred at pH 7.5 with percentage removal of almost 100 %. This combination technique has reduced the reagents consumption.

**Chemical Precipitation Combined with Other Methods**

There are also reports on the combination of chemical precipitation with other methods in the treatment of heavy metals. González-Muñoz et al. (2006) have studied precipitation of metals using sulfides followed by nanofiltration as a second step in order to optimise the reuse of water and the recovery of heavy metal ions (Zn, Se, As, Cd and Pb). This study showed a promising outcome whereby the heavy metals are being successfully precipitated and the resultant solution after these processes can be directly reused in the plant. Ghosh et al. (2011) investigated a stepwise combination of electro-Fenton process and chemical precipitation to treat rayon industry wastewater contains chemical oxygen demand (COD) and $Zn^{2+}$. The two-step treatment processes refer to electro-Fenton treatment for COD reduction, followed by removal of zinc by addition of lime, CaO. From the results obtained with industrial wastewater having a COD of 2,400 mg/L and 32 mg/L

$Zn^{2+}$, approximately 88 % COD was reduced by the electro-Fenton method but this step does not have any appreciable effect on zinc removal. Thus, CaO is added to precipitate zinc as zinc hydroxide, $Zn(OH)_2$ with removal efficiency of 99–99.3 % in the range of pH 9–10. Another study in combination of chemical precipitation and electrocoagulation to remove $Cu^{2+}$ from lithium bromide refrigerant was conducted by Cheng (2006). $Cu^{2+}$ removal from lithium bromide refrigerant was successfully achieved by pretreatment with $Ba(OH)_2$ solution followed by electrocoagulation using carbon steel plates as electrodes. Up to 98.5 % of $Cu^{2+}$ in the contaminated refrigerant could be removed by this combined treatment.

## 8.2.2   Ion Exchange

Ion exchange is the process through which an ion is removed out of an aqueous solution and is replaced by another ionic species. It has been continuously used in the treatment of heavy metals due to several advantages, such as high treatment capacity, high selectivity for certain heavy metal ions and most significantly, fast kinetics and most importantly it reduces the major problem faces in chemical precipitation – handling and disposal of large amount of sediments and sludge (Lacour et al. 2001; Abo-Farha et al. 2009, Al-Enezi et al. 2004). Dąbrowski et al. (2004) concluded that industrial wastewaters containing 0.1 g/L of $Cu^{2+}$, $Cd^{2+}$ or $Hg^{2+}$ compounds give 10-, 9- and 5-times larger amounts of sediments, respectively, and 6 kg of sediments are obtained from one kilogram of chromates.

Strongly acidic resins with sulfonic acid groups ($-SO_3H$) and weakly acid resins with carboxylic acid groups ($-COOH$) are the most common cation exchangers. Hydrogen ions in the sulfonic group or carboxylic group of the resin can serve as exchangeable ions with metal cations in the wastewater (Lacour et al. 2001; Fu and Wang 2011). The degree of exchange that occurs depends on the size and ionic charge of the metal cations, initial metal concentration, nature (both physical and chemical) of the ion exchange substance, pH and temperature (Lee et al. 2006a; Al-Enezi et al. 2004). Since resins have different swelling characteristics, the total exchange capacity on a volumetric basis ranged from 1.0 to 4.0 meq/mL. Abo-Farha et al. (2009) studied the adsorption behaviour of cation exchange resin purolite C100 with respect to $Ce^{4+}$, $Fe^{3+}$ and $Pb^{2+}$ from aqueous system. Purolite C100 is a polymer of polystyrene-divinyl benzene, with sulfonic acid groups as the functional groups. They found that the metal ions adsorption sequence can be given as $Ce^{4+} > Fe^{3+} > Pb^{2+}$. Another batch study conducted by Lacour et al. (2001) shows the potential usage of cation-exchange-textiles (CET) – a special class of grafted textiles carrying different functional groups namely carboxylate($R - COO^-$, $Na^+$), sulfonate ($R–SO_3^-$, $Na^+$) or phosphate ($R–O–PO_3^{2-}$, $2Na^+$) in removing $Cu^{2+}$ and $Cd^{2+}$ from the solution. The report reveals the adsorption behaviour of the CET towards $Cu^{2+}$ and $Cd^{2+}$ is influenced by parameters such as the counter-ion and functional group nature, the metal concentration and the presence of different competitors in the solution.

Besides synthetic resins, natural zeolites also show effective removing efficiency towards heavy metal from aqueous solutions under different experimental condition (Inglezakis and Grigoropoulou 2004; Petrus and Warchoł 2005; Barreira et al. 2009). Clinoptilolite is one of the most frequently studied natural zeolites that have received extensive attention due to its selectivity for heavy metals. (Inglezakis and Grigoropoulou 2004) reported that the uptake of $Pb^{2+}$, $Cu^{2+}$, $Fe^{3+}$ and $Cr^{3+}$ in aqueous solutions using natural clinoptilolite is influenced by different parameters following this order: metal cations concentration > volumetric flow rate > particle size > modification of the material. In fact, modification of the natural sample by NaCl has led to an increase of removal efficiency by 32–100 %. The study also focused on the fixed bed reactors design rather that batch systems because kinetics in beds is influenced by the whole equilibrium curve and diffusion rates in the material. This indicates the effect of operating conditions carry out in fixed beds is more representative than that obtained in batch systems. Warchoł and Petrus (2006) supported the column study of clinoptilolite by investigating heavy metals removal, ion-exchange equilibrium and column dynamics through mathematical modeling. In fact, the possibility of replacing expensive synthetic ion-exchangers by natural zeolites – low cost sorbents with abundant availability has added value to the application of this method in wastewater treatment.

## 8.2.3 Membrane Filtration

The ultimate objective of most of the traditional methods in heavy metals treatments is not the recovery of the metal but rather its elimination. However, recovery of heavy metals shows greater benefits. It allows re-use of the metals and thus provides further economic and environmental benefits by reducing the disposal costs and raw material requirements. In this case, membrane processes exert as an alternative for heavy metal recovery, as they can achieve high permeate fluxes and high rejection coefficients with low energy costs and under mild conditions. (Landaburu-Aguirre et al. 2006). Other advantages of this technique including fewer chemicals are used in the process, which helps to minimise the negative impacts of those chemicals on the whole process; produce more reliable performance; simple operation and convenience since it occupies less floor space in comparison to the conventional treatment systems (Paul Chen et al. 2006). Besides, there are many studies show that membrane processes can be easily combined with other separation processes for better removal efficiency (Blöcher et al. 2003; Mimoune and Amrani 2007; Rivas and Palencia 2011).

Membrane separation system consists of a membrane that can separate materials depending on their physical and chemical properties when a driving force, either a gradient of chemical potential (concentration or pressure gradient) or electrical potential, is applied across the membrane. A good membrane must have a narrow range of pore sizes, a high porosity, and a thin layer of material (Paul Chen et al. 2006). The membrane processes used for wastewater treatment containing

**Table 8.2** Pore size of the membrane processes

| Membrane process | Pore size |
|---|---|
| Microfiltration | 0.1–10 $\mu$m (>100 nm) |
| Ultrafiltration | 0.005–0.2 $\mu$m (5–200 nm) |
| Nanofiltration | 0.001–0.01 $\mu$m (<10 nm) |

heavy metals including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO) and electrodialysis (ED) depending on the size of the particle that can be retained (Table 8.2). The membrane techniques that are often used for heavy metals removal are UF, NF, RO and ED.

## Ultrafiltration

Ultrafiltration (UF) is a membrane technique used to separate heavy metals, organic pollutants or inorganic pollutants from aqueous solution. UF membranes are capable of retaining species in the range of 300–500,000 Da of molecular weight, with pore sizes ranging from 0.005 to 0.2 $\mu$m (5–200 nm) (Paul Chen et al. 2006). Since the pore sizes of UF membranes are larger than dissolved metal ions in the form of hydrated ions or as low molecular weight complexes, these ions would pass easily through UF membranes. Hence, two important techniques – micellar enhanced ultrafiltration (MEUF) and polymer enhanced ultrafiltration (PEUF) are proposed for effectively use in removal of metal ions (Table 8.3).

The combination of surfactants and membranes in separation of dilute polluted water has led to the development of MEUF. It is considered an economical alternative available to the conventional membrane separation process, because it reduces the requirement of higher pressure and high membrane costs. In this process, surfactants are added into the aqueous stream at levels equal to or higher than their critical micelle concentrations (CMCs). At this particular surfactant concentration, surfactant monomers will assemble and form aggregates called micelles. The anionic micelles, which are negatively charged, can bind to metal cations, which are positively charged. This micelle solution is then filtered through an ultrafiltration membrane whose pore size is smaller than the micelle size to reject the micelles. The metal cations that adsorbed onto the micelles can thus be removed from the polluted water (Mungray et al. 2012).

MEUF can be used to remove different heavy metals from wastewater containing single metal, mixture of metals or feed mixtures containing metals and organic material (Juang et al. 2003; Häyrynen et al. 2012; Rahmanian et al. 2012). As the binding of metal cations to oppositely charged micelle surface is primarily electrostatic in nature, it is expected that the ions with the same charge are removed with a nearly equal rejection in MEUF. Juang et al. (2003) reported that the rejection of $Sr^{2+}$, $Mn^{2+}$, $Co^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ are 99.1, 78.6, 80.6, 82.1 and 83.7 % respectively. The comparatively high rejection of $Sr^{2+}$ by the GE membrane in this system promotes the application of MEUF for selective separation of $Sr^{2+}$ from low-level waste effluents. Increases of surfactant (sodium dodecyl sulfate) to

**Table 8.3** Heavy metal removal by micellar enhanced ultrafiltration (MEUF) and polymer enhanced ultrafiltration (PEUF)

| UF type | Membrane material | Surfactant/complexing agent | Heavy metal | Initial concentration | Optimum pH | Removal efficiency (%) | Reference |
|---|---|---|---|---|---|---|---|
| MEUF | Polyamide, polyethersulfone | Sodium dodecyl sulfate | $Cs^+$, $Sr^{2+}$, $Mn^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cr^{3+}$ | 50 mg/L | 3–12 | 40.0, 99.1, 78.6, 80.6, 82.1, 83.7, 86.7 | Juang et al. (2003) |
| MEUF | Ceramic | Dodecylbenzenesulfonic acid, dodecylamine | $Pb^{2+}$, $AsO_4{}^-$ | 4.4–7.6 mg/L | 7.47 | >99.0, 19.0 | Ferella et al. (2007) |
| MEUF | Regenerated cellulose acetate | Sodium dodecyl sulfate | $Pb^{2+}$ | 50–150 | 7.84 | 99.5 | Rahmanian et al. (2012) |
| MEUF | Polyethersulfone | Sodium dodecyl sulfate | $Cd^{2+}$, $Cu^{2+}$ | 0.37, 0.41 | 2.7 | 85, 81 | Häyrynen et al. (2012) |
| PEUF | Ceramic | Chitosan | $Cd^{2+}$ | 10 mg/L | 7.5 | 98.5 | Llorens et al. (2004) |
| PEUF | Polysulfone | Chitosan, polyethyleneimine, pectin | $Cr^{3+}$, $Cr^{4+}$ | 10 ppm | 7–9 | >99, <50 | Aroua et al. (2007) |
| PEUF | Poly(vinyl alcohol) | Poly(ethyleneimine) | $Pb^{2+}$, $Cd^{2+}$, $Cu^{2+}$ | 100 ppm | 5.0 | 96.0, 99.5, 99.0 | Bessbousse et al. (2008) |
| PEUF | Polyethersulfone | Carboxy methyl cellulose | $Cu^{2+}$, $Cr^{3+}$, $Ni^{2+}$ | 10 mg/L | 7.0 | 97.6, 99.5, 99.1 | Barakat and Schmidt (2010) |

metal ratio increase the rejection because the sizes of micelles are relatively larger at higher concentration of surfactant compare to the UF membrane. However the metal rejection shows a decrease of 3–6 % at pH lower than 3 due to competition of $H^+$ trapped on micelle surface with metal ions. A combination process of micellar enhanced ultrafiltration and activated carbon fibre (ACF) shows nearly complete rejection of copper from the solution. The coupling of MEUF with ACF has the potential to overcome the problems caused by excessive surfactant concentration since activated carbon has proved to be a good adsorbent for surfactants that contained in the permeate (Bade and Lee 2007).

In PEUF, water soluble polymer is used to bind with metal ions to form macromolecular complexes which have a larger molecular weight and size than the membrane. As a result, these macromolecular complexes will be retained and removed while the non-complexed ions will pass through the membrane. So far, polymers with carboxylic or amine groups have been extensively studied for this purpose. PEUF process is more economically competitive because the non-protonated form of the polymers that bind to the metal ions, will release the metal ions when an acid is added. This has led to the regeneration and reuse of the polymer. Hence, pH plays an important role in the binding and removal of metal species from the solution. Llorens et al. (2004) and Aroua et al. (2007) had conducted studies on the removal of cadmium and chromium from aqueous solution using PEUF. For both of the studies, high rejections were obtained at pH 7 or higher and decrease when pH is lowered for all types of the polymers tested namely chitosan, polyethyleneimine and pectin.

## Reverse Osmosis

Reverse osmosis (RO) is one of the techniques able to remove a wide range of dissolved species from water. It is not only able to remove suspended solid $> 1\,\mu m$ (conventional filtration), but also dissolved solids, bacteria, viruses, and other germs contained in the water. In the application of RO, when the waste stream flows past the membrane, the solvent (e.g., water) is driven through the membrane (operation pressure ranges from 10 to 70 bars) while the remaining solutes (e.g., organic or inorganic components) do not pass through, and become increasingly concentrated on the feed side of the membrane. The general use of RO is desalination of seawater for its conversion into potable water. It is reported that more than 20 % of the world's desalination process is using this technique (Shahalam et al. 2002). The RO membranes have pore size ranging from approximate 0.5–1.5 nm (5–15 Å). The extremely small size of RO pores allows only the smallest organic molecules and unchanged solutes to pass through the semipermeable membrane along with the solvent (Paul Chen et al. 2006). Nowadays, many studies have been going on to utilise RO process in wastewater treatment.

A study by Ozaki et al. (2002) shows that it is successful to remove $Ni^{2+}$, $Cu^{2+}$ and $Cr^{6+}$ ions from industrial wastewater using RO membrane separation

at lower pressures (350–450 kPa). The rejections 99.03 % of $Ni^{2+}$, 98.75 % of $Cu^{2+}$ and 99.37 % of $Cr^{6+}$ were achieved at pH 7. Metal rejections are higher at high pH values (7–9) due to the formation of insoluble complexes with $OH^-$ ions, causing the solute changed to a larger molecule and precipitate onto the membrane surface. They also found that presence of co-ions such as calcium and magnesium in the industrial wastewater decreases the rejection. Meanwhile, a pilot-scale combination of membrane bioreactor (MBR) with RO separation is conducted by Dialynas and Diamadopoulos (2009). The combination technique shows high heavy metal removals, whereby Pb and Ni were removed completely, while Cr and Cu were removed by 89 % and 49 %, respectively. However, the performance of RO and its life-time are highly sensitive to wastewater properties. The accumulation of particulate and colloidal matters at the feed side of the membrane surface caused membrane fouling and permeate flux decline (Lee et al. 2006b).

**Nanofiltration**

Nanofiltration (NF) is the intermediate process between UF and RO and used to separate different fluids or ions. It has larger membrane pore structure, 0.001–0.01 $\mu$m (1–10 nm) as compared to the RO membranes, which allows more salt passage through the membrane. The advantages of NF compared to RO processes including operation at lower pressure (7–14 bar) but able to produce high recoveries, thereby conserving total water usage due to a lower concentrate stream flow rate (Paul Chen et al. 2006). Ortega et al. (2008) studied the removal of various metal ions from an acidic leachate solution by two different nanofiltration membranes (Desal5 DK and NF-270). The results show good metal ion rejection (62–100 %) where divalent ions were better rejected than monovalent ions. In recent years, Murthy and Chaudhari (2008) have conducted numerous studies on the removal of heavy metal ions from aqueous solution using NF membrane. They found that $Ni^{2+}$ can be removed by a thin-film composite polyamide NF membrane. Rejection of nickel ions increases with increase in feed pressure and decreases with increase in feed concentration at constant feed flow rate. The maximum rejection of the metal is found to be 98 % and 92 % for an initial feed concentration of 5 and 250 ppm, respectively (Murthy and Chaudhari 2008). At the same year, Bouranene et al. (2008) also proved that $Co^{2+}$ and $Pb^{2+}$ ions can be removed by polyamide NF membrane. The rejection rate of 97 % and 81 % were observed for cobalt and lead, respectively, at constant permeate flux of $122 \pm 2$ $Lh^{-1}$ $m^{-2}$ and ion concentration of 1,600 $mgL^{-1}$ in mixed-salt solution. In the following year, Murthy and Chaudhari (2009) continue to investigate removal efficiency of cadmium and nickel in a binary system. The maximum observed solute rejection of nickel and cadmium ions are 98.94 % and 82.69 %, respectively, for an initial feed concentration of 5 ppm.

**Electrodialysis**

Electrodialysis (ED), which requires electrical energy as a driving force, allows transportation of ions through ion permeable membranes from one solution to another due to the potential gradient that created. Since ED membranes have the ability to selectively transport ions having positive or negative charge and reject ions of the opposite charge, the removal or separation of ions/electrolytes can be achieved by this technique. Cifuentes et al. (2009) reported on the effectiveness of ED in separating $Cu^{2+}$ and $Fe^{2+}$ ions and subsequently water recovery in industrial copper electrowinning operations. Approximately 96.6 % of Cu and 99.5 % of Fe were separated from the working solution which constituted of initial concentration of 9 g/L and 5 g/L of $Cu^{2+}$ and $Fe^{2+}$, respectively. Removal rates for $Cu^{2+}$ and $Fe^{2+}$ from the working solution increased linearly with cell current density and electrolyte agitation. Removal of $Cr^{3+}$ from the tanning process in the leather industry is crucial due to the environmental issue and also to its industry. It is because presence of chromium ion has a negative effect on the quality of the tanned leather. Lambert et al. (2006) had proposed a two-step Cr(III) separation–concentration process. In the first step, a cation-exchange membrane, Nafion®117, modified by electrodeposition of polyethylenimine was used to separate successfully Cr(III) from NaCl solutions. In the second step, conventional electrodialysis with three different membranes permits to increase the concentration of Cr(III) in the solutions produced in the separation stage.

## 8.2.4 Adsorption

Adsorption is known as an efficient, convenient and economic method for wastewater treatment. The flexibility in design and operation, high removal efficiency and the possibility for most of the adsorbents to be regenerated and reuse by suitable desorption process have made adsorption process gaining its popularity (Krishnani et al. 2008; Elouear et al. 2008; Afkhami et al. 2010; Salehi et al. 2012). Adsorption is a mass transfer process whereby a substance is transferred from a gas or liquid phase to the surface of a solid and bound by physical and/or chemical and/or electrostatic interactions. Physical adsorption is caused by the van der Waals forces whereas chemical adsorption involved the electronic interactions between specific surface sites and solute molecules. This kind of electrostatic interaction is generally referred to coulombic attractive forces between ions and charged functional groups. The three steps involved in the adsorption process are: (i) the transport of the solute from the bulk solution to the sorbent surface; (ii) adsorption on the particle surface; and (iii) transport within the sorbent particle.

Activated carbon (AC) is being recognised as one of the most popular adsorbents used in the removal of heavy metal contaminants. AC has been used efficiently in both batch and column studies due to its large micropore and mesopore volumes. This kind of structure also resulted in a high surface area. However, the drawback of

AC is it remains as an expensive material because of depleting source of commercial coal-based AC. Higher cost is accounted for higher quality of activated carbon. Another limitation of AC adsorption in heavy metal removal is its high affinity towards organic molecules. As a result, these kinds of high molecular weight organic compounds will block the heavy metal ions from reaching the adsorbents bed.

The problems revealed by AC adsorption have led to the production of alternative adsorbents to replace the costly AC. Attention has been focused on various adsorbents that have metal-binding capacities, especially if it is a source that is readily abundant. Besides this, utilization of inexpensive sources is also being viewed as attractive alternatives. For this, many researchers have shown interest to convert carbonaceous materials into AC for heavy metals remediation. Momčilović et al. (2011) prepared activated carbon from the cones of the European Black pine to remove lead (II) ions from aqueous solutions. The maximum adsorption capacity of 27.53 mg/g is observed and the optimum adsorbent dosage was established to be 2.0 mg/l. Namasivayam et al. (2007) had converted jatropha husk, an agricultural solid waste, generated from bio-diesel industries, to jatropha husk activated carbon. This material is an effective adsorbent for the removal of toxic ions, dyes and organics. Besides AC, other adsorbents that are used for adsorption of heavy metal ions include natural minerals, modified biopolymers as well as low cost materials, which are derived from agricultural waste and industrial by products. Among these compounds, adsorbent from low cost materials have gained considerable attention because of their low cost and local availability, which will be elaborated further in next chapter.

## 8.2.5 Biological Treatment

The method of biological treatment was introduced in the early years of the twentieth century. It involved confining naturally occurring bacteria at very much higher concentrations in tanks. These bacteria, together with some protozoa and other microbes, are collectively referred to as activated sludge. The initial objective of biological treatment is to remove small organic carbon molecules by the bacteria. As a result, the bacteria grow, and the wastewater is cleansed. Lately, this method is applied in the removal of heavy metals from wastewater where biosorption phenomenon itself plays an important role. Biosorption is a metabolism-independent binding of heavy metals to living cells, non-living biomass, or microbial extracellular polymers (Volesky and Holan 1995).

Lipczynska-Kochany and Kochany (2009) have investigated the influence of humic substances (humate, HS) on the biological treatment of wastewater containing heavy metals (Cr, Cu, Fe, Mn, Ni, and Zn). Humic substances (HS) are naturally occurring compounds resulting from microbial and chemical transformation of organic debris. The removal of ammonia and metals was 99 % and over 90 %, respectively. Chipasa (2003) studied the accumulation and removal of heavy metals (Cd, Cu, Pb and Zn) through a biological wastewater treatment system. The results

revealed that the removal of heavy metals from wastewater is influenced by their initial contents in the influent. The reduction in heavy metal contents was reported to follow this sequence: Cd < Pb < Cu < Zn. Biological wastewater treatment is a complex process depending not only on many biological and physicochemical conditions but also on process operating conditions and design. Consequently, all these are also influential variables in controlling the removal efficiency of heavy metals.

## 8.3 Utilization of Low-Cost Adsorbents in Heavy Metals Removal

Heavy metals such as chromium, lead, copper, nickel, zinc and iron are not biodegradable and they are toxic to human health and environment. Due to their tendency to accumulate in organisms and cause various disorders and diseases, these heavy metals are recognized as an emergent class of human carcinogenic compounds. Besides, the presence of high concentration of these metals in the environment may lead to serious problems including water pollution and soil degradation. Obviously, an effective method in heavy metal removal is urgently needed to lessen the pollution loads before the discharge enters the surface water.

Techniques currently used for the removal of heavy metals from wastewaters are chemical precipitation, ion exchange, membrane filtration, electrolytic reduction, coagulation, solvent extraction and catalysis. Owing to the drawbacks found in these techniques: generation of residues, low efficiency, applicability to a wide range pollutants, need of pre-treatments and high cost, they may not be suitable solutions at industrial scale. Since the last decades, adsorption process has been viewed as a highly effective method for removal of various heavy metals due to ease of operation. In particular, high surface area materials like activated carbon, silica aerogel and other mesoporous materials have been proven for their excellent adsorption capability for heavy metals. Unfortunately, production of these adsorbents is usually costly, making them not attractive to be applied by small and medium scale industries. Thus, the use of low cost materials as adsorbent for metal ion removal from the wastewaters has been highlighted. In general, the low-cost adsorbent materials are referred to those available at free cost and exist abundantly in nature. They may also represent the unused resources after a manufacturing process with serious disposal problems. Apparently, utilization of naturally occurring material or locally available agricultural waste materials or industrial by-products as the adsorbents in removing heavy metals from wastewaters is not only cost effective in heavy metal removal, but it also contributes to a zero waste situation in the environment.

A numerous studies have been carried out to investigate as well as to further improve the usage of different low cost adsorbent materials for adsorption of individual or multiple heavy metals in an aqueous solution. These low cost adsorbents range from industrial by-products, agriculture waste to biosorbents, for example wood

bark, sawdust, mangoesteen peel, durian husk, oil palm empty fruit bunch, tea leaves and exhausted coffee etc. In this section, some of the intensively studied low cost adsorbents such as fly ash, rice husk, wheat straw, chitin and chitosan composites as well as algae and bacteria were discussed in terms of their efficiency for heavy metals removal. Recent reported adsorption capacities of the selected adsorbents are presented in Tables 8.4, 8.5, 8.6, 8.7, 8.8, and 8.9 to give some idea of adsorbent effectiveness. It is interesting to note that the reported values are strongly dependent on the experimental condition. Therefore it is advisable for the reader to refer to the original work in order to have a better understanding on the specific conditions that have been imposed on the adsorption process.

### 8.3.1  Fly Ash

Fly ash is a waste material produced during the combustion of various materials, such as coal, plant biomass and municipal solid waste in the electrical generation process. This industrial by-product is usually grey in color, abrasive, mostly alkaline and refractory in nature. It contains mainly silica ($SiO_2$) of $\sim$47 %, alumina ($Al_2O_3$) of $\sim$25 %, iron oxide ($Fe_2O_3$) of $\sim$16 %, and calcium oxide (CaO) of $\sim$5 % with varying amounts of carbon, calcium, magnesium and sulphur. The variations that are commonly found in fly ash, either in terms of their chemical composition or physical properties is most likely due to different sources of coal as well as diversity in the design of coal-fired boilers. However, an empirical formula for fly ash based on the dominance of certain key elements has been proposed as below (Iyer and Scott 2001): $Si_{1.0}Al_{0.45}Ca_{0.51}Na_{0.047}Fe_{0.039}Mg_{0.020}K_{0.013}Ti_{0.011}$.

Depending on the ratio of calcium to silica and ratio of alumina to iron in the ash, fly ash can be classified into two types: Type C and Type F. The Type C fly ash is normally produced from the burning of low-rank coals, such as lignites or sub-bituminous coals. This fly ash has cementitious properties as it is self-hardening upon reacted with $H_2O$. Meanwhile, the Type F fly ash is generated from the burning of higher-rank coals like bituminuous coals or anthracites. This type of fly ash is pozzolanic in nature where it is hardening when reacted with $Ca(OH)_2$ and $H_2O$.

In fact, the fly ash has been used effectively as raw material in cement and brick production, soil amendment and as filler in road works. Besides, the fly ash can be converted into zeolite, another versatile material which is widely used as catalyst and adsorbent due to its outstanding high surface area and porosity. However, all the fly ash produced from the power industry cannot be reused by these applications. Consequently, most of the fly ash generated is disposed of as landfill, a practice which is under examination for environmental concerns. Therefore, continuing research efforts have been made to explore other applications prior to discarding in order to lessen the environmental burden. Since fly ash is enriched with $SiO_2$ and contains a portion of unburned carbon, this material is potentially used as low cost adsorbent to remove various hazardous elements from wastewaters. A lot of

**Table 8.4** Adsorption capacities of metals by fly ash[a]

| Metals | Adsorbent | Adsorption capacity[b] | Temperature (°C) | References |
|---|---|---|---|---|
| As(III) | Fly ash coal-char | 3.7–89.2 | 25 | Pattanayak et al. (2000) |
| | Fly ash brown coal-char | 25.1–32.1 | 25 | Polowczyk et al. (2011) |
| As(V) | Fly ash | 7.7–27.8 | 20 | Diamadopoulos et al. (1993) |
| | Fly ash coal-char | 0.02–34.5 | 25 | Pattanayak et al. (2000) |
| Cd(II) | Fly ash | 1.6–8.0 | – | Ayala et al. (1998) |
| | Fly ash zeolite | 95.6 | 20 | Ayala et al. (1998) |
| | Fly ash | 0.67–0.83 | 20 | Bayat (2002a) |
| | Afsin-Elbistan fly ash | 0.08–0.29 | 20 | Bayat (2002b) |
| | Seyitomer fly ash | 0.0077–0.22 | 20 | Bayat (2002b) |
| | Fly ash | 198.2 | 25 | Apak et al. (1998) |
| | Fly ash-washed | 195.2 | 25 | Apak et al. (1998) |
| | Fly ash-acid | 180.4 | 25 | Apak et al. (1998) |
| | Bagasse fly ash | 1.24–2.0 | 30–50 | Gupta et al. (2003) |
| | Fly ash | 0.05 | 25 | Weng and Huang (1994) |
| | Coal Fly ash | 18.98 | 25 | Papandreou et al. (2007) |
| | Coal Fly ash pellets | 18.92 | – | Papandreou et al. (2007) |
| | Bagasse fly ash | 6.19 | – | Ho et al. (1989) |
| | Fly ash zeolite X | 97.78 | – | Apiratikul and Pavasant (2008b) |
| | NaOH activated fly ash | 30.21 | 20–25 | Visa et al. (2012) |
| Co(II) | Fly ash zeolite 4A | 13.72 | – | Hui et al. (2005) |
| Cr(III) | Fly ash | 52.6–106.4 | 20–40 | Cetin and Pehlivan (2007) |
| | Fly ash porous pellet | 22.88 | 25 | Papandreou et al. (2011) |
| | Bagasse fly ash | 4.35 | – | Gupta and Ali (2004) |
| | Fly ash zeolite 4A | 41.61 | – | Hui et al. (2005) |
| Cr(VI) | Fly ash + wollastonite | 2.92 | – | Panday et al. (1984) |
| | Fly ash + China clay | 0.31 | – | Panday et al. (1984) |
| | Fly ash-chitosan composite | 33.27 | 15 | Wen et al. (2011) |
| | Fly ash | 1.38 | 30–60 | Banerjee et al. (2004) |
| | Fe impregnated fly ash | 1.82 | 30–60 | Banerjee et al. (2004) |
| | Al impregnated fly ash | 1.67 | 30–60 | Banerjee et al. (2004) |
| | Afsin-Elbistan fly ash | 0.55 | 20 | Bayat (2002b) |
| | Seyitomer fly ash | 0.82 | 20 | Bayat (2002b) |
| | Bagasse fly ash | 4.25–4.35 | 30–50 | Gupta and Ali (2004) |
| | Fly ash | 23.86 | – | Bhattacharya et al. (2008) |
| Cs(I) | Fly ash zeolite | 443.9 | 25 | Mimura et al. (2001) |
| Cu(II) | Fly ash | 0.1825 | – | Salam et al. (2011) |
| | Fly ash | 1.39 | 30 | Panday et al. (1985) |
| | Fly ash + wollastonite | 1.18 | 30 | Panday et al. (1985) |
| | Fly ash | 1.7–8.1 | – | Ayala et al. (1998) |
| | Afsin-Elbistan fly ash | 0.34–1.35 | 20 | Bayat (2002c) |
| | Seyitomer fly ash | 0.09–1.25 | 20 | Bayat (2002c) |
| | Fly ash | 207.3 | 25 | Ricou et al. (1999) |
| | Fly ash-washed | 205.8 | 25 | Ricou et al. (1999) |

**Table 8.4** (continued)

| Metals | Adsorbent | Adsorption capacity[b] | Temperature (°C) | References |
|---|---|---|---|---|
| | Fly ash-acid | 198.5 | 25 | Ricou et al. (1999) |
| | Fly ash | 0.63–0.81 | 25 | Lin and Chang (2001) |
| | Bagasse fly ash | 2.26–2.36 | 30–50 | Gupta and Ali (2000) |
| | Fly ash | 0.76 | 32 | Rao et al. (2003) |
| | Fly ash | 7.5 | – | Ricou et al. (1999) |
| | Coal Fly ash pellets | 20.92 | 25 | Papandreou et al. (2007) |
| | Fly ash zeolite 4A | 50.45 | – | Hui et al. (2005) |
| | Fly ash | 7.0 | – | Hossain et al. (2005) |
| | Coal fly ash (CFA) | 178.5–249.1 | 30–60 | Hsu et al. (2008) |
| | CFA-600 | 126.4–214.1 | 30–60 | Hsu et al. (2008) |
| | CFA–NAOH | 76.7–137.1 | 30–60 | Hsu et al. (2008) |
| | Fly ash zeolite X | 90.86 | – | Apiratikul and Pavasant (2008b) |
| | Fly ash | 7.0 | – | Gupta (1998) |
| | Fly ash mixed willow saw dusk | 7.7160 ppm/g | – | Lucaci et al. (2011) |
| | Fly ash mixed white poplar saw dusk | 6.2305 ppm/g | – | Lucaci et al. (2011) |
| Hg(II) | Fly ash | 2.82 | 30 | Sen and De (1987) |
| | Fly ash | 11.0 | 30–60 | Banerjee et al. (2004) |
| | Fe impregnated fly ash | 12.5 | 30–60 | Banerjee et al. (2004) |
| | Al impregnated fly ash | 13.4 | 30–60 | Banerjee et al. (2004) |
| | Sulfo-calcic fly ash | 5.0 | 30 | Ricou et al. (1999) |
| | Silico-aluminous ashes | 3.2 | 30 | Ricou et al. (1999) |
| | Fly ash-C | 0.63–0.73 | 5–21 | Kapoor and Viraraghavan (1992) |
| Ni(II) | Fly ash | 9.0–14.0 | 30–60 | Banerjee et al. (2003) |
| | Fe impregnated fly ash | 9.8–14.93 | 30–60 | Banerjee et al. (2003) |
| | Al impregnated fly ash | 10–15.75 | 30–60 | Banerjee et al. (2003) |
| | Afsin-Elbistan fly ash | 0.40–0.98 | 20 | Bayat (2002c) |
| | Seyitomer fly ash | 0.06–1.16 | 20 | Bayat (2002c) |
| | Bagasse fly ash | 1.12–1.70 | 30–50 | Gupta et al. (2003) |
| | Fly ash | 3.9 | – | Ricou et al. (1999) |
| | Fly ash zeolite 4A | 8.96 | – | Hui et al. (2005) |
| | Afsin–Elbistan fly ash | 0.98 | – | Bayat (2002c) |
| | Seyitomer fly ash | 1.16 | – | Bayat (2002c) |
| | Bagasse fly ash | 6.48 | – | Ho et al. (1989) |
| | Fly ash | 0.03 | – | Rao et al. (2002) |
| Pb(II) | Fly ash zeolite | 70.6 | 20 | Gan (2000) |
| | Fly ash | 444.7 | 25 | Yadava et al. (1987) |
| | Fly ash-washed | 483.4 | 25 | Yadava et al. (1987) |
| | Fly ash-acid | 437.0 | 25 | Yadava et al. (1987) |
| | Fly ash | 753 | 32 | Yadava et al. (1987) |
| | Bagasse fly ash | 285–566 | 30–50 | Goswami and Das (2000) |
| | Fly ash | 18.8 | – | Diamadopoulos et al. (1993) |

(continued)

**Table 8.4** (continued)

| Metals | Adsorbent | Adsorption capacity[b] | Temperature (°C) | References |
|--------|-----------|------------------------|------------------|------------|
|        | Fly ash zeolite X | 420.61 | – | Apiratikul and Pavasant (2008b) |
|        | Fly ash porous pellet | 45.54 | 25 | Papandreou et al. (2011) |
|        | NaOH activated fly ash | 2,000.0 | 20–25 | Visa et al. (2012) |
| Zn(II) | Fly ash | 6.5–13.3 | 30–60 | Banerjee et al. (2003) |
|        | Fe impregnated fly ash | 7.5–15.5 | 30–60 | Banerjee et al. (2003) |
|        | Al impregnated fly ash | 7.0–15.4 | 30–60 | Banerjee et al. (2003) |
|        | NaOH activated fly ash | 18.87 | 20–25 | Visa et al. (2012) |
|        | Fly ash | 0.25–2.8 | 20 | Bayat (2002a) |
|        | Afsin-Elbistan fly ash | 0.25–1.19 | 20 | Bayat (2002c) |
|        | Seyitomer fly ash | 0.07–1.30 | 20 | Bayat (2002c) |
|        | Bagasse fly ash | 2.34–2.54 | 30–50 | Gupta and Ali (2000) |
|        | Bagasse fly ash | 13.21 | 30 | Gupta and Sharma (2003) |
|        | Fly ash | 4.64 | 23 | Weng and Huang (1990) |
|        | Fly ash | 0.27 | 25 | Weng and Huang (1994) |
|        | Fly ash | 0.068–0.75 | 0–55 | Weng and Huang (2004) |
|        | Fly ash | 3.4 | – | Ricou et al. (1999) |
|        | Fly ash porous pellet | 17.66 | 25 | Papandreou et al. (2011) |
|        | Fly ash zeolite 4A | 30.80 | – | Hui et al. (2005) |
|        | Bagasse fly ash | 7.03 | – | Hui et al. (1989) |
|        | Fly ash | 11.11 | – | Gupta (1998) |
|        | Rice husk ash | 14.30 | – | Bhattacharya et al. (2006) |
|        | Fly ash | 7.84 | – | Gupta (1998) |
|        | Fly ash | 0.1806 | – | Salam et al. (2011) |

[a]These reported adsorption capacities are values obtained under specific conditions. Readers are encouraged to refer to the original articles for information on experimental conditions
[b]In the unit of $(mg\ g^{-1})$ unless specified

investigations have been reported on usage of fly ash in adsorption of individual pollutants in an aqueous solution or flue gas. Overall, the results obtained when using these particular fly ashes are encouraging for the removal of heavy metals and organics from industrial wastewater. Some of the researches showed that the unburned carbon in fly ash is partly activated during the combustion process, resulting in a relatively high surface area and good porosity (Wang and Li 2007; Lu et al. 2008). These characteristics of unburned carbon are similar to that of activated carbon, hence fly ash could be a potential adsorbent and substitute of commercial activated carbon. Recently, biomass fly ash has attracted attention to be used as adsorbent in wastewaters treatment (Pengthamkeerati et al. 2008). It could be a better adsorbent as compared to other fly ash due to its higher percentage of unburned carbon.

In real practical, the fly ash is always submitted to heating treatment and/or modification before being applied as adsorbent. Adsorption capacities of metals by different fly ashes are provided in Table 8.4.

**Table 8.5** Adsorption capacities of metals by rice husk[a]

| Metals | Adsorbent | Adsorption capacity[b] | Temperature (°C) | References |
|---|---|---|---|---|
| As(III) | Copolymer of iron and aluminum impregnated with active silica derived from rice husk ash | 146 | – | Abo-El-Enein et al. (2009) |
| As(V) | Rice husk | 615.11 | – | Roy et al. (1993) |
| | Quaternized rice husk | 18.98 | – | Lee et al. (1999) |
| Au(I) | Rice husk | 64.10 | 40 | Nakbanpote et al. 2002) |
| | Rice husk | 50.50 | 30 | Nakbanpote et al. (2002) |
| | Rice husk | 39.84 | 20 | Nakbanpote et al. (2002) |
| | Rice husk ash | 21.2 | – | Nakbanpote et al. (2000) |
| Cd(II) | Partial alkali digested and autoclaved rice husk | 16.7 | – | Krishnani et al. (2008) |
| Cd(II) | Phosphate-treated rice husk | 103.09 | 20 | Ajmal et al. (2003) |
| | Rice husk | 73.96 | – | Ye et al. (2010) |
| | Rice husk | 21.36 | – | Roy et al. (1993) |
| | Rice husk | 4 | – | Tarley et al. (2004) |
| | Rice husk | $8.58 \pm 0.19$ | – | Kumar and Bandyopadhyay (2006) |
| | Rice husk | 0.16 | – | Munaf and Zein (1997) |
| | Rice husk | 0.32 | – | Marshall et al. (1993) |
| | NaOH activated rice husk | 125.94 | – | Ye et al. (2010) |
| | NaOH activated rice husk | 7 | – | Tarley et al. (2004) |
| | NaOH activated rice husk | $20.24 \pm 0.44$ | – | Kumar and Bandyopadhyay (2006) |
| | $NaHCO_3$ activated rice husk | $16.18 \pm 0.35$ | – | Kumar and Bandyopadhyay (2006) |
| | Epichlorohydrin treated rice husk | $11.12 \pm 0.24$ | – | Kumar and Bandyopadhyay (2006) |
| | Rice husk ash | 3.04 | – | Srivastava et al. (2008) |
| | Polyacrylamide grafted rice husk | 0.889 | – | Sharma et al. (2009) |
| | $HNO_3$, $K_2CO_3$ treated rice husk | $0.044 \pm 0.1$ $mmol\ g^{-1}$ | 30 | Akhtar et al. (2010) |
| | Partial alkali digested and autoclaved rice husk | 9.57 | – | Krishnani et al. (2008) |
| Cr(III) | Rice husk | 1.90 | – | Marshall et al. (1993) |
| | Rice husk ash | 240.22 | – | Wang et al. (2008) |
| Cr(VI) | Rice husk | 164.31 | – | Roy et al. (1993) |
| | Rice husk | 4.02 | – | Munaf and Zein (1997) |
| | Rice husk ash | 26.31 | – | Bhattacharya et al. (2008) |

(continued)

**Table 8.5** (continued)

| Metals | Adsorbent | Adsorption capacity[b] | Temperature (°C) | References |
|---|---|---|---|---|
| | Rice husk-based activated carbon | 14.2–31.5 | – | Guo et al. (2002) |
| | Formaldehyde treated rice husk | 10.4 | – | Bansal et al. (2009) |
| | Pre-boiled rice husk | 8.5 | – | Bansal et al. (2009) |
| Cu(II) | Rice husk | 1.21 | – | Marshall et al. (1993) |
| | Rice husk | 0.2 | – | Srivastava et al. (2008) |
| | Rice husk | 7.1 | – | Nakbanpote et al. (2007) |
| | Rice husk ash | 11.5191 | – | Feroze et al. (2011) |
| | Raw rice hush | 4.90 | – | Luo et al. (2011) |
| | Expending rice husk | 8.02 | – | Luo et al. (2011) |
| | RH-cellulose | 7.7 | – | Nakbanpote et al. (2007) |
| | Rice husk heated to 300 °C (RHA300) | 6.5 | – | Nakbanpote et al. (2007) |
| | Rice husk heated to 500 °C (RHA500) | 16.1 | – | Nakbanpote et al. (2007) |
| | $HNO_3$, $K_2CO_3$ treated rice husk | $0.036 \pm 0.2$ mmol g$^{-1}$ | 30 | Akhtar et al. (2010) |
| | Microwave incinerated rice husk ash (800 °C) | 3.497 | – | Johan et al. (2011) |
| | Microwave incinerated rice husk ash (500 °C) | 3.279 | – | Johan et al. (2011) |
| | Partial alkali digested and autoclaved rice husk | 10.9 | – | Krishnani et al. (2008) |
| | Tartaric acid modified rice husk | 29 | 27 | Wong et al. (2003a) |
| | Tartaric acid modified rice husk | 22 | 50 | Wong et al. (2003a) |
| | Tartaric acid modified rice husk | 18 | 70 | Wong et al. (2003a) |
| | Tartaric acid modified rice husk | 31.85 | – | Wong et al. (2003b) |
| | Carbonized rice husk | 42.1 | 15 | Ye et al. (2012) |
| | Carbonized rice husk | 45.5 | 25 | Ye et al. (2012) |
| | Carbonized rice husk | 55.2 | 35 | Ye et al. (2012) |
| Fe(II) | Copolymer of iron and aluminum impregnated with active silica derived from rice husk ash | 222 | – | Abo-El-Enein et al. (2009) |
| Hg(II) | Rice husk ash | 6.72 | 30 | Feng et al. (2004) |
| | Rice husk ash | 9.32 | 15 | Feng et al. (2004) |
| | Rice husk ash | 40.0–66.7 | – | Tiwari et al. (1995) |
| | Polyaniline/rice husk ash nanocomposite | Not determined | – | Ghorbani et al. (2011) |
| | Partial alkali digested and autoclaved rice husk | 36.1 | – | Krishnani et al. (2008) |
| Mn | Copolymer of iron and aluminum impregnated with active silica derived from rice husk ash | 158 | – | Abo-El-Enein et al. (2009) |
| | Partial alkali digested and autoclaved rice husk | 8.30 | – | Krishnani et al. (2008) |

**Table 8.5**  (continued)

| Metals | Adsorbent | Adsorption capacity[b] | Temperature (°C) | References |
|---|---|---|---|---|
| Ni(II) | Rice husk | 0.23 | – | Marshall et al. (1993) |
| | Rice husk ash | 4.71 | – | Srivastava et al. (2009) |
| | Microwave-irradiated rice husk (MIRH) | 1.17 | 30 | Pillai et al. (2009) |
| | Partial alkali digested and autoclaved rice husk | 5.52 | – | Krishnani et al. (2008) |
| Pb(II) | Rice husk ash | 12.61 | 30 | Feng et al. (2004) |
| | Rice husk ash | 12.35 | 15 | Feng et al. (2004) |
| | $HNO_3$, $K_2CO_3$ treated rice husk | $0.058 \pm 0.1$ mmol g$^{-1}$ | 30 | Akhtar et al. (2010) |
| | Rice husk ash | 207.50 | – | Wang et al. (2008) |
| | Rice husk ash | 91.74 | – | Naiya et al. (2009) |
| | Copolymer of iron and aluminum impregnated with active silica derived from rice husk ash | 416 | – | Abo-El-Enein et al. (2009) |
| | Tartaric acid modified rice husk | 120.48 | – | Wong et al. (2003b) |
| | Tartaric acid modified rice husk | 108 | 27 | Wong et al. (2003a) |
| | Tartaric acid modified rice husk | 105 | 50 | Wong et al. (2003a) |
| | Tartaric acid modified rice husk | 96 | 70 | Wong et al. (2003a) |
| | Partial alkali digested and autoclaved rice husk | 58.1 | – | Krishnani et al. (2008) |
| | Tartaric acid modified rice husk | 21.55 | – | Tarley et al. (2004) |
| | Rice husk | 6.385 | 25 | Surchi (2011) |
| | Rice husk | 5.69 | 30 | Zulkali et al. (2006) |
| | Rice husk | 45 | – | Tarley et al. (2004) |
| | Rice husk | 11.40 | – | Roy et al. (1993) |
| Zn(II) | $HNO_3$, $K_2CO_3$ treated rice husk | $0.037 \pm 0.2$ mmol g$^{-1}$ | 30 | Akhtar et al. (2010) |
| | Rice husk | 30.80 | 50 | Mishra et al. (1997) |
| | Rice husk | 29.69 | 40 | Mishra et al. (1997) |
| | Rice husk | 28.25 | 30 | Mishra et al. (1997) |
| | Rice husk | 26.94 | 20 | Mishra et al. (1997) |
| | Rice husk ash | 14.30 | – | Bhattacharya et al. (2006) |
| | Rice husk ash | 7.7221 | – | Feroze et al. (2011) |
| | Rice husk ash | 5.88 | – | Srivastava et al. (2008) |
| | Partial alkali digested and autoclaved rice husk | 8.14 | – | Krishnani et al. (2008) |
| | Rice husk | 0.75 | – | Marshall et al. (1993) |
| | Rice husk | 0.173 | – | Munaf and Zein (1997) |
| | Rice husk-polyaniline nanocomposite | 24.3 | – | Ghorbani et al. (2012) |

[a]These reported adsorption capacities are values obtained under specific conditions. Readers are encouraged to refer to the original articles for information on experimental conditions
[b]In the unit of (mg g$^{-1}$) unless specified

**Table 8.6** Adsorption capacities of metals by wheat based materials[a]

| Metals | Adsorbent | Adsorption capacity[b] | References |
|---|---|---|---|
| Cd(II) | Wheat straw | 39.22 | Farooq et al. (2011) |
| | Wheat straw | 14.56 | Dang et al. (2009) |
| | Wheat straw | 11.60 | Tan and Xiao (2009) |
| | Wheat straw | 40.48 | Dhir and Kumar (2010) |
| | Wheat bran | 51.58 | Nouri and Hamdaoui (2007) |
| | Wheat bran | 15.71 | Nouri et al. (2007) |
| | Wheat bran | 21.0 | Farajzadeh and Monji (2004) |
| | Wheat bran | 101 | Özer and Pirincci (2006) |
| Cr(III) | Wheat straw | 21.0 | Chojnacka (2006) |
| | Wheat bran | 93.0 | Farajzadeh and Monji (2004) |
| Cr(VI) | Wheat straw | 295.36 | Xu et al. (2011) |
| | Wheat straw | 47.16 | Dhir and Kumar (2010) |
| | Wheat bran | 35 | Dupont and Guillon (2003) |
| | Wheat bran | 40.8 | Wang et al. (2008) |
| | Wheat bran | 310.58 | Singh et al. (2009) |
| | Wheat bran | 0.942 | Nameni et al. (2008) |
| Cu(II) | Wheat straw | 11.43 | Dang et al. (2009) |
| | Wheat straw-citric acid treated | 78.13 | Gong et al. (2008) |
| | Wheat bran | 12.7 | Dupont et al. (2005) |
| | Wheat bran | 17.42 | Aydin et al. (2008) |
| | Wheat bran | 8.34 | Basci et al. (2004) |
| | Wheat bran | 6.85 | Wang et al. (2009) |
| | Wheat bran | 51.5 | Özer et al. (2004) |
| | Wheat bran | 15.0 | Farajzadeh and Monji (2004) |
| Hg(II) | Wheat bran | 70.0 | Farajzadeh and Monji (2004) |
| Ni(II) | Wheat straw | 41.84 | Dhir and Kumar (2010) |
| | Wheat bran | 12.0 | Farajzadeh and Monji (2004) |
| Pb(II) | Wheat bran | 87.0 | Bulut and Baysal (2006) |
| | Wheat bran | 62.0 | Farajzadeh and Monji (2004) |
| | Wheat bran | 79.4 | Özer and Pirincci (2006) |
| Zn(II) | Wheat bran | 16.4 | Dupont et al. (2005) |
| U(VI) | Wheat straw | 19.2–34.6 | Wang et al. (2011) |

[a]These reported adsorption capacities are values obtained under specific conditions. Readers are encouraged to refer to the original articles for information on experimental conditions
[b]In the unit of $(mg\ g^{-1})$

## 8.3.2 Rice Husk

Rice, a cereal grain, is the most important staple food for a large part of the world's human population, especially in Asia and the West Indies. In fact, the rice is grown on every continent except Antarctica and ranks second only to maize (corn) in terms of worldwide area and production in 2010. In rice milling industries, rice husk which is the hard outer shell of the rice grain is generated as the major by-product or waste,

**Table 8.7** Adsorption capacities of metals by chitosan and chitosan composites[a]

| Metal | Adsorbent | Adsorption capacity[b] | Temperature (°C) | References |
|---|---|---|---|---|
| As(III) | Chitosan/ceramic alumina | 56.50 | 25 | Veera et al. (2008b) |
| As(V) | Chitosan/ceramic alumina | 96.46 | 25 | Veera et al. (2008b) |
| Au(III) | Chitosan/cotton fibers (via Schiff base bond) | 76.82 | 25 | Qu et al. (2009b) |
| | Chitosan/cotton fibers (via C–N single bond) | 88.64 | 25 | Qu et al. (2009b) |
| Cd(II) | Chitosan/clay beads | 72.31 | 25 | Tirtom et al. (2012) |
| | Chitosan/cotton fibers (via Schiff base bond) | 15.74 | 25 | Zhang et al. (2008) |
| | Chitosan/glutaraldehyde | 32.9 | 25 | Vitali et al. (2008) |
| | Chitosan/perlite | 178.6 | 25 | Shameem et al. (2006) |
| | Chitosan/PVA | 142.9 | 50 | Kumar et al. (2009) |
| | Chitosan/xanthate | 357.14 | RT | Sankararamakrishnan et al. (2007) |
| Co(II) | Chitosan/clinoptilolite | 467.90 | 25 | Dinu and Dragan (2010) |
| Cr(III) | Chitosan/Reactive Blue 2 | 11.2 | 25 | Vasconcelos et al. (2007) |
| Cr(VI) | Chitosan coated acid treated oil palm shell charcoal (CCAB) | 60.25 | – | Nomanbhay and Palanisamy (2005) |
| | Chitosan coated oil palm shell charcoal (CCB) | 52.68 | – | Nomanbhay and Palanisamy (2005) |
| | Chitosan/cellulose | 13.05 | 25 | Sun et al. 2009 |
| | Chitosan/ceramic alumina | 153.8 | 25 | Veera et al. (2003) |
| | Magnetic chitosan | 69.40 | – | Huang et al. (2009) |
| | Chitosan/montmorillonite | 41.67 | 25 | Fan et al. (2006) |
| | Chitosan/perlite | 153.8 | 25 | Shameem et al. (2003) |
| Cu(II) | Chitosan/alginate | 67.66 | – | Wan Ngah and Fatinathan (2008) |
| | Chitosan/cellulose | 26.50 | 25 | Sun et al. (2009) |
| | Chitosan/ceramic alumina | 86.20 | 25 | Veera et al. (2008a) |
| | Chitosan/clinoptilolite | 574.49 | – | Dragan et al. (2010) |
| | Chitosan/clinoptilolite | 719.39 | 25 | Dinu and Dragan (2010) |
| | Chitosan/cotton fibers (via Schiff base bond) | 24.78 | 25 | Zhang et al. (2008) |
| | Chitosan/H2fmbme | 113.6 | 25 | Vasconcelos et al. (2008) |
| | Chitosan/heparin | 81.04 | 25 | Coelho et al. (2007) |
| | Chitosan/perlite | 196.07 | – | Kalyani et al. (2005) |
| | Chitosan/perlite | 104.0 | 25 | Shameem et al. (2008) |
| | Chitosan/PVA | 47.85 | – | Wan Ngah et al. (2004) |
| | Chitosan/PVC | 87.9 | – | Srinivasa et al. (2009) |
| | Chitosan/Reactive Blue 2 | 57.0 | 25 | Vasconcelos et al. (2007) |
| | Chitosan/sand | 10.87 | – | Wan et al. (2007) |
| | Chitosan/sand | 8.18 | – | Wan et al. (2010) |
| Fe(III) | Chitosan/nano-hydroxyapatite | 6.75 | – | Kousalya et al. (2010) |

(continued)

**Table 8.7** (continued)

| Metal | Adsorbent | Adsorption capacity[b] | Temperature (°C) | References |
|---|---|---|---|---|
| Hg(II) | Chitosan/cotton fibers (via Schiff base bond) | 104.31 | 35 | Qu et al. (2009a) |
| | Chitosan/cotton fibers (via C–N single bond) | 96.28 | 25 | Qu et al. (2009a) |
| | Chitosan/glutaraldehyde | 75.5 | RT | Vieira and Beppu (2006) |
| Ni(II) | Chitosan/clay beads | 32.36 | 25 | Tirtom et al. (2012) |
| | Chitosan/calcium alginate | 222.2 | – | Vijaya et al. (2008) |
| | Chitosan/cellulose | 13.21 | 25 | Sun et al. (2009) |
| | Chitosan/ceramic alumina | 78.10 | 25 | Veera et al. (2008a) |
| | Chitosan/clinoptilolite | 247.03 | 25 | Dinu and Dragan (2010) |
| | Chitosan/cotton fibers (via Schiff base bond) | 7.63 | 25 | Zhang et al. (2008) |
| | Chitosan/magnetite | 52.55 | – | Tran et al. (2010) |
| | Chitosan/perlite | 114.94 | – | Kalyani et al. (2005) |
| | Chitosan/PVC | 120.5 | – | Srinivasa et al. (2009) |
| | Chitosan/silica | 254.3 | – | Vijaya et al. (2008) |
| Pb(II) | Chitosan/cellulose | 26.31 | 25 | Sun et al. (2009) |
| | Chitosan/cotton fibers (via Schiff base bond) | 101.53 | 25 | Zhang et al. (2008) |
| | Chitosan/magnetite | 63.33 | – | Tran et al. (2010) |
| | Chitosan/sand | 12.32 | – | Wan et al. (2010) |
| Th(IV) | Poly(methacrylic acid) grafted-chitosan/bentonite | 110.5 | 30 | Thayyath et al. (2010) |
| Zn(II) | Chitosan/cellulose | 19.81 | 25 | Sun et al. (2009) |

[a]These reported adsorption capacities are values obtained under specific conditions. Readers are encouraged to refer to the original articles for information on experimental conditions
[b]In the unit of ($mg \, g^{-1}$)

accounting for almost 20 % of the rice production. Throughout the world, the annual generation of rice husk was approximately 120 million tonnes in 2009, leading to serious environmental problems as the rice husk is usually disposed in the end of the production.

In nature, rice husk is tough, woody, insoluble in water, has good chemical stability and high mechanical strength. It is considered as a lignocellulosic agricultural by-product that contains approximately 32 % cellulose, 21 % hemicelluloses, 21 % lignin, 20 % silica and 3 % crude proteins (Nadzi et al. 2007). After a complete combustion process, the resulted rice husk ash consists of about 96 % silica (Rahman and Ismail 1993). The high percentage of silica coupled with a large amount of the structural polymer, lignin has made the rice husk not only resistant to water penetration and fungal decomposition, but also resistant to the best efforts of man to dispose it since husk does not biodegrade easily.

Obviously, the utilization of this abundant scaly residue will yield economic as well as environmental dividends. Therefore, exploration of possible usage of

**Table 8.8** Adsorption capacities of metals by untreated and pretreated algae-based materials[a]

| Metals | Algae | Adsorption capacity[b] | References |
|---|---|---|---|
| Al(III) | Sargassum fluitans-NaOH treated | 0.950–3.740 | Lee and Volesky (1999) |
| Cd(II) | Ascophyllum nodosum (B) | 0.338–1.913 | Holan et al. (1993) |
| | Ascophyllum nodosum-CaCl₂ treated | 0.930 | Yu et al. (1999) |
| | Ascophyllum nodosum-Divinil sulfone treated | 1.139 | Holan et al. (1993) |
| | Ascophyllum nodosum-formaldehyde treated | 0.750–0.854 | Leusch et al. (1995), Chong and Volesky (1995) |
| | Ascophyllum nodosum-formaldehyde (3CdSO₄, H₂O) treated | 1.121 | Holan et al. (1993) |
| | Ascophyllum nodosum-formaldehyde + urea treated | 1.041 | Holan et al. (1993) |
| | Ascophyllum nodosum-formaldehyde Cd(CH₃COO)₂ treated | 1.326 | Holan et al. (1993) |
| | Ascophyllum nodosum-glutaraldehyde treated | 1.259 | Holan et al. (1993) |
| | Ascophyllum nodosum-glutaraldehyde treated | 0.463–0.480 | Leusch et al. (1995) |
| | Caulerpa lentillifera (G)-dried macroalgae | 0.026–0.042 | Pavasant et al. (2006) |
| | Caulerpa lentillifera (G)-dried macroalgae | 0.0381 | Apiratikul and Pavasant (2008a) |
| | Chaetomorha linum (G) | 0.48 | Hashim and Chu (2004) |
| | Chlorella vulgaris (G) | 0.30 | Klimmek et al. (2001) |
| | Corallina officinalis (R) | 0.2642 | Basso et al. (2002) |
| | Durvillaea potatorum (B) -CaCl₂ treated | 0.260 | Matheickal et al. (1999) |
| | Durvillaea potatorum-CaCl₂ treated | 1.100–1.130 | Matheickal et al. (1999) |
| | Codium fragile (G) | 0.0827 | Basso et al. (2002) |
| | Ecklonia maxima (B) -CaCl₂ treated | 1.150 | Yu et al. (1999) |
| | Ecklonia radiate (B) -CaCl₂ treated | 1.040 | Yu et al. (1999) |
| | Fucus vesiculosus (B) | 0.649 | Holan et al. (1993) |
| | Gracilaria edulis (R) | 0.24 | Hashim and Chu (2004) |
| | Gracilaria Salicornia (R) | 0.16 | Hashim and Chu (2004) |
| | Laminaria hyperbola (B)-treated CaCl₂ | 0.820 | Yu et al. (1999) |
| | Laminaria japonica (B)- treated CaCl₂ | 1.110 | Yu et al. (1999) |

(continued)

**Table 8.8** (continued)

| Metals | Algae | Adsorption capacity[b] | References |
|--------|-------|----------------------|------------|
| | Lessonia flavicans (B) -treated CaCl₂ | 1.160 | Yu et al. (1999) |
| | Lessonia nigresense (B)-treated CaCl₂ | 1.110 | Yu et al. (1999) |
| | Padina sp.(B) | 0.53 | Kaewsarn and Yu (2001) |
| | Padina sp-CaCl₂ treated | 0.52 | Kaewsarn and Yu (2001) |
| | Padina tetrastomatica | 0.53 | Hashim and Chu (2004) |
| | Porphira columbina (R) | 0.4048 | Basso et al. (2002) |
| | Sargassum baccularia (B) | 0.74 | Hashim and Chu (2004) |
| | Sargassum fluitans-formaldehyde treated | 0.9519 | Leusch et al. (1995) |
| | Sargassum fluitans-glutaraldehyde treated | 1.0676 | Leusch et al. (1995) |
| | Sargassum fluitans-protonated biomass | 0.710 | Davis et al. (2000) |
| | Sargassum sp. (B) | 1.40 | Tobin et al. (1984) |
| | Sargassum natans (B) | 1.174 | Holan et al. (1993) |
| | Sargassum siliquosum (M) | 0.73 | Hashim and Chu (2004) |
| | Sargassum vulgare-protonated biomass | 0.790 | Davis et al. (2000) |
| | Ulva lactuca (G) | 0.1.52 | Areco et al. (2012) |
| Cr(IV) | Chlorella vulgaris-artificial cultivation | 1.525 | Dönmez et al. (1999) |
| Cr(VI) | Chlorella vulgaris | 0.534 | Aksu et al. (1997) |
| | Chlorella vulgaris | 1.525 | Dönmez et al. (1999) |
| | Sargassum sp. | 1.3257 | Cossich et al. (2002) |
| | Sargassum sp. | 1.30 | Silva et al. (2003) |
| | Scenedesmus obliquus | 1.131 | Dönmez et al. (1999) |
| | Scenedesmus obliquus-artificial cultivation | 1.131 | Dönmez et al. (1999) |
| Cu(II) | Ascophyllum nodosum-CaCl₂ treated | 1.090 | Yu et al. (1999) |
| | Ascophyllum nodosum-formaldehyde treated | 0.990 | Chong and Volesky (1995) |
| | Ascophyllum nodosum-formaldehyde treated | 1.306–1.432 | Leusch et al. (1995) |
| | Ascophyllum nodosum-glutaraldehyde treated | 0.803–0.850 | Leusch et al. (1995) |
| | Caulerpa lentillifera (G)-dried macroalgae | 0.042–0.088 | Pavasant et al. (2006) |

(continued)

**Table 8.8** (continued)

| Metals | Algae | Adsorption capacity[b] | References |
|---|---|---|---|
| | Caulerpa lentillifera (G)-dried macroalgae | 0.112 | Apiratikul and Pavasant (2008b) |
| | Chlorella miniata (G) | 0.366 | Lau et al. (1999) |
| | Chlorella vulgaris | 0.295 | Lau et al. (1999) |
| | Chlorella vulgaris | 0.254–0.549 | Aksu et al. (1997) |
| | Chlorella vulgaris | 0.758 | Dönmez et al. (1999) |
| | Chlorella vulgaris-artificial cultivation | 0.759 | Dönmez et al. (1999) |
| | Durvillaea potatorum-CaCl$_2$ treated | 0.040–1.310 | Matheickal and Yu (1999) |
| | Ecklonia radiate-CaCl$_2$ treated | 0.070–1.110 | Matheickal and Yu (1999) |
| | Ecklonia maxima-CaCl$_2$ treated | 1.220 | Yu et al. (1999) |
| | Laminaria hyperbola-treated CaCl$_2$ | 1.220 | Yu et al. (1999) |
| | Laminaria japonica-treated CaCl$_2$ | 1.200 | Yu et al. (1999) |
| | Lessonia flavicans-treated CaCl$_2$ | 1.250 | Yu et al. (1999) |
| | Lessonia nigresense-treated CaCl$_2$ | 1.260 | Yu et al. (1999) |
| | Padina sp-CaCl$_2$ treated | 0.8 | Kaewsarn (2002) |
| | Sargassum sp. | 1.08 | Silva et al. (2003) |
| | Sargassum fluitans-glutaraldehyde treated | 1.574 | Leusch et al. (1995) |
| | Sargassum fluitans-formaldehyde treated | 1.7938 | Leusch et al. (1995) |
| | Sargassum fluitans-NaOH treated | 0.650–1.540 | Lee and Volesky (1999) |
| | Sargassum fluitans-protonated biomass | 0.800 | Davis et al. (2000) |
| | Sargassum vulgare-protonated biomass | 0.930 | Davis et al. (2000) |
| | Scenedesmus obliquus (G) | 0.524 | Dönmez et al. (1999) |
| | Scenedesmus obliquus-artificial cultivation | 0.524 | Dönmez et al. (1999) |
| | Ulva lactuca (G) | 0.34 | Areco et al. (2012) |
| Fe(III) | Chlorella vulgaris | 0.439 | Aksu et al. (1997) |
| Ni(II) | Ascophyllum nodosum | 1.346–2.316 | Holan and Volesky (1994) |
| | Ascophyllum nodosum-formaldehyde treated | 1.431–1.618 | Leusch et al. (1995) |

**Table 8.8** (continued)

| Metals | Algae | Adsorption capacity[b] | References |
|---|---|---|---|
| | Ascophyllum nodosum-formaldehyde + CH₃COOH treated | 0.409 | Holan and Volesky (1994) |
| | Ascophyllum nodosum-formaldehyde + urea treated | 0.511 | Holan and Volesky (1994) |
| | Ascophyllum nodosum-glutaraldehyde treated | 0.920–1.959 | Leusch et al. (1995) |
| | Chlorella miniata | 0.237 | Lau et al. (1999) |
| | Chlorella vulgaris | 0.205–1.017 | Klimmek et al. (2001) |
| | Chlorella vulgaris | 1.017 | Dönmez et al. (1999) |
| | Chlorella vulgaris | 0.205 | Lau et al. (1999) |
| | Chlorella vulgaris-artificial cultivation | 1.017 | Dönmez et al. (1999) |
| | Chondrus crispus (R) | 0.443 | Holan and Volesky (1994) |
| | Codium taylori (G) | 0.099 | Holan and Volesky (1994) |
| | Cystoserta Indica (B) | 0.76 | Pahlavanzadeh et al. (2010) |
| | Durvillaea potatorum-CaCl₂ treated | 0.17–1.13 | Yu and Kaewsarn (2000) |
| | Fucus vesiculosus | 0.392 | Holan and Volesky (1994) |
| | Fucus vesiculosus-formaldehyde treated | 0.559 | Holan and Volesky (1994) |
| | Galaxaura marginata (R) | 0.187 | Holan and Volesky (1994) |
| | Galaxaura marginata-CaCO₃ treated | 0.187 | Holan and Volesky (1994) |
| | Nizmuddinia Zanardini (B) | 0.76 | Pahlavanzadeh et al. (2010) |
| | Padina australis (B) | 0.37 | Pahlavanzadeh et al. (2010) |
| | Padina gymnospora (B) | 0.170 | Holan and Volesky (1994) |
| | Padina gymnospora-CaCO₃ treated | 0.238 | Holan and Volesky (1994) |
| | Sargassum fluitans (B) | 0.409 | Holan and Volesky (1994) |
| | Sargassum fluitans-epichlorohyridin treated | 0.337 | Holan and Volesky (1994) |
| | Sargassum fluitans-formaldehyde treated | 1.9932 | Leusch et al. (1995) |
| | Sargassum fluitans-formaldehyde + HCl treated | 0.749 | Holan and Volesky (1994) |
| | Sargassum fluitans-glutaraldehyde treated | 0.7337 | Leusch et al. (1995) |

**Table 8.8**  (continued)

| Metals | Algae | Adsorption capacity[b] | References |
|---|---|---|---|
| | Sargassum glaucescens (B) | 0.76 | Pahlavanzadeh et al. (2010) |
| | Sargassum natans | 0.409 | Holan and Volesky (1994) |
| | Sargassum vulgare (M) | 0.085 | Holan and Volesky (1994) |
| | Scenedesmus obliquus | 0.5145 | Dönmez et al. (1999) |
| | Scenedesmus obliquus-artificial cultivation | 0.514 | Dönmez et al. (1999) |
| Pb(II) | Ascophyllum nodosum | 1.313–2.307 | Holan and Volesky (1994) |
| | Ascophyllum nodosum-CaCl$_2$ treated | 1.150 | Yu et al. (1999) |
| | Ascophyllum nodosum-Bis(ethenil)sulfone treated | 1.733 | Holan and Volesky (1994) |
| | Ascophyllum nodosum-formaldehyde treated | 1.3755 | Leusch et al. (1995) |
| | Ascophyllum nodosum-formaldehyde + CH$_3$COOH treated | 1.308 | Holan and Volesky (1994) |
| | Ascophyllum nodosum-formaldehyde + urea treated | 0.854 | Holan and Volesky (1994) |
| | Ascophyllum nodosum-glutaraldehyde treated | 1.318 | Holan and Volesky (1994) |
| | Ascophyllum nodosum-glutaraldehyde treated | 0.816–0.898 | Leusch et al. (1995) |
| | Caulerpa lentillifera (G)-dried macroalgae | 0.076–0.139 | Pavasant et al. (2006) |
| | Caulerpa lentillifera (G)-dried macroalgae | 0.142 | Apiratikul and Pavasant (2008a) |
| | Chlorella vulgaris | 0.47 | Klimmek et al. (2001) |
| | Cladophora glomerata (G) | 0.355 | Jalali et al. (2002) |
| | Chondrus crispus treated with 1-chloro-2,3-epoxipropane | 1.009 | Holan and Volesky (1994) |
| | Chondrus crispus | 0.941 | Holan and Volesky (1994) |
| | Codium taylori | 1.815 | Holan and Volesky (1994) |
| | Durvillaea potatorum-CaCl$_2$ treated | 0.020–1.550 | Matheickal and Yu (1999) |
| | Ecklonia maxima-CaCl$_2$ treated | 1.400 | Yu et al. (1999) |
| | Ecklonia radiate-CaCl$_2$ treated | 0.050–1.260 | Matheickal and Yu (1999) |
| | Fucus vesiculosus | 1.105–2.896 | Holan and Volesky (1994) |

**Table 8.8** (continued)

| Metals | Algae | Adsorption capacity[b] | References |
|--------|-------|------------------------|------------|
| | Fucus vesiculosus-formaldehyde treated | 1.752 | Holan and Volesky (1994) |
| | Fucus vesiculosus-formaldehyde + HCl treated | 1.453 | Holan and Volesky (1994) |
| | Galaxaura marginata | 0.121 | Holan and Volesky (1994) |
| | Galaxaura marginata-CaCO$_3$ treated | 1.530 | Holan and Volesky (1994) |
| | Gracilaria corticata (R) | 0.2017–0.2606 | Jalali et al. (2002) |
| | Laminaria hyperbola-treated CaCl$_2$ | 1.350 | Yu et al. (1999) |
| | Laminaria japonica-treated CaCl$_2$ | 1.330 | Yu et al. (1999) |
| | Lessonia flavicans-treated CaCl$_2$ | 1.450 | Yu et al. (1999) |
| | Lessonia nigresense-treated CaCl$_2$ | 1.460 | Yu et al. (1999) |
| | Padina gymnospora | 0.314 | Holan and Volesky (1994) |
| | Padina gymnospora-CaCO$_3$ treated | 0.150 | Holan and Volesky (1994) |
| | Padina tetrastomatica (B) | 1.049 | Jalali et al. (2002) |
| | Polysiphonia violacea (R) | 0.4923 | Jalali et al. (2002) |
| | Sargassum fluitans | 1.594 | Holan and Volesky (1994) |
| | Sargassum fluitans-epichlorohyridin treated | 0.975 | Holan and Volesky (1994) |
| | Sargassum fluitans-formaldehyde treated | 1.8244 | Leusch et al. (1995) |
| | Sargassum fluitans-glutaraldehyde treated | 1.6603 | Leusch et al. (1995) |
| | Sargassum hystrix (B) | 1.3755 | Jalali et al. (2002) |
| | Sargassum natans | 1.221 | Holan and Volesky (1994) |
| | Sargassum natans | 1.1487 | Jalali et al. (2002) |
| | Sargassum vulgare | 1.100 | Holan and Volesky (1994) |
| | Ulva lactuca (G) | 0.61 | Jalali et al. (2002) |
| | Ulva lactuca (G) | 0.145 | Areco et al. (2012) |
| | Undaria pinnatifida (B) | 1.945 | Kim et al. (1999) |
| Zn(II) | Ascophyllum nodosum-formaldehyde treated | 0.680 | Chong and Volesky (1995) |
| | Ascophyllum nodosum-formaldehyde treated | 0.719 | Leusch et al. (1995) |

(continued)

**Table 8.8** (continued)

| Metals | Algae | Adsorption capacity[b] | References |
|---|---|---|---|
| | Ascophyllum nodosum-formaldehyde treated | 0.8718 | Leusch et al. (1995) |
| | Ascophyllum nodosum-glutaraldehyde treated | 0.138–0.367 | Leusch et al. (1995) |
| | Caulerpa lentillifera (G)-dried macroalgae | 0.021–0.141 | Pavasant et al. (2006) |
| | Chlorella vulgaris | 0.37 | Klimmek et al. (2001) |
| | Sargassum fluitans-formaldehyde treated | 0.9635 | Leusch et al. (1995) |
| | Sargassum fluitans-glutaraldehyde treated | 0.9942 | Leusch et al. (1995) |
| | Ulva lactuca (G) | 0.145 | Areco et al. (2012) |

*B* brown alga, *G* green alga, *R* red alga

[a]These reported adsorption capacities are values obtained under specific conditions. Readers are encouraged to refer to the original articles for information on experimental conditions

[b]In the unit of (mmol g$^{-1}$)

the rice husk is of great significance in many rice producing countries. It is quite unlikely to use rice husk as animal feed because of its relatively low content of total digestible nutrients (less than 10 %). Besides, the husk has a very low protein and available carbohydrates values, and yet, at the same time, high in crude fiber and crude ash. Due to its abrasive character, poor nutritive value, low bulk density and high ash content which would sometimes cause harmful effect, the rice husk cannot be considered as a safe and suitable animal feed.

Owing to its excellent properties including good heat insulation, ability in emitting smell or gases, and resistant to corrosive, the rice husk can be seen as a potential raw material used to insulate walls, floors and roof cavities. Unfortunately, the cost of building materials manufactured using rice husk as the aggregate is considerably high and not economic as compared to that of using other aggregates, hence its application as building material is not practical in real production. Recently, the rice husk ash has been used as silica source in synthesis of silica aerogel, MCM-41 and other silica- based mesoporous materials which have higher commercial value. Nevertheless, the quality of the resulted materials is hard to be controlled due to variation of silica content and presence of different elements in the rice husk ash depending on the rice species and the combustion process.

Thus, efforts have been made to use this cheap and readily available resource as a low cost adsorbent in the removal of heavy metals from aqueous environment. The high content of cellulose, a polymer contains three reactive hydroxyl groups has made rice husk a potential adsorbent material. However, both lignin and silica that present in rice husk tends to reduce the binding between accessible functional group

**Table 8.9** Adsorption capacities of metals by bacterials[a]

| Metals | Adsorbent | Adsorption capacity[b] | References |
|---|---|---|---|
| Ag(I) | Geobacillus thermodenitrificans | 37.86 | Chatterjee et al. (2010) |
| Cd(II) | Aeromonas caviae | 155.3 | Loukidou et al. (2004) |
| | Brevundimonas sp. ZF12 Strain | 49.01 | Masoudzadeh et al. (2011) |
| | Enterobacter sp. | 46.2 | Lu et al. (2006) |
| | Geobacillus thermodenitrificans | 42.9 | Chatterjee et al. (2010) |
| | Ochrobactrum anthropi | – | Ozdemir et al. (2003) |
| | Pseudomonas aeruginosa | 42.4 | Chang et al. (1997) |
| | Pseudomonas putida | 8.0 | Pardo et al. (2003) |
| | Pseudomonas putida | 500.00 | Hussein et al. (2004) |
| | Pseudomonas sp. | 278.0 | Ziagova et al. (2007) |
| | Sphingomonas paucimobilis | – | Tangaromsuk et al. (2002) |
| | Staphylococcus xylosus | 250.0 | Ziagova et al. (2007) |
| | Streptomyces pimprina | 30.4 | Puranik et al. (1995) |
| | Streptomyces rimosus | 64.9 | Selatnia et al. (2004a) |
| Co(II) | Geobacillus thermodenitrificans | 69.76 | Chatterjee et al. (2010) |
| Cr(III) | Geobacillus thermodenitrificans | 70.7 | Chatterjee et al. (2010) |
| Cr(VI) | Aeromonas caviae | 284.4 | Loukidou et al. (2004) |
| | Bacillus coagulans | 39.9 | Srinath et al. (2002) |
| | Bacillus megaterium | 30.7 | Srinath et al. (2002) |
| | Bacillus coagulans | 39.9 | Srinath et al. (2002) |
| | Bacillus licheniformis | 69.4 | Zhou et al. (2007) |
| | Bacillus megaterium | 30.7 | Srinath et al. (2002) |
| | Bacillus thuringiensis | 83.3 | Sahin and Öztürk (2005) |
| | Pseudomonas sp. | 95.0 | Ziagova et al. (2007) |
| | Pseudomonas fluorescens. | 111.11 | Hussein et al. (2004) |
| | Staphylococcus xylosus | 143.0 | Ziagova et al. (2007) |
| | Zoogloea ramigera | 2 | Nourbakhsh et al. (1994) |
| Cu(II) | Bacillus firmus | 381 | Salehizadeh and Shojaosadati (2003) |
| | Bacillus sp. | 16.3 | Tunali et al. (2006) |
| | Bacillus subtilis | 20.8 | Nakajima et al. (2001) |
| | Enterobacter sp. | 32.5 | Lu et al. (2006) |
| | Geobacillus thermodenitrificans | 50.0 | Chatterjee et al. (2010) |
| | Micrococcus luteus | 33.5 | Nakajima et al. (2001) |
| | Pseudomonas aeruginosa | 23.1 | Chang et al. (1997) |
| | Pseudomonas cepacia | 65.3 | Savvaidis et al. (2003) |
| | Pseudomonas putida | 6.6 | Pardo et al. (2003) |
| | Pseudomonas putida | 96.9 | Uslu and Tanyol (2006) |
| | Pseudomonas putida | 15.8 | Chen et al. (2005) |
| | Pseudomonas putida | 163.93 | Hussein et al. (2004) |
| | Pseudomonas stutzeri | 22.9 | Nakajima et al. (2001) |

**Table 8.9** (continued)

| Metals | Adsorbent | Adsorption capacity[b] | References |
|---|---|---|---|
| | Sphaerotilus natans | 60 | Beolchini et al. (2006) |
| | Sphaerotilus natans | 5.4 | Beolchini et al. (2006) |
| | Streptomyces coelicolor | 66.7 | Öztürk et al. (2004) |
| | Thiobacillus ferrooxidans | 39.8 | Liu et al. (2004) |
| Fe(III) | Geobacillus thermodenitrificans | 79.9 | Chatterjee et al. (2010) |
| | Streptomyces rimosus | 122.0 | Selatnia et al. (2004b) |
| Ni(II) | Bacillus thuringiensis | 45.9 | Öztürk (2007) |
| | Pseudomonas putida | 556 | Hussein et al. (2004) |
| | Streptomyces rimosus | 32.6 | Selatnia et al. (2004d) |
| Pb(II) | Bacillus sp. | 92.3 | Tunali et al. (2006) |
| | Bacillus firmus | 467 | Salehizadeh and Shojaosadati (2003) |
| | Corynebacterium glutamicum | 567.7 | Choi and Yun (2004) |
| | Enterobacter sp. | 50.9 | Lu et al. (2006) |
| | Geobacillus thermodenitrificans | 32.26 | Chatterjee et al. (2010) |
| | Pseudomonas aeruginosa | 79.5 | Chang et al. (1997) |
| | Pseudomonas aeruginosa | 0.7 | Lin and Lai (2006) |
| | Pseudomonas putida | 270.4 | Uslu and Tanyol (2006) |
| | Pseudomonas putida | 56.2 | Pardo et al. (2003) |
| | Streptomyces rimosus | 135.0 | Selatnia et al. (2004c) |
| Pd(II) | Desulfovibrio desulfuricans | 128.2 | De Vargas et al. (2004) |
| | Desulfovibrio fructosivorans | 119.8 | De Vargas et al. (2004) |
| | Desulfovibrio vulgaris | 106.3 | De Vargas et al. (2004) |
| Pt(IV) | Desulfovibrio desulfuricans | 62.5 | De Vargas et al. (2004) |
| | Desulfovibrio fructosivorans | 32.3 | De Vargas et al. (2004) |
| | Desulfovibrio vulgaris | 40.1 | De Vargas et al. (2004) |
| Th(IV) | Arthrobacter nicotianae | 75.9 | Nakajima and Tsuruta (2004) |
| | Bacillus licheniformis | 66.1 | Nakajima and Tsuruta (2004) |
| | Bacillus megaterium | 74.0 | Nakajima and Tsuruta (2004) |
| | Bacillus subtilis | 71.9 | Nakajima and Tsuruta (2004) |
| | Corynebacterium equi | 46.9 | Nakajima and Tsuruta (2004) |
| | Corynebacterium glutamicum | 36.2 | Nakajima and Tsuruta (2004) |
| | Micrococcus luteus | 77.0 | Nakajima and Tsuruta (2004) |
| | Zoogloea ramigera | 67.8 | Nakajima and Tsuruta (2004) |
| U(VI) | Arthrobacter nicotianae | 68.8 | Nakajima and Tsuruta (2004) |
| | Bacillus licheniformis | 45.9 | Nakajima and Tsuruta (2004) |
| | Bacillus megaterium | 37.8 | Nakajima and Tsuruta (2004) |
| | Bacillus subtilis | 52.4 | Nakajima and Tsuruta (2004) |
| | Corynebacterium equi | 21.4 | Nakajima and Tsuruta (2004) |
| | Corynebacterium glutamicum | 5.9 | Nakajima and Tsuruta (2004) |
| | Micrococcus luteus | 38.8 | Nakajima and Tsuruta (2004) |
| | Nocardia erythropolis | 51.2 | Nakajima and Tsuruta (2004) |
| | Zoogloea ramigera | 49.7 | Nakajima and Tsuruta (2004) |

(continued)

**Table 8.9** (continued)

| Metals | Adsorbent | Adsorption capacity[b] | References |
|---|---|---|---|
| Zn(II) | Aphanothece halophytica | 133.0 | Incharoensakdi and Kitjaharn (2002) |
| | Bacillus firmus | 418 | Salehizadeh and Shojaosadati (2003) |
| | Geobacillus thermodenitrificans | 48.26 | Chatterjee et al. (2010) |
| | Pseudomonas putida | 6.9 | Pardo et al. (2003) |
| | Pseudomonas putida | 17.7 | Chen et al. (2005) |
| | Streptomyces rimosus | 30.0 | Mameri et al. (1999) |
| | Streptomyces rimosus | 80.0 | Mameri et al. (1999) |
| | Streptoverticillium cinnamoneum | 21.3 | Puranik and Paknikar (1997) |
| | Thiobacillus ferrooxidans | 82.6 | Celaya et al. (2000) |
| | Thiobacillus ferrooxidans | 172.4 | Liu et al. (2004) |

[a]These reported adsorption capacities are values obtained under specific conditions. Readers are encouraged to refer to the original articles for information on experimental conditions
[b]In the unit of ($mg\ g^{-1}$)

on the surface of rice husk and adsorbate ions/molecules. Therefore, considerable researches have been carried out to modify or treat the rice husk via different methods prior to the application in heavy metals removal. Adsorption capacities of metals by untreated and treated rice husk are presented in Table 8.5.

### 8.3.3 Wheat Straw and Wheat Bran

Wheat (*Triticum aestivum*) is the major food crop of the world. The World Agricultural Supply and Demand Estimates (WASDE) has estimated the total consumption of wheat worldwide was 652 million tons for year 2010. Straw and bran are generated as by-products in the wheat milling industry. Every year, over 200 million tons of wheat straw are produced and every 100 million ton of wheat can create 25 million tons of bran. In addition to their use as feed and fuel, large amounts of redundant wheat straw and bran must be disposed of. Therefore, finding new applications for these abundantly available waste materials is a challenge to the wheat production countries.

The wheat straw is a lignocellulosic agricultural waste consisting of about 34–40 % cellulose, 20–35 % hemicellulose, 8–15 % lignin and sugars as well as other compounds carrying different functional groups like carboxyl, hydroxyl, sulphydryl, amide and amine. Although the substances are almost similar, the percentage composition of different substances may vary in different parts of the world depending on the wheat species and growing conditions. Wheat straw has been used as fodder and in paper industry to produce low quality boards or packing materials. The stems are usually burnt directly in some parts of the world for fuel supply, leading to serious atmospheric pollution and wastage of resources.

The wheat bran is the shell of the wheat seed that contains most nutrients of wheat. Despite its high nutritious value, this bran is always removed in the processing of wheat into flour and discarded as waste. Most recently, wheat bran is found to be a valuable material which is applicable in fermentation industry, pharmaceutics and biomedical research. This by-product can also serve as a potential nutritious and cheap raw material for fermentation industry. In addition, its amazing properties of anti-oxidative and anti-inflammatory can bring about a revolution in the fields of melanoma and cancer research. However, more researches and investigation should be carried out to make its applications realistic in human daily life.

As attempt to fully utilize these industry by-products, both wheat straw and wheat bran have been investigated for their adsorption behaviour towards metal ions (Table 8.6). The high adsorption capability of straw is closely related to high content of cellulose, presence of different functional groups and porosity of the material. The reported metal adsorption capacities of wheat based materials could depend on the structure of wheat bran used in different studies, along with other parameters.

### 8.3.4 Chitin, Chitosan and Chitosan Composites

Chitin is a naturally and abundant occurring mucopolysaccharide from exoskeletons of insects, crustaceans shells, fungi cellular walls, annelids and molluscs. It is the second most abundant polymer in nature after cellulose. Chitin contains 2-acetamido-2-deoxy-$\beta$-D-glucose through a $\beta$ $(1 \rightarrow 4)$ linkage. Through deacetylation process of chitin, a type of natural poly(aminosaccharide), so called chitosan is produced. The chitosan consists mainly of poly $(1 \rightarrow 4)$-2 amino-2-deoxy-D-glucose unit and this derivative biopolymer has been known as a promising polymeric material of great scientific interest due to its special characteristics, for instances, hydrophilicity, biocompatibility, biodegradability, non-toxicity, good adsorption properties and wide range of applications.

The chitosan has drawn particular attention for its feasible application in a variety of forms, from flake-types to gels, beads and fibers. Due to high content of amino and hydroxyl groups in chitosan, this by-product material has been found to be able on adsorbing several heavy metals, including copper, chromium, silver, platinum and lead physically or chemically. In fact, chitosan has binding capacities of more than 1 mmol/g for heavy metals which is far greater than that of activated carbon. The extend of metal adsorption depends strongly on the chitosan source, deacetylation degree, nature of metal ion, variations in crystallinity, amino content and solution pH.

Chitosan can either form gel or dissolve depending on the pH values. Its high sensitivity towards pH has constituted an obstacle to chitosan's performance as a biosorbent in wastewater treatment. To solve this problem, chemical modification of chitosan using cross-linking reagents, for instance glyoxal, formaldehyde, glutaraldehyde, epichlorohydrin, ethylene glycon diglycidyl ether and isocyanates

have been used to stabilize chitosan in acidic solutions. Several chemical changes are possible in the chitosan polymeric matrix because of the existence of reactive amino and hydroxyl groups. It has been demonstrated that the chemical modification does not only increase the chemical stability of the adsorbent in acidic media, and especially, decrease its solubility in most mineral and organic acids, but also enhances its mechanical properties. However, the adsorption ability of the modified chitosan may be reduced after the cross-linking procedure due to increment in the rigidity of the structural conformation of the polymer.

A great number of chitosan derivatives have been obtained by introducing selected functional groups into the polymeric matrix of chitosan in order to enhance its interaction with variety of metallic ions, thus increase the adsorption capacity and selectivity for metal ions in solution. The reported chitosan derivatives are those containing nitrogen, phosphorus and sulfur as heteroatoms, and other derivatives such as chitosan crown ethers and chitosan ethylenediaminetetraacetic acid (EDTA)/diethylenetriaminepentaacetic acid (DTPA) complexes. Apart from that, chitosan composites have also been extensively investigated for heavy metals removal. Various types of substance have been used to form composite with chitosan, including activated clay, bentonite, montmorillonite, polyurethane, poly vinyl alcohol, poly vinyl chloride, kaolinite, oil palm ash and perlite. Table 8.7 shows the heavy metal removal capacities through adsorption process using these modified chitosan materials.

### 8.3.5 Algae

The term "algae" refers to a large and diverse assemblage of organisms that contain chlorophyll and carry out oxygenic photosynthesis. Algae are simple plant-like organisms, ranging from unicellular to multicellular forms, which are easily found in aquatic habitats, freshwater, marine and moist soil. The largest and most complex marine forms are called seaweeds. This abundantly available biomass has been extensively studied for its variety applications as fertilizer, energy sources, pollution control, pigment, stabilizing substances, nutrition and biodegradable plastic production.

Algae can be classified according to several characteristics, including the nature of the chlorophyll(s) present, the carbon reserve polymers produced, the cell wall structure, and the motility type. Although all algae consist of chlorophyll a, there are some, which contain other chlorophylls that differ in minor ways from chlorophylls a. The presence of these additional chlorophylls is typical of particular algal groups. Chrysophyta (golden-brown algae and diatoms), Euglenophyta (euglenoids, is also considered as protozoa), Pyrrophyta (dino-flagellates), Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae) are some of the major groups of algae.

The algal cell is surrounded by thin, rigid cell walls which are usually made of multilayered microfibrillar framework generally consisting of cellulose and inter-

sperse with amorphous material (Madigan et al. 2000). These walls are essentially impermeable to larger molecules or to macromolecules. However, existence of pores about 3–5 nm wide on the algal cell wall has allowed low molecular-weight constituents such as water, ions, gases, and other nutrients to pass through freely for metabolism and growth.

Due to their high surface area to volume ratio, various algae have been investigated as potential materials for heavy metal removal in aqueous solutions. Actually, the metal biosorption by algae mainly depend on the components on the cell, especially through cell surface and the spatial structure of the cell wall. The cell walls of algae generally contain cellulose, alginic acid and sulphated polysaccharides. These compounds consist of several functional groups, such as carboxyl, hydroxyl, sulfate, phosphate, and amino groups which play a very important role in biosorption process of metal. The mechanism of binding metal ions by algal biomass is always affected by the biomass characteristics, species and ionic charges of the targeted metal ions, and other external environmental factors such as solution pH and temperature.

Among the three groups of algae (red, green and brown algae), brown alga is the most widely studied. This could be associated to the high sorption capability of the brown algae as compared to other two algae species (Romera et al. 2006; Brinza et al. 2007). Very often, the algae undergo treatment in different ways to enhance their sorption capacity (Romera et al. 2006). Adsorption capacities of metals by untreated and treated algae are presented in Table 8.8. In brief, algae have been proven to be both economic and eco-friendly because they are ubiquitous occurrence in nature, can be regenerated after use and have lesser volume of chemical and/or biological sludge to be disposed of.

### 8.3.6 Bacteria

Bacteria are a large domain of prokaryotic microorganisms. They exist at almost everywhere on the Earth, including in soil, acidic hot springs, radioactive waste, and deep in the Earth's crust, as well as in organic matter and the live bodies of animals and plants. There are about five nonillion ($5 \times 10^{30}$) bacteria present on the Earth, forming a biomass that exceeds that of all plants and animals. Bacteria have simple morphology and have a wide range of shapes, ranging from spheres or ovoid (coccus) to rods (bacillus, with a cylindrical shape) and spirals (spirillum). They are recognized as microscale organisms whose single cells have neither a membrane-bounded nucleus nor other membrane-bounded organelles like mitochondria and chloroplasts. Due to differences in genetics and ecology, however, bacteria may vary in size as much as in shape. The smallest bacteria are about 0.3 $\mu$m, and a few bacteria become fairly large, e.g. some spirochetes occasionally reach 500 $\mu$m in length.

In the past decades, there has been an increasing interest to use bacteria in degrading or mineralizing several textile dyes. Bacterial decolorization was attributed to the presence of oxidoreductive enzymes, such as laccase, NADH-DCIP reductase

and azoreducases in the bacteria. However, the effectiveness of decolorization using bacteria depends strongly on the adaptability and the activity of the selected bacterium.

Recently, the feasibility of bacteria as biosorbent in heavy metals was explored. It has been demonstrated that composition of cell wall of bacteria is one of the key factors influencing bacterial biosorbing properties. The anionic functional groups exist in the teichoic acids and peptidoglican of gram-positive bacteria, lipopolysaccharides and phospholipids of gram-negative bacteria are the important elements responsible for the metal binding ability of the cell wall. Besides, the metal binding ability of bacteria would be further increased with the involvement of extra-cellular polysaccharides. Usually, the availability of this compound depends on the bacterial species and growth conditions.

Advantages such as availability, small size, ability to grow under controlled conditions and resiliency to a wide range of environmental situations are some of the reasons that made bacteria gaining its popularity in the development of new biosorbents materials. Adsorption capacities of metals by bacterial are provided in Table 8.9.

## 8.4 Equilibrium, Continuous and Modelling Studies of Heavy Metals Adsorption

### 8.4.1 Batch Adsorption Experiments

An efficient contact between adsorbent and adsorbate for a period of time is desired in a batch adsorption experiment. This allows the concentration of adsorbate in solution to attain equilibrium with the adsorbate on the surface. In most of the cases, particle size, pH, concentration and agitation are the key factors for the time of equilibrium attainment. Since the last decades, porous adsorbents such as activated carbon and mesoporous silica materials are widely used in batch equilibrium operations for their extremely high surface area, bringing into more effective adsorbent-adsorbate contact and a reducing of diffusional resistance inside the pores. Separation process is always carried out after the adsorption process. The adsorbed adsorbate on the solid phase can be separated from the adsorbate residue in solution via several methods such as settling, filtration, or centrifugation. In industry, the used adsorbent is either regenerated for further use or disposed, depending on the cost involved. Both adsorption isotherm and kinetics modelling are commonly applied in discussion of batch adsorption studies.

### 8.4.2 Adsorption Isotherm Models in a Batch System

In general, the adsorption isotherms describe how adsorbates interact with adsorbents and therefore they are the basic as well as the most necessity in an adsorption

design. The adsorption isotherms are always referred to adsorption properties and equilibrium data obtained in an adsorption system. Apparently, establishing an appropriate and correct correlation for the equilibrium data is important to understand adsorbate-adsorbent interaction, subsequently contributing to improvement of an adsorption system. Besides, the data must fit to a mathematical model for a reliable prediction on adsorption parameters. The compliance of data would also allow a quantitative comparison on adsorption activities of variety adsorption systems under different operating conditions.

During an adsorption process, adsorbate is adsorbed onto the adsorbent. At the same time, desorption process of adsorbate from the adsorbent might also occur with a slower rate. When the rate of both adsorption and desorption processes is the same, the adsorption equilibrium is said to be achieved. Usually, the equilibrium condition is characterized in graph of adsorbate concentration in solid phase versus that in liquid phase. One or more isotherm models may be used to explain the distribution of adsorbate molecules between the adsorbent and the liquid phase, which provides information of equilibrium position in the adsorption process. The suitability, so-called 'favorable' degree of an isotherm model to the adsorption system is often verified from the shape generated in isotherm graph. The isotherm model is 'favored' by the adsorption system if the value of $R^2$ of graph slope is equal or very close to unity. Besides, the isotherm shape also gives qualitative information on the nature of the solute–surface interaction. In adsorption system, the performance of different adsorbents is always evaluated from their maximum adsorption capacity which can be obtained from the fitted adsorption isotherm. The capacity information also allows selection of the most appropriate adsorbent for a particular adsorption application under certain conditions.

Two- and three- parameter models are widely applied in correlating adsorption equilibria in liquid phase adsorption, even though they are originally used for gas phase adsorption. The thermodynamic assumptions made and different equation parameters used within these models often provide insight into surface properties and affinity of the adsorbent as well as the adsorption mechanism. Obviously, a proper correlation of equilibrium data is crucial for an optimized adsorption condition as well as improved adsorbent's activities.

## Two-Parameter Adsorption Isotherms

There are a number of available two-parameter adsorption isotherms for description of adsorption equilibria in liquid phase. However, the underlying assumptions within the model often limit the application of each model for an adsorption system. Among the models, Langmuir, Freundlich, and Brunauer-Emmet-Teller (BET) models appear as the most popular isotherms. Further, other two-parameter models such as Temkin and Dubinin-Radushkevich Jovanovic, Halsey and Hurkins-Jura are also presented in this text.

Langmuir Adsorption Isotherm

The Langmuir model has been intensively applied in quantifying the amount of adsorbate being adsorbed on an adsorbent as a function of concentration at a certain temperature in a homogenous adsorption system (Langmuir 1918). This isotherm assumes the adsorption process happens at specific homogeneous sites on the surface of adsorbate. Therefore, the Langmuir model is valid for homogenous adsorption and it is always referred to monolayer coverage of adsorbate over a homogenous adsorbent surface. For a biosorption process, all the sites on the adsorbent are equivalent and no further adsorption can occur at a site once the site has been occupied by an adsorbate molecule. Based on the assumption made, the adsorption is localized based on lattice model and no further adsorption is allowed at equilibrium where a saturation point is reached. In addition, the model makes assumption that the adsorption of each molecule has equal adsorption activation energy and adsorption enthalpy is independent of surface coverage. The Langmuir equation is written below:

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \tag{8.2}$$

where $q_e$ = mass of adsorbate adsorbed/mass of adsorbent at equilibrium

$q_{max}$ = mass of adsorbate adsorbed/mass of adsorbent for a complete monolayer

$K_L$ = Langmuir constant related to the enthalpy of adsorption

$C_e$ = concentration of adsorbate at equilibrium

Apparently, characteristic of the Langmuir isotherm can be represented by a plateau in the plot $q_e$ versus $C_e$. In addition, the Langmuir isotherm is traditionally used in quantifying the maximum uptake and estimating the adsorption capacity $q_{max}$ of different adsorbents. Since the Langmuir assumes all the surface sites possess equal affinity for the adsorbate, the $q_{max}$ should be temperature independent. In practical, however, the presence of functional groups in the adsorbent has brought to modest changes in adsorption capacity with temperature. Besides, some other factors including the number of active sites on the adsorbent, the chemical state of the sites, the accessibility of the adsorbate to the sites and the affinity between the sites such as binding strength has greatly affected the adsorption capacity of an adsorbent in real situation.

An adsorption process can be classified as physisorption or chemisorption. In physisorption, physical forces are dominant in the bonding between adsorbates and the surface. The forces become weaken with increasing of temperature. Therefore, the exothermal nature of the adsorption process can be proven from the decrease of $K_L$ value with elevating temperature (Ho and Ofomaja 2006; Shaker 2007; Padmavathy 2008; Djeribi and Hamdaoui 2008). On the other hand, the thermal energy is needed for the strong interaction between the adsorbate and the adsorbent in generating new types of electronic bonds in a chemisorption process. Thus, the rise in temperature is more favourable for the chemisorption which involves the binding of adsorbates to active sites, making it an endothermic process (Febrianto

et al. 2009). Usually, the van't Hoff plots are used to further verify the exothermal or endothermal nature of the adsorption process by considering the enthalpy of adsorption. An integrated van't Hoff equation relates the Langmuir constant, $K_L$ to the temperature as below.

$$K_L = K_0 \exp\left(-\frac{\Delta H}{RT}\right) \tag{8.3}$$

where $K_0$ = parameter of the van't Hoff equation

$H$ = enthalpy of adsorption

The determination of the adsorption parameters may not be easy since the Eq. 8.2 is in non-linear form. In order to overcome this problem, the Langmuir equation is often expressed in different linear forms as following:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max}}C_e + \frac{1}{K_L q_{\max}} \tag{8.4}$$

$$\frac{1}{q_e} = \left(\frac{1}{K_L q_{\max}}\right)\frac{1}{C_e} + \frac{1}{q_{\max}} \tag{8.5}$$

$$q_e = q_{\max} - \left(\frac{1}{K_L}\right)\frac{q_e}{C_e} \tag{8.6}$$

$$\frac{q_e}{C_e} = K_{Lq\max} - K_{Lq e} \tag{8.7}$$

As compared to the non-linear equation, the four Langmuir linear equations (Eqs. 8.4, 8.5, 8.6, and 8.7) are more favourable among the researchers due to their simplicity and convenience in acquiring isotherm data. Nevertheless, one should realise that the isotherm parameters obtained using the four Langmuir linear equations could be varied in some cases, but they are identical when the non-linear method is applied (Ho 2006).

In fact, the essential characteristics of the Langmuir adsorption isotherm can be expressed in terms of dimensionless constant equilibrium parameter, $R_L$ (Hall et al. 1966). The $R_L$ is also recognized as separation factor and it is defined as:

$$R_L = \frac{1}{1 + K_L C_o} \tag{8.8}$$

where $C_o$ = maximum initial concentration of adsorbate.

The isotherm shape can be interpreted according to the value of calculated $R_L$ (Table 8.10).

Even though the Langmuir adsorption is considered as conventional method in studying an adsorption system, it has suffered from some disadvantages as the model ignores adsorbate-adsorbent interactions. In fact, there are two types of adsorbate-adsorbent interaction, namely direct interaction and indirect interaction

**Table 8.10** Langmuir
adsorption isotherm constant
equilibrium parameter, $R_L$

| $R_L$ value | Type of isotherm |
|---|---|
| $R_L > 1$ | Unfavorable |
| $R_L = 1$ | Linear |
| $0 < R_L < 1$ | Favorable |
| $R_L = 1$ | Irreversible |

present in real system. In direct interactions, the adjacent adsorbed molecules tend to make adsorbing near another adsorbate molecule more or less favourable. Meanwhile, indirect interaction influences the adsorption behaviour of the nearly sites by changing surface around the adsorbed site. Since the existence of both adsorbate-adsorbent interactions has been evidenced in heat of adsorption data, they should be taken into consideration in proper estimation of the adsorption parameters. Furthermore, the Langmuir adsorption model considers only the adsorbate of smooth and homogenous surface, making it fails to account for the rough surface of the adsorbate. Since a rough inhomogeneous surface may create different types of sites and change the properties of the sites, such as heat of adsorption, a significant deviation from this model is expected in many cases.

Freundlich Adsorption Isotherm

Freundlich isotherm (Freundlich 1906) is the oldest of the non-linear isotherms. Unlike the Langmuir isotherm which assumes homogenous site energies and limited levels of adsorption, it suggests adsorption onto heterogeneous surface. Since the multilayer adsorption is allowed in this model, concentration of adsorbate on the adsorbent surface rises with increasing of adsorbate concentration in the system. The Freundlich equation relates the concentration of an adsorbate on the surface of an adsorbent to the concentration of the adsorbate in the liquid which it is in contact. The empirical Freundlich equation is mathematically written as:

$$q_e = K_F C_e^{1/n} \tag{8.9}$$

where $q_e$ = mass of adsorbate adsorbed/mass adsorbent
$\quad C_e$ = concentration of adsorbate at equilibrium
$\quad K_F$ = Freundlich constant related to adsorption capacity at particular temperature
$\quad n$ = Freundlich constant related to adsorption intensity at particular temperature, $(n > 1)$
$\quad$ Equation 8.9 can also be presented in a linearized logarithmic form:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \tag{8.10}$$

$K_F$ is important in providing insight on adsorption capacity which appears as the most significant property of an adsorbent. Usually, adsorption capacity is affected

by pH and temperature of the system as well as nature of the adsorbent, such as pore and particle size distribution, specific surface area, cation exchange capacity and surface functional groups. As shown in Eq. 8.10, both Freundlich constants of $K_F$ and $n$ can be calculated from the intercept and slope, respectively in the graph of log $q_e$ versus log $C_e$. The $K_F$ value depends on the units upon which $q_e$ and $C_e$ are expressed if $1/n \neq 1$. $K_F$ is equivalent to $q_e$ when $C_e$ equals to 1. In the case of $1/n$ equals unity, identical adsorption energy for all sites is observed associated to linear adsorption occurrence (Site 2001). The constant $n$ is the Freundlich equation exponent that characterizes quasi-Gaussian energetic heterogeneity of the adsorption surface (Bansal and Goyal 2005). Usually, larger value of $n$ implies a stronger adsorbent-adsorbate interaction. For a favourable adsorption, Freundlich constant $n$ should have values lying in the range of 1–10.

In fact, Freundlich isotherm is one of the most established isotherm models in biosorption study because it fits nearly all experimental adsorption–desorption data especially in highly heterogeneous adsorbent systems. Although Freundlich isotherm is inappropriate over a wide concentration range, this limitation is always ignored by researchers since moderate concentration is always used in most biosorption studies.

Brunauer–Emmer–Teller (BET) Model

In 1938, Stephen Brunauer, Paul Hugh Emmett, and Edward Teller had introduced the first isotherm for multimolecular layer adsorption (Brunauer et al. 1938). This adsorption theory, so called Brunauer–Emmer–Teller (BET) theory, is extension of Langmuir theory where it proposes multilayer adsorption of adsorbate on the surface of adsorbate. This BET model suggests a random distribution of sites covered by one or more adsorbate molecules by assuming the adsorbent surface consists of fixed individual sites and molecules can be adsorbed more than one layer on the surface of the adsorbent. Besides, Langmuir theory can be applied to each layer and the interaction between each adsorption layer is ignored. In other words, the same kinetics concept proposed by Langmuir is applicable to this multiple layering process, i.e., the rate of adsorption and desorption on any layer is equal.

The BET model is widely used in systems involving heterogeneous material and simple non-polar gases. However, it may not suitable for complex systems dealing with heterogeneous adsorbent such as biosorbents and adsorbates because of the assumption of all sites are energetically identical along with no horizontal interaction between adsorbed molecules. Therefore, this model should not be used in the interpretation of liquid phase adsorption data for complex solids. The simplified form of BET equation is given as following:

$$q_e = q_{\max} \frac{K_B C_e}{(C_e - C_s)\left[1 + (K_B - 1)(C_e/C_s)\right]} \qquad (8.11)$$

where $q_{max}$ = mass of adsorbate adsorbed/mass of adsorbent for a complete monolayer

$K_B$ = constant related to energy of adsorption

$C_s$ = concentration of adsorbate at saturation of all layers

After rearrangement, the Eq. 8.11 can be written into a linear form:

$$\frac{C_e}{(C_s - C_e)q} = \frac{1}{K_B q_{max}} + \left(\frac{K_B - 1}{K_B q_{max}}\right)\left(\frac{C_e}{C_s}\right) \quad (8.12)$$

Temkin Adsorption Isotherm

After considering the limitation of Langmuir adsorption isotherm, the Temkin model takes into accounts of indirect interactions of adsorbate-adsorbate molecules on adsorption isotherms (Temkin 1941). As the surface of adsorbent is occupied by adsorbate, the Temkin isotherm assumes that the heat of adsorption of all molecules in the layer would decrease linearly with coverage due to the indirect interactions. Thus, the Temkin model is dissimilar with Freundlich model which suggests a logarithmical decrease in adsorption. The Temkin equation is written as below:

$$q_e = \frac{RT}{b} \ln a\, C_e \quad (8.13)$$

where a = Temkin isotherm constant

b = Temkin constant related to heat of adsorption

The Temkin equation proposes the increase in degree of completion of the adsorption centres on an adsorbent may bring to a linear decrease of adsorption energy. The linearized form of Temkin equation is expressed below:

$$q_e = \frac{RT}{b} \ln a + \frac{RT}{b} \ln C_e \quad (8.14)$$

Obviously, both constants $a$ and $b$ can be determined from a plot of $q_e$ versus $\ln C_e$. The Temkin equation is useful to analyse the adsorption data at moderate concentration. Nevertheless, this equation which is originally applied in gas adsorption system, is not well-suited for a complex phenomenon involved in liquid phase adsorption. In liquid phase adsorption, the adsorbed molecules are not organized in a tightly packed structure with identical orientation like what happen in gas phase adsorption. The system becomes more complicated with the presence of solvent molecules and the formation of micelles from adsorbed molecules in liquid phase. Besides, liquid phase adsorption is also greatly influenced by other factors such as temperature, pH, solubility of adsorbate in the solvent and surface chemistry of the adsorbent. Therefore, the Temkin equation is seldom used for experimental data description of complex systems and it receives less attention as compared to three isotherms discussed previously.

Dubinin-Radushkevich Adsorption Isotherm

Dubinin and Radushkevich (D-R) adsorption isotherm has been widely used to express the adsorption isotherms in micropores (Dubinin and Radushkevich 1947). Based on the assumption made of inhomogeneous surface or constant adsorption potential, this model is applicable in estimation of porous structure of the adsorbent and the characteristics of adsorption. Besides, apparent free energy of the adsorption process can also be determined by using this model. The D-R isotherm is given by:

$$q_e = Q_m \exp(-K\varepsilon^2) \tag{8.15}$$

where $Q_m$ = adsorption capacity of adsorbent per unit mass
  $K$ = constant related to the affinity coefficient and the adsorption energy
  $\varepsilon$ = Polanyi's adsorption potential which is correlated to temperature
  The D-R equation can be also expressed in a linear form:

$$\ln q_e = \ln Q_m - K\varepsilon^2 \tag{8.16}$$

By plotting $\ln q_e$ versus $\varepsilon^2$, constant $K$ and adsorption capacity, $Q_m$ can be determined from the slope and the intercept, respectively. The constant $K$, defined as $1/\beta E$, is correlated to the affinity coefficient ($\beta$) which is closely related to the adsorbate-adsorbent interaction. It is also depends on mean free energy of adsorption ($E$) per mole of the adsorbate during the transportation process from infinite distance in solution to the surface of the adsorbent. Therefore, $E$ can be calculated from the $K$ value using the following equation:

$$E = \frac{1}{\sqrt{2K}} \tag{8.17}$$

Besides, the mean free energy can be computed using the following relationship:

$$\varepsilon = RT \ln \left(1 + \frac{1}{C_e}\right) \tag{8.18}$$

Since the D-R isotherm is temperature dependent, a characteristic curve with all the suitable data lying on the same curve is expected by plotting the adsorption data at varied temperatures ($\ln q_e$ vs. $\varepsilon^2$). In other words, the applicability of the D-R equation in expressing the adsorption equilibrium data is confirmed with the attainment of the identical curve. Thus, it would be doubtful on the validity of the ascertained parameters when deviation in the characteristic curve generated from analyzed data is observed, even though the fitting procedure gives high correction value. Practically, however, the examination on the characteristic curve of biosorption systems is rarely done as the experiments are usually conducted at particular temperature. The D-R isotherm is applicable for not only single adsorbate

but also bi-adsorbate adsorption system (Jaroniec and Derylo 1981). Unfortunately, the D-R isotherm is only appropriate for only an intermediate range of adsorbate concentrations since it may exhibit unrealistic asymptotic behavior.

Jovanovic Adsorption Isotherm

The Jovanovic model keeps the same assumptions contained in the Langmuir model which suggests monolayer adsorption on homogeneous solid surface. However, this model considers also the possibility of mechanical contact between the adsorbing and desorbing molecules (Wrudzinski and Wojciecbowski 1977). A general equation derived by Jovanovic is shown as below:

$$q_e = q_{max}[1 - \exp(-aCv)] \tag{8.19}$$

where $C$ = concentration of the adsorbate in the bulk

$v$ = the heterogeneity parameter (with $0 < v < 1$) which emphasizes the allowance for

$a$ = constant correlates to function of the sole temperature

The constant $a$ characterizes the magnitude of the adsorbate–adsorbent interaction energy (Quinones and Guichon 1998). It is defined as (Quinones et al. 1999):

$$a = K \exp(E_a/RT) \tag{8.20}$$

where $K$ = low-pressure equilibrium constant or Henry constant

$E_a$ = characteristic energy of the distribution

The Jovanovic adsorption isotherm emphases the surface binding vibrations of an adsorbed species where the rate of desorption is proportional to the adsorbate surface coverage which can be determined from $q_e/q_{max}$.

Halsey Adsorption Isotherm

The Halsey model is applicable to multilayer adsorption (Halsey 1948). Its ability to confirm the heteroporous nature of the adsorbent makes it differs from the Freundlich adsorption isotherm which is also used for multilayer adsorption. The Halsey equation is written as below:

$$q_e = \mathrm{Exp}\left(\frac{\ln k_H - \ln C_e}{n}\right) \tag{8.21}$$

where $k_H$ = Halsey isotherm constant

$n$ = Halsey isotherm exponent

Harkin-Jura Adsorption Isotherm

The Harkin-Jura (H-J) adsorption isotherm proposes the presence of a heterogeneous pore distribution in the adsorbent (Harkins and Jura 1944). Similar to Freundlich and Hasley adsorption isotherms, this model is useful in explanation of multilayer adsorption. The H-J isotherm is expressed as below:

$$q_e = \sqrt{\frac{A_H}{B_2 + \log C_e}} \tag{8.22}$$

where $A_H$ = isotherm parameter
  $B_2$ = isotherm constant

## Three-Parameter Adsorption Isotherms

In order to have better understanding in adsorbate-adsorbent interaction and adsorption behaviour, models involving more than two parameters are necessary to interpret the data. It is very often for researchers to make comparison studies between a series of two-parameter and three-parameter isotherms in describing the equilibrium data because more than one model might be needed to explain the biosorption mechanism in some cases. Some commonly used three-parameter isotherms for the prediction of biosorption experimental data are presented.

Redlich–Peterson Adsorption Isotherm

The Redlich-Peterson (R-P) isotherm is the combination of the Langmuir and Freundlich equations (Redlich and Peterson 1959). This model proposes a non ideal monolayer adsorption and the adsorption mechanism is a hybrid of the two models. The R-P model is applicable in adsorption equilibrium data description over a wide concentration range. The R-P equation is represented below:

$$q_e = \frac{K_{RP}C_e}{1 + a_{RP}C_e{}^{\beta}} \tag{8.23}$$

where $K_{RP}$, $a_{RP}$ and $\beta$ are the Redlich-Peterson parameters.
  The exponent $\beta$ ranges between 0 and 1. When $\beta = 0$, the equation resembles Henry's Law.

$$q_e = \frac{K_{RP}C_e}{1 + a_{RP}} \tag{8.24}$$

If $\beta = 1$, the R-P equation is similar to Langmuir equation.

$$q_e = \frac{K_{RP} C_e}{1 + a_{RP} C_e} \tag{8.25}$$

In most biosorption cases, the $\beta$ values are close to unity. Thus, the Langmuir adsorption is the better model to correlate the adsorption data.

Equation 8.23 can also be expressed in linear form as:

$$\ln\left(K_R \frac{C_e}{q_e} - 1\right) = \ln a_{RP} + \beta \ln C_e \tag{8.26}$$

Since R-P isotherm incorporates three parameters, it is not possible to obtain the parameters of the R-P isotherms from the linearized equation. To overcome this problem, the parameters of Eq. 8.26 have to be verified by maximizing the correlation coefficients between the experimental data points and those from theoretical model predictions with the solver add-in function of the Microsoft Excel. Nevertheless, the linear equation of R-P isotherm is useful in determination of the parameters of the Langmuir and Freundlich adsorption models.

Sips Adsorption Isotherm

Sips isotherm was proposed after considering the continuing increase in the adsorbed amount with rising concentration (Sips 1948). It has solved the constraint observed in Freundlich model. In fact, the Sips expression (Eq. 8.27) resembles the Freundlich adsorption isotherm, and differs only on the finite limit of adsorbed amount at adequately high concentration of adsorbate.

$$q_e = q_{max} \frac{(K_S C_e)^{\gamma}}{1 + (K_S C_e)^{\gamma}} \tag{8.27}$$

where $K_S$ = Sips isotherm constant

On the other hand, the Sips equation, Eq. 8.27 is similar to the Langmuir equation, Eq. 8.2. An additional parameter, $\gamma$ which describes heterogeneity of the system is present in the Sips equation. The heterogeneity could arise from the adsorbent or the adsorbate, or both of them. When $\gamma$ equals unity, Eq. 8.27 is same with Eq. 8.5.

Toth Adsorption Isotherm

Both Sips and Freundlich equations have their limitations in describing an adsorption data at certain concentration range. The Sips equation is inappropriate for adsorption equilibria data expression the low concentration end, while the

Freundlich equation is invalid at intense concentration. In order to solve this problem, Toth isotherm which was derived from the potential theory is proposed (Toth 1971). It makes assumption that most sites have adsorption energy less than the mean value due to an asymmetrical quasi-Gaussian energy distribution with its left-hand side widened. This model obeys Henry's at low concentration and reaches an adsorption maximum at high concentration. The Toth equation is written as:

$$q_e = q_{max} \frac{C_e}{\left[a_t + C_e^t\right]^{1/t}} \tag{8.28}$$

where $a_T$ = adsorptive potential constant

$t$ = heterogeneity coefficient of the adsorbent ($0 < t \leq 1$)

Toth equation is capable to characterize adsorption for heterogeneous systems as it possesses a heterogeneity coefficient, $t$. When $t = 1$, the surface of adsorbent is homogeneous and the Toth equation is reduced to the Langmuir equation.

### 8.4.3 Adsorption Kinetics Models in Batch System

The efficiency of the adsorption process is greatly affected by the rate of the adsorbate to be attached on surface of adsorbent. Obviously, an excellent adsorbent should possess not only high adsorption capacity but also fast adsorption rate. Therefore, kinetic studies involving reaction rate investigation are crucial in identifying the ideal adsorbent for a particular adsorbate. In fact, kinetic studies are also carried out to indentify factors affecting the adsorption process, leading to an optimized adsorption system.

In an adsorption process, the kinetic models are always used to examine the rate determining mechanism as well as the role of adsorption surface, chemical reaction involved and/or diffusion mechanisms. In general, the adsorption mechanism is illustrated as the following steps: (i) bulk diffusion which involves transportation of adsorbate from the bulk solution to the surface of the adsorbent; (ii) film diffusion of adsorbate through the boundary layer to the surface of the adsorbent; (iii) pore diffusion or intraparticle diffusion of adsorbate from the surface to within the particle's pores; lastly (iv) adsorption of adsorbate on the active sites that available on the internal surface of the pores. In most of the cases, the bulk diffusion in step (i) is negligible by providing sufficient stirring to avoid particle and solute gradients in the batch system. Thus, the adsorption dynamics can be approximated by three consecutive steps [(ii)–(iv)] only. A rapid uptake always occurs in adsorption process which is the step (iv) of the mechanism. This immeasurable fast process can be considered as an instantaneous process especially in the case of physical adsorption. As a result, the overall rate of adsorption process is determined by either film or intraparticle diffusion in steps (ii) and (iii), respectively, or a combination of both.

By considering the film diffusion is the rate-controlling step in adsorption mechanism, the change of adsorbate concentration with respect to time can be presented as follows:

$$\frac{dC}{dt} = -k_L A(C - C_s) \tag{8.29}$$

where $C$ = bulk liquid phase concentration of adsorbate at any time $t$

$C_s$ = surface concentration of adsorbate

$k_L$ = external mass transfer coefficient

$A$ = specific surface area for mass transfer

During the initial stage of adsorption, it is assumed that the transport is mainly due to film diffusion mechanism and the intraparticle resistance can be ignored. At $t = 0$, $C = C_0$ and the surface concentration of adsorbate, $C_s$ is negligible. With the assumptions made above, Eq. 8.29 can be simplified as:

$$\left[ \frac{d(C/C_0)}{dt} \right] = -k_L A \tag{8.30}$$

Unlike physical reactions, the adsorption rate may be controlled by its own kinetic rates in the case of chemical reactions. As the adsorption kinetics provide valuable insights to the practical application of the process design and operation control, a complete modeling of kinetics should consider the diffusion equations, boundary conditions and adsorption isotherm equation. It has caused a complicated system of equations. This problem is, however, possible to be resolved by separating the diffusion steps. The diffusion mechanisms can be considered independently by assuming initial adsorption rate is controlled by intraparticle diffusion and it is characterized by external diffusion.

The kinetic studies are carried out practically in batch reactions using various adsorbent dosages, particle sizes of adsorbent, initial adsorbate concentrations, agitation rate, pH values and temperatures along with different adsorbent and adsorbate types. A linear regression is used to determine the best-fitting kinetic rate equation. The compliance of experimental data to the kinetic rate equations is often confirmed using the linear least-squares method that applied to the linearly transformed kinetic rate equations. Lagergren model, pseudo-second-order kinetic model, Elovich model and intraparticle diffusion model are the four commonly applied kinetic models among the researchers.

## Lagergren Model

Lagergren model, which is also recognized as pseudo-first order kinetic model, appears as simplified kinetic model which has been widely used in adsorption kinetics examination (Lagergren 1898). The well fitting of experimental data to the

pseudo-first order kinetic model suggests film diffusion is the rate-limiting step. Based on solid capacity, the Lagergren first-order rate expression is presented as follows:

$$\frac{dq}{dt} = k_1(q_e - q_t) \tag{8.31}$$

where $q_e$ = adsorption capacity at equilibrium state
$\quad q_t$ = adsorption capacity at time t
$\quad k_1$ = rate constant of pseudo-first-order adsorption
At $t = 0$, $q_t = 0$, and at $t = t$, $q_t = q_t$, Eq. 8.31 can be integrated as:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \tag{8.32}$$

Equation 8.32 can be written in non-linear form as below:

$$q_t = q_e(1 - \exp(-k_1 t)) \tag{8.33}$$

A straight line of $\ln(q_e - q_t)$ versus $t$ is good indicator of the applicability of this kinetic model. The $q_e$ value obtained by this method should be compared with the experimental value to identify the deviation. A time lag resulted from external mass transfer or boundary layer diffusion at the beginning of the adsorption process may cause the difference in $q_e$ values. If a large discrepancy in the $q_e$ values is observed, even though the least square fitting process yields high correlation coefficient, a reaction still cannot be classified as first-order. In this case, $q_e$ and $k_1$ can be estimated via non-linear procedure fitting of Eq. 8.33. On the other hand, both rate constant and equilibrium adsorbate uptake can be determined from the straight-line plots of $\ln(q_e - q_t)$ against $t$ of Eq. 8.32 at different initial adsorbate concentrations.

**Pseudo-Second-Order Kinetic Model**

Ho and McKay introduced the pseudo-second order model on the basis of the adsorption capacity of the solid phase (Ho and McKay 1999). The well fitting of experimental data to the pseudo-second order kinetic model suggests chemisorption is the rate-limiting step. Similar to Lagergren model or pseudo-first order kinetic model, this model takes account of all the steps of adsorption including external film diffusion, intraparticle diffusion and adsorption. In particular, this pseudo-second order model is useful to the adsorption systems using various adsorbent dosages and adsorbate concentrations. This model is written as:

$$\frac{dq}{dt} = k_2(q_e - q_t)^2 \tag{8.34}$$

where $k_2$ = rate constant of pseudo-second-order adsorption

At $t = 0$, $q = 0$, and at $t = t$, $q_t = q_t$, integration of Eq. 8.34 with the boundary conditions yields

$$\frac{1}{q_e - q} = \frac{1}{q_e} + k_2 t \tag{8.35}$$

The linear form of Eq. 8.35 is shown as below:

$$\frac{t}{q} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2} \tag{8.36}$$

It is noteworthy that pseudo-second order model enables the determination of adsorption capacity, pseudo-second-order rate constant, and initial adsorption rate without prior knowledge of experimental parameters.

## Elovich Model

The Elovich model is a kinetic model for chemisorptions that has been widely used to describe the adsorption of gas onto solid systems (Zeldowitsch 1934; Rudzinski and Panczyk 2000). Since last decade, this model has also been applied to discuss the adsorption behaviour of heavy metal removal from aqueous solutions (Cheung et al. 2001). The Elovich equation is generally expressed as:

$$\frac{dq_t}{dt} = \alpha \exp(-\beta q_t) \tag{8.37}$$

Where $\alpha$ = initial adsorption rate
   $\beta$ = the desorption constant
   By assuming $\alpha \beta t \gg t$ and by applying the boundary conditions $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, Eq. 8.36 can be simplified into (Chien and Clayton 1980):

$$q_t = \frac{1}{\beta} \ln(\alpha \beta) + \frac{1}{\beta} \ln(t) \tag{8.38}$$

If the adsorption data fit the Elovich model, a plot of $q_t$ against $\ln(t)$ should yield a linear relationship. The $\beta$ and $\alpha$ can be calculated from the slope and intercept, respectively.

## Intraparticle Diffusion Model

By assuming intraparticle diffusion is the only rate determining step, the intraparticle diffusion model correlates adsorption capacity to effective diffusivity

of adsorbate within the particle (Weber and Morris 1963). The well fitting of experimental data to the intraparticle diffusion model suggests pore diffusion is the rate-limiting step. The model mathematically is expressed in the equation below:

$$q_t = f\left(\frac{Dt}{r_p^2}\right)^{1/2} = K_{WM}t^{1/2} \tag{8.39}$$

where $q_t$ = adsorption capacity at time $t$

$D$ = effective diffusivity of solutes within the particle

$r_p$ = particle radius

$K_{WM}$ = intraparticle diffusion rate constant

Theoretically, the plot of $q$ versus $t^{1/2}$ should give a straight line passing through the origin. The slope of the straight line provides the intraparticle diffusion rate constant $K_{WM}$. However, detection of multi linear plots from adsorption data may imply the occurrence of some other mechanisms during the adsorption process. Accordingly, a significant external resistance to mass transfer surrounding the particles in the early stage of adsorption is evidenced by the first shaper portion. Meanwhile, the intraparticle diffusion happens in the second linear portion, which is a steady adsorption stage. Eventually, the final equilibrium stage is achieved when the rate of intraparticle diffusion becomes slow associated to extremely low solute concentration in solution in the third portion. Apparently, a good correlation of rate data in this model is important to rationalize the adsorption mechanism, subsequently to determine $K_{WM}$ values by linearization the curve $q = f(t^{0.5})$.

The rate of diffusion within the particle is much slower compared to that of movement of the adsorbate from solution to the external solid surface. This is because of the restraining chemical attractions between adsorbate and adsorbent as well as the greater mechanical hindrance to movement exist at the surface molecules or surface layers. Usually, adsorbate molecules reach at the adsorbent surface more fast than they can diffuse into the solid adsorbent, resulting in accumulation of the adsorbate at the surface. Therefore, a (pseudo)-equilibrium may be established. Nevertheless, further adsorption of adsorbate can occur at the same rate as the surface concentration is reduced by inward adsorption.

### 8.4.4 Continuous Packed-Bed System in Biosorption of Heavy Metals

The batch adsorption method is only application for adsorption system involving small volumes of adsorbate. Thus, continuous flow treatments should be applied for large scale application of biosorption process. In this method, adsorbates in

solution are flown continuously from either the top or the bottom of a stationary bed of solid adsorbent which is more recognized as column. Since this method has closer simulation of commercial systems in industry, it is commonly used in the assessment of the suitability of an adsorbent for a particular adsorbate. After the adsorption process, the adsorbent can be easily separated from the effluent and the used adsorbent is usually regenerated. Among the different experimental set-ups, the packed-bed column is possibly the most effective device for continuous operations.

In a down-flow packed bed column, the adsorbate solution is in contact with adsorbent when it moves through the column. As flowing down the column, adsorption process occurs where most of the adsorbate is adsorbed gradually from liquid onto the adsorbent. As a result, concentration of adsorbate in the effluent is either very low or even untraceable at the end of the column. Depending on the value of adsorbate concentration desired for its lower boundary, the length of the adsorption zone in the column is somewhat random. When more adsorbate solution is fed into the column, concentration of adsorbate in the effluent increases slowly due to equilibrium and kinetic factors. Usually, the adsorption zone will shift down the column like a slowly moving wave when the upper portion of packing adsorbent is saturated with adsorbate. Ultimately, the lower edge of the adsorption zone reaches the bottom of the column, resulting in a significant rise in adsorbate concentration in the effluent. As little additional adsorption takes place with the entire bed approaching an equilibrium state with the feed at this breakthrough point, the flow is stopped. However, one should realize that the equilibrium between adsorption and desorption process is seldom achieved under this continuous flow condition. In most cases, the breakthrough point increases with increasing of bed height, reducing of adsorbent's particle size and deceasing of flow rate.

A breakthrough curve, which is plot of adsorbate effluent concentration versus time, is applicable for description of continuous packed bed performance. The general position of the breakthrough curve along the time or volume axis is indicative of loading behaviour of the adsorbate to be removed from a solution in a fixed bed. A normalized concentration as a function of time or volume of effluent ($V_{eff}$) for a given bed height is used to represent the breakthrough position. In real practical, the normalized concentration is referred to the ratio of effluent adsorbate concentration to inlet adsorbate concentration ($C/C_0$). An adsorption isotherm is favoured with infinite adsorption rate the breakthrough curve approaches a straight vertical line. The breakthrough curve becomes less sharp as decreasing of the mass transfer rate. Since mass transfer is always finite, a breakthrough curve of S-shape would be expected. Both the breakthrough point and breakthrough curve are greatly influenced by nature of the adsorbate and the adsorbent, geometry of the column and operating conditions.

In order to evaluate and predict the dynamic behavior of a continuous packed-bed system, a number of simple mathematical models underlying different assumptions have been established. Some commonly used models in characterization of the fixed bed performance for biosorption process are presented here.

**Adams–Bohart Model**

The Adams-Bohart model was developed by assuming the adsorption rate is proportional to adsorbent concentration and its residual capacity (Bohart and Adams 1920). This model was originally applied to describe the relationship between $C/C_o$ and $t$ for the adsorption of chlorine on charcoal in fixed bed column. With the assumption of the adsorption rate is limited by external mass transfer, the model equation is expressed below:

$$\ln \frac{C}{C_0} = k_{AB} C_0 t - k_{AB} N \frac{Z}{U_0} \tag{8.40}$$

where $C$ = adsorbate concentration remaining at each contact time
$C_0$ = initial adsorbate concentration
$k_{AB}$ = Adams–Bohart kinetic constant
$N$ = adsorbate concentration in the bulk liquid
$Z$ = bed depth of column
$U_0$ = linear velocity calculated by dividing the flow rate by the column's sectional area

Equation 8.40 is generally valid in the initial part of the breakthrough as it is derived based on the assumption of low concentration field where $C < 0.15 C_o$. Thus, this model is always utilized in describing the initial part of the breakthrough curve only. The plot of $\ln C/C_0$ against $t$ at a given bed height and flow rate provides values that characterizes operational parameters of the column. As increasing of $t$, concentration of metal in the bulk liquid tends to approach a saturation condition where maximum capacity of adsorption is achieved.

The Adams-Bohart model is applicable in fixed bed column of different biosorption applications due to its ability to provide solution of the differential equations for mass transfer rate in solid and liquid phases. Consequently, its overall approach is suitable for quantitative description of other systems regardless the phase of adsorbate.

**Bed Depth/Service Time Model**

The Bed-Depth-Service-Time (BDST) model is often used to examine the capacity of fixed bed at various breakthrough values (Bohart and Adams 1920). By assuming a direct adsorption of adsorbate onto the surface of the adsorbent, this model correlates the service time ($t$) with the process variables by ignoring intraparticle mass resistance and external film resistance. The BDST model states that the service time of a column is given by:

$$t = \frac{N_0}{C_0 U_0} Z - \frac{1}{K_a C_0} \ln \left( \frac{C_0}{C} - 1 \right) \tag{8.41}$$

where $N_0$ = adsorption capacity
$K_a$ = rate constant in BDST

At 50 % breakthrough $\left(\frac{C_o}{C}\right) = 2$, and $t = t_{0.5}$, the Eq. 8.41 can be written as

$$t_{0.5} = \left(\frac{N_o}{C_o U_o}\right) Z \qquad (8.42)$$

or

$$t_{0.5} = \text{constant} \times Z \qquad (8.43)$$

A straight line passing through the origin is expected in a plot of BDST at 50 % breakthrough against bed depth using Eq. 8.43 if the adsorption data fits the model. Obviously, this model has solved the constraint in Adams and Bohard model which is only appropriate for initial part of the breakthrough.

## Yoon–Nelson Model

Yoon-Nelson (Y-N) model suggests the decrease rate in the probability of adsorption for each adsorbate molecule is comparative to the probability of adsorption of adsorbate and the probability of adsorbate breakthrough on the adsorbent (Yoon and Nelson 1984). This model can be applied without knowing the detailed information about adsorbent type, characteristics of both adsorbate and adsorbent, and physical properties of adsorption bed adsorbent. Therefore, it appears as a reasonably simple theoretical model. Expression of the Y-N equation for a single-component system is given as:

$$\ln \frac{C}{C_0 - C} = k_{YN} t - \tau k_{YN} \qquad (8.44)$$

where $t =$ time required for 50 % adsorbate breakthrough
   $\tau =$ breakthrough (sampling) time
   $k_{YN} =$ Yoon and Nelson rate constant
   The parameters $\tau$ and $k_{YN}$ for the adsorbate can be obtained from the intercept and slope, respective of the $\ln C/(C_0 - C)$ versus sampling time ($t$) plot. These values are important for the calculation of theoretical breakthrough curves for a single-component system.

## Thomas Model

The Thomas model is widely used for determination of rate constant for the maximum adsorption capacity of an adsorbate in a column. This model is derived based on assumption of Langmuir kinetics of adsorption-desorption and no axial dispersion (Thomas 1944). The Thomas equation takes into account of the rate driving force obeys second order reversible reaction kinetics, and it is written as:

$$\frac{C}{C_0} = \frac{1}{1 + \exp(k_{Th}/Q(q_0 X - C_0 V_{eff}))} \tag{8.45}$$

Where $k_{Th} =$ Thomas rate constant
$Q =$ flow rate
$q_0 =$ maximum solid-phase concentration of the solute
$X =$ amount of adsorbent in the column
$V_{eff} =$ effluent volume
The Thomas equation can also be presented in linear form as below:

$$\ln\left(\frac{C_0}{C} - 1\right) = \frac{k_{Th} q_0 X}{Q} - \frac{k_{TH} C_0}{Q} V_{eff} \tag{8.46}$$

By plotting $\ln[(C_0/C) - 1]$ versus $t$ at a given flow rate, the kinetic coefficient $k_{Th}$ and the adsorption capacity of the bed $q_0$ can be determined. The Thomas model allows the prediction of the concentration-time profile or breakthrough curve for the effluent, hence contributing to a successful design of a column sorption process.

**Clark Model**

Clark (Clark 1987) proposed a new simulation for study of a continuous packed-bed system based on the (i) hypothesis of the flow is piston type (Hamdaoui 2006); and (ii) the combination of both mass transfer concept and Freundlich adsorption isotherm (Ayoob and Gupta 2007). By ignoring the phenomenon of dispersion, the equation is expressed as:

$$\frac{C}{C_0} = \left(\frac{1}{1 + Ae^{-rt}}\right)^{1/n-1} \tag{8.47}$$

with

$$A = \left(\frac{C_0^{n-1}}{C_{break}^{n-1}} - 1\right) e^{rt_{break}} \tag{8.48}$$

and

$$R(n-1) = r \text{ and } R = \frac{K_{Cl}}{U_0} v \tag{8.49}$$

where $C_{break} =$ outlet concentration at breakthrough (or limit effluent concentration)
$t_{break} =$ time at breakthrough
$n =$ Freundlich parameter
$k_{Cl} =$ Clark rate constant
$v =$ migration rate

Both constants of the model *A* and *r* of a particular adsorption process on a fixed bed and a selected treatment objective can be obtained from Eq. 8.49 by non-linear regression analysis. These values are critical for the prediction of the breakthrough curve according to the relationship between $C/C_0$ and $t$ in Eq. 8.47.

**Wolborska Model**

Wolborska model is a simplified adsorption representation which emphasizes on the general mass transfer for the diffusion mechanism for low concentration range only for breakthrough curve (Wolborska 1989). Due to its limitation in describing the whole adsorption process, this model is seldom applied by the researchers. The equation for the mass transfer in the fixed bed adsorption is as follow:

$$\ln \left( \frac{C}{C_0} \right) = \frac{\beta_a C_0}{N_0} t - \frac{\beta_a Z}{U_0} \tag{8.50}$$

with

$$\beta_a = \frac{U_0^2}{2D} \left[ \left( 1 + \frac{4\beta_0 D}{U_0} \right)^{1/2} - 1 \right] \tag{8.51}$$

where $\beta_a$ = Kinetics coefficient of the external mass transfer
  $N_0$ = saturation concentration
  $Z$ = height of column
  $U_0$ = specific velocity
  D = axial dispersion coefficient

Kinetics coefficient of the external mass transfer $\beta_a$ can be estimated through the breakthrough curve. When the flow rate of solution through the bed is high or in short beds, axial diffusion $D$ is negligible and $\beta_a = \beta_0$. The Wolborska model resembles Adams-Bohart model if the coefficient $\beta_a/N_0$ is equal to $k_{AB}$.

## 8.4.5   Response Surface Methodology

Response surface methodology (RSM) is a multivariate technique that mathematically fits the experimental domain studied in the theoretical design through a response function. It is used for designing experiments, building numerical models, evaluating the effects of variables and searching for the optimum combinations of factors for a system (Myers and Montgomery 2002).

Generally, the targeted response in a designed of experiments is governed by several independent variables. The response refers to the performance measure or quality characteristic of the product or process, whereas independent variables are

sometimes called input variables. An experiment is a series of tests, called runs, in which changes are made in the input variables in order to identify the reasons for changes in the output response.

Conventional methods of investigating a process by varying one factor whilst maintaining all other factors involved at constant levels does not describe the combined effect of all the factors involved. This method is also time consuming and of low efficiency as it requires a number of experiments to determine the optimum levels, which are unreliable. These limitations of a classical method can be eliminated by optimising all the affecting parameters collectively by statistical technique such as RSM (Elibol 2002).

Two commonly used designs in RSM are central composite design (CCD) and Box-Behnken design (BBD) (Garg et al. 2008; Fu et al. 2009; Cerino Córdova et al. 2011). BBD is an independent quadratic design that does not contain an embedded factorial or fractional factorial design. In this design, the treatment combinations are at the center and midpoints of edges of the process space. These designs are either rotatable or near rotatable and require three levels of each factor. BBD is considered as an efficient option in RSM and an ideal alternative to CCD because it involved less input variables. Because of fewer treatment combinations, BBD is also a more time and energy saving design as compared to CCD.

Whilst CCD contains an imbedded factorial or fractional factorial design with center points that is augmented with a group of 'star points' that allow estimation of curvature. The star points represent new extreme values (low and high) for each factor in the design. The CCD is rotatable and requires five levels for each factor.

The similarity in both designs is that it allows the estimation of all the regression parameters required and represent in quantitative form as linear (first-order polynomial model), quadratic or cubic function (higher order polynomial models). The equations below show the linear and quadratic models which are most commonly used in RSM. A first-order model with $n$ experimental runs carrying out on $k$ design variables and a single response $y$ can be expressed as follows:

$$y = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \ldots\ldots\ldots + \beta_k x_{ik} + \varepsilon_i (i = 1, 2, \ldots\ldots, n) \quad (8.52)$$

where $y$ (predicted response) is a function of the design variables $x_1, x_2, \ldots, x_j$, $\beta_0$ is the constant coefficient, $\beta_1, \beta_{2\ldots}, \beta_j$ is the linear coefficients, and $\varepsilon$ is the experimental error. This model is appropriate for describing a flat surface with or without tilted surfaces.

When there is a curvature in the response surface, the first-order model is insufficient to describe the response and in this case, a higher order polynomial such as the quadratic model may be used. This is represented by Eq. 8.53.

$$y = \beta_o + \sum_{i=0}^{n} \beta_i X_i + \sum \sum_{i<j} \beta_{ij} X_i X_j \sum_{i=1}^{n} \beta_{ii} X_i^2 + \in \quad (8.53)$$
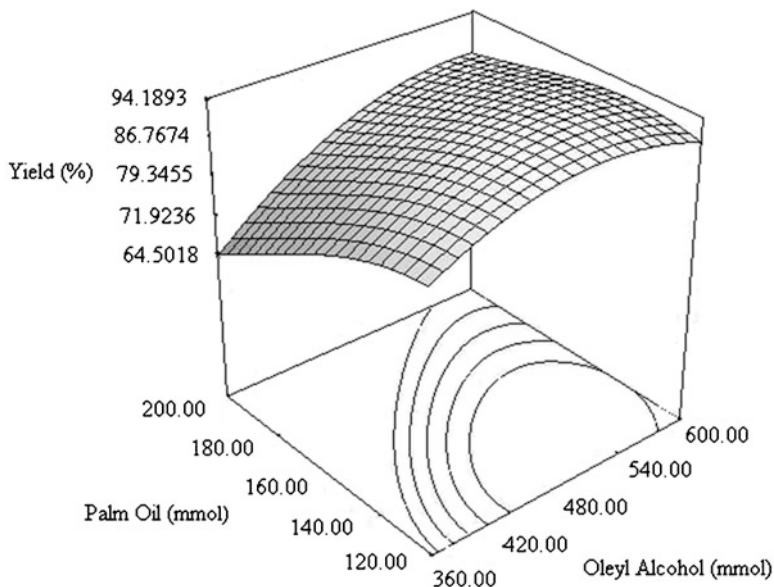
**Fig. 8.1** 3D response surface plot showing effect of amount of substrates on the percentage yield of palm-based wax ester production (Keng 2008)

where y is the predicted response, $\beta_0$ is the constant coefficients, $\beta_1$ is the linear coefficients of the input factor $x_i$, $\beta_{ij}$ is the quadratic coefficients between the input factor $x_i$ and $x_j$, $\beta_{ii}$ is the interaction coefficients of input factor $x_i$ and $\varepsilon$ is the experimental error. (Box and Hunter 1957). The second-order model is important to provide a good prediction throughout the region of interest. It is rotatable so the variance of the predicted response is the same at all points. Since the objective of RSM is optimisation and as the location of the optimum is unknown prior to running the experiment, it makes sense to use the design that provides equal precisions of estimation in all directions (Tan et al. 2008).

Generally, to perform experimental optimisation with RSM, it involves three major steps: (1) design and perform the experiments; (2) response surface modeling through regression analysis; (3) optimisation and checking the adequacy of the model. In the first step, important independent input variables and desired responses with the design constrains are identified and experimental design are adopted. Such designs are generated using computer software like Statistica, SAS or Design Expert. This is followed by regression analysis and statistical analysis of variance (ANOVA) of the experimental data in order to identify parameters that are significantly affects the desired response and develop a mathematical model having the best fit to the data obtained. Lastly, optimal combination of parameters is identified and confirmation experiments are conducted to verify the optimal parameters and mathematical model generated. For visualisation of the investigated factors, contour and response surface plots in 3D are generated that show the linear, interaction and quadratic effects of two or more parameters (Fig. 8.1).

## 8.5   Conclusion

Removal of heavy metals from aqueous environment is indeed a challenging problem in the control of environmental pollution. A wide range of treatment technologies such as chemical precipitation, ion exchange, membrane filtration and adsorption can be employed to remove heavy metals but these treatment methods still suffer from some inherent limitations. For instance, chemical precipitation, which has been traditionally and widely used to remove heavy metals from contaminated sources, is often associated with huge sludge production. Ion exchange process is generally applicable to treat or remove heavy metals at low concentration. As for membrane filtration, problems such as membrane fouling, high maintenance cost and low permeate flux are often unavoidable. Taking all these into considerations, the adsorption process thus appeared as one of the most promising methods.

Various low cost adsorbents have been investigated intensively in order to avoid the usage of costly activated carbon. It is evident that in most of the reported works, the study is still being centralised in the maximum adsorption capacities of the materials. While most of the low cost adsorbents have shown its potential in heavy metals removal without any modifications, but it is interesting to note that their removal capabilities can generally be improved through certain chemical modifications. Also, more than one factors such as pH, contact time, initial influent concentration, temperature, adsorbent dosage and etc. can significantly affect the adsorption process.

Numerous equations based on two-parameter or three parameter adsorption isotherms have been employed to describe the adsorption process under equilibrium condition. These include Langmuir, Freundlich, Brunauer-Emmet-Teller (BET), Temkin, Dubinin-Radushkevich, Jovanovic, Hasley, Harkin-Jura, Redlich-Peterson, Sips and Toth models. As for the kinetics studies, the two commonly used rate expressions are the pseudo-first and pseudo-second order kinetic models. In most of the cases, especially those involve chemisorption, pseudo-second order kinetic model is able to provide a better description than the pseudo-first order kinetic model as the former takes the interaction of adsorbent-adsorbate through valency forces into consideration.

To overcome the limitation of batch condition which is applicable only to the treatment under equilibrium condition, it is necessary to carry out flow tests using columns to obtain a design models which would be applicable to commercial systems. Besides this, developing an appropriate mathematical model through statistical approach is also important to obtain the optimum operating condition of the adsorption system.

Several low cost adsorbents can be seen as attractive alternatives for the removal of heavy metals based on their outstanding removal ability. However, in most of the cases, attention seems to be focused on the maximum adsorption capacity of the low cost adsorbents and the model pollutants are mainly synthetic types. In order to fully utilize the application of these materials in wastewater treatment, their

removal efficiency should be further tested in effluents from industries. Besides, the characterization of the adsorbents as well as the study of the sorption mechanism is equally important. More effort is required to provide insight into the direction of modeling, the effects of adsorbent recycling and regeneration and the recovery of heavy metals from wastewater. The possibility of immobilized these adsorbents can also be viewed as a feasible approach as suspended system requires a filtration step which is not suited for practical applications. If modification is needed, it is desirable to have a material with a wide range of metal affinities as this will be particularly useful for industrial effluents which carry more than one type of metals. Further research involving the combination of methodologies/system should also be encouraged as this would minimize or overcome the problem associated with a single treatment process.

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# Chapter 9
# Water Quality Monitoring by Aquatic Bryophytes

**Gana Gecheva and Lilyana Yurukova**

**Abstract** Bryophytes are non-vascular plants that are in a close relationship with their immediate environment. They often have large biomass in freshwater ecosystem and high level of production. Moreover, their tissues contain elevated amount of C, N and P, and cell walls have high cation exchange capacity. Aquatic bryophytes can be used to assess freshwater pollution as indicators – presence or absence of species – or as monitors for accumulating elements. Consumption of metals and other substances by aquatic bryophytes is an important exposure pathway for consumers. The use of bryophytes for water quality assessment is well documented, but different techniques and approaches prevent standardization and their applicability on the European scale. Thus we review major findings in 'bryomonitoring'. Data were reviewed from a range of countries, mainly in Europe, illustrating the advantages of low cost methods for monitoring water quality.

Here we introduce the term 'bryomonitoring' as a method to assess alterations of the environment. Biomonitoring can be split into passive – observation and analysis of native bryophytes, and active biomonitoring – based on species transplantation for a fixed exposure period. Two widespread northern hemisphere aquatic mosses, *Fontinalis antipyretica* and *Platyhypnidium riparioides,* are the most commonly used biomonitors for river quality assessment. For passive biomonitoring key issues are background and reference level determination, and proper selection of sampling sites. For active monitoring, upper segments of a same age from a reference region should be applied. The actual analytical techniques give in general similar results, but not completely interchangeable.

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Aquatic bryophytes are used to assess the ecological status. They are a stress-tolerant and various species have a wide trophic range. *Fontinalis antipyretica* and *Platyhypnidium ripariodes* have all criteria for biota monitoring in rivers for heavy metals.

Standardization of sampling procedures and analytical techniques in aquatic bryomonitoring is further needed. The number of samples should be fixed based on sampling area surface. Period of exposure time for active biomonitoring should be specified in general. Background levels and ambient metal concentrations have to be observed in parallel.

**Keywords** Bryophytes • Pollution monitoring • Water pollution • Cs • U • Ra • Th • Hg • Pb • Heavy metals

## Contents

## Abbreviations

| | |
|---|---|
| AAS | Atomic Absorption Spectrophotometry |
| BAF | Biota Accumulation Factor |
| BCF | Bioconcentration Factor |
| BMF | Biomagnification Factor |
| BQE | Biological Quality Element |
| C | Element concentration |
| $C_{bckg}$ | Background concentration |
| CF | Contamination Factor |
| $C_o$ | Element concentration in an organism |
| $C_{w/s}$ | Element concentration in water/sediment |
| DEHP | Di(2-ethylhexyl)-phthalate |
| DL | Detection Limit |
| EA | Environmental Alteration |
| EQR | Ecological Quality Ratio |
| EQS | Ecological Quality Standard |

| EU | European Union |
|---|---|
| FAAS | Furnace Atomic Absorption Spectrophotometry |
| GEA | Global Environmental Alteration |
| GFAAS | Graphite Furnace Atomic Absorption Spectrophotometry |
| HCH | Hexachlorocyclohexanes |
| HPLC | High Pressure Liquid Chromatography |
| IBMR | Macrophyte Biological Index for Rivers |
| ICP-AES (ICP-OES) | Inductively Coupled Plasma Atomic Emission Spectroscopy (Inductively Coupled Plasma Optical Emission Spectrometry) |
| ICP-MS | Inductively Coupled Plasma Mass Spectrometry |
| MAC | Macrophyte Assessment and Classification |
| MACPACS | MACrophyte Prediction And Classification System |
| MLD | Methodological Limit of Determination |
| MTR | Mean Trophic Rank |
| NAA | Neutron Activation Analysis |
| PAH | Polyaromatic Hydrocarbons |
| PCB | Polychlorinated Biphenyls |
| pH | Acidity |
| RHS | River Habitat Survey |
| RSD% | Relative Standard Deviation in percents |
| TIM | Trophic Index of Macrophytes |
| WFD | Water Framework Directive |

## 9.1 Introduction

Pollution is a major environmental issue affecting freshwater habitats and consequently human health. Lotic and lentic ecosystems constantly react to external and internal changes. Their recovery after human alterations depends on a variety of factors, among them living organisms, including bryophyte communities. Aquatic bryophytes as primary producers and habitat providers are important component of freshwater ecosystems and influence both biodiversity and water chemistry. They affect biodiversity by changing environmental conditions and resource availability and providing suitable habitats for new species and species already present. Nutrient dynamics can also be influenced by bryophytes (Stream Bryophyte Group 1999). In mountain sites (especially spring habitats) bryophytes usually cover large areas; sometimes they are able to modify water flow and can be considered as ecosystem engineers (Jones et al. 1994).

A review on monitoring studies of heavy metals with freshwater plants was published by Whitton (2003), comprising, among all phototrophs, data on bryophyte researches during the period 1969 and 2001. Extensive reviews on both terrestrial and aquatic bryophytes as monitors were also presented by Burton (1990) and Tyler (1990).

A variety of assessment methods based on aquatic bryophytes has been proposed, based both on field and/or laboratory data for broad surveys or the investigation of point-source contamination. Research results and outcomes were reviewed from a range of countries, mainly in Europe. Among them numerous laboratory studies on separate heavy metals bioaccumulation kinetics and intra-, extra- and intercellular distribution in recent years have been published (Samson et al. 1998; Vázquez et al. 1999; Vieira et al. 2009), as well as studies on photosynthetic pigments (Cruz de Carvalho et al. 2011; López and Carballeira 1989; Martinez-Abaigar and Núñez-Olivera 1998; Peñuelas 1984; Spitale 2009), and detoxification mechanisms (Dazy et al. 2008) but they will be reviewed in future paper, focusing on exposure of bryophytes under laboratory conditions.

## 9.2   Biomonitoring: Methodology and Application

Although there are many interpretations of the term, probably most essential is that biomonitoring is a system for long-term observation, assessment and forecast of possible environmental alterations based on biological objects (Martin and Coughtrey 1982). On the other hand, as a scientific term, bioindication has introduced literature at the end of 1960s of the past century. Bioindication is a time-dependent sensitive response of biological measurable parameters to anthropogenic pressure (Stöcker 1980). Thus bioindicator is an organism (or part of an organism or a group of organisms) that contains information on the quality of the environment (or a part of the environment) (Markert 2008). Haseloff (1982) divided them as visual, chemical and physico-biochemical bioindicators: (1) the first type includes species presence, reduced growth, leaf decoloration and population changes, (2) chemical bioindicators are characterized by accumulation of substances, (3) physico-biochemical bioindicators are characterized by alterations of enzymatic activity and physiological functions. Bioindicators were proposed for long-term observations, as well as for planning and management the effects of human activities (Hertz 1991).

In general, the difference between bioindicators and biomonitors is that the former gives a qualitative, and the last one quantitative assessment of the quality of the environment (Manning and Feder 1980; Martin and Coughtrey 1982; Markert 1991; Markert et al. 2003). A biomonitor is always a bioindicator as well, but a bioindicator does not necessarily meet the requirements for a biomonitor (Markert 2008). Biomonitors can be considered as sensitive and accumulative (Steubing 1976; Stöcker 1980). Sensitive biomonitors applied in aquatic ecosystems provide early warning system (Cairns and van der Schalie 1980). Accumulative biomonitors receive major attention towards heavy metal pollution. Tyler (1972) showed that dead organic matter, lichens and especially mosses as low-level plants, accumulate high heavy metals amounts. The main reason is the high stability of the chemical complexes between heavy metal ions and negative charged organic groups. Burton

(1990) underlined the possibility of bryophytes to produce information for reaction towards ecological factors, as well as element concentrations.

It is useful to distinguish between bioindicator and biomonitors (accumulators), but regardless differences in definitions, bioindication and biomonitoring must supply information on the extent of pollution and degradation of freshwater ecosystems.

Both passive and active biomonitoring received widespread popularity and their advantages and disadvantages are profoundly presented by Martin and Coughtrey (1982). Passive biomonitoring consists in observation and analysis of native bryophytes, while active biomonitoring is based on species transplantation for a fixed exposure period.

Numerous studies have reported that aquatic plants often accumulate heavy metals in concentrations much higher than those reached in their aqueous environment, even when those metals are not essential for metabolism or they are potentially toxic. The metal accumulated by a plant gives a better indication of the metal fraction in the environment likely to affect an aquatic ecosystem than most types of direct chemical analysis (Empain et al. 1980). In aquatic systems, metals exist both as free ions and as complexed forms. For many metals it is the free ionic form which is believed to be responsible for toxicity because the possibility for uptake is increased. Thus plant bioaccumulation is the basis for evaluating indirect exposure to other organisms including humans. Metal accumulation in plants is also pointed as common investigated for the biomonitoring of aquatic pollution in the review of Zhou et al. (2008).

In the current review we apply the term bryomonitoring in the context of biomonitoring and underlying the organisms (i.e. bryophytes) by which environmental quality is determined.

Bryomonitoring is founded on two basic approaches: species distribution and chemical analysis of bryophyte tissues (Burton 1986). Bryophytes can accumulate extremely high levels of heavy metals based on their high cation exchange tissue capacity, lack of cuticle and high surface to volume ratio (Tyler 1990). Metal ions are accumulated mainly through the passive ion exchange. Main tolerance mechanism is cell wall considerable efficiency to immobilize heavy metal ions and thus bryophytes are able to accumulate metals to remarkable concentrations. Large interspecific differences in accumulation levels were found in studies carried out at streams with severe contamination (Burton 1990).

In general, bryophyte high accumulative capacity led to their implementation mainly as monitors in regional and local discharges of heavy metals. The simplest way to use information on metal concentrations for monitoring is to compare values for specimens of a particular species at different sites, such as in a river downstream of an effluent or at a range of sites within a particular catchment or geographical region (Whitton 2003). Furthermore, several studies showed aquatic mosses are also suitable for monitoring radioactive contamination (Hongve et al. 2002) and pollution from organic compounds like oxolinic acid, flumequine or oxytetracycline, normally used as antibacterial agents (Delépée et al. 2004), monitoring of polychlorinated biphenyls and hexachlorocyclohexanes (Mouvet et al. 1985), and of polycyclic aromatic hydrocarbons (Roy et al. 1996).

Whatever is the analyzed substance, knowing its bioavailability is crucial, since bioavailability of its forms is tied to the potential effects on living organisms, man included Bioconcentration factors (BCF) or biota/water accumulation factors (BAF) are typically used to describe ratios of contaminants in tissues versus water for aquatic species and they are used to quantify contaminant uptake efficiency. The concept makes assumptions that the environment and the receptor are in pseudo-steady-state conditions, and the ratio is usually normalized for lipid and total organic carbon content of samples. The bioconcentration factor (BCF) is exceptionally high in bryophytes (Vanderpoorten and Goffinet 2009). The equation has the general form below:

$$BCF = C_o/C_{w/s}$$

where: $C_o$ = contaminant concentration in the organism

$C_{w/s}$ = contaminant concentration in the water/sediment

Mouvet et al. (1986) proposed a method for the evaluation of metal contamination based on a Contamination Factor (CF), defined as the ratio of metal concentration in the indicator to the background level in that species. On the basis of CF obtained, Mouvet et al. (1986) suggested five categories of contamination:

– No contamination, up to 2 times the background level;
– Suspected contamination, between 2 and 6 times the background level;
– Moderate contamination, between 6 and 18 times the background level;
– Severe contamination, between 18 and 54 times, and
– Extreme contamination, more than 54 times.

### 9.2.1 Passive Biomonitoring

In this review the nomenclature accepted in Grolle and Long (2000) for liverworts and Hill et al. (2006) for mosses was presented at first appearance of a species and then taxa are cited following original studies.

High accumulation capacity and vast distribution of some bryophyte species has led to intensive growth of publications dealing with passive biomonitoring. Most of the studies covered only species element content without additional data on the surrounding environment (water and/or sediments).

Among aquatic bryomonitors, *Fontinalis* is the most studied genus (Table 9.1). It has sensitivity to Cu, exhibiting tip chlorosis, while it is insensitive to Cd (Glime 2003). The ecological characteristics of *Fontinalis antipyretica* Hedw. are described in details by Say and Whitton (1983). Species shows considerable potential as monitor of heavy metals. Moreover, it has the ability to colonize variety of substrates and to grow under various flow regimes. Two centimeters tips were suggested to reflect recent events and for long-term surveillance, while whole plants were found more useful for preliminary studies to detect water quality.

**Table 9.1** Passive biomonitoring – examples

| Species | Sample preparation | Substances | Analysis | Number of studied sites, country | Duration of study | Reference |
|---|---|---|---|---|---|---|
| *Rhynchostegium riparioides* | Drying at 105 °C, Digestion: 2 M $HNO_3$ | Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Ba, Pb | | 105, England | 6 weeks | Wehr and Whitton (1983a) |
| *Fontinalis antipyretica* | Drying at 105 °C, Digestion: 2 M $HNO_3$ | Ca, Mn, Fe, Cu, Zn, Cd, Pb | | 52, Belgium and England | | Say and Whitton (1983) |
| *Rhynchostegium riparioides, Scapania undulata, Hygrohypnum duriusculum, Schistidium agassizii, Philonotis seriata* | Dried sample, Digestion: 15 ml $HNO_3$, 5 ml $H_2O_2$ | P, K, Ca, Mg, S, Fe, Al, Mn, Na, Zn, Cu, Pb, Cd, Co, Ni, As, Se, Cr | ICP-AES | 10 rivers, 3 lakes, Bulgaria | 3 months | Yurukova et al. (1996) |
| *Platyhypnidium riparioides, Scapania sp., Fontinalis antipyretica* | Dried at 40 °C, Digestion: $HNO_3$ and $H_2O_2$ | Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, V, Zn, Ca, Mg, K | ICP-AES/AAS (for Cd, Cu, Pb) | 41 sites, Germany | | Samecka-Cymerman et al. (2002) |
| *Plagiochila porelloides, Scapania undulata, Atrichum undulatum, Bryum pseudotriquetrum, Rhizomnium punctatum, Fontinalis antipyretica, Amblystegium riparium, Sanionia uncinata, Warnsorfia exannulata, Brachythecium velutinum, B. plumosum, Rhynchostegium riparioides* | Dried at 40 °C, Digestion: $HNO_3$ and $H_2O_2$ | N, P, K, Ca, S, Mg, Na, Fe, Al, Mn, Co, Ni, Cu, Zn, Pb, Cd, As | ICP-AES/Nitrogen after Kjeldahl method | 23 sites, Bulgaria | 5 years | Yurukova and Gecheva (2004) |

(continued)

**Table 9.1** (continued)

| Species | Sample preparation | Substances | Analysis | Number of studied sites, country | Duration of study | Reference |
|---|---|---|---|---|---|---|
| *F. antipyretica, F. squamosa, P. riparioides* | Digestion: 10 ml $HNO_3$ in a microwave oven | Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Se Zn | FAAS/GFAAS (for As, Cd, Hg, Pb and Se) | Biomonitoring network of 121 sites, Spain | 1 year survey | Vázquez et al. (2007) |
| *Sanionia uncinata* | Digestion: 300 mg dw of moss samples with $HNO_3$ and $HClO_4$ | Na, Cd, Co, Cr Cu, Fe, Mn, Ni, Pb, V, Zn | FAAS (Fe, Mn and Zn)/GFAAS (Cd, Co, Cr, Cu, Ni, Pb and V)/flame photometer (Na) | 29 sites, West Spitsbergen (Svalbard) | Summer season | Samecka-Cymerman et al. (2011) |

*Fontinalis antipyretica* is proved biomonitor of many macro- and microelements in European freshwater ecosystems: Belgium (Empain 1976, 1977; Wehr et al. 1983), Bulgaria (Yurukova et al. 1997), Hungary (Kovács and Podani 1986; Kovács 1992), England (Say and Whitton 1983), Germany (Dietz 1972; Bruns et al. 1995), France (Empain 1976; Mouvet 1984), Poland (Samecka-Cymerman and Kempers 1992, 1993). Reported BCFs are extremely high, for example 3,200 for Pb and 9,400 for Zn (Dietz 1972).

Another intensively studied species is *Platyhypnidium riparioides* (Hedw.) Dixon (=*Eurhynchium riparioides* (Hedw.) P.W. Richards, *E. rusciforme* Milde, *P. rusciforme* (*Schimp*.) M. Fleisch., *Rhynchostegium riparioides* (Hedw.) Cardot, *R. rusciforme* Schimp.). It was confirmed as geographically and ecologically widespread, and as excellent species to monitor heavy metals (Wher and Whitton 1983a). Low pH and calcium values prevent species development. This moss was broadly included in biomonitoring researches in Belgium (Wehr et al. 1983), Bulgaria (Gecheva et al. 2011), England (Jackson et al. 1991), Spain (García-Álvaro et al. 2000).

Among liverworts *Scapania undulata* (L.) Dumort. received major attention for biomonitoring purposes. Species is absent in eutrophic waters and dominates streams which combine very low nutrients with very high heavy metal levels (Wher and Whitton 1983b). It was reported as tolerant to heavy metal contamination and as suitable biomonitor (McLean and Jones 1975; Burton and Peterson 1979; Satake et al. 1989). Thus *Scapania undulata* was implemented in many monitoring programmes (Whitton et al. 1982). Terminal shoots were analyzed from sites influenced by past or present mining activities in England, France, Germany and Ireland. Statistical analyses suggest that elevated pH and/or Ca lead to increased accumulation of Zn and Cd and that probably pH reflect also Pb accumulation. Species indicated the presence of perspective polymetallic deposits in Poland (Sudeten Mts) and had considerable mean values of Cd – 68 mg kg$^{-1}$, Co – 174 mg kg$^{-1}$, Ni – 232 mg kg$^{-1}$, Fe – 130,000 mg kg$^{-1}$, Mn – 19,900 mg kg$^{-1}$, as maximum accumulated levels near barite zones in the Sowie Mts were for As 2,190 mg kg$^{-1}$, B 10,100 mg kg$^{-1}$, Zn 550 mg kg$^{-1}$, Ni 150 mg kg$^{-1}$, Co 1,700 mg kg$^{-1}$, Ge 4,000 mg kg$^{-1}$, Pb 3,100 mg kg$^{-1}$, Sn 200 mg kg$^{-1}$, V 1,300 mg kg$^{-1}$ (Samecka-Cymerman 1991; Samecka-Cymerman and Kempers 1993).

Studies involving several bryophyte species and/or focusing on interspecific differences are increasing. Metal concentration detected in *Rhynchostegium riparioides*, *Fontinalis antipyretica* and *Cinclidotus danubicus* Schiffn. & Baumgartner reflected Cu and Cr fluctuations (Mouvet et al. 1986). *Fontinalis antipyretica*, *Rhynchostegium riparioides*, *Brachythecium rivulare* Schimp.*, Plagiothecium ruthei* Limpr. (=*Plagiothecium denticulatum* (Hedw.) Schimp.), *Pellia fabbroniana* (=*Pellia endiviifolia* (Dicks.) Dumort.), *Scapania undulata* and other species were found useful in biogeochemical prospecting for minerals (Samecka-Cymerman and Kempers 1992, 1993; Pirc 2003). Relationships between Zn, Cd and Pb concentrations in algae, liverwort *Scapania undulata* and three mosses *Amblystegium riparium* (=*Leptodictyum riparium* (Hedw.) Warnst.), *Fontinalis antipyretica*

and *Rhynchostegium riparioides* were established in Belgium, France, Germany, Ireland, Italy and Great Britain (Kelly and Whitton 1989). López and Carballeira (1993) studied interspecific differences in metal accumulation among *Fontinalis antipyretica*, *Brachythecium rivulare*, *Rhynchostegium riparioides* and *Scapania undulata* to accumulate metals. *Scapania undulata* and *Rhynchostegium riparioides* showed the highest BCFs. Physico-chemical variables with major influence on metal accumulation were sulphate concentration, pH, nitrite, ammonia and filterable reactive phosphate. Yurukova et al. (1996) applied five aquatic bryophytes (*Rhynchostegium riparioides*, *Scapania undulata*, *Hygrohypnum duriusculum* (De Not.) D.W. Jamieson, *Schistidium agassizii* Sull. & Lesq., *Philonotis seriata* Mitt.) as bioconcentrators of 19 macro- and microelements (N, P, K, Ca, Mg, S, Fe, Al, Mn, Na, Zn, Cu, Pb, Cd, Co, Ni, As, Se, Cr), both at river and lake stations in Rila Mountain.

Positive correlation between copper levels in mosses and in ambient river water ($BCF > 10^3$) was established (Empain 1988). Connection between heavy metal concentrations in bryophytes (*Scapania undulata*, *Fontinalis squamosa* Hedw., *Rhynchostegium riparioides*) and water was studied in Scotland (Caines et al. 1985). Increased hydrogen ion concentrations due to soluble organic compounds and rain waters decrease Al, Mn and Zn bioaccumulation in *Scapania undulata*; moreover, accumulated Mn and Al can be released from bryophyte tissues at low pH level ($<5.5$). *Fontinalis antipyretica* and *Leptodictyum riparium* were suggested to monitor sites disturbed by multiple pollution sources, as in industrial and urban areas (Gecheva et al. 2011). Physico-chemical water variables represent the major component differentiating bryophyte assemblages at affected sites but the relative importance of environmental factors underlying community compositions differed strongly. Thus, in the assessment of surface water quality, bryophyte species composition was found as representative of river hydromorphology, while the content of elements in bryophyte tissue of water chemistry. The concentrations of elements in water and *F. antipyretica* collected in the same station were poorly correlated (Vázquez et al. 2004). *Fontinalis antipyretica* and *F. squamosa* appeared to avoid sites with low pH and high levels of Ca and Mg (pH, Ca and Mg were highly significantly and positively correlated), while prefer high concentrations of Cl, Na, K and Si – also significantly and positively correlated (Vázquez et al. 2007). Nevertheless, Mg and Ca play protective role for plants and reduce the toxic influence of heavy metals (Samecka-Cymerman and Kempers 1994).

Studies on both moss species and sediments are scarce. Natural background levels for Cd, Cr, Cu, Pb and Zn in four moss species (*Fontinalis antipyretica*, *Rhynchostegium riparioides*, *Amblystegium riparium* and *Fontinalis squamosa*) and sediments were reported from Portugal (Gonçalves et al. 1992). Cadmium and zinc were accumulated 107 and 70 times respectively higher than their background levels. Aquatic mosses appeared to reflect more recent conditions in freshwaters than sediments which incorporate particulate matter and are under unknown influence upstream.

An integrated research incorporated aquatic mosses (*Fontinalis squamosa* and *Rhynchostegium riparioides*), river water and sediments (Say et al. 1981).

*Fontinalis antipyretica* was successfully used in radiological monitoring of $Cs^{137}$, $Cs^{134}$, $U^{235}$, $Ra^{236}$, $Th^{232}$, $K^{40}$ in Bulgarian montane river (Mishev et al. 1996). *Cinclidotus danubicus* was applied as monitor of radionuclides in France (Kirchmann and Lambinon 1973) and *Fontinalis* sp. in the U.K. (M A F F 1967; Hunt 1983).

Selecting sampling sites for passive biomonitoring is an important issue. Proper criteria for extensive biomonitoring network were given by Vázquez et al. (2007). When a single site was located in a drainage basin ($\approx$100 km$^2$), it was sited in the mid–low stretch of the river to integrate as far as possible the inputs received throughout the basin. When two sites were located in a drainage basin ($\approx$300 km$^2$), one was located at the head of the river and the other close to the mouth of the river. When more than two sites were located in a drainage basin (500 km$^2$), they were distributed in the different stretches of the main flow and of the main tributaries, covering different sub-basins. Location of sites in stretches possibly affected by reservoirs or immediately downstream of contamination foci (centres of population, industrial areas, etc.) was avoided. Each sampling site consists of a stretch of river approximately 100 m long and a sample was collected from at least five mats of one of the selected species.

Another key issue is background or reference level determination, as the metal bioavailability depends on metal form and concentration in environment. At the same time, the evaluation of environmental contamination is based on calculated CFs, i.e. on the ratio of metal concentration in the indicator to the background level. The background levels are needed also to calculate the. Background levels of course should be assessed on regional level and per species, since substrate lithology significantly differs geographically. Data are available for background levels of 19 macro- and microelements in *Fontinalis antipyretica* and high mountain river water in Bulgaria (Yurukova et al. 1997). *Scapania undulata* and *Platyhypnidium riparioides* had higher reference concentrations of Cd, Cr, Cu, Co, Ni, Pb, Zn, Fe and Mn in comparison with *Fontinalis antipyretica* and *Fissidens polyphyllus* collected from rivers in Spain (Carballeira and López 1997). Background levels of the former two species showed no significant difference with those in *Brachythecium rivulare*. Background concentrations in liverwort *Chiloscyphus* sp., *Fontinalis antipyretica* and *Platyhypnidium rusciforme* collected in French streams embedded in basaltic rocks were presented by Samecka-Cymerman and Kempers (1999). Background levels in *Platyhypnidium riparioides* for Ni, Cr, Co, Zn, Mn, Pb, Cd, Cu, Ba, Al and V were reported also by Samecka-Cymerman et al. (2002). Background levels in *P. riparioides* for Cd, Co, Cr, Fe, Mn, Ni, Pb and Zn presented by Cesa et al. (2010) for Italian River Bacchiglione basin (calcareous upper and alluvial lowland basin, respectively) were similar to those in dominated with limestone and dolomites in Tatra National Park (Samecka-Cymerman et al. 2007), and vice versa background levels in siliceous substrate are higher. Background levels for 25 elements in *F. antipyretica* and in addition element and site specific CFs were obtained by Vázquez et al. (2004).

It could be summarized that *F. antipyretica* and *P. riparioides* are the most commonly used and proven biomonitors in river quality assessment, especially in chronic exposures or long-term effects of chemical pollutants.

### 9.2.2  Active Biomonitoring

When no bryophyte records could be found in cases of severe pollution or environmental factors, active monitoring approach is applied. In general, mainly alive specimens collected from unaffected habitats, put in nylon cage are exposed at contamination for a period of several days or months. Major problem with moss transplantation appear to be their survival in habitats where local climate or pollution conditions do not meet species optima (Tyler 1990). Such sensitivity was known for *Fontinalis hypnoides* Hartm. (Gimeno and Puche 1999).

Although a great variety of experimental details exists (Table 9.2), the defined time period of exposition is a major advantage (Siebert et al. 1996). Despite equal metal accumulation in alive and dead material, the last can be associated with decay in plants. The number of moss bags per area, their size and attached or suspended exposition are most likely to result in different outcomes. Exposition period range has to be at least 24 h (Kelly et al. 1987), while 1 month is probably the upper limit at highly contaminated freshwaters. According to López et al. (1994) exposition should be at least for 5 days.

Already in 1970 Benson-Evans and Williams (1976) applied aquatic bryophytes to detect river pollution in Great Britain. *Fontinalis antipyretica* and *Eurhynchium riparioides* were transplanted from their natural environment at six selected stations (Table 9.2). *Fontinalis squamosa* and *Scapania undulata* were studied for heavy metal accumulation from McLean and Jones (1975). Elevated levels of Pb, Cu, Zn and Mn were reached after 6 weeks, and after 18 weeks a decay process was observed. At the same time *Scapania undulata* survived after transplantation at region with lower contamination and no considerable differences in metal content were found. Thus authors recommended selecting the commonest species in the survey area for metal monitoring in rivers. When none is characteristic for monitored river sites, then transplants can be applied under the conditions that same-age specimens of a selected bryomonitor are used and additionally physico-chemical parameters influencing accumulation (pH, light conditions and metal ambient concentrations) are monitored. Growth form is also linked to the process. *Fontinalis* has long branches, while *Scapania* formed solid tufts and thus the former species is more constantly exposed to free metal ions and respectively more rapidly accumulates metals. Bryophyte tolerance is considered to be effective until metals are included in complexes or are isolated in safe areas as cell walls (McLean and Jones 1975). When saturation is reached, any increase in metal concentration lead to damages on cell metabolism, and finally death.

Kelly et al. (1987) applied 2 cm apical stems of *Rhynchostegium riparioides* and *Fontinalis antipyretica* in monitoring of Zn, Cd, Pb in England. Alive specimens of

**Table 9.2** Active biomonitoring – examples

| Species | Moss-bag [g ww] | Sample preparation | Substances | Analysis | Number of studied sites, country | Exposure time | Reference |
|---|---|---|---|---|---|---|---|
| *Eurhynchium riparioides, Fontinalis antipyretica* | – | Perspex cylinder in a nylon mesh cage | – | Mosses were assigned to one of 5 breakdown categories – macroscopically and microscopically for morphology and structure | 6, Great Britain | 1, 2, 4, 6, 12, and 22 days | Benson-Evans and Williams (1976) |
| *Cinclidotus danubicus* | | Plastic mesh grids (1 × 1 cm) | PCBs and α-, β, and γ-HCH | gas chromatography | France | 13, 24 and 51 days | Mouvet et al. (1985) |
| *Fontinalis antipyretica* | 500 | Containers of plastic mesh with 2.5 × 3.5 mm pore size | Zn, Cu, Pb, Ni, Cr | | 4 sites, Hungary | 3 months | Kovács (1992) |
| *Fontinalis antipyretica* | 10 | | Cd, Cu, Cr, Pb, Sn | | 10 sites | 15-24-28 days | Mersch and Pihan (1993) |
| *Hygrohypnum ochraceum* | 8 | Plastic mesh bags | Cd, Mn, Pb, Al | Oven-dried at 40 °C for 24 h/9 ml 4 M HNO₃/AAS | 13 (including native stream), France | 1 month | Claveri et al. (1995) |
| *Fontinalis antipyretica* | 20 | Glass fiber bags | PAHs | | 5 sites, Lake Kallavesi, Finland | 35 days | Roy et al. (1996) |
| *Fontinalis antipyretica* | 10 | 2–4 cm long sections in plastic nets | Cd, Pb, Zn, Cu | Dried at 80 °C/microwave digestion/ICP-MS | 18, Germany | 12, 14, 16 and 21 days (182 days for a lost sample) | Bruns et al. (1997) |

(continued)

**Table 9.2** (continued)

| Species | Moss-bag [g ww] | Sample preparation | Substances | Analysis | Number of studied sites, country | Exposure time | Reference |
|---|---|---|---|---|---|---|---|
| R. riparioides, F. antipyretica, Cinclidotus danubicus | 20 | | Zn, Pb, Cu, Cr, Ni | | 5 sites | 11 and 16 days | Mersch and Reichard (1998) |
| Fontinalis antipyretica | | Cages (15 × 15 × 15 cm) made from plastic coated, stainless steel wire (1.5 × 3 mm holes) | As, Cr, Cu | Samples dried at air temperature for 3–5 days, followed by drying at 40 °C for 3–4 days. Digestion: $HNO_3$; ICP-AES/AAS (for As) | Norway | 24, 48, 72 h, 6 days | Rasmussen and Andersen (1999) |
| F. antipyretica | 10 | Nylon net, opening light of 1.3 mm | Cd, Cr, Cu, Pb, Hg | Digestion: 2.5 ml $HNO_3$; AAS (Cd, Pb, Cu, Cr)/CVAAS (Hg) | 9 sites (lake and river), Italy | 14 and 28 days | Cenci (2000) |
| Fontinalis antipyretica | 10–12 | Plastic 1 × 1 cm mesh bags | Al, Co, Cu, Ni, Zn/K, Mg, Ca | Intracellular, extracellular and particulate fractioning/AAS | 4 sites (including control station), Spain | 1, 4, 11, 21, 35 days (1, 4, 13, 27, 48 days at control station) | Vázquez et al. (2000) |
| Fontinalis antipyretica | 500 | Attached to the buoy | Ca, Mn, Cr, Pb, Zn, Fe, Cu, Cd | Dried at 60 °C | 1 site, Korea | 2 days | Lee et al. (2002) |
| Fontinalis antipyretica | 5 g dry weight | Nylon nets (10 × 10 cm; 1 mm² gaps) | N, P, K, Ca, S, Mg, Na, Fe, Al, Mn, Co, Ni, Cu, Zn, Pb, Cd, As | ICP-AES/Nitrogen after Kjeldahl method | 1 site, Bulgaria | 31, 30, 31, 61 days (5 months in total) | Yurukova and Gecheva (2003, 2004) |

| Species | Number | Method | Elements/compounds | Analytical method | Location | Exposure time | Reference |
|---|---|---|---|---|---|---|---|
| *Fontinalis antipyretica* | | Plastic tube, 150 mm long and 12 mm Diameter. Via lateral cut along the tube, only the basal part of the moss is introduced, allowing the remainder of the plant to float free | Cu, Zn, Pb, Fe, Ni, Ca, K, Mg | | 10 sites, Portugal | | Figueira and Ribeiro (2005) |
| *F. antipyretica* | 5 | Spin-dried, nylon mesh bags (4 mm) | N, P, K, Ca, Mg, S, Fe, Al, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, Zn | | 15 sites, Poland | 60 days | Samecka-Cymerman et al. (2005) |
| *Rhynchostegium riparioides* | 25–30 | 30 × 30 cm nylon bags with 7 mm holes | As, Cd, Cr, Cu, Mn, Ni, Pb, Zn | Digestion: 3.5 ml $HNO_3$, 0.1 ml HCl and 2 ml $H_2O_2$; AAS | 3 sites, Italy | 20, 34, 48 and 62 days | Cesa et al. (2006) |
| *R. riparioides* | 20–30 | Plastic net (4 mm holes) | Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, V, Zn | 2.5 ml $HNO_3$ and 0.5 ml $H_2O_2$; AAS | 119 stations placed in 64 sites, Italy | 4 weeks | Cesa et al. (2010) |
| *Fontinalis antipyretica* | 100 | Collected from a natural stream | 10 metals 16 priority PAHs | | 4 streams, Lisbon, Portugal | 3 months | Augusto et al. (2011) |
| *Fontinalis antipyretica* | 20 | 0.5 cm mesh size plastic bag | Zn, Cu, Ni, Pb | Mineralization in a microwave furnace with $HNO_3$ and H2O2; FAAS | 3 sites, Morava River, Czech Republic | 28 days | Diviš et al. (2012) |

*Fontinalis antipyretica*, collected from reference region and putted in nylon mesh at depth of 10–20 cm successfully reflected heavy metal contamination (Kovács 1992). Similar results were obtained with the same species during a 28-day exposure by López et al. (1994). Metal uptake kinetic reached an equilibrium phase which is mostly correlated to concentration in water. Retransplantation in an unpolluted site led to metal release occurring with a two-phase, mainly regulated by metal concentration in moss tissue at the beginning of the recovery. *F. antipyretica* was used as active biomonitor in Spain (Vázquez et al. 1999, 2000). High acidity did not appear to influence uptake by intracellular structures. During recovery significant changes were noted in extracellular metal levels. The species was recommended to be applied as active biomonitor. Claveri et al. (1995) applied transplants of *Hygrohypnum ochraceum* (Turner ex Wilson) Loeske for monitoring Al, Mn, Pb, Cd. Good correlation was observed at increasing Al and Pb values in water and moss.

As active biomonitor *F. antipyretica* showed good accumulation properties for all metals (Bruns et al. 1997). Authors recommended use of freely suspended samples in the water and noted that during autumn and winter season highest accumulation were observed. A detailed description of use of *F. antipyretica*, including sampling and conditioning was presented by Cenci (2000), and the method was recommended for detection of high risk situation in European water bodies. Transplants of *Fontinalis antipyretica* were used to assess the contamination of an industrial effluent discharge on a river located in south Portugal for 2 years (Figueira and Ribeiro 2005). An increase of water contamination by Cu, Zn was verified with extra and intracellular moss fractions. An interesting transplantation device was proposed in the study (Table 9.2).

*F. antipyretica* was applied simultaneously in passive and active biomonitoring of heavy metals (Samecka-Cymerman et al. 2005). Transplants accumulated significantly higher amounts of Al, Cr, Cu, Pb, V and Zn than native mosses, while Co and Mn concentrations were higher in the native specimens.

Relatively obscure are data concerning experiments with dead (initially cleaned and dried) material. Moss-bags with *Fontinalis antipyretica* of about 5 g dry weight were studied for a maximum period of 28 days along river sites in Luxembourg (Mersch and Pihan 1993), and for 5 months at impacted river site in Bulgaria respectively(Yurukova and Gecheva 2003, 2004).

Most common species applied for transplants is *Fontinalis antipyretica* not only for heavy metals but also for polycyclic aromatic hydrocarbons (Roy et al. 1996). Glass fiber bags for 35 days were applied at Lake Kallavesi (Finland) to investigate the cause-effect relationship between bioaccumulation of PAHs and the responses of antioxidant enzymes in aquatic moss. Higher activities of antioxidant enzymes and activated forms of oxygen were observed in moss transplanted near the harbor in comparison with moss-bags located upstream at two reference sites.

Regardless dead or alive are transplants, water acidity has significant influence on metal accumulation. Moss-bags accumulated Al, Pb, Fe and first two elements showed strong dependence on pH level (Mersch and Pihan 1993). Release of Cu and Zn was observed and commented in connection with high acidity. A negative

correlation between water acidity and metal accumulation was also reported by Vázquez et al. (2000) during exposure of *Fontinalis antipyretica* at affected stations along Spanish rivers. Release of total Ca and Mg, as well as intracellular K at pH < 5 was established. Carballeira et al. (2001) suggested upper shoots of *Fontinalis antipyretica* could be used for assessment of temporal pH decreasing on the basis of preloaded Cd release.

The most intensive work with moss bags in recent years was done with *Rhynchostegium riparioides* in Italy (Cesa et al. 2006, 2008, 2009a, b, 2010). Transplanted moss confirmed ability to detect spatial patterns of bioaccumulation, to reveal Pb and Cu chronic contamination and Cr, Zn and Ni intermittent contamination, and to localize emission sources. Highest uptake ratios were observed for Al, Cu, Cr, Hg, and Pb under laboratory conditions.

Combined technique incorporating transplants of *Cinclidotus nigricans* for short regular periods and mussels towards long-term investigations was proposed (Mersch and Johansson 1993). Mosses and mussels appeared to be complementary in biomonitring due to specific uptake and depuration kinetics. Transfer technique was evaluated as preferable to assess the recent pollution situation in comparison with the native *Fontinalis antipyretica*.

Active biomonitoring has proven its effectiveness, especially in industrial and urban areas, although a great variety of moss-bag size, exposure periods, used material (dead or alive), and biomonitor species was tested. Thus selection of a particular species, upper segments of a same age, from reference region with known level of anthropogenic impact, is recommended. A control moss-bag at a reference station is advisable.

## 9.2.3 Analyses of Elements, Priority Substances and Additional Pollutants

Whole plants are recommended to be analyzed in broad studies and in tracing contamination sources, while apical shoots (2–4 cm) are more suitable in regular surveys at particular sites (Wehr and Whitton 1983a; López and Carballeira 1993; García-Álvaro et al. 2000; Cesa et al. 2010).

Due to seasonal fluctuation in metal content in bryophyte tissues and in environmental factors, it is advisable to not rely only up on samples collected during winter. Despite bryophytes grow all around the year and have nutrient uptake less seasonal than vascular plants, most species have reduced biomass during winter and this could lead to an increase in metal concentration within moss tissues. *Fontinalis antipyretica*, *Leptodictyum riparium*, and *Platyhypnidium riparioides* have vital stems throughout the year but seasonal variation of metals between species and habitats was reported (Wehr and Whitton 1983b). Found increased accumulation of Pb in the period autumn-winter was a consequence of the lower aqueous concentrations of reactive phosphate and Mg due to extended period of high flows.

Nevertheless, the results showed that seasonal influences on metal accumulation are negligible and changes which appear seasonal are probably due usually to correlated changes in aqueous chemistry.

Environmental factors influence metal uptake and affect the concentration in the plant at the time of sampling (Whitton 2003). Among measured parameters of ambient water pH should be obligatory. As observed by early works, pH of water influences metal ion accumulation, as in lower pH values the accumulation decreases (Caines et al. 1985; Lingsten 1991). In addition, aqueous Ca was pointed out as the principal non-heavy-metal factor reducing the accumulation of Zn and Cd (Kelly and Whitton 1989), while phosphates reduce Zn and Pb accumulation (Wehr and Whitton 1983a).

Chemical analyses of bryophytes should be preceded by removal of organic particles and additional fragments at field. Washing with tap water can alter element levels through cation exchange and therefore the possible cellular locations of the studied elements, this is to be considered before sampling and extraction method to be applied (Bates 2008). Washing by use of demineralized water is strongly recommended.

The representative samples of aquatic mosses by species should be carefully cleaned from other organic matter and mineral particles before analysis. The laboratories are advised to carry out homogenization of mosses according to their normal routines. The use a sample weight of at least 0.5 g, dried at 40 °C moss material, is credential, and the laboratory staff is recommended to use wet-ashing methods, preferably with concentrated or 1:1 nitric acid (with or without hydrogen peroxide or perchloric acid) or not dissolved before analysis (methodology in European Moss Surveys, Steinnes et al. 1997). Acid-digestion of mosses is performed on a hotplate or in a microwave oven using a range of temperatures. Triplicates of each sample are prepared independently.

The element concentrations could be determined by various analytical techniques, under the broad heading of atomic absorption spectrometry (flame or graphite furnace) (FAAS, GFAAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS), and neutron activation analysis (NAA).

In any case reference moss materials are applied in each moss series of analyses. A detection limit (DL) equivalent to three SD of the lowest instrumental measurements of the stock standard solutions, blanks – all reagents and all analytical procedures, but without biological material is recommended. The Methodological Limit of Determination (MLD) is calculated on the basis of not less than three measurements for each solution of the digested sample, dilution of digested solutions and weight of each sample, and corresponding blank. The last concentrations were determined according to all measurements and repetitions, dilution of solutions, weight of samples and blanks, usually in $mg/kg^{-1} = \mu g \ g^{-1} = ppm$. RSD% for all elements analysed is obligatory for each moss sample.

As Markert (1996) pointed out a strict differentiation between the terms 'precision' and 'accuracy' should be established in each analytical research with environmental samples. The precision of the data is connected with repeatedly

measuring of the analytical signal purposing to eliminate errors, 1–5 % RSD is sufficiently exact. The accuracy of data involves use of one or more plant reference materials with certified values, and use of independent analytical procedures, i.e. inter-laboratory analyses by a wide ring of laboratories.

No one of above mentioned analytical techniques is suitable for all important essential and trace elements accumulated in moss tissues. Inductively coupled plasma optical emission spectrometry is good to be applied for more than 20 elements, in cases with higher level of Cd, Pb, As in mosses; neutron activation analysis without decomposing of the sample gives the widest idea of inorganic content – moreover 46 elements (Au and Sb included), but without important heavy metals as Cd, Cu, Pb; inductively coupled plasma mass spectrometry, available since 1984, is the combination of multi-element capabilities with extremely wide linear dynamic range over nine orders of magnitude, and could be used for 25–30 elements without P, Al, and Mn, Ca, Fe, due to a lower sensitivity thresholds, especially for toxic Al and essential P.

Analyses carried out simultaneously by ICP-MS and ICP-OES in both water and in *F. antipyretica* provided quite similar results (Vázquez et al. 2004). In moss samples the concentrations of Al, Ba, Fe, Sr and Ti measured by both methods were equivalent ($p < 0.05$), whereas those of Mn were significantly different. For the remaining elements the differences were small, obtaining higher values by ICP-OES than by ICP-MS for Co, Cr, Cu, Ni and Zn, and slightly lower for Ca levels. With respect to water samples, both techniques gave equivalent results for Ba, Ca and Zn. The remaining elements (Fe, Mn, Sr and Al), showed values obtained by ICP-OES lower than those obtained by ICP-MS, finding the greatest difference between techniques in the levels of Al measured. Thus, it can be stated that the results obtained with these two analytical methods were in general similar or equal, but not completely interchangeable.

Procedures for the analysis of organic contaminants in bryophytes include extraction from wet or freeze-dried samples with organic solvents, removal or destruction of lipids, clean-up, fractionation, high pressure liquid chromatography (HPLC) or gas chromatographic separation and different kinds of detection, e.g. fluorimetric, electron capture or MS (European Commission 2010). The total fat weight can be determined and used to normalise analytical results; this procedure should be considered as an alternative to weight normalisation.

## 9.3 Aquatic Ecosystem Assessment with Bryophytes Under the Water Framework Directive

The Water Framework Directive 2000/60/EC (WFD) (European Union 2000) requires Member States of the European Union (EU) to achieve good ecological status by 2015 in all water bodies. Individual water bodies are graded into one of five quality classes (high, good, moderate, poor or bad) reflecting Ecological Quality Ratio (EQR) which evaluates water quality in a score ranging from 0 (worst status) to 1 (reference status).

The WFD requires comparability of the EQR scales between the different EU countries in order to have a common understanding of the good ecological status of surface waters. This will ensure comparability of the classification results derived by various monitoring systems and reliability of the results produced by each classification tool. Biological quality elements (BQE), among them aquatic flora, are the key parameters on which the assessment is based. High ecological status is thus determined via dominance of reference species in type specific vegetation density. The status observed at the monitored station is compared to the status expected under reference/near natural conditions.

### 9.3.1 Aquatic Bryophytes as Bioindicators in the Context of Macrophyte Metrics

Local habitat characteristics determine river macrophyte communities, particularly light availability, current velocity, sediment patterns and nutrient supply (Birk and Willby 2010).

Compositional patterns of aquatic bryophytes are sensitive to a number of factors such as water flow velocity and level, eutrophication, pollution, and additional pressures. Substrate type also directly affects macrophyte development. Rocks and hard, immobile substrates are associated with bryophytes (Janauer and Dokulil 2006). Coarse substrate and variable flow regime contribute both to the success of bryophytes and to the exclusion of vascular hydrophytes (Scarlett and O'Hare 2006). Bryophytes are a dominant component in lotic ecosystems, especially in undisturbed conditions and their relations with environmental factors were studied (Glime and Vitt 1987; Suren 1996; Duncan et al. 1999; Suren and Duncan 1999; Suren et al. 2000; Scarlett and O'Hare 2006). Combined influence of underlying geology, water physico-chemistry, current velocity and substrate morphology on 17 bryophyte species was investigated in three minimally impacted high-latitude headwater streams in Scotland (Lang and Murphy 2012).

Anthropogenic influence results in reduction of species richness and fragmentation of populations, at the same time tolerant macrophyte taxa exhibit high growth capacity. Thus presence and abundance of aquatic macrophytes can indicate specific water characteristics such as trophic status, ion content, etc.

Aquatic plants were classified based on trophic status already 30 years ago, and for example *Sphagnum* species were allocated to plants of dystrophic, poor-in-nutrients type (Haslam 1982).

In Eastern Europe and Balkan region Papp and Rajczy (1995) suggested bryophytes as indicators of ecosystem alterations along Danube River. Correlation between the bryophyte assemblages and water quality along the Hungarian Danube section (Papp and Rajczy 1998a) and relation between assemblages of various streams in Hungary to the chemical parameters of water were studied (Papp and Rajczy 1998b). These studies report that species composition of the

aquatic-riparian bryophyte vegetation and the abundance-frequency values reflect water quality along rivers. Similarly changes in aquatic bryophyte assemblages were applied for assessment of trophic level (Vanderpoorten and Palm 1998). Phosphates appeared not to influence bryophytes at lowland river sites (Scarlett and O'Hare 2006; Gecheva et al. 2010). It was found also that *Leptodictyum riparium* and *Brachythecium rivulare* are associated with high levels of phosphate. Bryophyte species composition of some Greek streams and their correlation with environmental conditions are described in Papp et al. (1998).

Most macrophyte-based assessment systems in Europe evaluate river ecological status focusing on the potential of species to detect eutrophication as main pressure (Birk et al. 2006). As part of the broad group of aquatic macrophytes, bryophytes are included to several assessment methods: French "Indice Biologique Macrophytique en Riviere" (IBMR), German Reference Index, British Mean Trophic Rank and Dutch Macrophyte Score. *Leptodictyum riparium*, *Brachythecium rivulare* and *Fontinalis antipyretica* are listed in Mean Trophic Rank (MTR) and Macrophyte Biological Index for Rivers (IBMR) with species scores: 1.0 and 2.5; 8 and 7.5; 5 and 5 respectively (Szoszkiewicz et al. 2006). *Platyhypnidium riparioides* has score (5) only in MTR.

In summary, *L. riparium* is accepted as tolerant to organic pollution by both indices. MTR examines *F. antipyretica* and *P. riparioides* as species with intermediate tolerance to nutrient enrichment, and *B. rivulare* with considerably low tolerance. In contrary, IBMR evaluates *F. antipyretica* and *B. rivulare* as tolerant to organic pollution.

Four moss species above were not included in Trophic Index of Macrophytes (TIM) since it was designed to indicate the trophic status of rivers as whole ecosystems and combining water and sediment nutrients.

These broadly used biomonitors were also listed as type specific species according Reference Index (Schaumburg et al. 2004), which classifies rivers by regional approach and reflects different kinds of ecological stresses, as well as river pollution.

Mosses and liverworts were also included in development of predictive models to evaluate the streams ecological quality: MACPACS (MACrophyte Prediction And Classification System), MAC (Macrophyte Assessment and Classification) and AQUAFLORA (Aguiar et al. 2011; Feio et al. 2012).

Seven national macrophyte assessment methods were processed to an international data set that covered three European stream types and common reference conditions were defined (Birk and Willby 2010). Type-specific reference community for siliceous mountain brooks includes liverworts (*Scapania undulata* or *Chiloscyphus polyanthus*, and less frequently, *Marsupella emarginata* or *Jungermannia atrovirens*; thallose *Pellia epiphylla*) and acrocarpous mosses (most notably *Racomitrium aciculare*, plus smaller quantities of marginal species, such as *Philonotis fontana* and *Dicranella palustris*, *Fissidens crassipes* and *F. rufulus*). These taxa occur against a backdrop of extensive growths of a range of pleurocarpous mosses, including *Rhynchostegium riparioides*, *Fontinalis squamosa*, *F. antipyretica*, *Hygrophypnum ochraceum* (and occasionally *H. luridum*), *Brachythecium rivulare*, *B. plumosum*, *Hyocomium armoricum*, *Thamnobryum alopecurum* and

*Amblystegium fluviatile*. Several of these species persist in the lowest quality sites, most notably *Fontinalis antipyretica* and *Rhynchostegium riparioides*, but most bryophytes are replaced by *Amblystegium riparium*.

*F. antipyretica* and *P. riparioides* were found to form the "core" of bryophyte communities in more stable stream habitats (Lang and Murphy 2012). Benchmark bryophyte community for upland headwater streams of reference conditions was identified. *Scapania undulata* and *Hygrohypnum ochraceum* were established as indicators for oligotrophic upland streams, and *Chiloscyphus polyanthus* and *Hygrohypnum luridum* for calcareous and mineral-rich streams.

Specification of aquatic plant composition and percentage cover at different stages of eutrophication were given in Hime et al. (2009). *Rhynchostegium riparoides*, *Leptodictyum fluviatile* and *Fontinalis antipyretica* accounted for above 65 % of the total cover in highest quality.

*Fontinalis antipyretica* is among dominant species in lowland rivers, associated with higher quality, while it is pointed as a negative indicator in mountain streams (Birk and Willby 2010). Scoring bryophytes in the context of macrophyte-based metrics should assume wide trophic range of several moss species, including *F. antipyretica* (Vanderpoorten et al. 1999). Moreover, *F. antipyretica* has different ecological preferences in particular regions probably due to the existence of different ecotypes (Vázquez et al. 2007). As pointed out by Glime (2007) osmotic effect plays a major role in bryophyte nutrient needs and toxicity. Except some thallose taxa, bryophytes lack epidermis and waxy in their cuticles, and are especially susceptible to osmotic shock. Thus the same species can respond quite differently under different concentrations of nutrients and heavy metals, i.e. if a plant has grown from spores at a certain nutrient/ion level, then its osmotic potential is more likely to be adjusted.

Studies on effects of hydro-morphological degradation underlined the importance of bryophytes in stream ecosystems and increased knowledge about the effects of flow regulations. Taxonomic richness was found to decrease at flow regulated sites probably due to increasing substrate stability and dominance of strong competitors (Englund et al. 1997). Additional factor for lower richness was suggested by Downes et al. (2003). Regulated streams have limited ranges of water level and as a consequence, large rocks have only narrow zones that are subject to a variety of wetted conditions, which were assumed as more suitable for bryophyte growth and colonization than constant submergence.

Bryophyte species composition is also suitable for evaluating the impact of hydromorphological alteration. Unstable river sites are characterized by small-sized species or by absence of bryophytes in strongly affected habitats such as those amenable to erosion (Gecheva et al. 2011). Quantification of the impact of small hydroelectric schemes on bryophytes and lichens showed that they are largely under-recorded in small streams where small hydroelectric schemes are likely to be developed (Demars and Britton 2011).

River Habitat Survey (RHS) method was developed in the United Kingdom and carried out in several European countries to assess, in broad terms, the physical character of freshwater streams and rivers (Raven et al. 1998). RHS only records

plants growing by the riverside and the results show distribution in riparian habitats, in particular hygrophyte bryophyte species.

The potential for a macrophyte tool indicative of hydro-morphological impact is discussed by O'Hare et al. (2006). The abundance of liverworts and mosses was in negative correlation with homogeneity of water depth, deep water (the dominant depth) and fine particle substrate (the dominant substrate). Presence of *Fontinalis antipyretica* and metrics such as the presence of the group 'liverworts/mosses/lichens' may indicate that a site is unimpacted by hydro-morphological degradation.

Mosses *Fontinalis antipyretica*, *Amblystegium riparium* and *Rhynchostegium riparioides* reach their highest abundance in high stream power and coarse bed sediments in British rivers (Gurnell et al. 2009). Mosses can tolerate and may even prefer the most disturbed physical environment (O'Hare et al. 2011). Thus mosses are considered a stress-tolerant group and so they are not disadvantaged by the low nutrient and carbon conditions associated with high specific stream power and coarse substrate.

## 9.3.2 Establishing Environmental Quality Standards

Directive 2008/105/EC (Environmental Quality Standards Directive) defines the good chemical status also to be achieved by all Member States in 2015 and gives, together with the WFD, the legal basis for the monitoring of priority substances in sediment and biota (European Union 2008). Good chemical status refers to a list of 41 pollutants: 33 priority substances (PSs) and 8 other pollutants. Member States should have the possibility to establish EQS for sediment and/or biota at national level and to provide long-term trend analysis of concentrations of those substances listed in Part A of Annex I, giving particular consideration to Anthracene, Brominated diphenylether, Cadmium and its compounds, C10-13 Chloroalkanes, Di(2-ethylhexyl)-phthalate (DEHP), Fluoranthene, Hexachloro-benzene, Hexachloro-butadiene, Hexachloro-cyclohexane, Lead and its compounds, Mercury and its compounds, Pentachloro-benzene, Polyaromatic hydrocarbons (PAH) and Tributyltin compounds. The frequency of the monitoring has to provide sufficient data for a reliable long-term trend analysis and should take place every 3 years. In this context, perennial and long-lived shuttle bryophyte species are particularly suitable, since their life-strategy is based on 3–4 years (During 1992).

For organic substances, monitoring in biota should be performed when the biomagnification factor (BMF) is $>1$ or when the bioconcentration factor (BCF) is $>100$ (European Commission 2010). Biomagnification referring to absorption of the substances via the epithelia of the intestines, is limited to heterotrophic organisms (Markert et al. 2003). Metals from highly volatile compounds as Hg and As, are taken through the respiratory organs. In the selection of biota species, consideration should be given to the main purposes of the EQS Directive: trend monitoring and compliance with EQS.

Under the review of the existing approaches, active monitoring with transplants was evaluated as more suitable (Besse et al. 2012). Macroinvertebrates were chosen as most appropriate organisms in the active approach, as they enable robust control of biotic factors, by using size-homogenous and sex-homogenous indicator species, that lend themselves well to practical, easy-to-handle, infield caging systems. In the same article bryophytes were pointed out as useful monitors but not reliable for checking compliance with biota EQRs. We suppose that the main reason for that statement is that WFD requires biota EQSs to protect humans, top predators such as birds and mammals, and benthic and pelagic predators. Nevertheless, aquatic bryophytes meet all nine criteria stated in the Guidance on chemical monitoring of sediment and biota under the Water Framework Directive (European Commission 2010):

– "A relationship exists between contaminant concentrations in the species and average concentrations in the surrounding environment;
– The sampled organism is a potential food for predatory organisms or humans;
– The species accumulates the contaminants;
– The species is sedentary (migrating species should be avoided) and thus represents the sampling location, and does not originate e.g. from aquaculture plants;
– The species is widespread and abundant in the study region, to allow comparisons between different areas;
– The species lives long enough so that more than 1 year-class can be sampled, if desired;
– The species is large enough to yield sufficient tissue for analysis;
– The species is easy to collect and hardy enough to survive unfavourable conditions;
– The species is easy to identify."

Aquatic bryophyte species such as *Fontinalis antipyretica* and *Platyhypnidium riparioides* comply with all the above criteria. The Guidance also pointed out that "candidate species for biota monitoring in rivers include: . . . the aquatic bryophytes (e.g. genera *Fontinalis*) for heavy metals."

Plants uptake metals and other substances through tissues or organs as bioconcentrators. Unlike the most common route of metal exposure in plants through the roots, bryophytes provide direct exposure pathway (water to bryophytes) which can be used to evaluate exposure to higher trophic levels to which can cause harm. Moreover, plants give an indication of the soluble metal fraction in the environment which is likely to affect major compartments of the aquatic ecosystem. Therefore, when a heavy metal or toxic element is not detected (concentration is below MLD) in the bryophytes analyzed, indicating a low bioavailability, then water quality could be evaluated as "very good".

In addition, experimental and modelling results suggest that cationic composition of water have significant implication in the interpretation of autochthonous aquatic mosses contamination levels (Ferreira et al. 2009).

It should be emphasized that bryophytes can make an important contribution to stream metabolism and influence the distribution of key groups, such as in-

vertebrates. Invertebrate fauna abundance is strongly correlated with bryophyte biomass, since invertebrates rely on detrital or periphyton biomass associated with the plants (Suren 1993). Most bryophyte monitoring surveys are with heavy metals, but organic contaminants were also monitored. Spatial insecticide contamination was revealed by HCH and PCBs accumulated in *Cinclidotus danubicus* moss bags (Mouvet et al. 1985). Concentration factors of 10,000 for PCBs and 300 for gamma-HCH were reported for *Platyhypnidium riparioides* (Frisque et al. 1983). Data are available that *Fontinalis antipyretica* and *F. squamosa* were affected in their photosynthetic production at 10 $\mu$g l$^{-1}$ of herbicide atrazine after 24 h and 20 days, respectively, *F. hypnoides* exhibited a much greater reduction (90 %) in net photosynthesis within 24-h at an exposure of only 2 $\mu$g l$^{-1}$ (Hofmann and Winkler 1990).

Integrated research on concordance among fish, benthic macroinvertebrates and bryophytes in Finland, showed three groups responded to different environment factors (Paavola et al. 2003). Fish community structure correlated with depth, substrate size and oxygen, macroinvertebrate community with stream size and pH, and bryophytes with water colour, nutrients and habitat variability. The last group is strongly regulated by large substrate availability and by reach-scale factors as flow variability.

According to the guidance document no 25 (European Commission 2010) transplants or so called caged organisms provide a time-integrated assessment of environmental quality over the 4-week transplantation period. In that context an Index (Palladio) for trace element alteration was proposed by Cesa et al. (2010) in the absence of autochthonous bryophytes, offering characterization of 13 elements in five classes. The metric environmental alteration (EA) based on transplanted mosses was presented according to the definition of contamination factor (CF) introduced by Mouvet (1986) for autochthonous bryophytes:

$$EA = C_{(i)}/C_{bckg}$$

EA is the ratio between the element concentration (C) at station i and the background concentration ($C_{bckg}$). A novel interpretation scale was applied, inspired to Mersch and Claveri (1998) and Mouvet (1986):

1. EA < 2 condition of naturality, no evidence of alteration (blue)
2. $2 \leq EA < 4$ suspect of alteration (green)
3. $4 \leq EA < 12$ sure alteration (yellow)
4. $12 \leq EA < 24$ severe alteration (orange)
5. $EA \geq 24$ extreme alteration (red)

Elements producing a suspected, sure, severe or extreme alteration established the condition of global environmental alteration (GEA) for each station, except for Al and Fe since their prevalent terrigenous origin.

Five classes of global environmental alteration are as follows:

– I: no evidence of environmental alteration (EA < 2), absolute naturality (blue)

**Fig. 9.1** Five classes of global environmental alteration (GEA) and relation to the classification of ecological status according to the water framework directive (WFD)
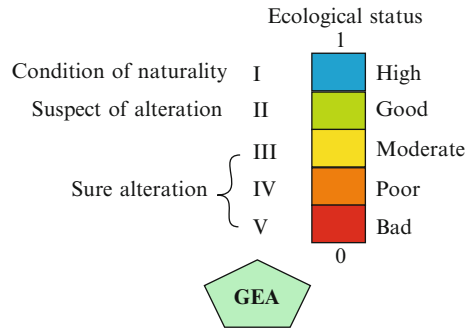


- – II: suspect of alteration ($2 \leq EA < 4$) for some elements (green)
- – III: sure alteration ($EA \geq 4$) for one to two elements (yellow)
- – IV: sure alteration ($EA \geq 4$) for three to four elements (orange)
- – V: sure alteration ($EA \geq 4$) for five or more elements (red)

Among many advantages of the Index Palladio, representation of each class of environmental alteration per station in coloured maps reflecting the WFD five ecological status classes, has to be underlined (Fig. 9.1). The Index can be easily integrated as a tool for environmental monitoring from Public Authorities.

## 9.4 Bryophytes: Cost-Effective and Rapid Ecological Assessment Tool?

It can be summarized that indigenous and/or transplanted bryophytes as biomonitors have important advantages: suitable sample material can be found throughout the year, low cost and rapid sampling, applicability in all freshwater habitat types, simple process of dissolution and analysis, and possibility to reveal past history (passive monitoring) of contamination or deposition during different time periods (active biomonitoring).

In unstable environment bryophytes often are the only biota representatives. As postulated by Slack and Glime (1985), many aquatic species are to some extent opportunists, since they must withstand currents and abrasion, must survive desiccation and flooding, must spread by vegetative or sexual reproduction.

Additional advantage of bryomonitoring is that most aquatic bryophytes resist freezing (up to – 10 °C) and have low temperature optima for growth (Stream Bryophyte Group 1999). Unlike vascular plants, bryophyte biomass is not grazed by most of the herbivores and thus presents opportunity for representative sample collection throughout the whole year. Moreover, bryophytes are shade-tolerant and can grow under high light intensity. Most species have very low light compensation (the light level at which net photosynthesis is zero) and light saturation points (Glime 2007).

Recent research demonstrated both in field and in laboratory that *F. antipyretica* exhibits partial desiccation tolerance which implies its significant desiccation rate and consequently survival (Cruz de Carvalho et al. 2011).

## 9.5 Conclusion

Aquatic bryophytes have been studied and used as biological indicators and monitors of water quality for more than 30 years. Freshwater quality can be reflected in individual species abundance as well as by the structure of bryophyte communities. Many species exhibit a high tolerance to contaminants, allowing bioaccumulation. Moreover it is well known that water sample element concentrations are often low and under the detection limits of analytical techniques. Thus bryomonitoring is used to measure bioaccumulation and bioavailability, and to assess the linkages to impacts. In the simplest experiments bioaccumulation can be characterized by measuring concentrations of metals in plant tissues and link exposure and bioaccumulation to potential impacts.

Standardization of sampling procedures in a space- and time-dependent manner and analytical techniques in aquatic bryomonitoring is needed. The number of samples should be fixed based on sampling area surface. Period of exposure time for active biomonitoring should be specified in general. Background levels and ambient metal concentrations have to be observed in parallel.

Two widespread throughout the northern hemisphere, considerably easy to recognize species are recommended in future researches: *Fontinalis antipyretica* and *Platyhypnidium riparioides* both in passive and active biomonitoring. The former is the most easily recognized aquatic species and the last is most tolerant to all kinds of pollution.

Inductively coupled plasma optical emission spectrometry (ICP-OES) could be suggested as more suitable technique in comparison with inductively coupled plasma mass spectrometry (ICP-MS) due to possibility to detect in moss tissues the macroelements (P, K, Ca and S) important for plant physiology.

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# Chapter 10
# Halogenated PAH Contamination in Urban Soils

**Takeshi Ohura, Teru Yamamoto, Kazuo Higashino, and Yuko Sasaki**

**Abstract** Halogenated polycyclic aromatic hydrocarbons (HPAH) such as Cl-PAH and Br-PAH are new ubiquitous environmental aromatic contaminants. We found HPAH in urban soils and river sediments in Japan. We developed the analysis of HPAH by gas chromatography-electron ionization-tandem mass spectrometry. 20 Cl-PAH, 11 Br-PAH, and 16 PAH were analyzed. We found concentrations from 55.6 to 534,000 ng/g for Cl-PAH, 3.14–914 ng/g for Br-PAH and 19,800–1,080,000 ng/g for PAH. Similar compositions of HPAH were observed among some soil samples, suggesting that common emission sources. The highest contaminations of Cl-PAH were observed in old chlor-alkali plant soils. To test HPAH origin from the industry, electrolysis of brine in the presence or absence of pitch were carried out in the laboratory. We found that congener and homolog profiles of HPAH produced by electrolysis are consistent with those of soils from old chlor-alkali plant sites. Consequently, chlor-alkali production facilities including electrolysis of brine in the presence of graphite could be an unintentional source of HPAH in the environment.

**Keywords** PAH • Cl-PAH • Urban soil pollution • PCB • Br-PAH • GC/MS • Brine electrolysis • Sediments

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**Contents**

## 10.1   Introduction

Many organohalogen compounds (OHC) have been used extensively as chemical materials including pesticide and insulator, and are raising our standard of living (Tanabe and Minh 2010). On the other hand, a portion of such compounds emitted in the environment are possible to create various health menaces due to their toxicities (Schecter et al. 2006; Van den Berg et al. 1998). In fact, there are environmental quality standards for organohalogen compounds in the air, water, and soil, in Japan. As one of the hazardous organohalogen compounds, certain polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), and polychlorinated biphenyls (PCB) have been adequately recognized by their global distribution, persistence and potent toxicities. (Schecter et al. 2006; Van den Berg et al. 1998). They are therefore concerned to cause problems on adverse effects of not only human health but ecological integrity. The structural characteristics caused by steric hindrance and lipophilicity would be attributed main factors of environmental persistent and bioaccumulative potential.

   Polycyclic aromatic hydrocarbons (PAH) have been also known as typical environmental hazardous pollutants because certain species of them possess strong mutagenicity/carcinogenicity and endocrine-disrupting effects, like dioxins and PCBs (Boström et al. 2002). The wide distribution of PAH in the environment could be due to easily productivity during combustion process of organic materials. That is, there are some common features including the sources and toxicities between dioxins and PAH, so that halogenated PAH (HPAH) having structural combination of dioxins and PAH are possibly present in the environment. Based on such hypothesis, author and co-workers have tried to examine the environmental occurrence of HPAH and actually detected many HPAH in the air (Kitazawa et al. 2006; Ohura et al. 2005, 2008, 2009). HPAH in the air showed the behaviors as expected. For example, the concentrations of certain chlorinated PAH (Cl-PAH) associated with particles showed the typical seasonal trend observed in the case of PAH: elevated in winter and decreased in summer (Ohura et al. 2005, 2008). The concentrations of Cl-PAH also showed the significant correlations to those of corresponding parent PAH. These facts suggest that the Cl-PAH in the air are possibly derived from common sources of PAH. Furthermore, much of Cl-PAH

showed that the photostability increased with increasing the number of chlorine substituted on the PAH skeleton (Ohura et al. 2004, 2005). It indicates that Cl-PAH may show a higher environmental persistence rather than PAH.

The study on environmental analysis of HPAH except in air and soils is further limited. Recently, the occurrences of HPAH in rice in China were reported and estimated the exposure risks (Ding et al. 2012). Although it suggests that the cancer risk values were below priority risk level ($10^{-4}$), the overall exposure processes of HPAH remained unclear. There is an incomplete understanding of HPAH's environmental occurrences and emission sources at present, so that lack of knowledge of HPAH would discourage to evaluate the environmental and biological effects. Here, we introduce the occurrences of HPAH contaminations in urban surface soils. We also discuss the possible sources of HPAH.

## 10.2 Halogenated Polycyclic Aromatic Hydrocarbons Analysis by GC/MS/MS

Although HPAH analysis has been developed using GC/MS in EI mode, those methods were applied to samples collected from air (Ohura et al. 2008, 2009). In general contaminants in air samples including gas and particles contents could be lower than those in soil and sediment samples, so that strict removal of contaminants, i.e. cleanup process, from such environmental samples will need to analyze effectively using GC/MS.

In the case of analysis of dioxins in soils, treatments using sulfuric acid and reduced copper in the cleanup process are frequently performed to reduce pigment compositions from the samples. Although the stabilities of Cl-PAH during the reduced copper treatment are unclear, the sulfuric acid treatment degrades dramatically not only PAH but also Cl-PAH (data not shown). Therefore cleanup media of Cl-PAH should be adopted that used in the case of PAH such as silica gel and alumina (Liu et al. 2007; Ohura et al. 2005, 2008). Indeed, for analysis of Cl-PAH in air, the pretreatment process used silica gel column showed reasonable recovery rates of 92–114 % (Ohura et al. 2005). As other cleanup procedures for PAH analysis in highly contaminated samples, GPC and dialysis used semipermeable membrane devices are also adopted (Czuczwa and Alford-Stevens 1989; Williamson et al. 2002). Although the adjustability for HPAH has been not investigated yet, high selectivity and condensation may be expected to employ those procedures. As instruments in support of the cleanup processes, usage of GC-tandem MS (GC/MS/MS) will be an efficient tool for analysis of samples including matrix such as soils because of possessing the high sensitivity and selectivity. Here, we introduce a new analytical method of HPAH by using GC/MS/MS.

Twenty species of Cl-PAH (Fig. 10.1) and 11 species of Br-PAH (Fig. 10.2) were used as HPAH standard compounds. The HPAH were analyzed on a gas chromatograph (Agilent HP7890A) interfaced with a tandem quadrupole mass
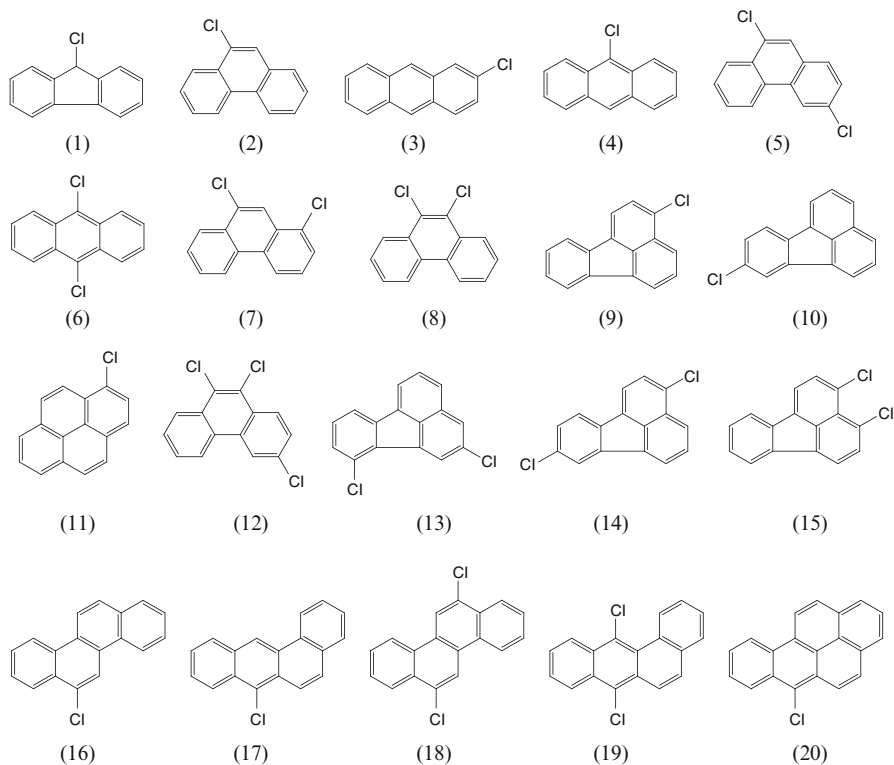
**Fig. 10.1** Chemical structures of monitored Cl-PAH. *PAH* polycyclic aromatic hydrocarbons
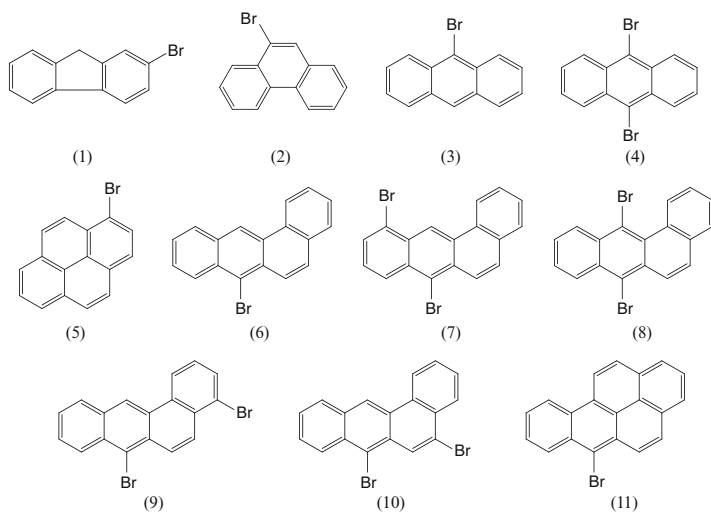


**Fig. 10.2** Chemical structures of monitored Br-PAH. *PAH* polycyclic aromatic hydrocarbons

spectrometer (Waters Quattro micro GC), operating in EI mode (70 eV). The analytical conditions were as follows: for both Cl-PAH and Br-PAH, injection temperature was 250 °C and 5 μL of extracts were injected in the system using pulsed splitless mode at pulse time of 2 min and pulsed pressure of 20 psi. An DB-5 ms capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, Agilent) was used. A constant flow of helium at 1.0 mL/min was used as the carrier gas. The GC column temperature was programmed from 100 (initial equilibrium time 1 min) to 200 °C via a ramp of 25 °C/min, 200–300 °C via a ramp of 5 °C/min and maintained at 300 °C for 5 min. GC interface and ionization source temperatures were both 300 °C and the system operated in MS/MS mode using argon as collision gas at a pressure of $2.8 \times 10^{-3}$ mbar in the collision cell. The developed multiple reaction monitoring (MRM) conditions of targeted HPAH and the detection limits were listed in Table 10.1. The GC/MS/MS chromatograms of each standard mixture of Cl-PAH and Br-PAH were represented in Figs. 10.3 and 10.4, respectively, which achieved good separations.

## 10.3   Halogenated Polycyclic Aromatic Hydrocarbons in Sediments

There are a few reports that the concentrations of Cl-PAH were investigated in sediment samples (Horii et al. 2009; Ma et al. 2009, Sun et al., 2011). Horii et al. (2009) investigated the residual concentrations and profiles of Cl-PAH in sediments from water bodies near industrialized areas, Tokyo Bay, Japan; the Saginaw River watershed, Michigan, USA; a former chlor-alkali plant, Georgia, USA; and the New Bedford Harbor Superfund site, Massachusetts, USA. Among the soil samples, a high mean concentration of Cl-PAH was observed in sediment collected near a former chlor-alkali plant (8.82 ng/g) in comparison with other sediments affecting multiple sources such as New Bedford Harbor (1.88 ng/g) and Saginaw River watershed (1.14 ng/g). It suggests that chlor-alkali plant could be concerned with formation of Cl-PAH. In addition, Cl-PAH profile observed in the chlor-alkali plant showed that the dominant components were 6-Cl-BaP and 1-Cl-Py. Such profiles have been often observed in those of ambient air samples. This fact suggests that sources of Cl-PAH in the sediment samples are significantly related to those in air samples. Sun et al. (2011) recently reported that the occurrences of Cl-PAH and Br-PAH in surface sediments in Shenzhen, South China. Despite a small number of targeted Cl-PAH (9,10-dichloroanthracene, 9-chlorophenanthrene, and 2-chloroanthracene), the mean concentration of Cl-PAH was 27.6 ng/g, which was >3-times higher than those in the above study. Since no correlation was observed between the concentrations of Cl-PAH and parent PAH, the formation of Cl-PAH may have no relation of that of PAH. Although the authors suggested that the regional urbanization account for increase in HPAH levels in sediment, the direct emission sources remained unclear.

**Table 10.1** GC/MS/MS analytical conditions of 20 Cl-PAH and 11 Br-PAH. The running number of compound is corresponded to Fig. 10.1 (Cl-PAH) and 2 (Br-PAH). *GC* gas chromatography, *MS* mass spectrometry, *PAH* polycyclic aromatic hydrocarbons

| No | Compound | Abbrev. | RT | MRM1 | MRM2 | MRM3 | MRM4 | MRM5 | DL (pg) |
|----|----------|---------|-----|------|------|------|------|------|---------|
| | *ClPAH* | | | | | | | | |
| 1 | 9-chlorofluorene | 9-ClFlu | 8.03 | 202 > 165 (10) | 165 > 164 (16) | 165 > 163 (28) | 165 > 115 (24) | 165 > 139 (24) | 16.7 |
| 2 | 9-chlorophenanthrene | 9-ClPhe | 9.97 | 212 > 176 (24) | 212 > 177 (12) | 212 > 151 (28) | 212 > 150 (44) | 214 > 177 (12) | 10.6 |
| 3 | 2-chloroanthracene | 2-ClAnt | 10.12 | 212 > 176 (24) | 212 > 177 (12) | 212 > 151 (28) | 212 > 150 (44) | 214 > 177 (12) | 10.5 |
| 4 | 9-chloroanthracene | 9-ClAnt | 10.16 | 212 > 176 (24) | 212 > 177 (12) | 212 > 151 (28) | 212 > 150 (44) | 214 > 177 (12) | 4.8 |
| 5 | 3,9-dichlorophenanthrene | 3,9-ClPhe | 12.17 | 246 > 176 (26) | 248 > 176 (26) | 176 > 150 (18) | 246 > 211 (12) | 176 > 175 (16) | 23.3 |
| 6 | 9,10-dichloroanthracene | 9,10-Cl2Ant | 12.43 | 246 > 176 (26) | 248 > 176 (26) | 176 > 150 (18) | 246 > 211 (12) | 176 > 175 (16) | 13.2 |
| 7 | 1,9-dichlorophenanthrene | 1,9-Cl2Phe | 12.43 | 246 > 176 (26) | 248 > 176 (26) | 176 > 150 (18) | 246 > 211 (12) | 176 > 175 (16) | 8.5 |
| 8 | 9,10-dichlorophenanthrene | 9,10-Cl2Phe | 12.64 | 246 > 176 (26) | 248 > 176 (26) | 176 > 150 (18) | 246 > 211 (12) | 176 > 175 (16) | 6.2 |
| 9 | 3-chlorofluoranthene | 2-ClFluor | 13.46 | 236 > 201 (18) | 236 > 200 (32) | 238 > 201 (18) | 238 > 200 (32) | 200 > 199 (16) | 20.4 |
| 10 | 8-chlorofluoranthene | 8-ClFluor | 13.55 | 236 > 201 (18) | 236 > 200 (32) | 238 > 201 (18) | 238 > 200 (32) | 200 > 199 (16) | 28.5 |
| 11 | 1-chloropyrene | 1-ClPy | 14.26 | 236 > 201 (18) | 236 > 200 (32) | 238 > 201 (18) | 238 > 200 (32) | 200 > 199 (16) | 13.2 |
| 12 | 3,9,10-trichlorophenanthrene | 3,9,10-Cl3Phe | 14.95 | 280 > 210 (24) | 282 > 210 (24) | 210 > 175 (16) | 282 > 175 (42) | 282 > 212 (24) | 12.4 |
| 13 | 5,7-dichlorofluoranthene | 5,7-Cl2Fluor | 15.65 | 270 > 235 (16) | 270 > 200 (28) | 272 > 200 (28) | 200 > 199 (16) | 200 > 198 (28) | 20.7 |
| 14 | 3,8-dichlorofluoranthene | 3,8-Cl2Fluor | 16.41 | 270 > 235 (16) | 270 > 200 (28) | 272 > 200 (28) | 200 > 199 (16) | 200 > 198 (28) | 7.2 |
| 15 | 3,4-dichlorofluoranthene | 3,4-Cl2Fluor | 17.28 | 270 > 235 (16) | 270 > 200 (28) | 272 > 200 (28) | 200 > 199 (16) | 200 > 198 (28) | 35.7 |
| 16 | 6-chlorochrysene | 6-ClChry | 18.76 | 262 > 226 (26) | 264 > 226 (22) | 226 > 224 (22) | 226 > 225 (18) | 224 > 223 (18) | 25.2 |
| 17 | 7-chlorobenz[a]anthracene | 7-ClBaA | 18.94 | 262 > 226 (26) | 264 > 226 (22) | 226 > 224 (30) | 226 > 225 (18) | 224 > 223 (18) | 23.4 |
| 18 | 6,12-dichlorochrysene | 6,12-Cl2Chry | 21.37 | 296 > 226 (26) | 298 > 226 (26) | 226 > 224 (28) | 226 > 225 (18) | 224 > 223 (18) | 6.1 |
| 19 | 7,12-dichlorobenz[a]anthracene | 7,12-Cl2BaA | 21.49 | 296 > 226 (26) | 298 > 226 (26) | 226 > 224 (28) | 226 > 225 (18) | 224 > 223 (16) | 8.7 |
| 20 | 6-chlorobenzo[a]pyrene | 6-ClBaP | 24.33 | 286 > 250 (26) | 288 > 250 (32) | 250 > 248 (28) | 250 > 249 (18) | 250 > 224 (22) | 11.9 |

| | BrPAH | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2-Bromofluorene | 2-BrFlu | 27.31 | 244 > 165 (15) | 246 > 165 (15) | 165 > 164 (15) | 165 > 163 (25) | 165 > 115 (20) | 174.5 |
| 2 | 9-Bromophenanthrene | 9-BrPhe | 31.12 | 258 > 176 (25) | 256 > 177 (15) | 258 > 177 (15) | 256 > 176 (25) | 258 > 151 (30) | 148.6 |
| 3 | 9-Bromoanthracene | 9-BrAnt | 31.47 | 258 > 176 (25) | 256 > 177 (15) | 258 > 177 (15) | 256 > 176 (25) | 258 > 151 (30) | 120.2 |
| 4 | 9,10-Dibromoanthracene | 9,10-Br2Ant | 37.01 | 335.9 > 176 (25) | 333.9 > 176 (30) | 337.9 > 176 (30) | 176 > 150 (20) | 176 > 175 (15) | 145.7 |
| 5 | 1-Bromopyrene | 1-BrPy | 37.27 | 280 > 201 (20) | 282 > 201 (20) | 201 > 200 (15) | 280 > 200 (40) | 282 > 200 (40) | 185.4 |
| 6 | 7-Bromobenz[a]anthracene | 7-BrBaA | 42.58 | 306 > 226 (30) | 308 > 226 (25) | 226 > 224 (25) | 226 > 225 (15) | 306 > 227 (10) | 144.6 |
| 7 | 7,11-Dibromobenz[a]anthracene | 7,11-Br2BaA | 47.45 | 383.9 > 226 (30) | 387.9 > 226 (30) | 226 > 225 (15) | 226 > 224 (25) | n/a | 75.7 |
| 8 | 7,12-Dibromobenz[a]anthracene | 7,12-Br2BaA | 47.89 | 383.9 > 226 (30) | 387.9 > 226 (30) | 226 > 225 (15) | 226 > 224 (25) | n/a | 60 |
| 9 | 4,7-Dibromobenz[a]anthracene | 4,7-Br2BaA | 48.72 | 383.9 > 226 (30) | 387.9 > 226 (30) | 226 > 225 (15) | 226 > 224 (25) | n/a | 59.5 |
| 10 | 5,7-Dibromobenz[a]anthracene | 5,7-Br2BaA | 48.72 | 383.9 > 226 (30) | 387.9 > 226 (30) | 226 > 225 (15) | 226 > 224 (25) | n/a | 59.7 |
| 11 | 6-Bromobenzo[a]pyrene | 6-BrBaP | 50.53 | 330 > 250 (35) | 332 > 250 (35) | 330 > 251 (15) | 332 > 251 (15) | 250 > 248 (20) | 129.1 |
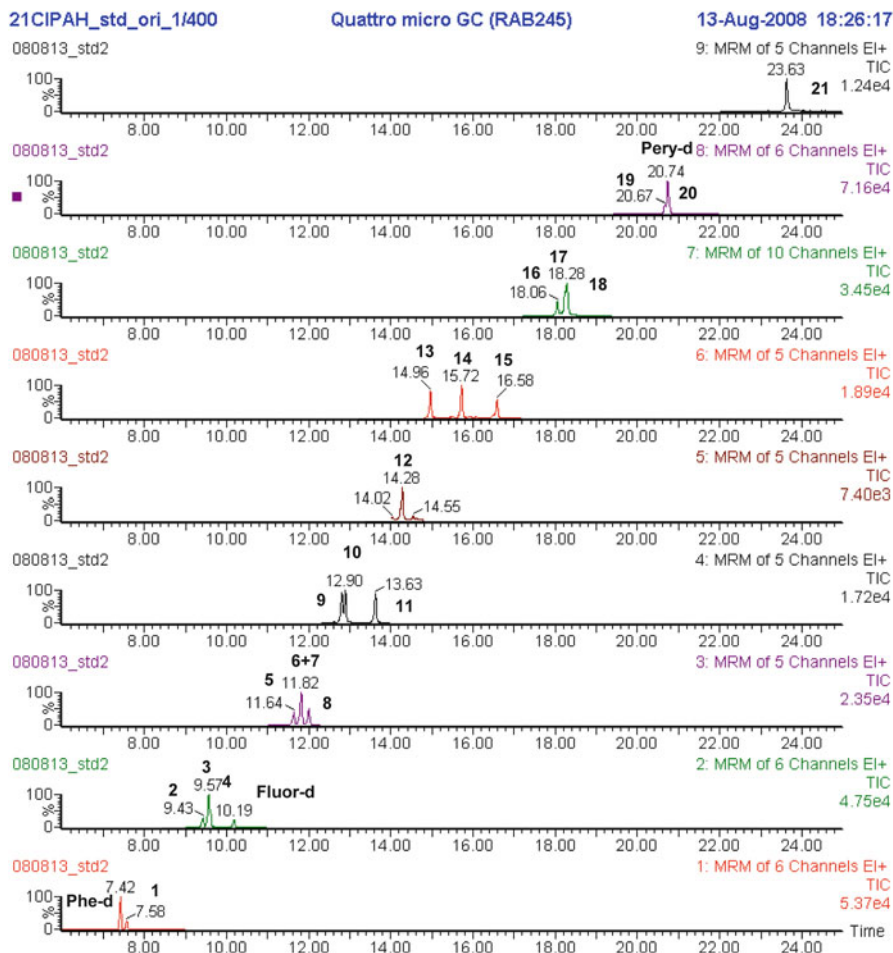
**Fig. 10.3** GC/MS/MS chromatogram of 20 Cl-PAH. The number noted in chromatogram is corresponded to Table 10.1 and Fig. 10.3. *PAH* polycyclic aromatic hydrocarbons, *GC* gas chromatography, *MS* mass spectrometry

## 10.4 Halogenated Polycyclic Aromatic Hydrocarbons in Surface Soils of Old-Factory Sites

The soil samples collected from three former factory sites in a megacity Tokyo, Japan were previously known to be highly polluted $(2,900 \sim 2,000,000$ pg-TEQ/g) by polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofuran (PCDD/F) (Yamamoto et al. 2009). In addition, one river sediment sample was also highly polluted by PCDD/F (5,900 pg-TEQ/g), whereas the possible sources remained unclear. Those highly polluted soil and sediment samples collected previously were
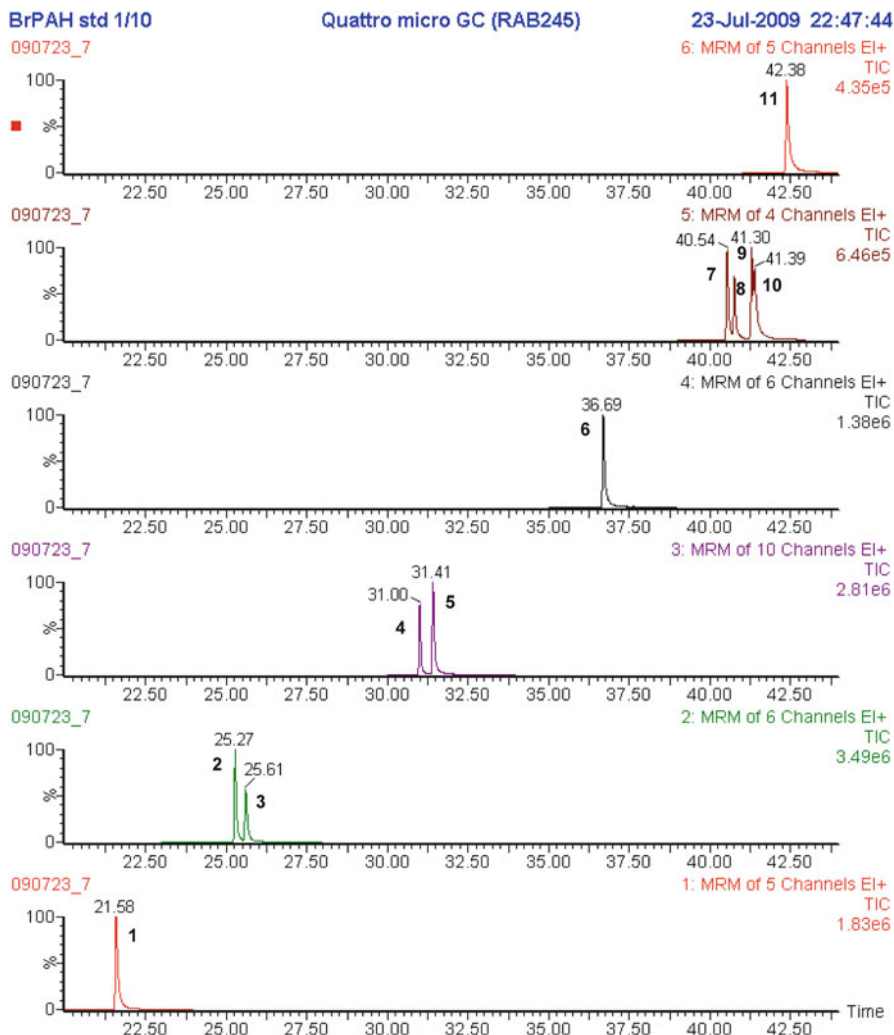
**Fig. 10.4** GC/MS/MS chromatogram of 11 Br-PAH. The number noted in chromatogram is corresponded to Table 10.1 and Fig. 10.4. *PAH* polycyclic aromatic hydrocarbons

used in this study, followed by extraction and cleanup. One to ten gram of the dried soil and sediment samples weighted and subsequently soxhlet extraction using toluene for 16 h. Note that the surrogate standards are not added here in the samples because the extracts could be used in other study such as biological assays. The extracts were concentrated at 50 mL, and a part of the solution was subsequently cleaned up using Sep-Pak silica gel (Waters), previously conditioned with 10 mL hexane. The sample extract was eluted with 15 mL of hexane. When the eluate

**Table 10.2** Total concentrations and the contributions of 16 species of PAH, 20 species of Cl-PAH, and 11 species of Br-PAH in the samples of surface soils (A-C) and a urban river sediment (D). *PAH* polycyclic aromatic hydrocarbons

| Sample code | Sample information | Main factory | Concentration,[a] ng/g (contribution, %) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Σ16PAH | | Σ20Cl-PAH | | Σ11Br-PAH | |
| A | Soil from landfill | Unknown | 117,000 | (95.3) | 5,770 | (4.7) | 13.5 | (0.01) |
| B | Soil from former factory site | Metal and rubber processing factory | 73,000 | (71.2) | 29,500 | (28.8) | 33.4 | (0.03) |
| C | Soil from former factory site | Chlor-alkari plant | 1,080,000 | (66.9) | 534,000 | (33.1) | 914 | (0.06) |
| D | Sediment from river | Unknown | 19,800 | (99.7) | 55.6 | (0.3) | 3.14 | (0.02) |

[a]Concentrations of sample A and C show the average values of two samples

is colored, the decoloration will be successfully accomplished with repeating the cleanup. The eluent was evaporated until ca. l mL under nitrogen at 40 °C, followed by addition of internal standards as syringe spike.

Measurements of targeted HPAH and priority PAH were performed in five soils collected from three kinds of surface soil and one sediment samples (Table 10.2). In brief, those soils were from landfill, former metal and rubber processing factory site and former chlor-alkali plant site, and sediment was collected from river nearby previous landfill.

Most of targeted PAH and Cl-PAH rather than Br-PAH were found in the every sample (Table 10.2). The total concentrations of Cl-PAH, Br-PAH, and PAH ranged from 55.6 to 534,000 ng/g, from 3.14 to 914 ng/g, and from 19,800 to 1,080,000 ng/g, respectively. The contributions of PAH were abundant at the range from 66.9 % to 99.7 % in contrast to those of Br-PAH (0.01 ~ 0.06 %). In addition, the total concentrations of each HPAH in the samples showed significant correlations to those of PAH (Fig. 10.5). It indicates that those compounds could be produced at a time even if the emission sources are different among the collected sites.

The relatively high contributions of Cl-PAH were observed in the sample of soil B and C that were collected from former industrial plants. For both Cl-PAH and Br-PAH, the most polluted soil was soil C, followed by soil B, soil A, and sediment D, whereas the extent of PAH as follows: C > A > B > D. Since the soil C was collected from a former chlor-alkari plant site, the industrial activity could be concerned to contamination of PAH and HPAH. Horii et al. (2009) reported that Cl-PAH were detected at relatively high concentration (24.1 ng/g) from marsh sediment collected near a former chlor-alkali plant in Georgia, USA. Comparing Cl-PAH concentrations between two samples corresponding to chlor-alkali plant in Japan and USA, the current data was approximately 20,000 times higher than the sediment sample in USA. Although there was significant difference in the both concentrations, the incontrovertible fact is that chlor-alkali plant could be significant emission sources of not only PCDD/F but also Cl-PAH in the environment.
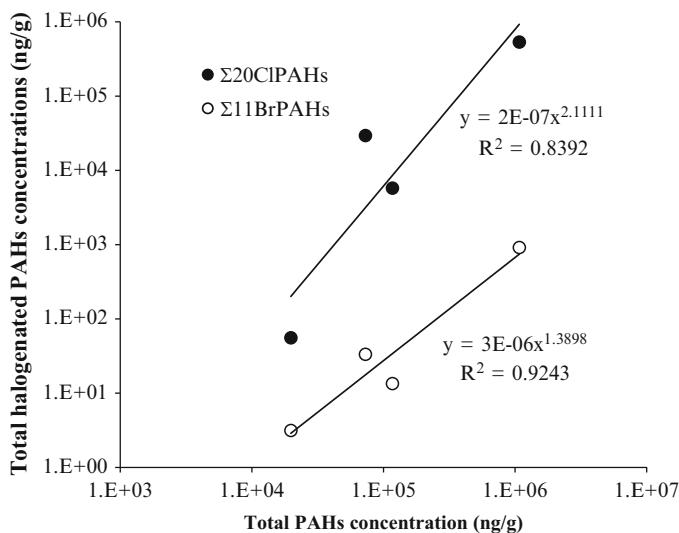
**Fig. 10.5** Relationships of total concentrations of Cl-PAH or Br-PAH and PAH in soil and sediment samples. *PAH* polycyclic aromatic hydrocarbons

In our previous studies, the concentrations of Cl-PAH in the air were approximately two orders lower than those of PAH (Ohura et al. 2008). Interestingly, the current case that the content of Cl-PAH in soils is relatively high, suggests that the soils polluted by industrial activities could include relatively high levels of Cl-PAH.

## 10.5 Halogenated Polycyclic Aromatic Hydrocarbons Production by Brine Electrolysis

As suggested above, we believe that brine electrolysis performed in clor-alkali plant is a crucial emission source of not only Cl-PAH but also Br-PAH. Halogenations of PAH could be occurred during electrochemical treatment of saline aqueous solutions including PAH, which become a serious problem (Muff and Søgaard, 2011). Vizard et al. (2006) reported that the disposal of graphite electrode sludges from brine electrolysis used in old chemical works could provoke high contaminations of PCDD/F in the soils. Indeed, Yamamoto et al. (2009) confirmed the production of PCDD/F during brine electrolysis containing pitch used as the binding agent of graphite electrode. So we investigated whether HPAH are produced by brine electrolysis. For the electrode, artificial graphite was used as anode in the electrolytic cell because it had been typically used in the past industrial chro-alkali process. Furthermore solid pitch used as a material of graphite electrode binder was also added in the brine solution supplied to anode cell. So, the laboratory unit of brine
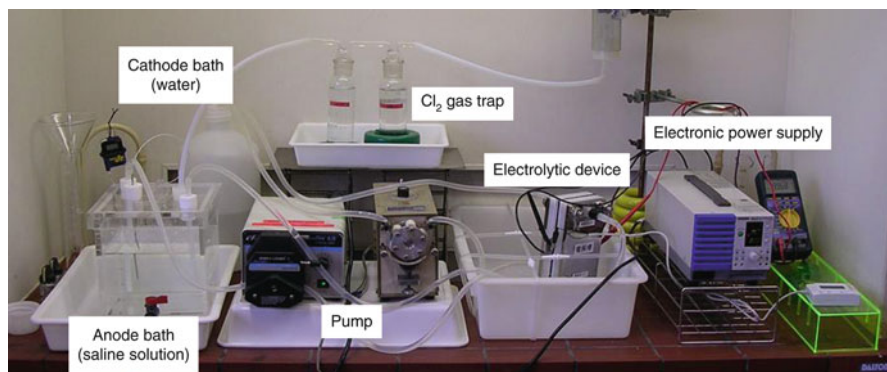
**Fig. 10.6** The laboratory unit of brine electrolysis by flow cell system consisted of electrolytic cell, anode/cathode feeder and circulating water system to supply solution

electrolysis by flow cell system was used to investigate the possibility of production of HPAH during the brine electrolysis. The apparatus consisted of the electrolytic cell, anode/cathode feeder and circulating water system to supply solution was installed in an exhaust chamber. Inside the electrolytic cell, an artificial graphite electrode or a titanium electrode used as anode and SUS304 as cathode were separated two compartments by ion-exchange membrane made by perfluorosulfonic acid-based films (Fig. 10.6). Graphite electrode fixed in anode cell was used as in the past typically industrial chlor-alkali processes. Saturated brine solution supplied to anode cell made chlorine ion form chlorine, and hypochlorous acid and hydrochloric acid were also formed depending on pH and temperature condition of analyte. There was also some sodium hydroxide formed by the reaction of sodium ions passing through the separator with hydroxyl ion. Gaseous chlorine formed from anode cell was removed by $Cl_2$ recovery system. The full scale of experimental apparatus of brine electrolysis is 1.6 m × 0.6 m × 1.1 m (L × W × H) size. Electrolysis was performed in acid during the operation. After dechlorination, the analyte solution included suspended solid was extracted by Empore disk (C18). These were combined and extracted over 16 h with toluene by Soxhlet extraction.

The brine electrolysis was performed at 2.5 kA/m$^2$ of current density at 48 h. After that, the formations of HPAH were analyzed by using extracts from NaCl solution and added pitch. The toluene extracts from NaCl solution before electrolysis contained PAH with 39,500 ng/L and Cl-PAH with 481 ng/L, but not detection of Br-PAH (Table 10.3). However, after electrolysis, the toluene extracts contained considerably amounts of Cl-PAH and PAH in addition to Br-PAH. The concentrations of Cl-PAH were the highest contribution with 76.2 %, indicated that brine electrolysis induce the formation of Cl-PAH and minimal Br-PAH. A significant amount of PAH included in the pitch dispersed in NaCl solution, so that Cl-PAH could be produced by chlorination of PAH eluted from pitch throughout electrolysis. Similarly, minor amounts of bromine contained in the brine also could induce the bromination of PAH, resulted in production of Br-PAH. Comparing the

**Table 10.3** PAH and Cl/Br-PAH concentrations (ng/l) of dichloromethane extracts from NaCl solution (4 L) dispersed pitch (5 g) before (E-1) or after electrolysis at 2.5 kA/m$^2$, 48 h (E-2), and the concentrations (ng/g) of toluene extracts from the pitch (0.021 g) before electrolysis (E-3). PAH: polycyclic aromatic hydrocarbons

| Sample code | Sample information | Σ16PAH | % | Σ20Cl-PAH | % | Σ11Br-PAH | % |
|---|---|---|---|---|---|---|---|
| E-1 | Before electrolysis | 39,500 | 98.8 | 481 | 1.2 | 0 | 0 |
| E-2 | After electrolysis | 802,000 | 23.2 | 2,640,000 | 76.2 | 21,100 | 0.6 |
| E-3 | Pitch extracts | 158,000,000 | 99.9 | 1,280 | <0.1 | 0 | 0 |
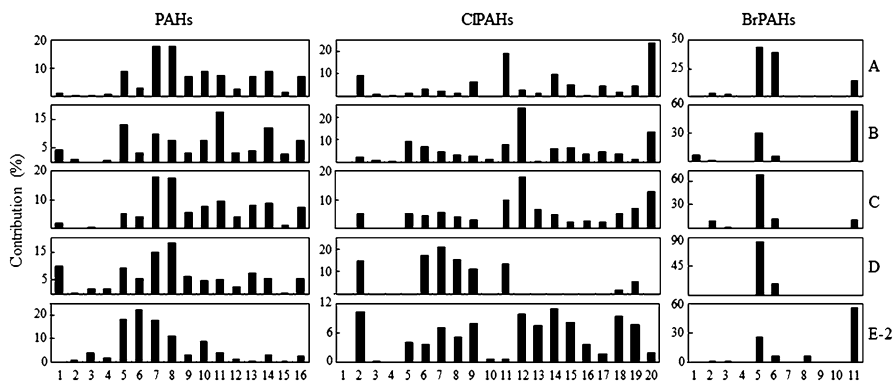


**Fig. 10.7** Contributions of individual PAH, Cl-PAH, and Br-PAH in former industrial soils, a urban river sediment, and extract from the NaCl solution after brine electrolysis. The number noted in Cl/Br-PAH is corresponded to Table 10.1. The number noted in PAH is as follows: 1: naphthalene, 2: acenaphthene, 3: acenaphthylene, 4: fluorine, 5: phenanthrene, 6: anthracene, 7: fluoranthene, 8: pyrene, 9: benz[*a*]anthracene, 10: chrysene, 11: benzo[*b*]fluoranthene, 12: benzo[*k*]fluoranthene, 13: benzo[*a*]pyrene, 14: indeno[1,2,3-*cd*]pyrene, 15: dibenz[*a,h*]anthracene, 16: benzo[*ghi*]perylene. *PAH* polycyclic aromatic hydrocarbons

compositions of PAH and HPAH in sample (E-2) obtained from the electrolysis, specific distributions were observed (Fig. 10.7). The compositions of PAH and Cl-PAH detected from the pitch after electrolysis (E-2) were showed slight different patterns to those of environmental samples (A ∼ D), but similar pattern for Br-PAH. As further emission source analysis, we here performed cluster analysis using the PAH and HPAH concentrations obtained from environmental samples and electrolytic solution.

## 10.6 Profiles of Halogenated Polycyclic Aromatic Hydrocarbons in Soils

The homologue profiles of certain compounds in the environment give us useful information such as the emission sources, environmental behaviors and fate. Here the profiles of PAH, Cl-PAH, and Br-PAH group were compared among the samples,

because there were significant differences of the concentration levels among the compound group (Fig. 10.7). The profiles of PAH showed similar pattern among the samples of A, C and D, and the main contributors were fluoranthene (#7 in Fig. 10.7) and pyrene (#8). The profiles of sample B showed relative high contributions of high molecular weights PAH such as benzo[*b*]fluoranthene (#11) and indeno[1,2,3-*cd*]pyrene (#14) in compared to the other samples. The relative high compositions of such PAH have been observed in other urban surface soils (Zhang and Wang 2011). PAH pollution at urban sites could be mainly caused by various anthropogenic sources such as traffic and industrial activities. In addition, the occurrence in soils are significantly affected by not only the directly exposures and wet/dry deposition from air but also decay processes such as biodegradation, photodegradation, and effluent. Therefore, it could be difficult to evaluate the sources from profile of PAH in surface soils.

Of Cl-PAH, the profiles showed characteristic pattern rather than those of PAH (Fig. 10.7). The profiles of sample B and C were similar, which illustrating the relative high contributions of 3,9,10-trichlorophenanthrene (#12) and 6-chlorobenzo[*a*]pyrene (#20). It also suggests that those soils may be polluted by the common sources and/or production mechanisms. The profile of Cl-PAH on sample A was highly contributed by 1-chloropyrene (#11) and 6-chlorobenzo[*a*]pyrene (#20). A similar profile was also observed in the surveys of Cl-PAH in particulate matter in urban air, Japan. It suggests that Cl-PAH in soil A and urban air may be emitted from common sources. The profile of sediment sample E was obviously different from those of above soil samples, and high contributions of high molecular weights Cl-PAH such as 3,9,10-trichlorophenanthrene (#12), 3,8-dichlorofluoranthene (#14) and 6,12-dichlorochrysene (#18) were observed. The profile analyzed in sediment of Saginaw river (USA) also showed relatively high contribution of 3,8-dichlorofluoranthene and 6,12- dichlorochrysene. Horii et al. reported that the sediment of Saginaw river receive discharges from multiple industrial sources. Therefore, the pollution of Cl-PAH in sediment E could also be affected by multiple sources rather than a specific source.

For Br-PAH, small amounts were detected in all soils collected with relatively high contributions of 1-bromopyrene (#5), 7-bromobenz[*a*]anthracene (#6), and 6-bromobenzo[*a*]pyrene (#11). The similar trend has been observed in the past study that was surveyed in Tokyo Bay sediment core. It suggests that those selected Br-PAH may be relatively stable, resulted abundant in soils.

In order to clarify the relationship among the soil samples, cluster analysis was performed by using the concentrations of Cl-PAH. The dendrogram indicated that the profiles between sample A and D, and B and C were similar to each other (Fig. 10.8). It suggests that those soils are possible to be polluted by the common sources. Furthermore, the profiles of sample B and C were also similar to the profile of pitch solution after electrolysis (E-2). This fact indicates that sample B and C could be affected by brine electrolysis. Indeed, sample C was collected from a former clor-alkali plant (Table 10.2), elucidating Cl-PAH as useful indicator
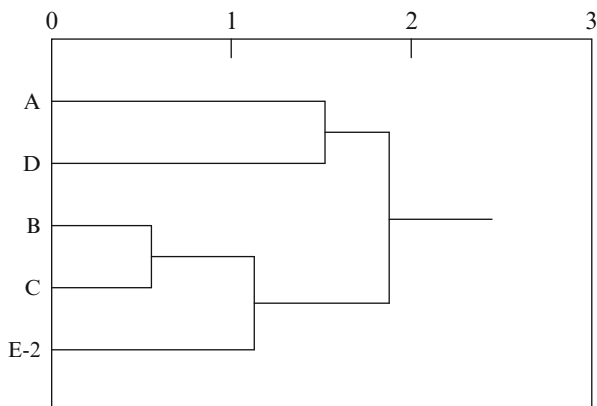
**Fig. 10.8** Hierarchical dendrogram of samples contaminated by Cl-PAH. *PAH* polycyclic aromatic hydrocarbons

for these emission sources. The differences in compositions observed among environmental and electrolytic solution samples may be due to the environmental degradation, volatilization, runoff, and so on. Consequently, brine electrolysis is possible to produce HPAH in the process, so that chlor-alkari plant might be one of significant emission source of HPAH in the environment. Since the environmental impact and behavior of HPAH is not adequately evaluated, further investigations on the environmental fate are urgently required.

The concentration ratios of certain PAH have been used as a tool exploring the emission sources and environmental behaviors. If the analysis is performed by other persistent pollutants, relative stable and/or source specific species should be used to avoid environmental losses. Here we introduce the suitability of Cl-PAH as chemical marker. In a previous work we indicated that 3-chlorofluoranthene (3-ClFluor) is ubiquitously present in the environment, and is relative stable to photo-irradiation among ClPAH species. It was also indicated that 6-chlorobenzo[*a*]pyrene (6-ClBaP) in the air exhibit characteristic behaviors compared to other Cl-PAH; its concentration is elevated in summer, and decreased in winter. So we used those Cl-PAH as the indicator to define the profiles among soil samples. Table 10.4 shows the ratios of frequently observed ClPAH to 3-ClFluor. There were no significant differences of each concentration ratio normalized to 3-ClFluor among the soil samples, whereas the ratios normalized to 6-ClBaP and 3,9,10-trichlorophenanthrene (3,9,10-Cl3Phe) showed great variability among the samples. Considering those profile analysis and the source information, the concentration ratio of 3,9,10-Cl3Phe/3,9,10-Cl3Phe + 1-chloropyrene (1-ClPy) could be an appropriate indicator to evaluate the possible sources. It also means that those ClPAH may be useful species to distinguish the characteristics such as sources, origins, and fates in environmental samples.

**Table 10.4** Concentration ratios of selected Cl-PAH normalized to 3-Cl-Fluor, 6-Cl-BaP, and 3,9,10-Cl-3Phe and 1-Cl-Py in former industrial soils and urban river sediment, soil samples from an e-waste recycling facility and a chemical industrial complex. *PAH* polycyclic aromatic hydrocarbons

| Samples | 9-ClPhe/ 3-Fluor | 3,9,10-Cl3Phe/ 3-ClFluor | 6-ClBaP/ 3-Fluor | 9-ClPhe/ 6-ClBaP | 3,9,10-Cl3Phe/ 6-ClBaP | 1-ClPy/6-ClBaP | 3,9,10-Cl3Phe/ 3,9,10-Cl3Phe + 1-ClPy | Reference |
|---|---|---|---|---|---|---|---|---|
| A | 2.38 | 0.58 | 0.84 | 0.44 | 0.26 | 0.90 | 0.23 | This study |
| B | 0.90 | 0.90 | 0.84 | 0.17 | 1.76 | 0.57 | 0.76 | This study |
| C | 1.75 | 0.85 | 0.39 | 2.76 | 8.64 | 1.50 | 0.85 | This study |
| D | 1.37 | nd | nd | nd | *nd* | nd | nd | This study |
| E-2 | 1.28 | 0.55 | 0.20 | 5.18 | 4.97 | 0.39 | 0.93 | This study |
| e-waste soil[a] | 1.00 | 3.82 | 32.7 | 0.03 | 0.12 | 0.25 | 0.32 | Ma |
| Chemical soils[b] | nd | 0.27 | 110 | nd | <0.01 | 0.06 | 0.04 | Ma |

*nd* not determined

[a]Soils from an e-waste recycling facility

[b]Soils from a chemical industrial complex

## 10.7 Conclusion

Halogenated PAH are grouped in relatively new contaminants in the environment, so that the environmental behaviors remain unclear. In particular, there is only a few reports that HPAH were surveyed in soils and sediments. In the current chapter, we introduced the analytical method suitable for HPAH analysis in soils and sediment, i.e. GC/MS/MS. The method was applied to the surveys of HPAH in former industrial soils and river sediment in an urban city, Japan. The extends of HPAH contaminations varied in kinds of collected soils, suggesting that the significant sources could be present in the sites collected soil samples. The compositions of HPAH also showed specific patterns in the each sample. Since the soils collected from a former chlor-alkari plant site was included in the sample, the contribution to HPAH contaminants was also investigated. Using lab-scale electrolysis apparatus, brine electrolysis containing pitch induced the production of HPAH in the NaCl solutions. Indeed the profiles of Cl-PAH obtained from a former chlor-alkari plant site showed affinity of those obtained from the electrolysis. These findings suggest that Cl-PAH could become indicator to recognize the sources in various environmental contaminants.

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