Abid A. Ansari · Sarvajeet Singh Gill
Ritu Gill · Guy R. Lanza
Lee Newman *Editors*

Phytoremediation

Management of Environmental Contaminants, Volume 4

Springer

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Preface

"*Live as if you were to die tomorrow, learn as if you were to live forever*"

Mahatma Gandhi

Volume 4 of this 5 volume series adds various studies on phytoremediation of organic contaminants from terrestrial and aquatic ecosystems. In this volume, some examples on applications of phytoremediation in wastewater engineering technology have been provided. Various studies on natural and constructed wetlands for phytoremediation have also been included in this volume. The importance of phytoremediation in reclamation and restoration of terrestrial and aquatic ecosystems has been described. Information on uptake, tolerance mechanisms and the role of grasses in phytoremediation of various organic contaminants has also been provided. Plant microbe interactions, bio-retention systems, phenolic compounds and enzymatic applications in phytoremediation of contaminated soil and water have been described in different chapters of this volume. The chapters in volume 4 illustrate how phytoremediation applications using constructed wetlands can also serve in the removal of pathogenic bacteria from contaminated waters. Volume 4 of this book series provides additional accounts of some selected phytoremediation research projects and case histories from specific sites and/or laboratories. The editors and contributing authors hope that one result of publishing this book will be to provide a wide range of useful experimental data derived from global applications of phytoremediation. Hopefully, like the previous three volumes of this book series this volume can also provide new insights into the advantages and disadvantages of phytoremediation to manage the continuing threat of ecosystem degradation resulting from anthropogenic inputs of environmental contaminants.

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Part I
Phytoremediation of Organic Contaminants

Phytoremediation of PCBs and PAHs by Grasses: A Critical Perspective

Esmaeil Shahsavari, Arturo Aburto-Medina, Mohamed Taha, and Andrew S. Ball

Abstract Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are two major environmental contaminants which threaten our health and environment. The removal of these key environmental pollutants from the environment is therefore paramount. Among the cleanup methods currently being used, traditional methods such as chemical and physical treatments tend to be expensive, laborious and may cause secondary contamination. Phytoremediation, the use of plants and associated microorganisms, represents a promising, nondestructive and cost-effective in situ technology for the degradation or removal of contaminants. Grasses belonging to the Poaceae family have drawn significant attention in this regard due to their fast growth, dense, fibrous root systems, and the demonstrated fast removal of PAH and PCB compounds from soils in which these plants have been grown. In this review, we review research on the use of grasses for the degradation of PAHs and PCBs and highlight the benefits of this phytoremediation approach.

Keywords Phytoremediation • Grass • Polycyclic aromatic hydrocarbons • Polychlorinated biphenyls • Plant roots • Endophytes

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1 Introduction

Since the start of the industrial revolution, there has been a steady impact of human activities on the environment. This has resulted in large environmental problems that threaten both environmental and human health. The constant increase in human activities is related to the exponential growth of the world population in the last century, now expected to exceed 8.9 billion by 2050 (UN). Such an increase in the global population also means a faster depletion of natural resources and ever-increasing pressure on the environment, resulting in increasing amounts of chemical and radioactive pollutants into the environment. In addition, inhabitants of major cities are commonly affected by air pollution generated by heavy industries and motor vehicles; the World Health Organization (WHO) estimates that these emissions account for the death of three million people per year worldwide [1].

Water and soil pollution is a huge environmental problem caused by the vast amount of waste generated by human kind; in the European Union (EU) alone, around three million sites of contamination are suspected and 250,000 are known as contaminated sites which need to be cleaned up [2]. In China, it is estimated that 1×10^4 ha of land are contaminated with petroleum hydrocarbons [3]. The situation in aquatic systems is not much better; around 1.7–8.8 million metric tonnes of oil goes into aquatic environments [4] every year. Polycyclic aromatic hydrocarbons (PAHs), a key component of oil have been produced in huge amounts from anthropogenic activities such as oil refining and during incomplete fuel combustion [5], resulting in soil contamination between 1 μg/kg and 300 g/kg [6]. PAHs are of great concern since these compounds have carcinogenic and mutagenic properties and can enter the food chain because of their high persistence in the environment and their ability to bioaccumulate [7, 8].

Soils are also commonly polluted with hydrophobic man-made compounds that tend to be recalcitrant, they are called persistent organic pollutants (POPs) of which PAH are included [9]. These POPs are listed in the Stockholm convention (2004) and include aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene, polychlorinated biphenyls (PCB), DDT, dioxins, and furans. The following nine were added to the list in 2010: chlordecone, lindane, hexabromobiphenyl, pentachlorobenzene, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride (PFOS), tetrabromodiphenyl ether and pentabromodiphenylether ('commercial pentabromodiphenyl ether'), hexabromodiphenyl ether and heptabromodiphenyl ether ('commercial octabromdiphenyl ether') [10]. Many of these compounds were used for plant protection and as pest control chemicals and now are some of the most serious contaminants.

As a result of the threats to the environment from these organic contaminants, there is now an urgent need to remove them from the environment. There are a range of traditional removal techniques such as chemical oxidation, thermal treatment, and solvent washing for PAHs and PCBs; these methods are expensive (varying between \$50 and \$500 per tonne of soil) and further post-labor treatments are required. Therefore, increased attention has been paid to more economic and environmentally friendly remediation approaches such as phytoremediation. The aim of this chapter is to describe and assess the potential of phytoremediation of organic contaminants by grasses.

2 PAH and PCB Compounds

Polycyclic aromatic hydrocarbons (PAHs) are defined as a group of organic compounds, formed of two or more 2–6 aromatic rings (Fig. 1). Fossil fuel refining, timber products processing, iron and steel manufacturing, textile mills, vehicle exhausts, forest fires, and volcanoes are important sources of PAHs [11]; however, the primary source of PAHs is fuel combustion [9]. All PAHs exhibit toxicity properties; the high-molecular-weight PAHs are also potentially carcinogenic.

The 2015 ranking of PAHs in the United States Agency for Toxic Substances and Disease Registry (ATSDR) was 9 [12], while one particular PAH, benzo[*a*]pyrene, ranked 8. The US Environmental Protection Agency (US-EPA) lists 16 PAHs as

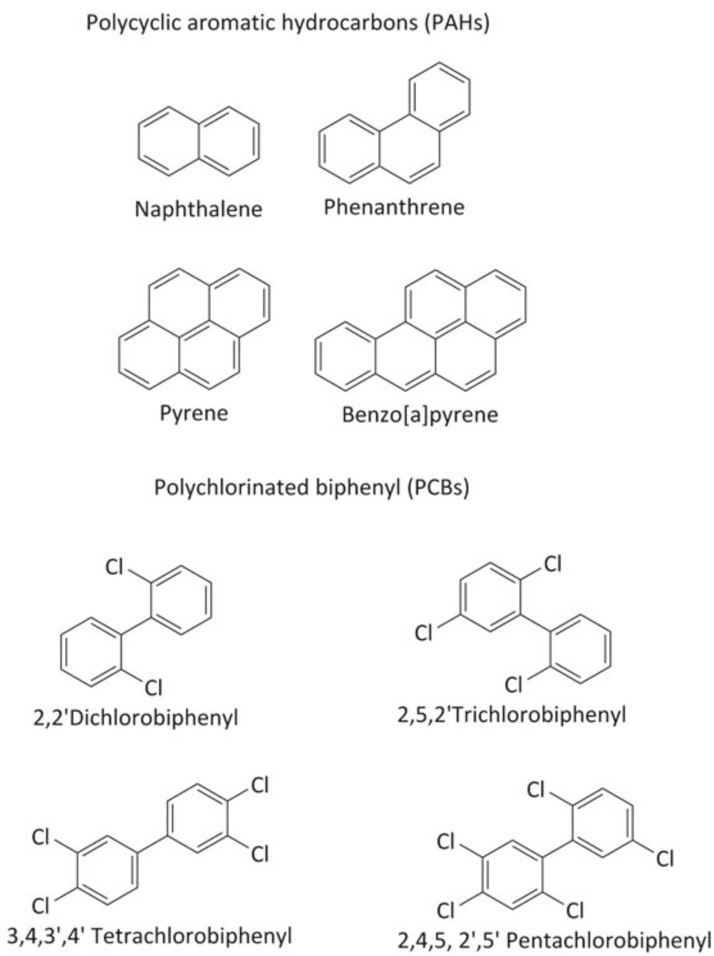

Fig. 1 Representative structure of PAHs and PCBs

priority pollutants. These 16 PAHs include naphthalene, fluorene, acenaphthene, acenaphthylene (2 ring), fluoranthene, phenanthrene, anthracene (3 ring), chrysene, pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene (4 ring), benzo[*a*]pyrene, indeno[1,2,3-*c*,*d*]pyrene, dibenzo[*a*,*h*]anthracene (5 ring), and benzo[*g*,*h*,*i*]perylene (6 ring) [13].

Polychlorinated biphenyls (PCBs) represent a major group of persistent organic pollutants (POPs), which contain 1–10 chlorine atoms and comprise 209 different congeners (Fig. 1). PCBs are widely used as coolants and lubricants in transformers, capacitors, heat exchange fluids, paint additives, carbonless copy paper, and plastic. It is estimated that about 1.5 million tons of PCBs were manufactured over 40 years between the1930s and 1970s. The usage of PCBs was banned in the late 1970s, however there is still substantial amounts of PCBs released into the environment from different sources (e.g. old electrical equipment) in conjunction with poor handling and storage, spills, and improper disposal in the past [14–16].

PCBs are classified as carcinogenic to humans. Exposure to PCBs leads to neurological, reproductive, endocrine, and cutaneous disorders. It has also been shown that PCBs are strongly linked to some metabolic diseases, including type 2 diabetes, obesity, metabolic syndrome, and non-alcoholic fatty liver disease [17]. Like PAHs, PCBs are listed as USEPA Priority Pollutants and were ranked 5 by the United States Agency for Toxic Substances and Disease Registry (ATSDR) in the year 2015 [12]. Although PAHs and PCBs are recalcitrant with low aqueous solubility, low bioavailability, and high stability in environments, both are subjected to biological degradation by bacteria, fungi, and plants. For more information regarding PAH degradation by bacteria and fungi, see articles by Bamforth and Singleton [6], Haritash and Kaushik [7], and Mougin [18]; and for a recent review for PCBs, see Passatore et al. [17].

3 Phytoremediation Technique

Phytoremediation is defined as the use of plants or associated microorganisms to remediate contaminated soils, sediments, and water [19, 20]. The term "phytoremediation" contains two words: Greek *phyto* (meaning plant) and Latin *remedium* (meaning to correct or remove an evil) [21]. This method is relatively recent and it has attracted significant attention on the basis of current research. However, like other methods, phytoremediation has both advantages and disadvantages as outlined in Table 1.

Many studies have shown phytoremediation to be very efficient for the removal of a wide range of contaminants such as metals [22–24], POPs [25–29], and hydrocarbons [30–36]. Furthermore, excellent reviews have also previously assessed the potential of phytoremediation [9, 33, 37, 38]. Phytoremediation is a general name and encompasses different techniques. These include phytoextraction, phytofiltration, phytostabilization, phytovolatilization, phytodegradation, rhizodegradation, and phytodesalination. The definition and applications of different methods of phytoremediation are presented in Table 2. It is important to note that each of these

Table 1 Advantages and disadvantages of phytoremediation of organic pollutants [90, 91]

Advantages	Disadvantages
Less disruptive to the environment (*in situ*)	Growth limitation due to environmental toxicity
No need for disposal sites	Taking a longer time than other methods
High public acceptance	Results in greater environmental damage and/or pollutant migration due to enhanced solubility of some contaminants
Avoids excavation and heavy traffic	Accumulation of pollutants in firewood
Useful for the treatment of several hazardous materials	Limited by certain climatic and geological conditions
Can be used in combination with other methods	Potential for the rerelease of pollutants to the environment during litter fall
Inexpensive and solar driven	Not successful for all pollutants

Table 2 Different approaches of phytoremediation using grasses [19, 21, 37, 92]

Phytoremediation method	Definition	Suitable contaminants	Usage of grasses
Phytoextraction	Contaminants accumulate in harvestable biomass (e.g. shoot)	Metals	
Phytofiltration	Contaminants from aquatic system are removed by plants	Metals	
Phytostabilization	Contaminants mobility and bioavailability are eliminated or reduced	Metals	Recommended
Phytovolatilization	Contaminants are taken up by the plants and released into the atmosphere	Volatile organic compounds such as MTBE, methyl-tert-butyl ether	
Phytodegradation	Organic contaminants are degraded by plant enzymes inside plant tissues	Herbicides and TNT	Recommended
Rhizodegradation	Contaminants are degraded by microbes associated with plant roots	PAHs	Recommended
Phytodesalination	Surplus salt is removed from saline soils by halophytes	Salt	Recommended

methods is suitable for different types of contaminants. There is a growing number of studies showing successful phytoremediation of POPs, PAHs and other contaminants using different types of grasses [22, 39–47], and some of the studies are very recent [48–50] suggesting the use of grasses is popular and effective.

4 Advantages of Grasses Used for Phytoremediation of Organic Compounds

Plants are the main agents of phytoremediation, and selection of appropriate plants for specific contaminants is a crucial step. This is because all plants do not show the same potential for phytoremediation as a result of different morphology, physiology, genetic background and root exudates. Irrespective of the fate of organic compounds in plants, the first step in dealing with most organic contaminants by phytoremediation is through the use of plants with a fibrous root system. Grass root systems show the highest root surface area per m^3 of soil relative to other plants and can be developed up to 3 m in the soils, providing a very large surface area for microbial colonization by soil microorganisms and ample space for the interaction of contaminants and microorganisms. In addition, the genetic diversity of grasses helps them survive in unfavorable soil conditions such as contaminated soils [51]. Merkl et al. [52] performed a pre-selection of 57 native species of plants containing 18 legumes, 19 grasses, 3 sedges, and 17 other herbaceous species. The authors found that the most extensive root system belonged to some grasses and sedges.

Moreover, grasses are fast growing and cover the contaminated area quickly, preventing the leaching of contaminants from the soil. In addition, the lower maintenance cost for grasses (e.g. lower fertilizer requirements) make them good candidates for the phytoremediation of organic compounds. Whilst it can be argued that the decontamination of sites by phytoremediation requires more time compared to other methods, the interaction of plants, especially grasses with endophytic microorganisms may lead to enhanced remediation beyond that of other technologies [39, 53]. Many grasses benefit from endophytic partnerships with both bacteria and fungi. Endophytes are defined as microorganisms (mostly bacteria, fungi, and actinobacteria) which live inside the plants without showing any disease symptoms [54]. Some cool season grasses such as tall fescue, perennial ryegrass, and meadow fescue infected with a fungal endophyte (*Neotyphodium* sp.) exhibit enhanced tolerance to abiotic and biotic stress [55].

It has been shown that *Neotyphodium* endophytes may enhance the phytoremediation of metals [56–58], salt [59], and petroleum hydrocarbons [45]. Aged petroleum-contaminated soil has been shown to be effectively remediated using *Festuca arundinacea* and *Festuca pratensis* containing the endophytic fungi *Neotyphodium coenophialum* and *Neotyphodium uncinatum*. Grasses infected with endophytic fungi showed a larger percentage of degradation of the total petroleum hydrocarbons (TPH), suggesting they may be more efficient for TPH removal [45]. However, our knowledge about the effects of *Neotyphodium* fungi on the degradation of other organic contaminants (e.g. PCBs) is limited. Endophytic bacteria from grasses have also alone showed significant potential for the bioremediation of contaminants [60–62]. Extensive reviews about the benefits of endophytic bacteria in the phytoremediation of contaminants have been recently published [53, 63–65].

5 Disadvantages of Grasses Used for the Phytoremediation of Organic Compounds

Apart from the benefits of grasses in phytoremediation applications, there are some disadvantages of using grasses in phytoremediation. Unlike legumes, grasses cannot fix nitrogen and this would be a disadvantage relative to legumes in regard to phytoremediation of contaminated soil with PAHs and PCBs as many of these soils already exhibit poor nutrition status (low nitrogen).

In addition, seed dormancy, quality and lifespan of seeds, low emergence, and germinations rate represent additional disadvantages when grasses are used for phytoremediation. Merkl et al. [52] reported that native grasses showed the poorest germination rate relative to legumes in pre-screen tests for selection for use in eastern Venezuela for phytoremediation of petroleum hydrocarbons. Also, the grasses could not propagate effectively when compared to legumes. Gaskin et al. [66] screened nine perennial Australian native grasses in a soil contaminated with 60:40 diesel/oil mixture at concentrations of 1 % (w/w) and 0.5 % (w/w). Their results showed that while at least three of the grasses showed the potential for phytoremediation of hydrocarbons, seedling emergence of all grasses was low.

6 Phytoremediation of PAHs and PCBs by Grasses

Plants can absorb the organic compounds, take up, translocate or metabolize them. The fate of the organic chemicals in plants depends on some factors such as lipophilicity, expressed as octanol–water partition coefficient (log K_{ow}), acidity constant (pKa), aqueous solubility (Sw), octanol solubility (So), and concentration of the contaminants. However, overall the log K_{ow} plays the most significant role [63]. It is generally believed that compounds with log K_{ow} values between 0.5 and 3 can be taken up by plants while compounds with values higher than log K_{ow} 4 are not easily taken up by the plant root system [19]. Rhizodegradation represents the main mechanism for the phytoremediation of PAHs and PCBs as many of them generally have log $K_{ow} > 4$, suggesting that plants are incapable of uptake in significant quantities. Like other plant roots, the presence of grass roots enhances microbial activity through the release of nutrients, root exudates, and oxygen into the contaminated soil [35]. In brief, the main effects of the plant rhizosphere are:

- Enhancing bioavailability of contaminants.
- Improving soil aeration and soil quality.
- Enhancing co-metabolism and genetic induction of some functional genes involved in the degradation of contaminants.
- Increasing the population of biosurfactant-producing microorganisms.

Many review papers have shown how the plant roots can enhance microbial ability as well as increase the degradation rate of organic contaminants [15, 33, 67]. Several studies have also shown the successful degradation of PAHs in soils by grasses (Table 3). The primary work by Aprill and Sims [68] showed that when eight

Table 3 Grasses used in some phytoremediation studies of PAHs and PCBs

Grass type	Contaminants	Conditions	References
PAHs			
Eight prairie grasses	Chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a*, *h*)] anthracene	Greenhouse/controlled environments	Aprill and Sims [68]
Tall fescue (*Festuca arundinacea*)	Benzo[*a*]pyrene	Greenhouse/controlled environments	Epuri and Sorensen [69]
Prairie buffalograss (*Buchloe dactyloides*)	PAHs	Field	Qiu et al. [78]
Kleingrass (*Panicum coloratum*)			
Mixture of 12 other warm- and cool-season grasses			
Switchgrass (*Panicum virgatum*)	PAHs (from manufactured gas plant)	Greenhouse/controlled environments	Pradhan et al. [93]
Little Bluestem grass (*Schizachyrium scoparium*)			
Tall fescue (*Festuca arundinacea*)	Benzo[*a*]pyrene	Greenhouse/controlled environments	Banks et al. [70]
Slender oat grass (*Avena barbata*)	Phenanthrene	Greenhouse/controlled environments	Miya and Firestone [94]
Tall fescue (*Festuca arundinacea*)	PAHs	Greenhouse/controlled environments	Ho and Banks [95]
Tall fescue (*Festuca arundinacea*)	Phenanthrene and pyrene	Greenhouse/controlled environments	Cheema et al. [75]
Perennial rye grass (*Lolium perenne*)	Pyrene	Greenhouse/controlled environments	D'Orazio et al.[74)
Tall fescue (*Festuca arundinacea*)	Pyrene and metals	Greenhouse/controlled environments	Lu et al. [96]
Commercial pasture seed mixture, composed of annual ryegrass, legumes and vetches and *Avena strigos*	PAHs	Field	Pizarro-Tobías et al. [76]
PCBs			
Tall fescue (*Festuca arundinacea*)	Aroclor 1260	Greenhouse/controlled environments	Epuri and Sorensen [69]
Tall fescue (*Festuca arundinacea*)	Aroclor 1248	Greenhouse/controlled environments	Chekol et al. [83]
Rye grass (*Lolium multiflorum*)			
Rye grass (*Lolium multiflorum*)	Aroclor 1242	Greenhouse/controlled environments	Ding et al. [85]
Ryegrass (*Lolium perenne*)	PCBs	Greenhouse/controlled environments	Lu et al. [97]
Tall fescue (*Festuca arundinacea*)	PCBs	Greenhouse/controlled environments	Li et al. [86]
Switchgrass (*Panicum virgatum*)	PCB congeners (PCB 52, PCB 77 and PCB 153)	Greenhouse/controlled environments	Liang et al. [89]

prairie grasses were planted in soil, increased degradation of chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*)]anthracene was observed compared with the control. Epuri and Sorensen [69] reported that the planting of tall fescue in contaminated soil led to a decrease in benzo[*a*]pyrene volatilization, enhanced mineralization, and increased solvent extractability after 180 days of plant incubation.

Banks et al. [70] investigated the effects of tall fescue plants on highly adsorbed, recalcitrant benzo[*a*]pyrene degradation. The result from that study showed that the level of residual benzo[*a*]pyrene in vegetated soil was lower (44 %) than in control soils (53 %). However, the authors did not observe any difference in the bacterial community in planted and unplanted soils. Chen and Banks [71] also showed that tall fescue plants enhanced the degradation of pyrene relative to the control in a greenhouse study. The pyrene level decreased from 758 mg/kg to below detection limit after 91 d of plant incubation compared to 82 mg/kg for the unplanted control after 147 days. Phenanthrene and pyrene have been used widely as model PAHs in many studies based on grass phytoremediation [72–75]. Cheema et al. [75] performed a greenhouse experiment to investigate the impact of tall fescue in soil spiked with different concentrations of phenanthrene (11–344 mg/kg) and pyrene (15–335 mg/kg). The results showed that the presence of phenanthrene and pyrene did not affect plant biomass at lower concentrations; however, biomass reduction was observed when the concentration of PAHs was increased in the soil. The authors also observed higher microbial viable counts, water-soluble phenolic compounds, and dehydrogenase activity in planted soil compared with unvegetated soil. In terms of PAHs removal, PAHs degradation rates were higher for phenanthrene (1.88–3.19 %) and pyrene (8.85–20.69 %) compared to degradation rates in unplanted soil.

Only limited studies on the phytoremediation of PAH-contaminated soils using grasses have been conducted in the field [76–78]. Pizarro-Tobías et al. [76] applied bioremediation (*Pseudomonas putida* strains) and rhizoremediation (annual grasses) methodologies for soil restoration in a field-scale trial in a protected Mediterranean ecosystem after a controlled fire. Their results showed that the site had returned to pre-fire status after 8 months of monitoring, with PAH concentrations falling from 398 mg/kg down to 36.8 mg/kg in planted soil treatments. Like PAHs, several studies have shown the degradation of PCBs in soils planted with grasses (Table 3) relative to unplanted soils [69, 79–82]. In regard to the phytoremediation of PCBs by grasses, Epuri and Sorensen [69] evaluated the effect of tall fescue plants on Aroclor 1260 (hexachlorobiphenyl)-contaminated loamy sand during 180 day experiments. The authors found that while the tall fescue plants had no effect on hexachlorobiphenyl volatilization and soil binding, the plants increased the mineralization as well as decreased extractability of Aroclor 1260.

Reed canary grass, switch grass, tall fescue, and deer tongue were all tested, among other plants (alfalfa, flat pea, *Sericea lespedeza*) in terms of the phytoremediation of PCB-contaminated soil. Approximately 62 % removal of PCB was observed in treated soil while only around 18 % was removed from the unplanted control soils. Greatest contaminant removal for grasses was observed in alfalfa and canary grass for legumes and grasses respectively after 4 months of growth in PCB-amended soil. The biodegradation of Aroclor 1248 was influenced by the presence of plants and plant–bacterial interactions [83]. PCB-contaminated soil, specifically contaminated with Aroclor 1260 at concentrations of 90–4200 μg/g, was treated with several grasses such as *Festuca arundinaceae*, *Glycine max*, *Medicago sativa*, *Phalaris arundinacea*, *Lolium multiflorum*, *Carex normalis*, and three varieties of *Cucurbita pepo* under controlled greenhouse conditions in Canada [84]. The authors reported that varieties of *C. pepo* extracted more PCBs from the soil compared to the other plants. All plants only showed signs of stress when the concentration of PCBs was 4200 μg/g (highest tested); however, at two lower concentrations of PCBs (250 and 90 μg/g) no effect on plant heath was observed. Overall, the results indicated that the planted soil did not enhance the degradation of PCBs.

Another study evaluated the microbial communities in planted or unplanted soil with ryegrass (*Lolium multiflorum* L.) in a PCB, Aroclor 1242-contaminated soil (8 mg /kg). At the end of 90 days, the presence of plants significantly enhanced Aroclor 1242 degradation compared with soils without ryegrass [85]). Phospholipid fatty acids (PLFAs) profiling showed that the distance from the rhizosphere impacted the PLFA profiles, confirming a distance-dependent selective enrichment of competent microorganisms involved in the degradation of this PCB. Li et al. [86] used tall fescue and alfalfa alone or in combination to evaluate the phytoremediation of PCB-contaminated soil in a greenhouse experiment. The results showed that the highest removal of PCBs was found in tall fescue single plant treatment, followed by a tall fescue and alfalfa combination. The authors concluded that tall fescue on its own produced greatest biomass and could extract more PCBs from soil relative to mixed plants. However, the highest gene copies of *bphA*, *bphD.1.B*, *bphD.2.A*, and *bphD.2.A*/*B* genes (i.e. genes involved in degradation of PCBs) as well as total bacteria counts and dehydrogenase enzyme activity was observed in the tall fescue/alfalfa treatment.

7 Future Aspects

Many studies have been carried out on the phytoremediation of PAHs and PCBs using grasses, but major gaps in our knowledge remains due to the complexity of contaminants, microbes, grasses, as well as environmental factors. Therefore, further work needs to be performed and a suggestion of the future research requirements is shown in Fig. 2. The literature shows that most of the phytoremediation of PAHs and PCBs studies have been carried out in greenhouse or controlled environments (Table 3). To our knowledge, there are very few reports in the literature involving field experiments of PCBs degradation by grasses. It is obvious that many of PAH- and PCB-contaminated sites are also co-contaminated with other pollutants (e.g. metals); in addition, the climatic conditions and other soil factors are more complex in the field rather than greenhouse. To further assess the potential of the grass phytoremediation strategy, more studies need to be performed in the field. In addition, many of the studies on grass phytoremediation are based on spiking the soils with PAHs and

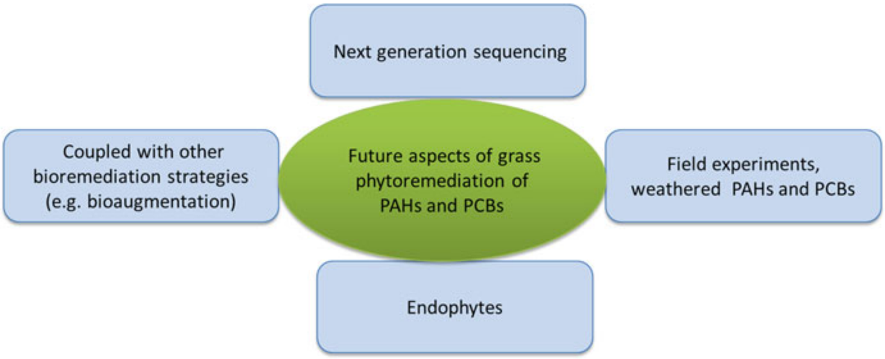

Fig. 2 Future aspects of grass phytoremediation of PAHs and PCBs

PCBs; in this case, bioavailability is not a limitation to phytoremediation. In reality, bioavailability of weathered PAHs and PCBs is one of the main limitations, since PAHs and PCBs tend to strongly attach to the soil particles. Therefore, more studies on weathered PAH- and PCB-contaminated soils are required.

Microbial communities play an important role in the degradation of both PAHs and PCBs and should be monitored during the phytoremediation of PAHs and PCBs to elucidate their microbial dynamics and to identify the microorganisms responsible for the degradation. Next-generation sequencing (NGS), metagenomics opens a new horizon to investigate different aspects of the microbial communities such as species richness and distribution as well as information on the functional genes present in the microbial communities. Furthermore, no prior knowledge of the organisms or specific genes is required in order to evaluate whole microbial communities [87]. Further studies using metagenomics can lead to a better understanding of active microorganisms involved in PAHs and PCBs degradation in the rhizosphere of grasses. As mentioned earlier, plant endophytes can represent a practical solution in the degradation of PAHs and PCBs. Some of the advantages of using endophytic bacteria include [53]:

– Endophytic bacteria are less affected by biotic and abiotic stresses than rhizosphere bacteria.
– The population of endophyte degraders is higher than rhizosphere bacteria.
– Genetic manipulation (genetic engineering) of endophytic bacteria is much easier than plants (in this case, grasses) where genetic engineering of PAHs and PCBs degradation pathways is needed.
– Symbiosis of endophytic PAH and PCB degraders with grasses leads to the degradation of contaminants inside the plants, resulting in reduced toxicity to other organisms and any subsequent biomagnification.
– Many endophytes contribute to enhanced plant growth, resulting in increased stress resistance of plants to contaminants such as PAHs and PCBs.

Our current understanding of the role of endophytic bacteria in grasses used for the phytoremediation of PAHs and PCBs is still incomplete. Only a few endophytic bacteria involved in degradation of PAHs and PCBs have been isolated and investigated. Recently Khan et al. [88] isolated an endophytic bacteria (*Pseudomonas putida*, PD1) from poplar which showed phenanthrene degrading ability when inoculated in willow and perennial ryegrass. The results of this study showed that the presence of PD1 not only increased (by 25–40 %) the removal rate of phenanthrene by willow and grasses but also the PD1 strain promoted root and shoot growth.

Toxicity associated with PAHs and PCBs is the main constraint to grass phytoremediation. Therefore, phytoremediation is not always successful; consequently, a combination of other bioremediation methods can represent long-term solutions in PAH- and PCB-contaminated soils. In one study, polychlorinated biphenyl (PCB) congeners (PCB 52, 77, and 153) were subjected to switch grass phytoremediation and bioaugmentation with *Burkholderia xenovorans* LB400Y [89]. The results showed that total PCB removal was greatest, with an average of 47.3 % in switch grass/LB400Y-treated soil. In addition, the presence of switch grass supported LB400Y survival in the soil. The authors concluded that the use of phytoremediation in conjunction with bioaugmentation might represent a sustainable approach to eliminate or degrade recalcitrant PCB congeners in soils. Our understanding about the interaction of grass with other microorganisms is not clear and remains to be elucidated in future studies.

In conclusion, grass phytoremediation is a promising, cost-effective, and environmental-friendly strategy to degrade or remediate PAH- and PCB-contaminated soils. However, most of the results reported to date have been obtained from the phytoremediation of PAHs and PCBs by grasses in greenhouses or spiked soil. There is now an urgent need to move to field studies. Is grass phytoremediation going to be a successful strategy in the real PAH- and PCB-contaminated environments? The answer to this question will be addressed through the application of field studies and the development of new molecular microbial and environmental analytical techniques.

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Organic Soil Amendments in the Phytoremediation Process

Anna Grobelak

Abstract Land application of biosolids, such as sewage sludge or compost, has a great incentive in view of its fertilizer and soil amendment values, unless they contain toxic elements. The heterogeneous nature of biosolids produced in different processes necessitates knowledge of the chemical and biological properties of biosolids prior to the land application. Plant wastes are being increasingly used to produce compost, which is an important amendment to improve the properties of degraded soils. Some soil amendments can be used directly for the remediation of degraded areas and to fertilize the soil. One of the challenges of environment management is connection in usage as many resources towards achieving maximum benefit with minimum damage to the environment and even with achieving the improvement of the soil conditions. The biomass, land, and wastes are extremely important resources in the green economy. The biomass becomes an increasingly important raw material that can be produced using a wide group of wastes and by-products during the soil reclamation process. The main objective of this study was to estimate the effectiveness of the conjugation of three processes: waste, land, and biomass management. The pot and field studies were conducted on degraded area, using by-products and organic waste, in order to achieve soil phytoremediation effect. The study was conducted using biosolids, e.g., compost from municipal sewage sludge, sewage sludge, and lacustrine chalk and two plants species, for wood biomass—pine (*Pinus sylvestris* L.) and for green biomass as energy crops giant miscanthus (*Miscanthus giganteus*).

Keywords Biosolids • Phytoremediation • Soil amendments • Trace elements • Soil reclamation • Compost • Sewage sludge

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1 Introduction

The organic soil amendments due to their high variability in chemical, physical, and sanitary parameters must be subjected to appropriate examination before their use. Introducing the organic additives into the soil can result in decreased mobility of heavy metals [1, 2]. Organic additives, obtained mainly from plants, are characterized by great variation in the content of biogenic elements such as nitrogen, phosphorus, and potassium. They contain large amounts of carbon and other elements, which are part of the main organic substances. The quality of plant substrate for the production of compost is influenced by: the content of organic material and fertilizers and the ratio of carbon to nitrogen. Sawdust, wood chips, bark, straw, plant wastes, and food waste from households are commonly used to produce compost [3]. Sewage sludge must also be seen as a valuable organic fertilizer. Sewage sludge contains trace elements and easily degradable organic substances. Sewage sludge contains also important nutrients such as nitrogen and significant amounts of phosphorus but low amount of potassium [4].

The use of sewage sludge in reclamation processes contributes to the possibility of valuable elements recovery, for example, nitrogen, phosphorus, and other nutrients which are important for plant growth [5]. The contents of individual components in sewage sludge result from the processes of wastewater treatment and the composition of influent [6]. The use of sewage sludge for fertilization and remediation is associated with certain limitations, which are caused by, e.g., the presence of hazardous substances, microorganisms in sewage sludge, and pathogenic micropollutants and undesirable odor. Despite such limitations, sewage sludge improves soil structure by the generation of large amounts of humus. For this reason, sewage sludge plays an important role in the phytoremediation. In the heavy metal binding process, both inorganic substances (sulfides, phosphates, hydroxides and oxides of noncrystalline iron and aluminum, and manganese) and organic substances (living microorganisms, organic and mineral remains of dead organic compounds) are involved [7].

Mechanisms playing a significant role in the binding of heavy metals in sewage sludge and composts are: ion exchange, precipitation and co-precipitation reactions, and adsorption of contaminants on the outer and inner surface of minerals [8]. The sewage sludge and composts that contain even a small content of heavy metals has a positive effect on the growth of the microbial biomass and microorganisms present in the soil [9]. Composts and sewage sludge used for the restoration of degraded land are involved in the processes such as: chemophytostabilization (the formation of stable metal salts), immobilization (immobilization of metals with functional groups of fulvic and humic acids which have available negatively charged loads), phytoremediation (phytoextraction and phytostabilization), bioaugmentation (application into the environment of some microorganisms), and biostimulation (addition of nutrients to stimulate the activity of soil microflora) [10, 11]. Composts, containing sewage sludge from municipal wastewater sources, may contain excessive amounts of heavy metals, such as Sn, Zn, Cd, Pb, Mn, Fe, Co, and Si, which in high concentrations can be toxic. In this case, the use of sewage sludge is not suitable for reclamation of degraded areas. Too much toxic elements in sewage may be leached into groundwater and surface water, creating threats for the whole ecosystem [12].

2 Sewage Sludge and Compost Soil Application: The Laws and Regulations of the European Union

The ever-growing need to protect the environment from degradation requires rationalization of sewage sludge management. The legislation of the European Union concerning the disposal of sewage waste is included in the Council Directive 86/278/EEC on environmental protection of 12 June 1986 (the so-called Sludge Directive). The 2000/60/EC of the European Parliament and Council of Europe of 23 October 2000 sets the norms of joint Community action in the field of Water Policy (Official Journal EC L 327 of 22 December 2000). The Water Framework Directive (WFP) defines sludge not as waste material, but as a “product” of sewage treatment.

The operational directive of the Water Framework Directive is the Directive 91/271/EEC of 21 May 1991 concerning the treatment of municipal sewage. The Directive obliges to monitor and report municipal sewage treatment and final disposal of municipal sewage sludge for agglomerations. Article 14 of Council Directive 91/271/EEC refers to sludge produced in course of sewage treatment and states that sewage has to be reused in every appropriate case, provided that adverse effects to the environment are prevented at all times. Implementation of this Operational Directive by the end of 2015 will increase the stream of sewage sludge, but on the other hand it will enable other methods of sludge reuse. Limits regarding storage of sludge are introduced by Directive 99/31/EC of 26 April 1999 on sludge storage, called the Landfill Directive.

Sewage sludge is subject to European Parliament and Council Directive 2008/98/EC of 19 November 2008 on sewage that is the Waste Framework Directive which regulates waste recycling including sewage sludge. According to the above-mentioned Directive, sludge defined as waste is subject to the procedure assigned for waste treatment. The Directive states that prevention of waste production is the first priority, the next being preparation of waste for reuse, recycling, or other forms of recovery and finally waste disposal. It is not possible to prevent the production of sewage waste. That is why other steps of dealing with waste are very important, that is preparation for reuse, understood as sludge reprocessing (including possible energy recovery or organic recycling).

Directive 2010/75/EC of 24 November 2010 on industrial emission officially updates and combines other directives, including Directive 2008/1/EC on integrated prevention of pollution and its control (IPPC), Directive 2001/80/EC on reduction of air pollutants emission from LCP’s another document, the Technical Report for End-of-Waste Criteria on Biodegradable Waste Subject to Biological treatment—Third Working Document (September 2012) places sewage sludge on the positive waste list and allows clean sludge to be used as fertilizer and gives way to qualify it as a waste product. The use of organic wastes as amendments to improve soil organic matter level and long-term soil fertility and productivity is gaining importance. The disposal of the large quantity of organic wastes produced by the municipal, agricultural, and agro-industrial activities is causing energetic, economic, and environmental impacts. Sewage sludge composting process for use in agriculture should be given a priority for its disposal. These organic amendments should not be treated as a waste but a valuable non-farm source of organic matter to soil. The composting process is a useful method of producing organic matter that can be used as a source of nutrients and soil amendments. Land application of sewage sludge has a great incentive in view of its fertilizer and soil amendment values, unless it contains toxic elements. The heterogeneous composition of biosolids produced in different wastewater treatment plants requires the chemical and biological investigation prior to land application [13, 14].

For the legal issues, in Europe the sludge (in agriculture) directive (86/278/CEE), the landfill directive (99/31/UE), and the waste incineration directive (CEC, 2000) are relevant to the fate of biosolids. Other relevant instruments are the urban wastewater treatment directive, nitrates directive, water framework directive, and the hazardous substances regulations that have controlled the production and use of substances such as PCBs and brominated flame retardants. These have affected the quantity and quality of biosolids. For example, by harmonizing requirements for phosphate removal during wastewater treatment, the urban wastewater treatment directive has increased the quantity of sludge produced and also increased phosphate content of sewage sludge. Regulations have reduced the concentrations in biosolids of the substances they regulate. The portal to EU legislation is available at http://eur-lex.europa.eu/en/index.htm.

3 Impact of Compost Initial Conditions on the Phytoremediation Process of Contaminated Soils

Compost is considered a multifunctional soil improver. It is, therefore, used in agriculture and horticulture as well as in producing topsoil for landscaping or land restoration. The application of compost usually improves the physical, biological, and chemical properties of soil. Repeated application of compost leads to an increase in soil organic matter, it often helps to reduce erosion, it increases the water retention capacity and pH buffer capacity, and it improves the physical structure of soil (aggregate stability, density, pore size). Composts may also improve the biological activity of the soil. Compost is often considered an organic fertilizer although the fertilizer function of compost (supply of nutrients) is, in many cases, less pronounced than the general soil improvement function [15–18]. The presence of heavy metals in the environment, and in areas used for agriculture, is an important issue for environmental concerns. Trace elements can be included in the food chain and biological circulation. A characteristic feature of heavy metals is also their capability to remain in the environment for long period of time, as well as resistance to chemical and biological degradation [19]. For this reason, it should be assured that potential soil biosolids contain as little heavy metal as possible [20]. The second issue is the problem of large areas, mainly post industrial, that are contaminated with heavy metals [11].

One of the methods for remediation of such areas is phytoremediation technique, which involves the plants for the treatment of soil from organic and inorganic contaminants (e.g., heavy metals). Phytoremediation method is economically justified, least environmentally invasive and generally acceptable by society. An additional advantage of phytoremediation of soils is the possibility of using waste and by-products (e.g., sewage sludge, composts, organic wastes, organic fertilizers, coal mules, lacustrine chalk) as soil additives to support this process and at the same time to affect the recycling and disposal of a significant amount of waste substances. Also the large areas of post-mining land like lignite mine dumping sites are characterized with poor organic and biogenic compounds. Soil organic additives are a source of organic matter, carbon, and biogenic elements which provide a significant improvement in the quality of the soil environment. Moreover, they have the sorption ability related to immobilization of contaminants in soil, e.g., heavy metals.

4 Case Study: Comparison of Composts for Remediation Purposes

Selected composts based on the organic wastes were prepared (Table 1) and tested for their suitability as a biosolid for improved phytoremediation. Compost samples were analyzed (Tables 2 and 3) for their chemical composition, dry matter content, organic carbon, total Kjeldahl nitrogen, total P, heavy metals, *Salmonella* spp. according to standard methods [14].

During the composting process, organic matter is decomposed and transformed to stable humic compounds. Humic substances have a capacity to interact with metal ions and the ability to buffer pH and to act as a potential source of nutrients for plants. The heavy metal concentration in the final product deserves consideration

Table 1 Composition of composts I–VI

Compost	Municipal sewage sludge (%)	Bulking agent (%)	Green plants (%)	Organic fraction of municipal solid wastes (%)	Organic home wastes (%)
I	65	5			30
II vermicompost	65	5			30
III	81	5		14	
IV vermicompost	81	5		14	
V	40	5	55 rape		
VI	40	5	55 grass		

Table 2 Composts physical and chemical parameters of different composts I–VI

Parameter	I	II	III	IV	V	VI
Organic matter [%]	65.7 ± 2.3	72.6 ± 2.3	74.9 ± 2.9	71.1 ± 2.7	65 ± 6.0	75 ± 4.2
C [% d.m.]	31.1 ± 0.8	33.3 ± 0.6	30.1 ± 0.9	26.7 ± 0.8	29.5 ± 1.3	44 ± 2.1
Kjeldahl N [% d.m.]	1.4 ± 0.06	1.7 ± 0.0	2.1 ± 0.1	1.9 ± 0.05	1.3 ± 0.1	1.4 ± 0.02
P [% d.m.]	2.21 ± 0.0	2.43 ± 0.0	1.8 ± 0.0	1.65 ± 0.0	1.7 ± 0.52	0.18 ± 0.01
K [% d.m.]	5.24 ± 0.4	5.71 ± 0.5	7.39 ± 0.7	4.99 ± 0.4	4.32 ± 0.3	1.05 ± 0.1
C/N ratio	22.2 ± 0.8	19.6 ± 0.9	14.3 ± 0.8	14.05 ± 0.5	22.68 ± 2	31.42 ± 1

Table 3 The heavy metals concentration in the composts I–VI

Metal [mg/kg d.m.]	Limit[a]	I	II	III	IV	V	VI
Cd	5	2.0 ± 0.2	1.4 ± 0.15	1.6 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	0.49 ± 0.1
Cr	100	17.9 ± 1.8	15.5 ± 1.6	13.5 ± 1.4	22.3 ± 2.2	55 ± 3.1	4. 9 ± 0.8
Ni	60	5.3 ± 0.6	5.5 ± 0.6	4.5 ± 0.5	7.2 ± 0.7	19.51 ± 1.3	2 ± 0.20
Pb	140	20.8 ± 2.1	22.5 ± 2.3	26.9 ± 3.1	25.1 ± 2.5	20.29 ± 2.10	5.14 ± 0.3
Hg	2	0.30 ± 0.001	0.20 ± 0.001	0.10 ± 0.001	0.10 ± 0.001	0.20 ± 0.01	0.10 ± 0.001

[a]Limits imposed by Polish regulations for soil organic amendment

since they may enter the food chain when the biosolids are applied on land. The obtained results show (Table 3) that the concentrations of heavy metals in all composts were within the limits of regulation for soil organic amendments. One of the issues encountered by the direct use of composted sewage sludge and organic fraction of municipal solid waste in agriculture is the risk of plant and human contamination by pathogens in addition to heavy metals. During the composting process, *Salmonella* and a number of live helminth eggs were removed from the final product, what confirms that composting is a proper method for biosolids production and the method of disposal of organic waste. It was found that composting process of different wastes resulted in obtaining a valuable source of organic amendment, which can be safely used for remediation purposes.

5 Trees in the Process of Phytoremediation of Degraded Areas

Phytoremediation is a green technology to remove environmental pollutants. Of the growing interest in the processes of phytoremediation of contaminated sites are perennial plants with high biomass, i.e., trees. Their extensive and deep root system enables purification of ground–water environment and the possibility of reaching the roots in a place inaccessible to smaller green plants. In addition, these plants are characterized by high resistance to adverse environmental conditions such as lack of nutrients and water scarcity. Trees used in phytoremediation process should have a resistance to high levels of toxic substances, high capacity for the collection and storage of pollutants and should produce a large amount of biomass [21]. Trees such as poplar, pine, or spruce are species that have the ability to actively respond to the presence of high concentrations of heavy metals in contaminated soil. Due to exudates release to the soil in rhizosphere layer (organic acids), roots are able to extract the trace elements to the roots and to aboveground parts of plants.

A more effective method of metal detoxification is their binding in the roots until reaching a maximum concentration. The most popular trees exhibiting a high capacity to accumulate heavy metals are: silver birch (*Betula pendula*), alder (*Alnus tenuifolia*), black locust (*Robinia pseudoacacia*), willow (*Salix* sp.), and conifer trees [22]. Pine (*Pinus sylvestris* L.) is an important element in the whole forest ecosystem with a very wide range of prevalence in the world. It is one of the most important tree species in Central Europe and Scandinavia. These species, take up nutrients including heavy metals, from soil. They are used for afforestation of areas and in the process of phytoremediation of soils contaminated with heavy metals. The heavy metals are stored mainly in the root system. The trees development over the years has secured them against the loss of genotypes responsible for tolerance to high concentrations of pollutants, and slower adaptation of selected species on degraded areas. A characteristic feature of heavily degraded areas is the lack of any shrubs and trees.

However, the immune mechanism created by the selected species of trees allow for their development on such areas without toxic symptoms [23]. The key mechanism used in phytoremediation is the limited ability of trees to capture and take up the pollutants from the root zone, and then the slow transport to upper plant parts. An important feature of these trees is their ability to propagate roots into soil layers which contain a lower amount of pollutants, thus increasing the chances of their survival under unfavorable conditions [24]. The tree species differ in the ability to immobilize the heavy metal in the cells, tissues, and organs. A method for binding metals depends not only on the age of the trees, environmental conditions, but also on the properties of the trace elements. The bioconcentration index of heavy metals by trees is diverse, often they do not demonstrate the ability to take up metals by the roots of trees (the index is low, the tree is not capable of accumulating contaminants). On strongly contaminated soils, the roots of trees cannot cope with high levels of heavy metals and toxic substances. As a result, excess contaminant is accumulated in the organs and tissues of trees, showing toxic effects [23, 25].

6 The Use of Selected Organic Amendments for Improved Phytoremediation

6.1 Experiment Description

For improved phytoremediation process, a growth chamber study was conducted using organic soil amendments under controlled conditions of phytotron chamber. Soil material used in the study was collected from two sites (Poland) (Table 4): from

Table 4 The physical and chemical properties of soil materials (modified after Dusza [33])

Parameter	Soil material from zinc smelter area (MS)	Soil material from lignite mine dumping site (B)
pH in H_2O	5.49 ± 0.02	8.11 ± 0.04
CEC [cmol(+) kg^{-1} d.m.]	3.18 ± 0.12	23.93 ± 0.21
C total [g kg^{-1} d.m.]	12.91 ± 0.02	4.05 ± 0. 05
N Kjeldhal [mg kg^{-1} d.m.]	577.50 ± 18.12	108.50 ± 11
P total [mg kg^{-1} d.m.]	176.55 ± 1.34	132.16 ± 1.11
Zn [mg kg^{-1} d.m.]	751.60 ± 57.49	15.22 ± 0.12
Cd [mg kg^{-1} d.m.]	28.78 ± 1.23	0.38 ± 0.01
Pb [mg kg^{-1} d.m.]	1696.20 ± 87.13	4.84 ± 0.08

the area of zinc smelter influence located in Silesia region and also from lignite mine dumping site near Belchatow. In this experiment, (1-year-old seedlings of *Pinus sylvestris* were used. The amendments used in the study were different types of organic amendments: compost (K), sewage sludge (OS). The following parameters were investigated for the soil subsamples collected : pH in H_2O and in KCl suspension, total Kjeldahl N, total carbon, total and available P, CEC (Cation Exchange Capacity), available heavy metals, total heavy metals using inductively coupled plasma/optical emission spectrometry (ICP-OES; Thermo apparatus). Both at the beginning of the experiment and after a year (12 months, $t = 1$), the analysis of physical, chemical parameters and changes in the content of elements and compounds in the soil material in all the pots was done.

The resulting biomass during the experiment was collected, dried, ground, and treated with digestion, and then the elemental analysis was performed. Plants aboveground biomass was harvested after 12 months of growth and also analyzed for the content of elements. Tested soil (Table 4) obtained from the area near zinc smelter (MS) was characterized by a high concentration of heavy metals (mainly Cd, Pb, and Zn), low pH, low sorption capacity, and low levels of organic matter and trace nutrients, i.e., N and P. It is a sandy soil, anthropogenically altered by the operation of the zinc smelter. The area of the lignite mine (B) is mainly podsol. Soil material from the lignite mine dumping site was characterized by a lack of soil profile (soil material is selected during operation of the mine from different deposits), alkaline pH, low humidity, low concentration of nutrients, and low concentrations of heavy metals.

Sewage sludge (OS) used in the study originated from an anaerobic co-digestion process. The compost (K) used in the experiment was obtained from the processing of municipal waste, which include: sewage sludge from anaerobic co-digestion process, plant biomass (grasses and rape grown on degraded soil), sawdust, and the organic fraction of municipal waste (OFMW). The composting process was conducted in a bioreactor according to standard procedure. Sewage sludge and municipal compost used in the pot experiment were characterized by a relatively neutral pH, relatively large hydration and the high content of nutrients (N and P) and carbon (Table 5) and additionally low concentrations of heavy metals. Due to the lower content of selected metallic elements, they fulfill the low criteria and can therefore be used in the restoration of degraded land and agriculture.

Table 5 The physical and chemical properties of organic amendments (modified after Dusza [33])

Parameter	Sewage sludge (OS)	Compost (K)
pH in H_2O	6.07 ± 0.01	6.86 ± 0.14
C total [g kg^{-1} d.m.]	401.1 ± 0.4	295.05 ± 6.15
N Kjeldhal [mg kg^{-1} d.m.]	4025 ± 247.49	13,545 ± 148.49
P total [mg kg^{-1} d.m.]	5197.39 ± 15.56	5207.29 ± 15.24
Cd [mg kg^{-1} d.m.]	0.6613 ± 0.03	1.443 ± 0.85
Pb [mg kg^{-1} d.m.]	6.249 ± 0.28	40.29 ± 2.93
Zn [mg kg^{-1} d.m.]	259.8 ± 11.24	453.2 ± 14.17

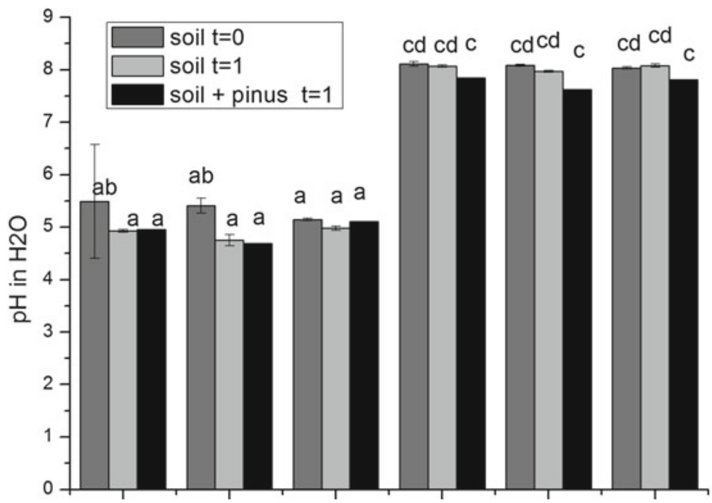

Fig. 1 Changes of soil pH value during the experiment (modified after Dusza [33])

6.2 *Results*

6.2.1 Changes of the Parameters of Soil Material and Plants Biomass

In the experiment, the pH value was determined in soil both at the beginning ($t=0$) of the experiment and after 12 months ($t=1$) (Fig. 1).

The soil material obtained in the affected zone located in zinc smelter (MS) was characterized by acidity and soil originating from the lignite mine dumping site (B) characterized by an alkaline pH. In both types of soils, similar trend of a slight decrease in pH after the application of sewage sludge was observed. The compost applied to metal contaminated soil (MS) resulted in halting the decline of pH. For every treatment, the vegetation of a Scots pine to soil contributed to lower pH of only lignite mine soil, due to root exudates of trees, i.e., organic acids. Decrease of soil pH after sewage sludge application is very slight and only marginally reduces this value, what was also confirmed by Ahmed et al. [26]. More pronounced effect was noted after Scots pine growth for alkaline soil. Another important parameter is sorption capacity, which is an important indicator for assessing the soil degradation. Some organic amendments like biochar can increase the sorption capacity of soil significantly [27].

Moreover, another advantage of biochar application is that unlike compost and manure, biochar does not need to be applied repeatedly. Usually, the soil organic matter, pH, phosphorus, and CEC generally increase after treatment with biochar, what confirms that fairly large amounts of carbon and exchangeable cations are introduced by biochar application. The main disadvantage of biochar is the energy input that is necessary to convert the biomass into biochar [28]. The less energy-consuming process is using organic amendments like compost, vermicompost, or sewage sludge directly into soil. It was found that the used organic additives significantly contributed to the increased sorption capacity of the soil from the area of lignite mine dumping site at the beginning of the experiment. However, after half a year, this effect was not observed.

Moreover, there was no effect on sorption capacity increase for soil which was strongly contaminated and with low pH. In the conducted field study, this parameter remained very low even after biosolids addition. The carbon content in the soil proves the soil condition and the ability to proper growth and development of plants. In the conducted study (Fig. 2), there was an increase in the total carbon content after the addition of compost and sewage sludge into the soil from the area of zinc smelter, as well as in the soil from lignite mine dumping site. The growth of pine caused a decrease in the content of total carbon only in soil with low pH.

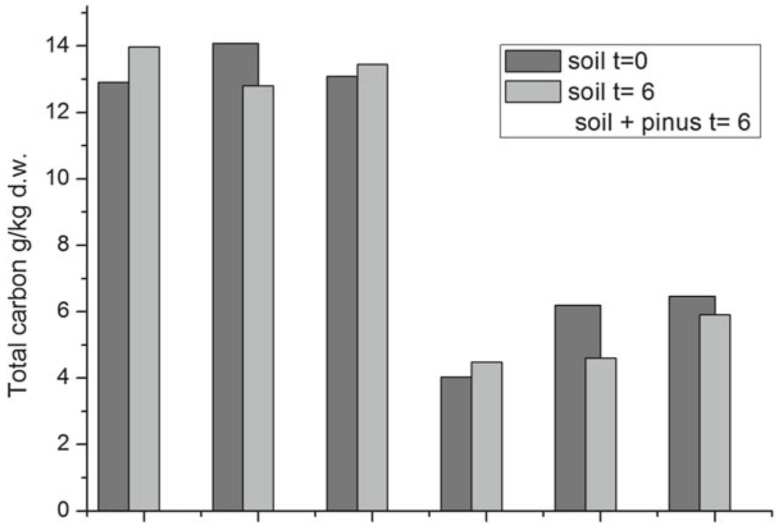

Fig. 2 The impact of organic additives and pine trees on the total carbon content in two types of degraded soils; B—lignite mine dumping site, MS—zinc smelter area, K—compost, OS—sewage sludge

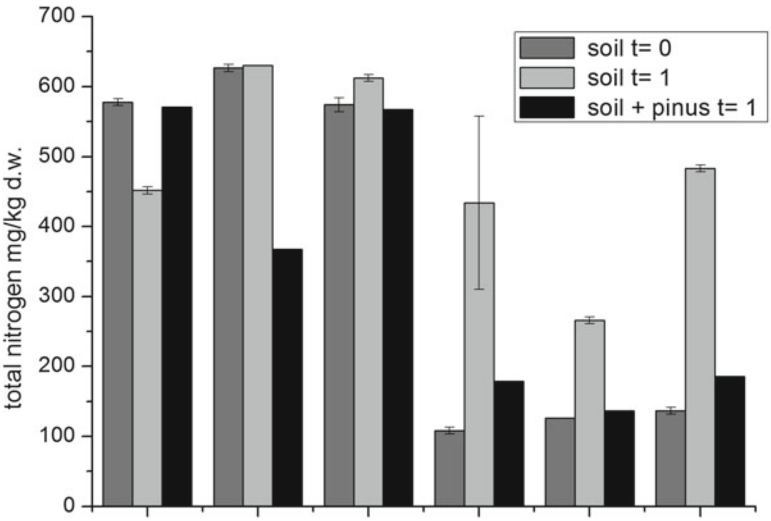

Fig. 3 The **e**ffect of organic additives and pine trees on the change of the total nitrogen content in two types of degraded soils; B—lignite mine dumping site, MS—zinc smelter area, K—compost, OS—sewage sludge

The nitrogen concentration is a very important indicator of soil fertility and crop yield. Based on the amount of the element in the soil material, plant growth, and development in the certain area, the effectiveness of the biological remediation can be estimated. The changes in the level of nitrogen in the soil are included in Fig. 3.

The use of organic additives caused an increase in nitrogen content in both investigated soils (Fig. 3). After 12 months of the experiment, nitrogen content in the soil increased in both degraded soils and amended with biosolids. However, in soils with pine, nitrogen decreased in both treatments with soil alone and with additions of biosolids. Probably, this is the result of extensive leaching processes of strongly acidic soils as also indicated by Suárez-Abelenda et al. [29]. Phosphorus performs a key role in plant growth, and therefore its contents in the soil have a significant impact on the efficiency of phytoremediation processes of degraded soils. Changes of total phosphorus content in the soil occurring during experiment are shown in Fig. 4.

At the beginning of the experiment, low levels of phosphorus were reported in soils from both sites of degraded areas. However, the amount of this element in the soil slightly increased after the application of organic additives (Fig. 4). After half a year of pot experiment, the increase in the concentration of this element in the soils with the addition of organic matter was observed. Also, the amount of phosphorus in the soil with additives and with plants increased compared with the control. Another significant parameter is the soil organic matter content presented in Fig. 5.

It was found that organic matter content was highest for the samples with the addition of compost. After a year of experiment, the highest recorded organic matter content in the soil from MS with sewage sludge was recorded. Organic substances were used in the pot experiment in order to supply organic matter and biogenic

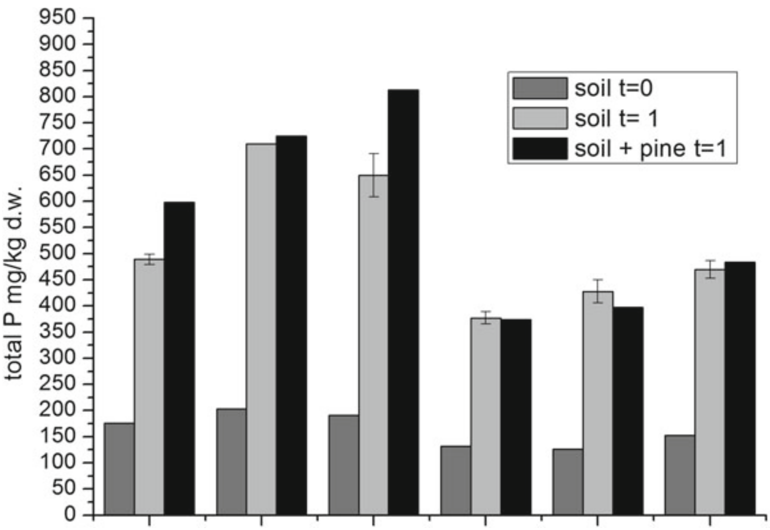

Fig. 4 The effect of organic additives and plants on the changes of the total phosphorus content in two types of degraded soils; B—lignite mine dumping site, MS—zinc smelter area, K—compost, OS—sewage sludge

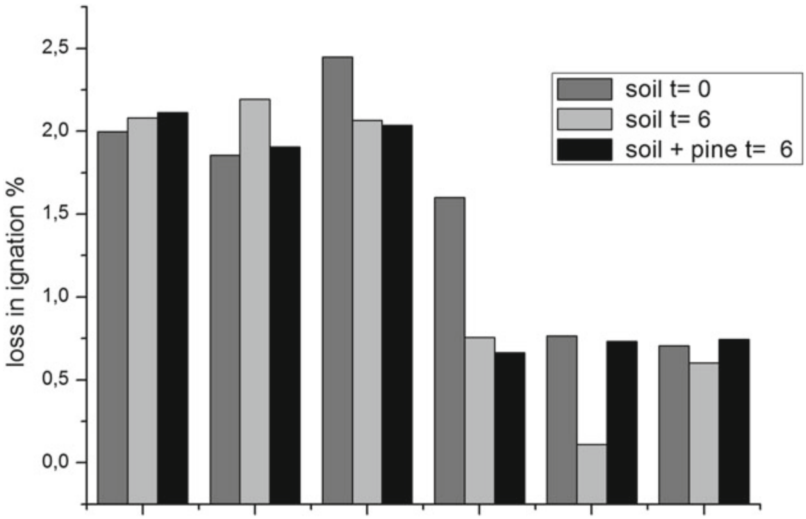

Fig. 5 The effect of organic additives and plants on the changes of the content of organic matter (expressed as losses on ignition) in two types of degraded soils; B—lignite mine dumping site, MS—zinc smelter area, K—compost, OS—sewage sludge

elements to the soil, limit the mobility of heavy metals and improve its water conditions. To support the phyto-sequestration process of soils, another five organic additives were used: the sewage sludge from the food industry, compost from the biodegradable fraction of municipal waste, compost from sewage sludge from household wastewater treatment plant, coal sludge, and lacustrine chalk. The experiment was carried out in the growth chamber for 12 months. This chapter focuses on the analysis of carbon and organic matter, including TOC (total organic carbon) OC (organic carbon) humic acids, in pore water and soil after different treatments. Studies have indicated that composts and coal mules were characterized by a similar content of organic carbon and total carbon.

By contrast, lacustrine chalk and sewage sludge contained much lower content of organic carbon than the total. In the experiment, an increase in OC content in combination with composts, coal mules, and plants were noted. However, there was a decrease of TOC in the soil after application of sewage sludge on the acidic soil. Whereas for compost used in acid soil, a high content of TOC after a year of research was still remained. For the neutral soil, this effect was not observed, on the contrary, the used additives resulted in higher total carbon content of soil. TOC analysis in pore water showed decreasing releasing of carbon into the soil solution. Furthermore, the lowest amount of carbon in the mobile form was observed after application of coal mules. Humic acids content after the application of additives was significantly higher compared to the control samples. However, after one year humic acids content decreased slightly in all combinations (also in controls) in the acidified soil.

Moreover, in the neutral soil humic acids content increased after one year of the experiment. Entering the organic additives, i.e., compost and sewage sludge are not justified on acidic soils because they do not cause permanent increase in the content of OC in these soils. However, such action is justified on neutral soil. The highest concentration of OC in the soil provides composts and coal mules. Lacustrine chalk and coal mules can be successfully used on acidic soils. For neutral soil, the content of exogenous carbon increases with time. In acidic soil, the organic matter content just after the application of additives increases, however 1 year after its content decreases. Additionally, acidic soils are not retaining introduced exogenous humic acids. The addition of compost compared to the sewage sludge results in higher retention of total carbon in the soil.

The content of available forms of heavy metals was examined, and the results are presented in Figs. 6 and 7. It was found that shortly after the application of organic additives ($t = 0$), an increase in the content of bioavailable Zn and Pb occurred. However, the bioavailable Pb decreased after the application of sewage sludge. In contrast, organic amendments did not affect the content of bioavailable Cd.

During the pot experiment, the content of available heavy metals in soils with Scots pine was determined. Content of available Zn, Pb, and Cd in the soil decreased significantly for all treatments (Fig. 7). This effect confirms the strong leaching process for acidic soils. The effect of immobilization process by organic amendments is dominated by leaching process in this study conditions. Fertilization of heavy metals contaminated soil with organic wastes and the pine vegetation contributed to the immobilization of metal elements in the soil and the rhizosphere and caused the transfer of some metal parts to the aboveground parts of plants (Fig. 8). The applied organic additives resulted in a reduction of Pb and Zn in plant biomass (Fig. 8). The applied sewage sludge caused a slight reduction of Ag, Co, Cr, Cu, Ni, and Tl in plant biomass. Compost influenced the reduction of Cd and Ni content slightly. The lowest Cd and Ni content were in plants fertilized with sewage sludge (Fig. 9).

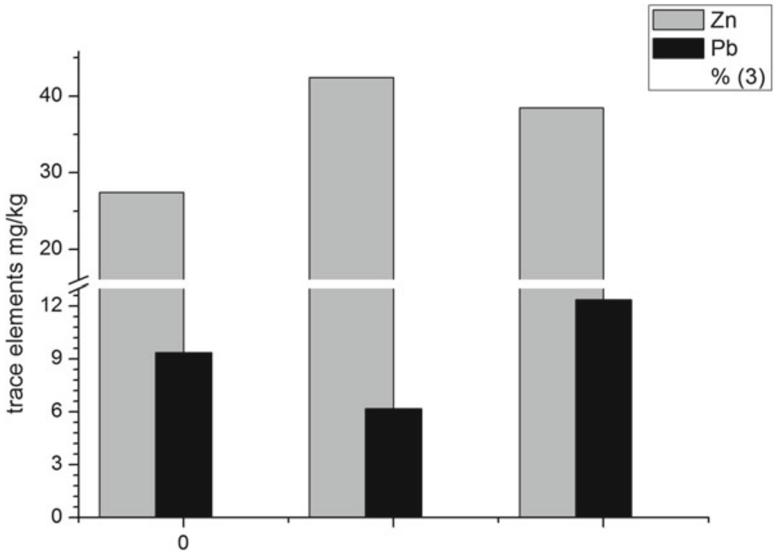

Fig. 6 The influence of organic additives on the content of trace elements (bioavailable) in the soil from the zinc smelter area for $t=0$; MS—zinc smelter area, K—compost, OS—sewage sludge

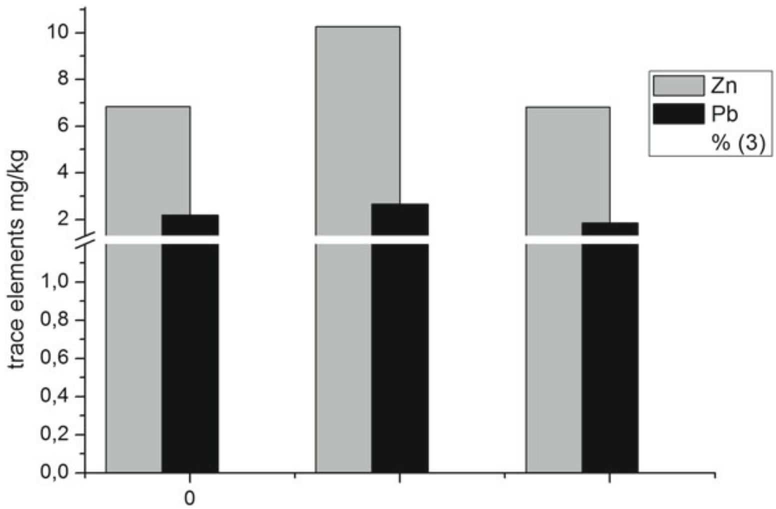

Fig. 7 The influence of organic additives on the change of the content of trace elements (bioavailable) in the soil with pine trees from the zinc smelter area for $t=1$; MS—zinc smelter area, K—compost, OS—sewage sludge

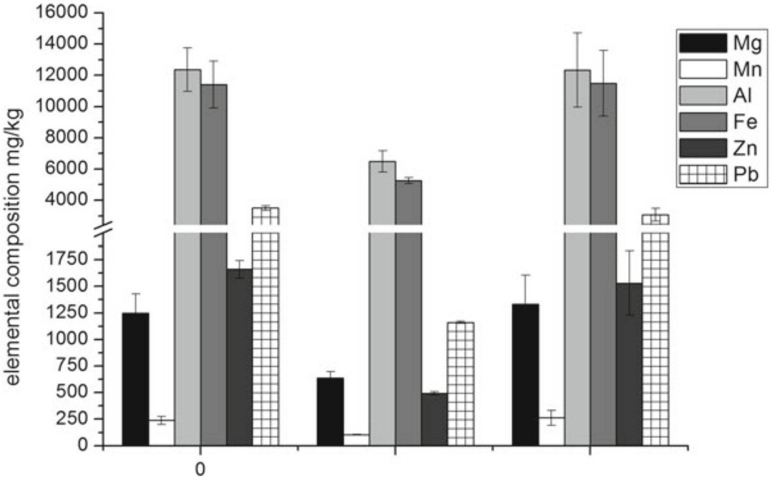

Fig. 8 The impact of organic additives on the metal content in the biomass of Scots pine on degraded soil from the zinc smelter area; $t=1$; MS—zinc smelter area, K—compost, OS—sewage sludge

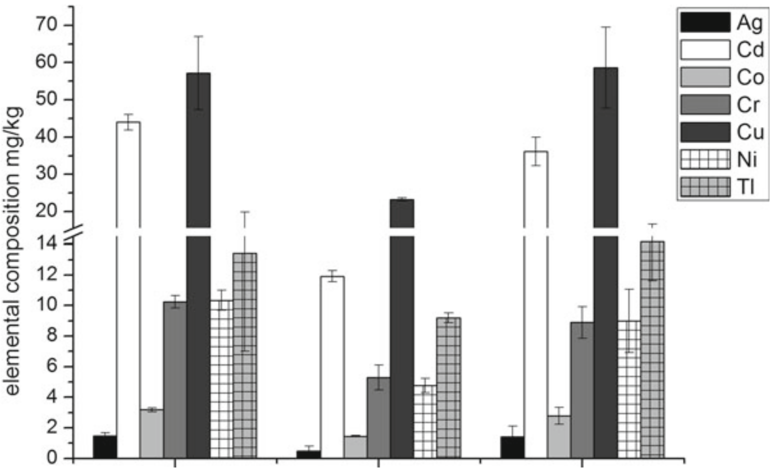

Fig. 9 The elemental composition of pine biomass growing on soil from zinc smelter area $t=1$; MS—zinc smelter area, K—compost, OS—sewage sludge

7 A Field Study

A field study was conducted using selected soil amendments and biosolids: sewage sludge-based compost, sewage sludge, and lacustrine chalk. The two plants species planted were pine (*Pinus sylvestris* L.) and energy crops (*Miscanthus giganteus*) on the area surrounding zinc smelter and post-mining land lignite mine dumping site. This experiment was conducted using two doses of biosolids. The dose 16 Mg/ha of organic amendments was applied for the 1 year experiment and the dose of 45 Mg/ha of organic amendments were applied for the 3 years experiment. After two vegetation periods, soil samples were collected and analyzed; moreover, the bioavailable (dissolved) compounds were also determined. The following parameters were investigated for the soil subsamples: pH, available (dissolved) P, dissolved N, dissolved organic carbon (DOC), IC (inorganic carbon).

For zinc smelter soil it was found that after two vegetation periods with sewage sludge application the dissolved compounds and biogenic elements were quite similar in soil solution compared to control without any biosolid treatment. Especially, it was noticed for dissolved organic carbon. Compost application gave similar results. Only when biosolids were used together with lacustrine chalk, the values of dissolved organic carbon are still high. This indicates that biosolids should be used only together with substances increasing pH value of soil. Three times higher dose of amendments did not increase the soil biogenic compounds concentration. These compounds were infiltrated into deeper soil layers. For soil from lignite mine dumping site, it was found that DOC was at similar level in control and biosolid-treated soils. Only soils treated together with biosolids and lacustrine chalk contained still high values of DOC. Moreover, the serious issue is the migration of nitrogen compounds into soil profile. In this research, it was found that for the zinc smelter soil (heavy metal contaminated), despite similar initial nitrogen content, much higher dissolved nitrogen was found for sewage sludge-treated soil than to compost treated. This indicates the more safety and beneficial usage of sewage-based compost than only sewage sludge. For dissolved nitrogen, the lacustrine chalk in addition with biosolids had no impact on total nitrogen in the soil solution. For soil from lignite mine dumping site, the high levels of dissolved nitrogen were found only for sewage sludge treated soil also with lacustrine chalk. And moreover, extremely high levels were found the second dose of sewage sludge.

8 Summary

The use of organic wastes as amendments to improve soil organic matter level and long-term soil fertility and productivity is gaining importance. The disposal of the large quantity of organic wastes produced by the municipal, agricultural, and agro-industrial activities is causing energetic, economic, and environmental problems. Sludge composting for use in agriculture should be given a priority for its disposal. Sludge should not be treated as a waste but a valuable non-farm source of organic matter to soil [30]. Land application of biosolids, such as sewage sludge or compost, has a great incentive in view of its fertilizer and soil amendment values, unless they contain toxic elements. The heterogeneous nature of biosolids produced in different processes necessitates knowledge of the chemical and biological properties of biosolids prior to the land application [31].

One of the methods for remediation of such areas is phytoremediation technique, which involves the plants for the treatment of soil from organic and inorganic contaminants (e.g., heavy metals). Phytoremediation is economically justified and generally acceptable by society method. An additional advantage of phytoremediation of soils is the possibility of using waste and by-products (e.g., sewage sludge, composts) as soil additives to support this process and at the same time to affect the recycling and disposal of a significant amount of waste substances [11]. Also the large areas of post-mining land lignite mine dump are characterized with poor organic and biogenic compounds. Soil organic additives are a source of organic matter, carbon, and biogenic elements which provide a significant improvement in the quality of the soil environment [2]. Moreover, they have the sorption ability related to immobilization of contaminants in soil, e.g., heavy metals [32]. Revitalization of industrial and mining areas, if only because of the large surface area, is often a matter of great importance for planning sustainable development at local and regional levels.

To achieve a successful revitalization of even smaller projects with lower complexity of remediation, it is essential to strategically integrate analysis of planning, legal, cultural, and economic aspects. Only the analysis of the kind of degradation or contamination and contaminants migration in the environment or impact on the ecosystem is not sufficient. The standard approach to revitalization will not produce appropriate results for the remediation of large areas, as is the case for opencast mining of lignite, or degradation as a result of metalliferous dust emissions from steel mills. Therefore, the type and extent of the required procedures for the remediation and redevelopment costs must be included in the expected benefits of the intended use of the land. The results of the study indicate, that taking into account the protection of soil and water environment, it is not justified the use of such high permitted doses of biosolids. Especially, large threat carries application of sewage sludge, while the application of compost-based sewage sludge is much more safety. It was also found that application of biosolids to different soils can give also not fully predicted results.

For some cases, the concomitant use of biosolids and calcium amendments is highly justified, when considering the soil protection. It was found that the effect of bio-waste on the promotion of plant growth and biomass increment in shoot and roots was significant. Moreover, most of the applied organic substances improved the condition of degraded soils. The results obtained lead to the following conclusions: "Application of sewage sludge, municipal compost and lacustrine chalk improved the condition of degraded soil and lead to increased production of plant biomass. The growth improvement and visible quality enhancement of aboveground biomass was recorded in rape and miscanthus after the application of organic substances to degraded soils. The release of nutrients from sewage sludge into the soil may pose a potential threat by contamination of surface and ground waters with main biogenic compounds".

Moreover, the chosen organic soil additives, derived from organic by-products, enable the development of plants on degraded lands and the establishment of vegetation cover, thus reducing water and wind erosion. The mobility of heavy metals in the soil and from soil to soil solution can be decreased by the application of organic additives such as: compost from organic fraction of municipal solid waste and lacustrine chalk. Selected energy crops, like *Miscanthus giganteus*, have excellent adaptability to change habitat conditions and the possibility to gradual reclamation of degraded land and the ability to prevent the migration of heavy metals into the soil and groundwater. Thus, this plant can be used in the remediation of soil and of devastated areas as pioneering plants. Moreover, as a biosolid, the treated sewage sludge should be used in the form of compost to achieve significant efficiency of carbon phyto-sequestration. Conducted research on degraded areas, deprived of humus layer and vegetation, allowed exploring the relationship between the application of organic substances and selected plant species on improving the quality of soil. It was also found that selected soil amendments and plants species improve the soil organic carbon sequestration (SOC). This indicates that biosolids should be used only together with substances increasing pH value of soil. Three times higher dose of amendments did not increase the soil biogenic compounds concentration. These compounds were infiltrated into deeper soil layers.

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Phytoremediation of Crude Oil-Contaminated Soil Using *Cynodon dactylon* (L.) Pers.

Budhadev Basumatary and Sabitry Bordoloi

Abstract Contamination of soil with petroleum hydrocarbons (PHCs) has become a serious problem in Upper Assam, India. For rehabilitation of oil-contaminated sites, phytoremediation represents a promising technology whereby plants are used to enhance biodegradation processes in soil. The aim of this experiment was to evaluate the efficiency of a native species *Cynodon dactylon* (L.) Pers. that could be effective in phytoremediation of petroleum hydrocarbon-contaminated soil. Experiments were conducted in net house to determine the tolerance of this species to crude oil-contaminated soil samples with application of two fertilizer levels. Plants were monitored for 180 days to analyze the reduction of petroleum hydrocarbon concentration if any in soil. In the presence of contaminants, plant biomass and height were reduced up to 33.8 % and 21.9 % respectively. As for fertilization, the lower fertilizer level led to higher biomass production. The root growth was reduced under the effects of petroleum hydrocarbon concentration in soil. *C. dactylon* was found to tolerate crude oil contamination in a concentration of 7.5 % (w/w). The estimation of total oil and grease in soil of the tested plants revealed that *C. dactylon* could decrease crude oil up to 46.7 % in low fertilizer level (200N, 100P, 100K) and 38.2 % in high fertilizer level (240N, 120P, 120K) in comparison to 11.5 % in low fertilizer level and 10 % in high fertilizer level in control pots without plants. The present investigation reveals that *C. dactylon* can serve as a low-cost alternative for removal of hydrocarbon contaminants from soil.

Keywords Phytoremediation • Crude oil • Biodegradation • Fertilizer levels • *C. dactylon* • Total oil and grease • Oil contaminations

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1 Introduction

Contamination of water and soil by crude oil as a result of exploration, production, maintenance, transportation, storage, and accidental release has caused significant environmental impacts and presents substantial hazards to human health. Phytoremediation is the use of plants to remediate contaminated matrices such as soil, sediment, surface, and groundwater. Plants can remove, degrade, and inactivate contaminants, thereby reduce remedial costs, restoring natural habitat and clean up contamination *in situ* [1–3]. Apart from bio-augmentation with oil-degrading microorganisms, phytoremediation is applied to provide long-term rehabilitation of the residual oil contamination [4].

Searching for the most effective plant species for remediation of a particular compound is a critical step in phytoremediation trials. Native and indigenous plant species may be preferred for long-term restoration of contaminated sites for ecological and economic reasons, as they may not require long-term maintenance and they are better adapted in the environment [5–7]. Many studies on the phytoremediation of petroleum hydrocarbon-contaminated soil reported the use of grasses (*Poaceae*) and legumes (*Leguminosae*) (e.g., [2, 8–14]). Grasses have proved to be most effective in enhancing degradation rates of organic contaminants in soils because their fibrous root system offers an increased root surface for microbial growth and activity [15, 16]. Burken and Schnoor [17], Nichols et al. [18], Siciliano et al. [19], and Bordoloi and Basumatary [20] reported that dissipation of contaminants in the rhizosphere is most likely due to enhanced microbial degradation. In the present study, an attempt was made to study (i) the influence of oil on plant growth (crude oil-contaminated and uncontaminated soil), (ii) the effect of plants on oil degradation (crude oil-contaminated soil with and without plants), and (iii) the influence of different fertilizer levels on plant growth and oil degradation (crude oil-contaminated soil with two fertilizer concentrations).

2 Materials and Methods

The experiment was conducted at the Institute of Advanced Study in Science and Technology, India (May to October 2010) (91°41′1.1″ Eastern longitude and 26°06′34.8″ Northern latitude lying at 48.47 m above the sea level). The soil used in the experiment was collected from a depth of 0–30 cm from the crude oil-contaminated pit of Jorajan, Duliajan, Assam (India) (95°28′57.92″ Eastern longitude and 27°20′39.43″ Northern latitude and elevation 135.95 m above the sea level) (Table 1). The air-dried soil was sieved through a 2-mm screen. Finally, the soil was mixed by hand with the Duliajan heavy crude oil (Table 2) in the concentration of 7.5 % of dry soil weight. The soil mixture was filled in cylindrical plastic pots (20-cm diameter) to a soil column of 45-cm height (approximately 15 kg soil per pot). Five transplants of *C. dactylon* were planted 15 days after soil contamination with crude oil (Table 3). Prior to this, the plants were propagated in the net

Table 1 Characteristic of the soil used in the experiment

Parameters	Mean values ± SD
Sand [wt%]	75 ± 1
Silt [wt%]	12 ± 0.25
Clay [wt%]	13 ± 0.14
Texture	Sandy loam
pH	4.5 ± 0.09
Organic carbon [wt%]	1.2 ± 0.05
Total N [wt%]	0.1 ± 0.01
Total P [ppm]	6.5 ± 0.05
K [ppm]	17 ± 0.06
Ca [ppm]	79 ± 0.45
Mg [ppm]	16 ± 0.06
Electrical conductivity (μS)	255 ± 1.55
Total dissolved solids (ppm)	70 ± 1.05

Table 2 Crude oil fractions [wt%] used in the experiment

Parameters	Mean values ± S.D.
Saturates	26.5 ± 0.34
Aromatics	43 ± 0.38
Asphaltenes	2.5 ± 0.08
Resins	28 ± 0.32

Table 3 Experimental details

Treatment	Plants[a]	Oil[b]	Level	Fertilizer (mg kg^{-1} soil)
A	+	+	Low	220N, 110P, 110K
B	+	+	High	300N, 150P, 150K
C	–	+	Low	220N, 110P, 110K
D	–	+	High	300N, 150P, 150K
E	+	–	Basic	120N, 60P, 60K

[a]+ 5 *C. dactylon* transplants/pot, – no plants
[b]+ Crude oil-contaminated soil, – uncontaminated soil

house (area covered with nylon net having 75 % light penetration). A total of 45 pots were maintained in the net house. The average monthly temperature in the net house was 22–25 °C (from a daily minimum average of 19 °C to maximum of 38 °C). The mean monthly relative humidity was 71–76.6 % (from a daily minimum average of 19 °C to maximum 38 °C). The daily photoperiod was characterized by 12 h of daylight with low variation during the experimental period. Replacement of plants with healthy transplants was done in case plants died in 7 days. Soil moisture was maintained approximately at 60 % water-holding capacity.

Plants in uncontaminated soil (treatment E) received basic doses of nitrogen (N), phosphorus (P), and potassium (K): 120, 60, and 60 mg kg^{-1} soil respectively in order to support good plant growth. The crude oil-contaminated soils (treatments A–D) were fertilized at low (mg kg^{-1} soil: 220N, 110P, and 110K) and high (mg kg^{-1} soil: 300N, 150P, and 150K) levels (Table 3). The calculation was based on [21]. The fertilizer was composed of urea, potassium nitrate, and urea phosphate. The total fertilizer amounts were split into four applications (7, 25, 65, and 150 days after planting).

Initial soil samples were taken before planting (after mixing crude oil). A weekly rating of plant mortality was done during the first 2 months of growth to know about the toxicity of crude oil and the effect of the fertilizer level. Three destructive samplings were carried out by using three pots from all the treatments (A, B, C, D, E) at 2 months interval (up to 6 months). Concerning the treatments with plants (A, B, E), shoots were cut at their base and number and lengths of tillers were determined. Roots were carefully separated from soil and rinsed. Both shoots and roots were dried at 65 °C for 3 days and the dry weight of the biomass was determined. The soil of each pot (all treatments) was homogenized and one 400-g composite sample was stored at 4 °C for 1–2 weeks prior to analysis.

Measurements of the soil pH were done on saturated extract [22], organic carbon percent by Walkley and Black method [23], and electrical conductivity by Rhoades [24]. Measurements of the soil N were done by Kjeldal method [25], available phosphorus by Olsen method [26], available potassium by normal acetate ammonium method [27], and texture of the soil by hydrometric method [28]. The water-holding capacity was measured on water-saturated soil samples in a brass box and left to stand overnight to drain freely and was defined by differences in weight. Calcium and magnesium were determined as per the method given in USDA Handbook No. 60 [29].

Total oil and grease (TOG) was determined by soxhlet extraction method using a modification of EPA method 3540B [30]. Of each sample, three 20 g replicates were analyzed. Saturates, aromatics, and polar fractions were performed by thin-layer chromatography combined with a flame ionization detector (Iatroscan). The chromarods were run with n-heptane to separate the saturated hydrocarbons and subsequently with toluene to collect the aromatic hydrocarbons in a single band. Both fractions, which include resins and asphaltenes, were calculated by difference. Bacterial counts were determined by performing the dilution plate-count method [31]. The statistical analyses were conducted using the Superior Performance Software System (SPSS) 15.0 for Windows. Concentration of TOG in soil, plant dry weight, root and shoot length were subjected to one-way analysis of variance to test for significant difference between treatments ($P<0.05$). Moreover, the bivariate correlation between TOG in soil, plant dry weight, root and shoot length in the contaminated treatments with plants were evaluated with Pearson's correlation coefficient for normal-distributed variables at $p=0.05$. The figures were drawn by Excel software (2003).

3 Results and Discussion

The mortality rates exhibited by *C. dactylon* transplants were 30 % for low fertilizer level (treatment A) and 40 % for the high fertilizer level (treatment B) in contaminated soils. The uncontaminated soil (treatment E) showed good plant growth.

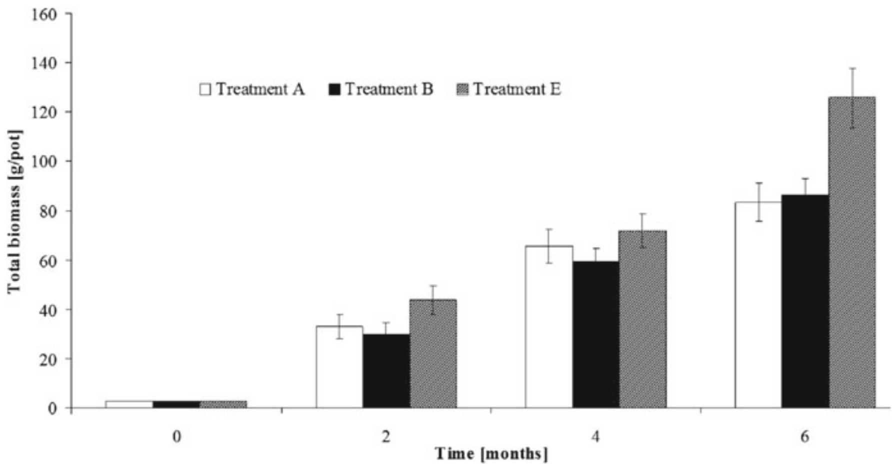

Fig. 1 Total biomass (dry weight) of *C. dactylon* as a function of time and soil treatment. Treatment A: crude oil-contaminated soil + low fertilizer level; Treatment B: crude oil-contaminated soil + high fertilizer level; and Treatment E: uncontaminated soil + basic fertilizer level

Mortality of transplants in treatments A and B is due to the toxic effect and inhibited water and nutrient uptake due to the hydrophobic character of the crude oil [32]. The effects of crude oil on plants are attributed to phytotoxicity, which depends on several factors: concentration of oil in soil, oil type and its content of phytotoxic compounds (e.g., polycyclic aromatic hydrocarbons, which includes most phytotoxic substances), environmental conditions, and plant species [33]. The growths of plants were weak after transplantation but exhibited a good growth and adaptation to the toxic environment, as shown by the good height and biomass production. Total biomass yield per pot increased in all treatments over the course of the experiment (Fig. 1).

The yields were more in uncontaminated soil than in contaminated soil (A, B) and differences were significant ($p = 0.05$) during 180 days. At the beginning of the experiment, all the plants had a height of 12 cm. Generally, growth of plants in contaminated soil was slower and tillers were shorter and stunted than uncontaminated soil (Fig. 2). Differences between the contaminated and uncontaminated soil were significant throughout the study ($p = 0.05$). The root growth reduced possibly due to the toxicity of hydrocarbon. Generally, the roots growing in uncontaminated soil were longer, finer, and more extensive than those growing in contaminated soil. In crude oil-contaminated soil, mean reduction of total plant biomass was 33 % in treatment A and 31 % in treatment B respectively, in comparison to uncontaminated soil. The mean reduction of plant height was 15 % in treatment A and 21 % in treatment B. High rates of plant mortality and reduction in height and biomass are typical reactions caused by oil contamination [34].

Despite inhibition of plant growth and root development, the decrease of crude oil was more in vegetated soil compared to unvegetated soil. This might be due to biodegradation of lower molecular weight hydrocarbons in the rhizosphere of *C. dactylon* in the presence of some rhizosphere or rhizospheric microorganisms.

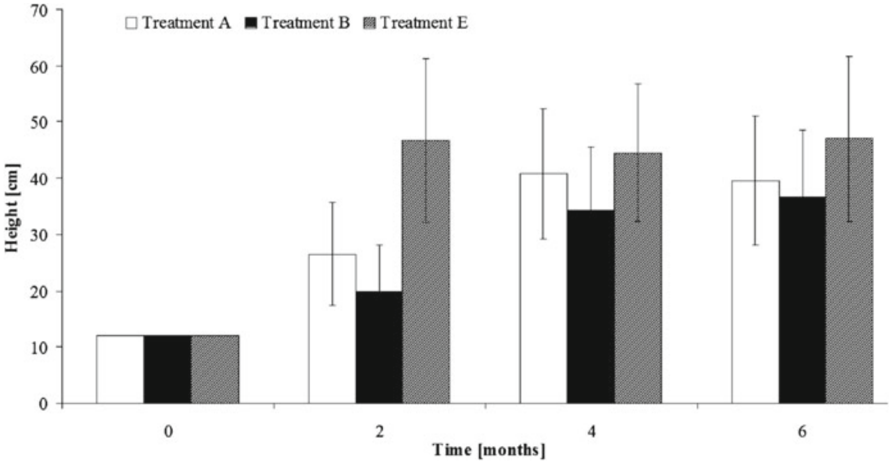

Fig. 2 Plant height of *C. dactylon* as a function of time and soil treatment. Treatment A: crude oil-contaminated soil + low fertilizer level; Treatment B: crude oil-contaminated soil + high fertilizer level; and Treatment E: uncontaminated soil + basic fertilizer level

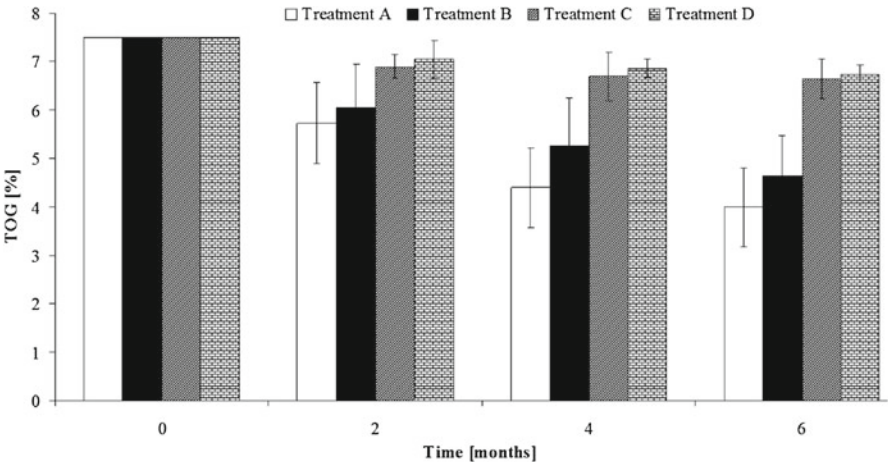

Fig. 3 TOG in soil (sample size: 20 g) as a function of time and soil treatment. Treatment A: crude oil-contaminated soil + low fertilizer level; Treatment B: crude oil-contaminated soil + high fertilizer level; and Treatment C: no plants + low fertilizer level; Treatment D: no plants + high fertilizer level

As roots grow, they penetrate through the soil, exposing entrapped contaminants that have been previously inaccessible, increasing their availability to degradation [35, 36]. Moreover, the root increases soil aeration, reducing soil moisture content and changing physicochemical and biological characteristics [37, 38]. The initial TOG content was 7.5 % of the total soil dry weight. A decrease of TOG was found over the course of the experiment in all contamination treatments (Fig. 3). The percentages of decrease in 2, 4, and 6 months in low fertilizer level were 23 %, 41 %,

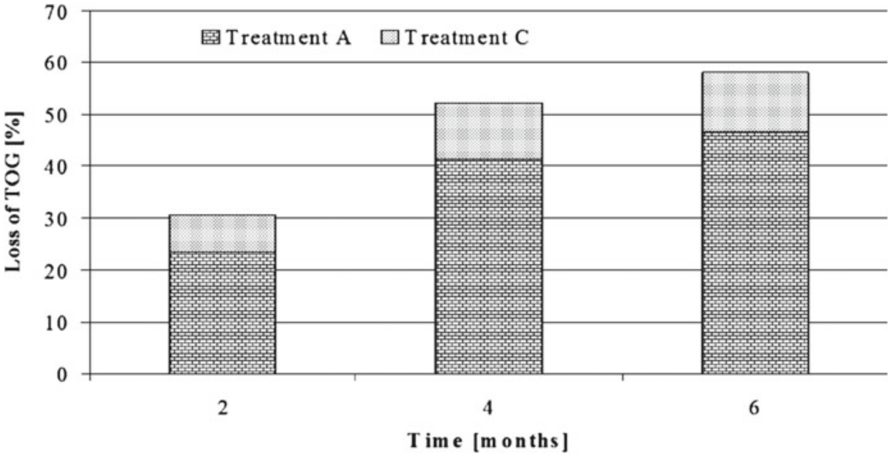

Fig. 4 Percent loss of TOG in soil in low fertilizer level: Treatment: A (crude oil-contaminated soil with plants) and Treatment: C (crude oil-contaminated soil without plants)

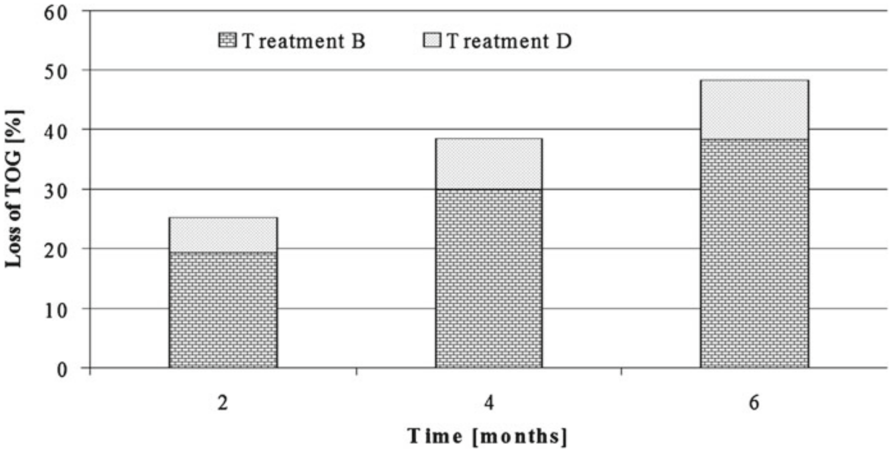

Fig. 5 Percent loss of TOG in soil in high fertilizer level Treatment: B (crude oil-contaminated soil with plants) and Treatment: D (crude oil-contaminated soil without plants)

and 46 % in vegetated pots (A) in comparison to 7 %, 10 %, and 11 % in unvegetated pots (C) (Fig. 4). Whereas, the percentages of decrease in high fertilizer level were 19 %, 30 %, and 38 % in vegetated pots (B) in comparison to 6 %, 8 %, and 10 % respectively in unvegetated pots (D) during 2, 4, and 6 months (Fig. 5). However, the differences in TOG decrease between vegetated and unvegetated treatments were significant after 6 months.

Merkl et al. [2] also showed enhanced degradation of crude oil under the influence of a tropical grass after only a few months. Huang et al. [39] reported approximately 30 % removal of persistent TPH in soil by *Festuca arundinaceae* in 120 days.

Table 4 Bacteria colony forming units (CFU) per gram dried soil in 10^5 dilutions in different treatments

	Treatments			
Time (month)	TA	TB	TC	TD
2	$4.25 \times 10^5 \pm 1.5$	$3.95 \times 10^5 \pm 1.67$	–	–
4	$6.55 \times 10^5 \pm 2.54$	$5.5 \times 10^5 \pm 1.55$	–	–
6	$6.94 \times 10^5 \pm 2.35$	$6.5 \times 10^5 \pm 1.65$	–	–

Results represent mean ± standard deviation of three replicates

Muratova et al. [40] showed TPH reduction up to 52 % during 3 years of rye cultivation. Diab [41] recorded 30 %, 16.8 %, and 13.8 % reduction of TPH in rhizosphere soil of broad bean, corn, and wheat respectively. Peng et al. [42] noted 41.61–63.2 % removal of TPH by *Mirabilis jalapa*. In addition, Razmjoo and Adavi [43] found 40 % of TPH reduction by Bermuda grass cultivars in petroleum-contaminated soils in 6 months study period.

The bacterial colony-forming units (CFU) increased with time in treatment A and treatment B. No significant difference was observed among the population of bacteria in treatment A (6.94×10^5) and treatment B (6.5×10^5) during 6 months (Table 4). In treatment C and treatment D, no bacterial population was detected in 10^5 dilutions. Fertilizers increase plant growth in oil-polluted soils in the case of nutrient deficiency [21, 34]. However, over fertilizing usually leads to yield depressions. Differential application of fertilizer level showed that low fertilizer level was more conducive for reduction of crude oil (Treatment A: 46 %) than high fertilizer level (Treatment B: 38 %). Graham et al. [44] assessed an array of N/P amendments for hexadecane biodegradation and suggested amendments above stoichiometric requirements can lead to diminished rate of degradation. Palmroth et al. [45] observed no improved degradation of diesel fuel with nutrient amendments during phytoremediation with pine, poplar, or grasses. However, excessive nitrogen amendments result in an increase in soil salinity and this increases the osmotic stress and suppresses the activity of hydrocarbon-degrading organisms [46]. A significant influence of fertilizer levels on oil degradation could not be detected in this study. In the present experiment, bivariate correlation between TOG degradation and root biomass in treatment A and B was significant.

Apart from biodegradation, a potential weathering process of petroleum hydrocarbon (PHC) in soil is volatilization of low molecular weight, aliphatic, and aromatic compounds [47]. Chaîneau et al. [48] also reported a decrease of 18 % in the initial fuel oil concentration in soil by volatilization. In the study reported here, the rapidly biodegradable and low molecular weight hydrocarbon fractions might have been reduced from the soil. Rhizosphere microbial populations may enhance plant's adaptation to PHCs by detoxifying contaminated soils through direct mineralization of these organic contaminants. Microorganisms also have a strong influence on the health conditions of plants. In our work, a plant-promoted degradation of hydrocarbon may be due to the complexity of plant–microorganism interactions.

4 Conclusion

The present study demonstrated that plant biomass and height of *C. dactylon* were reduced in the presence of crude oil contamination in soil. However, the species was found to have a high potential to adapt to the toxic environment, due to its vegetative reproductive ability. Therefore, it should be viewed as a species with definitive phytoremediation potential. Concerning fertilization, an NPK concentration as in low level (mg/kg soil: 200N, 100P, and 100K) is considered to be adequate for *C. dactylon* growing in the oil-polluted soil of North eastern India. Application of this plant species in the hydrocarbon-contaminated sites however would help in polishing soil and thus, prevent contaminants from surface spreading.

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A Study on Degradation of Heavy Metals in Crude Oil-Contaminated Soil Using *Cyperus rotundus*

Sabitry Bordoloi and Budhadev Basumatary

Abstract Study on degradation of heavy metals was carried out in crude oil-contaminated soil over a period of one year by using *Cyperus rotundus* as a candidate species. The study revealed significant ($p<0.05$) degradation of some heavy metals in soil and accumulation of certain heavy metals in roots and shoots during the study period. The metals that showed highest degradation were Lead (43.8 %), Mn (27 %), and Cd (31.3 %). Fe, Zn, and Cu were found to be least degraded. Analysis of roots showed significantly higher ($p<0.05$) accumulation of Pb (3.5 ± 0.5 mg kg^{-1}) followed by Fe, Cu, Pb, Cd, and Cu. Pb (2.5 ± 0.5) was found to be significantly ($p<0.05$) highest accumulated in shoot followed by Fe, Cr, Pb, Cd, and Cu. Therefore, it can be concluded that *C. rotundus* can significantly degrade and accumulate Pb, and so, it can be utilized for phytoremediation of Pb-contaminated soil.

Keywords Phytoremediation • Heavy metals • *C. rotundus*

1 Introduction

Anthropogenic sources of hydrocarbons and heavy metals are polluting the environment [1]. Heavy metal pollution from the biosphere has accelerated rapidly since the onset of the industrial revolution and heavy metal toxicity poses major environmental problems [2]. Drilling and mining activities generate large amounts of waste rocks and tailings, which are deposited on the land surface [3]. These wastes are often very unstable and become source of pollution of air, soil, and water. This may

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eventually lead to a loss of biodiversity, amenity, economic wealth [4, 5], and human health. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury, and arsenic [5]. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO [6]. When the elements necessary for human beings, flora, and the environment exceed a certain level, they may have toxic effects. There is a potential threat when these contaminants enter in to the food chain through bioaccumulation [7]. It is, therefore, very important to assess the extent of heavy metal pollution in contaminated areas and also to remove heavy metal from the contaminated land [8], and an understanding of reactive transport in porous media is necessary to predict the fate of pollutants in soils and aquifers [9].

The aim of present study is an attempt to determine the phytoremediation of lead, chromium, zinc, copper, cadmium, nickel, mercury, iron, manganese, selenium, and arsenic in crude oil-contaminated soil. *C. rotundus* is a native species to India, and this plant is found to be growing in hydrocarbon-contaminated soil in and around the oil fields of Assam, India [10]. They propagate by rhizome and seeds; grow in wet, disturbed, and altered areas; and could be vegetatively established in contaminated soil. They have fibrous, perennial root system with vigorous deep root rhizomes which can control soil erosion and surface spreading of hydrocarbon contaminants [7]. It makes economic sense to evaluate the potentiality of these plant species for use in phytoremediation of certain heavy metals. The most important contribution of this investigation is that these plants could be effectively used as pioneer of plant vegetation and decontamination of certain heavy metal pollutants in the crude oil-contaminated soil.

2 Materials and Methods

The soil used for this experimental study was collected from the oil-contaminated field of Duliajan (27°20′39.11″N 95°28.57′88″E), Assam (India), containing crude oil concentration of 1.8–4 % (w/w). The soil was taken from a depth of ~1 m using a spade. The soil was placed in buckets, transported to the Institute of Advanced Study in Science and Technology (IASST) laboratory, and kept safely before use. Elements (Pb, Cd, Mn, Cr, Fe, Se, As, Hg, Zn, Ni, Cu) were purchased from Merck and added to crude oil-contaminated soil. The elements are added and homogenized in such a way that all the elements are in same percentage (30 mg kg^{-1}). The soil mixture was allowed to stay for 1 month for making the mixture homogeneous. Analysis of crude oil-contaminated soil showed 30 mg kg^{-1} concentrations of elements.

2.1 Planting

Seeds of *C. rotundus* were collected from hydrocarbon-contaminated soil and were germinated and grown for 2 weeks in a growth chamber and then transplanted in plastic pots. A total of 40 pots were prepared for the study. Each plastic pot

(18-cm diameter) contained 12 kg of soil-crude oil mixture. Plants of equal heights were selected, and 20 plants were planted in each pot. The pots were maintained in replicates for statistical validity. Control pots were also maintained to study the degradation of heavy metals in unplanted ones. Uncontaminated control pots were also maintained in order to study the biomass difference between the plants grown in contaminated and uncontaminated ones. All pots were kept in a net house. The mean monthly temperatures were 22–25 °C (from a daily minimum average of 19 °C to maximum 38 °C) with 12 h photoperiod. The mean monthly relative humidity was 71–77 % (from a daily minimum average of 35 % to a maximum of 98 %). Replacement of plants with healthy transplants was done if plants died within 10 days. The soil was watered as needed based on visual inspection (approximately 100 mL every alternate day).

2.2 Analytical Methods

During 360 days, soil samples from each treatment were collected, air-dried, and sieved through 2 mm screen. Subsamples of 0.5 g of each soil sample were digested with 5 mL nitric acid (69 % HNO_3) and impurities removed by filtration [11]. For the analysis of chromium, zinc, lead, copper, cadmium, nickel, mercury, iron, manganese, selenium, and arsenic, 1 g from each soil sample was acid digested (4:2:1; H_2SO_4:HCl:HNO_3) and the readings were taken in atomic absorption spectrometer (Thermofisher Scientific iCE 3000 series). Arsenic, selenium, and mercury were analyzed with VP 100 vapor system (Thermo Scientific). Three replicates were analyzed from each sample.

For the analysis of heavy metal accumulation in plant root and shoots, plant samples were harvested during 360 days. Plants were washed properly with deionized water to remove the soil materials and then used for heavy metal measurements. For this purpose, plant tops were harvested by cutting the stem, just above the soil surface. The plant roots were harvested after soaking the pots and their contents in a water bath and gently washing the soil from the roots. To understand the heavy metal distribution patterns within the plant, plants were divided into two parts: top (including stem, leaves, flowers, and seeds) and root. The roots and shoots were dried at 60 °C for 2 days for constant weight and biomass determined. The roots and shoots are then ground in a platinum-coated grinder. Approximately, 5 g of finely ground plant tissues and ashes were placed in a muffle furnace at 550 °C for 2 h [12]. About 0.5 g of ash was digested with conc. HNO_3, [11] and metal concentrations in roots and shoots were determined by atomic absorption spectrometry (AAS). Arsenic, selenium, and mercury were analyzed with VP 100 vapor system (Thermo Scientific). Five replicates were analyzed from each sample. Statistical analysis was conducted using the Superior Performance Software System (SPSS) 15.0 for Windows. The concentration of plant biomass, heavy metals in soil, and heavy metal accumulation in root and shoot were subjected to one-way analysis of variance (ANOVA) to test for significant differences between treatments ($P < 0.05$).

3 Results and Discussion

3.1 Plant Biomass

The result of plant biomass is presented in Table 1. The plant biomass of *C. rotundus* was found to be 83 g during 360 days of experiment. Root biomass was 32 g and shoot biomass was 51 g, respectively. Which represents good biomass productivity even though the plant was in toxic condition? The uncontaminated pots have significantly higher biomass productivity than the contaminated ones (Total biomass 120 g). However, there was biomass reduction in contaminated pots and high biomass production in uncontaminated soil that can be attributed to the absence toxic metals [13].

3.2 Heavy Metals Degradation in Soil

Results of heavy metal analysis in the soil planted with *C. rotundus* are presented in Table 2. Decrease of heavy metal was highest for Pb (43.8 % in 360), followed by Mn (27 % in 360 days), Cd (31.3 % in 360 days), and Cr (16.6 % in 360 days). Fe (15.7 % in 360 days) and Zn (29.4 % in 360 days) also showed moderate

Table 1 Biomass production of *C. rotundus*

Plant species	Root biomass (g)	Shoot biomass (g)	Total biomass (g)
Contaminated soil	32 ± 4.5	51 ± 5	83 ± 5.4
Control soil	46 ± 3.8	74 ± 5.3	120 ± 5.5

Values are means ± SD of three replicates

Table 2 Results showing average degradation of heavy metals in hydrocarbon-contaminated soil in comparison to control by *C. rotundus*

Elements	Concentration in 0 day (mg kg^{-1})	Concentration in 360 days (mg kg^{-1})	Removal percentage in 360 days (%)	Concentration in control in 360 days (mg kg^{-1})
Mn	30	8.3 ± 1	27	29.99 ± 1.2
Pb	30	13.12 ± 1.5	43.8	29.9 ± 1
Cd	30	9.38 ± 1	31.3	30 ± 0.85
Cr	30	5 ± 0.5	16.6	29.99 ± 1.1
Fe	30	5.3 ± 0.4	17.7	29.8 ± 1.5
Se	30	0.6 ± 0.02	2	29.99 ± 1.1
As	30	0.6 ± 0.02	2	29.89 ± 1.4
Hg	30	0.9 ± 0.03	3	29.97 ± 1.5
Zn	30	8.8 ± 1.5	29.4	29.9 ± 1
Ni	30	1.4 ± 0.3	4.6	30 ± 1
Cu	30	2 ± 0.03	6.6	29.96 ± 1.2

Values represent mean ± SD of three replicates degradation. *C. rotundus* showed least degradation of Cu 6.6 % in 360 days and 13.3 % in 720 days. Degradation of Ni, Hg, Se, and As were minimal in soil in the presence of *C. rotundus*. The control pots showed negligible decrease of the heavy metals.

The high rate of metal removal from the soil in the planted treatments can be attributed to high concentration of the metals in the soil. This high rate of removal of metal ions have significant correlation with plant growth parameters, as it was noted that plants with higher biomass showed a higher metal removal. Plant species clearly correlated with pollutant concentrations, but effects only occurred high in relation to removal of metals in case of Pb. The least degradation of heavy metals in un-vegetated soil can be attributed to the absence of plants. Significant difference in the concentrations of metals between vegetated and un-vegetated pots for these species were rare, with un-vegetated soil removing high proportions of metals. Read et al. [14] also found similar result in their study of variation among plant species in pollutant removal from storm water in biofiltration systems. Other studies have also shown that the soil medium in vegetated and non-vegetated biofilters removes high proportions (e.g., >90 % on average) of influent metals, including Pb, Cu, and Zn [15–17]. Although some metals are required by plants in trace amounts, they are substantially less important than N and P, and concentrations are typically low in plant tissue, other than in specialists that hyperaccumulate some metals [18]. The biofilter media therefore play the dominant role in metal uptake. This study shows how plant species in their growth and morphology may influence effective pollutant removal in phytoremediation and, consequently, inform species selection for efficient phytoremediation design [7]. However, there are still substantial gaps in our knowledge. The trends found in this study require investigation across a broader range of plant species and under a range of environmental conditions.

3.3 Heavy Metal Analysis in Plant Tissues

3.3.1 Roots

The results of heavy metals found in the roots of *C. rotundus* during 360 days of growth are presented in Table 3. The presence (mg kg^{-1}) of heavy metals in the roots of this plant was 0.024 Cr, 0.02 Pb, 0.012 Cd, 0.04 Fe, 3.5 Zn, and 0.001 Cu. Heavy metals As, Se, Hg, Mn, and Ni were not found in the roots of *C. rotundus*.

Table 3 Heavy metal (mg kg^{-1}) analysis in the roots of *C. rotundus* during 360 days

Elements	Cr	Zn	Cd	As	Fe	Pb	Cu	Se	Hg	Mn	Ni
Concentration in root (mg kg^{-1})	0.024 ±0.01	0.02 ±0.01	0.012 ±0.01	ND	0.04 ±0.002	3.5 ±0.5	0.001 ±0.001	ND	ND	ND	ND

Results represent mean ± SD of three replicates. *ND* not detected

3.3.2 Shoots

Table 4 represents the heavy metals found in the shoots of *C. rotundus* during 360 days of plant growth. In the shoots of *C. rotundus*, Cr (0.023), Pb (0.012), Cd (0.012), Fe (0.03), Zn (2.5), and Cu (0.001) were present. Heavy metals As, Se, Hg, Mn, and Ni were not found in the shoots of *C. rotundus*. Concentrations of metal in plant roots and shoots were almost same. No significant difference of metal concentration was observed between shoots and roots. The results obtained from this study suggest that crude oil-contaminated soil significantly influenced metal degradation and accumulation in *C. rotundus*. The higher accumulation of metals in the tissues of the plant further explains the rapid metal removal in the soil. It is well documented that free Fe oxides are the dominant soil constituents responsible for metal sorption [19], and soil organic matter can also adsorb metals, thus reducing its availability [20, 21].

C. rotundus possesses good capacity to degrade and accumulate some heavy metals. There is positive relationship between the heavy metal concentrations of the soils with the plants. The uptake of heavy metals by these plant species may be more dependent on the adaptive physiological characteristics of the plant and the mobility and other properties of the metal itself than on the metal concentrations [7]. Cu and Zn are essential micronutrients to the plants, while Pb is nonessential and a toxic metal. Adaptive strategies including rejection, metabolization, and excretion of the heavy metals would be adopted selectively by the plant to regulate the heavy metals when growing in polluted environments. Deng et al. [22] reported that the metal tolerance mechanism in plants may be related to the special root anatomy in plants, the alleviated metal toxicity by the reduced rooting conditions, and the relatively high innate metal tolerance in some species. It also indicated that metal concentrations in plant parts were significantly different amongst plants [23]. The results indicated that the *C. rotundus* has the good ability to accumulate heavy metals under the present net house conditions. The plant species are tolerant to heavy metals; this tolerance may come from the plant's good capability to transfer metals, with higher heavy metal concentration.

Ye et al. [24] investigated metal concentrations in shoots and in roots of seedlings of four different ecotype populations of *Phragmites australis* cultivated in the MPCS substrate and found that the shoots contained 47–60 mg kg^{-1} Zn and 2.5–4.0 mg kg^{-1} Pb, while the roots contained 100–164 mg kg^{-1} Zn and 8.4–13 mg kg^{-1} Pb, which shows the accumulation ability of root is much higher than that of shoot, but the leaves have not been mentioned in it. In our study, there was no significant difference of heavy metal accumulation in roots in comparison to shoots. Some

Table 4 Heavy metal (mg kg^{-1}) analysis in the shoots of *C. rotundus* during 360 days

Elements	Cr	Zn	Cd	As	Fe	Pb	Cu	Se	Hg	Mn	Ni
Concentration in root (mg kg^{-1})	0.023 ±0.01	0.012 ±0.01	0.012 ±0.01	ND	0.03 ±0.002	2.5 ±0.5	0.001 ±0.001	ND	ND	ND	ND

Results represent mean ± SD of three replicates. *ND* not detected metals may be re-translocated and selectively stored in the vacuoles of older leaves [25]. This study showed that *C. rotundus* can accumulate Cu, Zn, and Pb, either for essential elements or for nonessential elements, at a high level. This indicates that there are different mechanisms operating to control variations in tolerance to heavy metals in plant species. The tolerance mechanism is probably related to the ecological and physiological behavior. This mechanism remains to be further elucidated. The phytoremediation potential of a plant is determined not only by its capacity to absorb high metal concentration but also by its ability to translocate the metal from roots to aerial parts and produce simultaneously a high biomass.

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Polycyclic Aromatic Hydrocarbons and Heavy Metal Contaminated Sites: Phytoremediation as a Strategy for Addressing the Complexity of Pollution

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Abstract Since the industrial revolution, soil has been increasingly subjected to continuous negative pressure, largely determined by human activities, which have dispersed heavy metals and many persistent organic compounds causing severe soil contamination. Among pollutants, heavy metals and polycyclic aromatic hydrocarbons (PAHs), which are ubiquitous and generated also from natural resources, are of particular concern. The simultaneous presence of both kinds of pollutants is very common in brownfield sites, and the clean-up of these areas presents technical difficulties and requires appropriate solutions at a reasonable cost. Remediation technologies have often used invasive processes that greatly damage soil characteristics, causing the deterioration of this important resource. In this chapter, the objectives are to briefly examine the processes involved in heavy metal and PAH reactions in soil in order to evaluate the best possible cost-effective remediation strategies for maintaining a high quality of soil and surrounding environment.

Keywords Soil • Remediation technologies • PAHs • Heavy metals • Phytoremediation

1 Introduction

Man is closely dependent on soil functions. Healthy soil ensures clean water, abundant crops and carries out essential functions such as the regulation of the cycle of nutrients and other elements, as well as the flow of water and solutes necessary for the survival of plants and animals. Soil supports the growth of higher plants and biodiversity being an ecological habitat for many organisms. Soil with its high

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buffer capacity works as living filter for waste products. Moreover, soil has the ability to maintain its porous structure to allow the passage of water and air, counteracting the erosive processes [1]. Since the industrial revolution, soil has been increasingly subjected to continuous environmental pressure, largely determined by human activities, which have dispersed heavy metals and many persistent organic compounds causing severe soil contamination. All these activities impair the natural ability of soil to perform the abovementioned functions. Among pollutants, heavy metals and polycyclic aromatic hydrocarbons (PAHs), which are ubiquitous, generated also from natural resources, are of particular concern. The simultaneous presence of both kinds of pollutants is very common in brownfield sites, and the cleanup of these areas presents technical difficulties and requires appropriate solutions at a reasonable cost. Remediation technologies have often used invasive processes that greatly damage soil characteristics, causing the deterioration of this important resource. In this chapter, the objectives are to briefly examine the processes involved in heavy metal and PAH reactions in soil in order to evaluate the best possible cost-effective remediation strategies for maintaining a high quality of soil and surrounding environment.

1.1 Polycyclic Aromatic Hydrocarbons (PAHs)

In the environment, PAH formation can occur as a result of incomplete combustion of organic materials, such as coal, oil, gas, waste, and other organic substances. In addition to these types of contribution, PAHs can also be released into the environment as a result of volcanic activity, forest fires, and burning coal. In soil, high levels of PAHs have been discovered at nearly all industrial sites where fossil fuels have been used in the production processes, including energy generation. The greatest contamination often occurs at former manufactured gas plant sites where generally heavy metals are also present in soils. The molecules of PAHs consist of two or more condensed aromatic rings, fused together via a pair of shared carbon atoms. The placement of the rings can determine linear, angular, or cluster forms. The physical-chemical properties of PAHs mainly depend on the molecular weight and the reactivity of the π electrons. When there is an increase in number of benzene rings and conjugated bonds occur, the delocalization of π electrons increases. The reactivity of the carbon atoms differs depending on the position, as regards both electrophilic substitution and the redox reactions. The reactive positions vary depending on the size of the molecules. PAHs are chemically rather stable; their reactivity is influenced not only by the molecular weight but also by numerous environmental factors such as temperature, light, and oxygen levels which favor the formation of numerous oxidation products and presence of co-pollutants and of materials capable of adsorbing them.

The distribution of PAHs in the environment depends greatly on their chemical–physical characteristics. All PAHs have high melting points and boiling points, low vapor pressure, which is inversely proportional to the number of rings, and low solubility in water, which decreases in the presence of high ions concentration: “salting out" effect. PAHs are extremely lipophilic, and this feature strongly influences their bioaccumulation.

In soil, the hydrophobic substances tend to reach equilibrium between the solid and the aqueous phases depending on numerous factors including temperature, concentration, amounts of solutes, amount of organic substances, and the characteristics of the contaminant. Their distribution between the liquid and solid soil phases is described by the partition coefficient K_d:

$$K_d = C_S / C_W$$

where C_S is the concentration of the substance in solid phase and C_W concentration of the substance in the aqueous phase. Since the tendency of a hydrophobic substance to distribute in soil between the two phases depends especially on the amount of total organic carbon, K_d can be replaced by K_{OW} the partition coefficient between water and octanol, the organic solvent with similar behavior to that of the organic matter:

$$K_{OW} = C_O / C_W$$

with C_O solubility of the compound in octanol and C_W solubility in water. Generally, $\log K_{OW}$ is inversely related to water solubility and directly proportional to molecular weight of PAHs.

K_{OW} allows evaluating the retention and release of organic compounds in soil and their tendency to bioaccumulate in human and animal tissues through the food chain.

Some properties of the most dangerous PAHs are reported in Table 1.

1.2 Toxicity Effects

It is known that PAHs have negative effects on the environment and human health. The risk to human health is associated with their toxic, mutagenic, genotoxic, teratogenic, and carcinogenic properties [2–4]. Numerous studies have shown that the compounds with 1, 2, and 3 rings are extremely toxic [5], while the higher molecular weight PAHs are considered to be genotoxic [6–8]. Although it has been estimated that 75 % of the total amount of PAHs assimilated enters the body through absorption in the epidermis [9], contamination of the food chain plays an important role in their accumulation since, once ingested, they are rapidly absorbed from the gastrointestinal tract due to their high lipid solubility [10]. Inhalation is another way of ingesting PAHs, following entry into the respiratory system of the particulate matter on which they are adsorbed.

Once in human body, PAHs undergo biotransformation reactions; their elimination depends on the ability to convert them into water-soluble metabolites. However, the formation of reactive metabolic products may determine the mutagenic and

Table 1 Physical-chemical properties of the 16 polycyclic aromatic hydrocarbons selected as priority pollutants by the U.S. Environmental Protection Agency

PAH compounds	PAHs abbreviation	Molecular weight (g mol^{-1})	Solubility at 25 °C (μg L^{-1})	log K_{OW}	log K_{OC}
Naphthalene	Nap	128	12,500	3.30	2.44
Acenaphthylene	AcPy	152	3420	3.94	3.40
Acenaphtene	AcP	154	4000	3.92	3.66
Fluorene	Flu	166	800	4.18	3.86
Phenenatrene	Phe	178	435	4.46	4.15
Anthracene	Ant	178	59	4.54	4.15
Fluoroanthene	FL	202	260	5.20	4.58
Pyrene	Py	202	133	5.18	4.58
Benzo[*a*]anthracene	BaA	228	11	5.76	5.30
Chrysene	Chr	228	1.9	5.81	5.30
Benzo[*b*]fluoroanthene	BbFL	252	2.4	5.80	5.74
Benzo[*k*]fluoroanthene	BkFL	252	0.8	6.00	4.98
Benzo[*a*]pyrene	BaP	252	3.8	6.13	5.74
Benzo[*g*,*h*,*i*]perylene	BghiP	276	0.3	6.63	6.20
Indeno[1.2.3-*c*,*d*] pyrene	InP	276	0.2	6.70	6.20
Dibenzo[*a*,*h*] anthracene	DBA	278	0.4	6.75	6.52

carcinogenic effects in mammals. Following an enzymatic reaction mediated by the cytochrome P450 monooxygenases, the aromatic rings are oxidized giving rise to intermediate epoxide, dihydrodiolepoxide [9]. These intermediates, in particular the diol-epoxides, combine covalently through a nucleophilic attack with the DNA molecules, generating distortions in the structure of the genetic material causing mutations and, therefore, a greater probability of carcinogenesis. Not all PAHs generate damage at the genetic level since not all are the precursors of these reaction intermediates. In particular, most of the PAHs that show carcinogenic properties are formed by more than three benzene rings [11]. The International Agency for Research on Cancer (IARC) has classified many PAHs as "probable or possible human carcinogen" (Group 2A and 2B, respectively), whereas benzo[*a*]pyrene was classified as Group 1 "carcinogenic to humans." In the environment, PAHs are usually found in mixtures and not as single compounds. This makes difficult to define the consequences for human health, due to possible synergistic effects, that make the toxicity of the mixture greater than the sum of the toxicity of individual compounds. Benzo[*a*]pyrene is used as an indicator for assessing levels of contamination and carcinogenic risk, since its carcinogenicity is higher than that of the other PAHs [12].

1.3 PAHs in Soil: Behavior and Effects

In soil, increasing concentrations of PAHs may impair the structure of the microbial community, reducing biomass and inhibiting certain metabolic activities [13]. The negative effects of PAHs differ depending on the compounds; for example, phenanthrene is more toxic than pyrene for the microbial community due to its greater accessibility. Also enzymatic activities are influenced by PAH contamination; dehydrogenase activity appears to be the biological parameter most sensitive to these contaminants in different types of soil [14]. Once in soil, the fate of PAHs is determined [15] by their distribution among the solid, liquid, and gaseous soil phases, which strongly influence the processes of migration and degradation. PAHs of low molecular weight may volatilize into the atmosphere or be leached along the soil profile. Those of high molecular weight may be strongly adsorbed to clay materials and humic substances of the soil. In addition, these compounds can undergo redox reactions of abiotic origin and can be absorbed and biodegraded by microorganisms in the soil.

The role of soil organic matter is of paramount importance in determining the fate of PAHs in soil; due to similarity of PAHs to humic substances, they are strongly adsorbed by soil organic matter. Interactions among PAHs and organic matter have been described according to different models; in particular the distributed reactivity model (DRM) and the dual-mode model (DMM) describe organic matter as a multidomain material, showing either linear or nonlinear sorption characteristics [16]. PAH sorption can be described by a dual-mode sorption composed of absorption by amorphous humic materials and adsorption to carbonaceous materials such as black carbon [17]. Due to the high affinity, the sorption process is often nonreversible, and it can be considered one of the main factors responsible for the aging process that greatly reduces PAH bioavailability [18]. Therefore, PAHs that are less volatile and less soluble in water accumulate in the soil.

Persistence in the soil depends on the overall result of all the mechanisms of transport and degradation above mentioned [15]. Water solubility should be considered one of the most important physical-chemical properties for PAH biodegradation. Hydrophobicity increases with increased number of fused benzene rings. Thus, low molecular weight PAHs are more quickly released from soil surfaces and therefore are more available for microbial degradation [19]; as a consequence many organisms are able to degrade 2 and 3 ring PAHs, while relatively few have been discovered to degrade 4, 5, and 6 ring PAHs. Because of their chemical–physical characteristics PAHs persist in the environment for long periods and are thus considered persistent organic pollutants (POPs). Nevertheless, these compounds can undergo transformation and degradation processes due to biotic and abiotic reactions. Photodegradation processes have an important relevance in the degradation process; PAHs can be degraded via two mechanisms: direct photolysis by ultraviolet radiation ($\lambda < 290$ nm), and indirect photolysis and photooxidation, due to the action of oxidizing agents (•OH radicals, O_3, NO_x). However, these reactions can result in the production of molecules far more dangerous to the environment, as in the case of nitro derivatives formed by interaction with NO_x [20]. Other processes of abiotic transformation of these organic contaminants may be derived from the oxidizing action of metal ions such as manganese and iron [21].

Microbial degradation is considered one of the principal mechanisms of PAH removal from soil [10, 22–24]. The metabolic processes involved in the degradation of the PAHs are predominantly aerobic based on oxidation reactions with oxygen or nitrate as electron acceptor. These processes are based on the cleavage by oxidation of the aromatic ring, with consequent formation of metabolites and carbon dioxide. Following exposure to hydrocarbons, the oxidation potential of microbial communities may increase due to adaptation processes [25] that produce an increase or a decrease of specific enzymes. Moreover, new metabolic abilities may develop after selective enrichment of organisms able to transform these pollutants [25–27]. The ability of microorganisms to degrade PAHs may be ascribable to the synthesis and subsequent excretion of enzymes characterized by oxide reductase activities [28, 29]. These enzymes are involved in the degradation process of recalcitrant organic compounds, such as lignin, a complex organic polymer consisting of multiple phenyl propane units. These enzymes (phenoloxidase and peroxidase) are able to oxidize PAHs [30] due both to their low substrate specificity and to the structural similarity of PAHs with lignin, resulting from their aromatic character.

Fungi, especially white-rot fungi (belonging to the group of Basidiomycetes and to a lesser extent of the Ascomycetes), are the organisms mostly involved in the degradation of lignin as oxide reductase producers, mainly peroxidases and laccases. Even brown-rot fungi possess "PAH-degrading" enzymes, which have shown the ability to use PAHs as a sole source of carbon [31]. Also many kinds of saprotrophic bacteria in soil synthesize enzymes with phenol oxidase activity [32, 33]. The degradation has been also promoted by sporogenic bacteria such as *Bacillus* and proteobacteria including *Pseudomonas* [34]. Microbial communities in soil have a very high potential to degrade PAHs. Synergy between various microbial groups promotes complete degradation; for example, by-products generated from the oxidation of PAHs by fungi can be further used by bacteria until complete decomposition. The action of specific bacteria ligninolytic and non-ligninolytic fungi has been reported in detail elsewhere [9]. In contaminated soil, the presence of readily biodegradable substances may produce a reduction of available oxygen and, in these cases, also PAH anaerobic degradation by means of electron acceptors other than oxygen has been described [9].

2 Heavy Metals

Heavy metals are defined as metallic elements that have a relatively high density at least five times greater than that of water. The term is broadly used to also include certain elements such as arsenic, which cannot be formally considered a heavy metal. Heavy metals are naturally present in the soil environment deriving from the pedogenetic processes of parent materials. However, in industrialized countries, many activities contributed to increase in heavy metals concentration in soil such as industrial activities, mining, waste disposal, etc. Heavy metal soil pollution has been increasing since the beginning of the Industrial Revolution. Since metals are not biodegradable, they tend to persist and accumulate in soils; however, the risks to humans and the environment strictly depend on their bioavailability.

2.1 Toxicity Effects

Heavy metals have been used for thousands of years, and emissions into the environment occur via air, water, and soil. The main hazards to human health from heavy metals are derived from exposure to chromium, lead, cadmium, mercury, and arsenic, the last one being a metalloid associated for its toxicity to heavy metals [35]. The environmental exposure of humans to heavy metals involves a very high degree of complexity, especially near contaminated sites, where the population is frequently exposed to a wide variety of pollutants, whose biological effects may be synergetic. To evaluate the risks in the presence of a complex environmental contamination requires studying of the molecular mechanisms of action of each contaminant and the identification of possible interactions between different biological effects. Regarding heavy metals, the association between environmental exposure and increased incidence of cancer is well known and widely documented for various metals by the International Agency for Research on Cancer. Specifically, chromium (Cr), cadmium (Cd), and nickel (Ni) are considered in Class 1 human carcinogens based on sufficient evidence of a carcinogenic effect on humans [12].

Lead (Pb) and mercury (Hg), frequently associated with environmental contamination, are classified as possible carcinogens (Class 2B) only in some chemical forms. Arsenic, due to its mechanisms of interaction with biological material, is considered a carcinogenic Class 1 contaminant [12]. Heavy metals are able to interact with different stages of the process of carcinogenesis, producing DNA damage directly or indirectly, reducing the efficiency of the defensive systems of the cell. Thus, they act as cancer promoters, in some cases also by modulating the processes of cell adhesion with consequences for the ability to produce metastases. Heavy metals are able to interact with cell components, producing, directly or indirectly, DNA damage; thus, they act as cancer promoters [36, 37].

2.2 Heavy Metals in Soil: Behavior and Effects

Heavy metals from anthropogenic sources are generally more mobile and their fate and transport in soil strictly depend on soil characteristics, which determine the chemical form and speciation of the metal [38]. Once in the soil, heavy metals distribute into different soil phases by precipitation—dissolution and adsorption—desorption reactions. In soils characterized by high contents of humic acids and clay minerals, metals are strongly retained by complexation and adsorption reactions, which reduce their mobility. pH affects the concentrations of metals in soil solutions by regulating precipitation–dissolution, specific adsorption, and complexation processes thus determining the concentration of most metal ions in the soil pore water. Ion exchange and specific adsorption are the mechanisms by which clay minerals adsorb metal ions from the soil solution. Highly selective sorption occurs at the mineral edges, but differences exist between clay minerals in terms of their ability to retain heavy metals. Also hydrous iron and manganese oxides are particularly effective in influencing metal solubility under relatively oxidizing conditions.

They reduce metal concentrations in soil solutions by both specific adsorption reactions and precipitation. The organic matter in soil has a great influence on metal mobility and bioavailability due to the tendency of metals to form soluble or insoluble complexes with organic matter. The negative charges on soil surfaces, described by cation exchange capacity (CEC), may be pH dependent or permanent. Heavy metals can substitute alkaline cations on these surfaces by exchange reactions; specific adsorption promotes the retention of heavy metals, also by partially covalent bonds. Redox potential (Eh) in soil determines the reduction–oxidation reactions, which control the chemical forms of metals at different oxidation state. Well-aerated soils are characterized by high values of Eh, while soils subject to waterlogging tend to have lower Eh values [38, 39].

Transport and retention are the key processes that determine the fate and behavior of heavy metals in soil. Transport may occur through the soil solution by diffusion or by mass flow or convection. Retention of heavy metals on soil surfaces strongly determines metal release into soil solution and their transport to groundwater. The process of retention comprises chemical and physical adsorption and precipitation. The adsorption processes are essential for the evaluation of the soil as a protective barrier against heavy metals. The distribution of heavy metals between the solid phase and the soil solution is considered to be a fundamental factor in the assessment of the environmental consequences of the accumulation of metals in soil. The soil's ability to hold heavy metals in the solid phase is the fundamental mechanism by which soil protects other environmental matrices. Therefore, it is essential to assess the strength of this holding action and the nature of chemical bonds involved. Different kinds of forces retain metals on surfaces; these forces range from electrostatic to covalent with related bonding energies. Mechanisms that remove metal ions from solution include ion exchange and specific sorption [40]. Adsorbed on solid phases, heavy metals are usually unavailable to environmental processes, including plant uptake.

Thus, adsorption processes influencing the equilibrium between soluble and solid phases determine their fate in the soil environment. Several models have been used to describe the retention/release reactions of metals in soils. The adsorption equations theoretically refer to a state of equilibrium in which the rates of adsorption and desorption are equal. This implies a reversible process; however, some metal species are irreversibly held by the solid phase due to the formation of bonds, which are not exclusively electrostatic. Despite these theoretical limitations, several models are commonly used to describe heavy metals sorption in soil. The most frequently used equations in soil chemistry are the Langmuir and Freundlich equations. The Langmuir equation (1), although originally derived for gas adsorption on solids, has been used successfully to describe heavy metal adsorption in different kinds of soil [40]:

$$q = \frac{q_{\max} KC}{1 + KC} \tag{1}$$

where q is the amount of metal sorbed per unity of mass of soil, $q_{\max}$ is the maximum amount of metal adsorbed by the soil, C is the equilibrium metal concentration, and K is a constant. The Langmuir equation can be derived from the action mass law, whereas the Freundlich equation (2) derives from the assumption that there is a linear relationship between the surface energy and the sites occupied. The general equation is:

$$q = KC^n \tag{2}$$

where q is the amount of metal sorbed per unity of mass of soil, C is the equilibrium metal concentration, and K and n are Freundlich parameters related to the maximum amount of adsorbable metal and the energy of bonds with which the metal is retained. Many other equations have been used in studying sorption and release of heavy metals in soil and elsewhere reported [41].

Much effort has been spent to quantify heavy metals retained with different kinds of forces by soil surfaces; in particular, in contaminated soils attention has focused on mobile and bioavailable metals [38, 39]. In soil, the chemical forms of heavy metals can be various. Heavy metals can be present as simple or complex ions in the soil solution, adsorbed or precipitated on the solid phases from which they can be released. When not specifically sorbed, they can be replaced by a competing cation by exchange reactions. If the binding mechanisms involve complexation and adsorption, metals are specifically sorbed and they are linked by covalent bonds to soil components. Heavy metals can also be occluded and coprecipitated with oxides, carbonates, and phosphates from which they can be released under specific conditions. In the crystalline lattices of primary minerals, metals are present in chemical forms that are not involved in environmental processes.

Knowledge of the chemical forms of metals is the key to understanding the toxicity, environmental hazards, and possible remedial strategies. Heavy metals are essential for plant and animal life, but can become toxic at high concentrations. Their toxicity for living organisms is closely linked to the bioavailability. In contaminated soils, bioavailability can be assessed by biological and chemical assays. The chemical assays provide information about bioavailability, determining the quantity of metals in soluble form thus in the liquid phase of the soil or easily releasable from the solid phase, for example metals retained with electrostatic bonds. This quantity can be determined either by direct sampling of interstitial water in the soil or through extractions with suitable reagents, such as water or dilute solutions of alkali metals.

Table 2 Some technologies utilized for PAHs and heavy metals

Class type	Technologies	PAHs	Heavy metals
Physical	Soil washing/soil flushing	Yes	Yes
Chemical	Solvent extraction	Yes	Yes
	Supercritical fluid extraction	Yes	Yes
	Precipitation	No	Yes
	Chemical oxidation	Yes	No
	Photocatalytic degradation	Yes	No
	Electrokinetic	Yes	Yes
Thermal	Incineration	Yes	No
	Thermal desorption	Yes	Yes
Biological	Bioremediation	Yes	No
	Phytoremediation	Yes	Yes

Extractions with stronger agents can be used to assess the potential release of metals from the surface of the soil with time [39, 42]. The chemical extraction must be supported by a biological test, for example evaluating the metal content in plants growing in the polluted soil. Chemical and biological tests are unable to produce a direct measure of bioavailability but both provide information about the amount of bioavailable metal [43]. The mobility/bioavailability processes may be used in the choice of remediation technologies. The aim of these technologies may be to remove from the soil the mobile fractions of the metals or to convert them to permanently stable forms. In the first case, chemical additives, which increase the mobility, are used. Alternatively, procedures may be used that reduce the bioavailability and prevent movement of the pollutants from the soil to living organisms [44].

3 Remediation of Heavy Metal and PAH Contaminated Soils

Due to concerns over health risks, many remediation technologies of soils contaminated with heavy metals or PAHs have been proposed and used. When the soil is simultaneously polluted by both these contaminants, the process of remediation presents considerable technical and economic difficulties. In Table 2, some remediation technologies for PAHs and heavy metals are schematically reported. Remediation technologies can be formally classified in Physical, Chemical, Thermal, and Biological according to the different processes adopted. They can be applied "in situ" or "ex situ" after excavation of soil.

Only few technologies are applicable when both contaminants are simultaneously present in soil, since we have to consider that heavy metals can severely reduce the biodegradation of PAHs. Of course, strategies of train technology can be applied; however, with a view to saving of time and costs the use of the same technology represents the best choice wherever possible. Some remediation technologies able to address both heavy metals and PAH contaminated soils are synthetically reported.

3.1 Soil Washing

Soil washing (SW) is a physical/chemical "ex situ" treatment which exploits the tendency of contaminants to adhere to soil finest particles. The technology is based on the intimate vigorous mixing of excavated soil with a solution, typically water, followed by a classification step, which separates soil into different size fractions. Contaminants, which are attached to coarse fractions through forces of adhesion and compaction, are removed by abrasive scouring and scrubbing action and concentrate into a smaller volume of soil through particle size separation [45]. This technology has been successfully applied to concentrate the heavy metals into a reduced soil mass (typically 5–30 % of the original soil volume) for subsequent treatment, whereas clean or slightly polluted fractions can be returned to the site as fill, or otherwise used. The technology is highly practicable when metals are mostly present as water insoluble forms and in such case the technology should be strictly considered a physical process. In addition, it is essential that a relevant fraction of soil particles be of coarse size. Soil washing has been used also for PAHs [46], but although it is possible to address both kinds of contaminants the real application is extremely difficult due to the need to separate heavy metals in the solid phases and PAHs in the liquid one.

Treatment is often difficult due to the low solubility of the hydrophobic organic contaminants, such as PAHs, which, being strongly lipophilic, tend to be adsorbed to the organic substance present on the fine fractions of the soil (silt and clay). The efficiency of treatment is limited or reduced especially when these contaminants are present in high concentrations. However, if their concentration is high even on the larger particles, the washing of soil with surfactant agents can be a viable solution [47]. In this case, surfactants added to the wash solution, to a concentration of a few $g\ L^{-1}$, can increase the rate of desorption of PAHs and their transfer from the soil. Surfactants are capable of lowering the interfacial tension and to collect the PAHs by forming micelles [48, 49]. Surfactants are particularly attractive for remediation because they are characterized by low toxicity and high biodegradability and, thus, are more environmentally compatible than other systems based on organic solvents. However, the efficiency of physical soil washing has not been tested when these contaminant classes coexist.

Soil washing as a chemical technology has been recently investigated for mixed contamination by PAHs and heavy metals, also "in situ" (soil flushing). Sequential flushing using two chemical agents: a surfactant (5 % Igepal) and a chelant 0.2 M ethylenediaminetetraacetic acid (EDTA) have been evaluated for the remediation of soils contaminated by heavy metals and PAHs. Chelant released heavy metals, while PAHs were removed by surfactant flushing, but the process is highly dependent on site and contaminants' characteristics and requires further improvements for full scale application [50]. The same conclusions were also reported by the authors for different chemical agents [51]. Very positive results of this cleanup strategy have been reported following the use of carboxymethyl-β-cyclodextrin (50 g L^{-1}) and carboxymethyl chitosan (5 g L^{-1}). Repeated washing cycles using these solutions have been reported to efficiently remove about 90 % of total PAHs and heavy metals [52].

3.2 Electrokinetic Technology

Electrokinetic remediation technology (EKRT) is a treatment that was originally developed for soils with a high clay content, contaminated by heavy metals. The technology is based on the insertion of electrodes in the contaminated soil. A direct current with low electric potential is applied to the electrodes; as a result different contaminant transport mechanisms are generated:

- Electromigration, which involves the transport of ions and other polar complexes dissolved in the pore soil solution, caused by the electrical potential applied
- Electroosmosis, the transport of ions and dissolved contaminants due to the movement of the interstitial soil solution, generated by the presence of the electrical double layer on the charged surfaces of the solid phase. Nonionic species are also transported along with the induced water flow
- Electrophoresis, the movement inside pore solution of colloidal particles with surface charge caused by the applied electrical potential

Among the processes of treatment available, electrokinetic remediation is interesting because of the advantages, linked to the possibility of being employed in situ and in clay soils. Many studies have investigated the electrokinetic removal of heavy metals from contaminated soil [53]. To improve the removal of metals and reduce the time for cleaning, systems may be employed that aim to adequately control pH by increasing the movement of the acid front that promotes the release of metals from the solid phase of the soil and their migration. The use of additives such as EDTA produced conflicting results depending on the cases studied [54–57]. Metal ions are transported to the electrode with the opposite charge (electromigration). The electroosmotic flow that is generated provides a driving force for the movement of solubilized contaminants [58]. Moreover, the treatment is able to simultaneously remove heavy metals and organic compounds. Electrokinetics has been used to remove PAHs from contaminated soils. Often the technology has been applied in conjunction with other treatments such as ultrasounds, to enhance electroosmotic or Fenton processes to promote oxidative/reductive processes [59]. In other cases, to improve the efficiency of removing PAHs, surfactants are employed. Positive results have been obtained at laboratory scale on Manufactured Gas Plant (MGP) soils. The PAHs are solubilized by surfactants in the presence of cyclodextrins and migrate significantly to the cathode [60]. Cyclodextrins are cyclic oligosaccharides derived from the degradation of starch by bacteria that have the ability to solubilize both heavy metals and PAHs. In particular, it has been reported that a modified cyclodextrin, hydroxypropyl β-cyclodextrin (HPCD), is capable of solubilizing both some PAHs and heavy metals simultaneously [61].

Cyclodextrins are particularly effective for the removal of phenanthrene in clay soils [62]. Also in this case the compound is collected at the cathode, due to the electroosmotic flow [63, 64]. This study is particularly interesting because the technology is applied to both classes of contaminants present in the soil at the same time. These authors underline both the potential and the drawbacks of this technology. In particular, there is a need to produce higher electroosmotic flow with higher concentration HPCD to obtain significant phenanthrene removal efficiency. Moreover, it is necessary to adjust soil pH towards acidic values to increase nickel removal efficiency. The effectiveness of EKRT is closely dependent on soil properties such as its buffering capacity, organic matter content, heterogeneity, and presence of coarse material. These conclusions were confirmed by an accurate study of EKRT application to marine sediments simultaneously polluted by heavy metals and PAHs. The technology is not viable without the appropriate additives. Heavy metal removal was enhanced only if EDTA was applied at both sides of the electrokinetic cell, while even after surfactant Tween 80 addition the efficiency of the technology was not satisfactory for PAH removal [57].

3.3 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is based on using a gas as solvent in conditions of pressure and temperature higher than the critical values; in the supercritical state, the fluid exhibits the high density and low compressibility of a classic liquid solvent and the high diffusivity and low viscosity typical of a gas. In terms of power solvation, as this characteristic is directly dependent on density, for solutes of similar molecular polarity, the supercritical fluid may be considered a good solvent, capable of dissolving amount of substance comparable to those obtained with equal amounts of organic solvents. At the same time, its excellent transport capacity facilitates better penetration in the soil matrix allowing a near complete extraction of solutes with advantages in terms of extractive high yields and reduced extraction times [65].

In supercritical fluid extraction, the extracted contaminants are solubilized into the supercritical solvent from which can be separated by changing pressure and temperature conditions. Excavated soil extracted with a stream of SFE is not negatively affected and can be returned to the site, while the solvent can be recycled for further extraction. Although there are many substances that can be in supercritical conditions, in practice the fluid most commonly employed is carbon dioxide [66] because it has a critical point ($T_c = 31.08$ °C; $P_c = 73.8$ bar) which allows to work under relatively mild conditions of temperatures and pressures, such as those commonly used in industrial systems. This characteristic is particularly useful both in terms of energy and because of the possibility of reducing degradation in the case of extraction of thermolabile substances.

The technology can be used for heavy metals [67, 68] by adding as modifier a complexing agent which is able to react with the charged ions to form neutral complexes that can be dissolved in the supercritical CO_2 [69]. Soil pH, moisture, temperature, and chemical forms of the metal species in the soil [68, 70] largely influence the efficiency of the remediation of metal ions from various solid and liquid matrices [71]. PAHs also can be successfully treated [46, 72]. Efficiency of PAH remediation has been obtained by the use (in addition to CO_2) of modifiers such as pentane, acetone, and methanol [73]. The increased efficiency can be attributed to the modifier's ability to break strong hydrophobic interactions between the soil matrix and the PAHs [74, 75]. The SFE can be used as a first step in train technology with the aim of extracting contaminants; the contaminants and any used solvent can be further treated more cost effectively. Furthermore, in the separation step, it is possible to bring back carbon dioxide under gaseous conditions, allowing the total release of this nontoxic, chemically inert gas. In comparison with conventional solvent extraction, SFE requires shorter extraction times and reduced solvent usage without leaving toxic residues in the soil [71]; however, further improvements are necessary for the extraction of both PAHs and heavy metals from multi-contaminated soils.

3.4 Phytoremediation

Phytoremediation is a technology that uses the natural biological processes of plants and rhizosphere microorganisms for removal or transformation of contaminants in soil. The technology is applied "in situ" and is characterized by its positive impacts on the environment and the low cost. Phytoremediation can be employed for the treatment of organic contaminants including PAHs and inorganics such as heavy metals. Depending on the interaction between plants and the soil to be treated and the physiological action that the plant exerts on the pollutants, the technology has been formally divided into different subcategories according to the remediation mechanisms: degradation, extraction, and stabilization. Although phytoremediation has greater economic and ecological benefits in comparison with conventional methods, it also has limitations. The main advantages of this methodology are its low cost, its non-invasiveness, landscape restoration, increased activity and diversity of soil microorganisms, and decreased human exposure to polluted substrates.

The main disadvantages include the long time required for completion of the reclamation due to slow growth of the plants, the poor efficiency in contaminants removal when present at low bioavailability, and the inability of the roots to reach the contaminant at considerable depths. Although increasing attention is been focused on this decontamination technology, its full scale application is still in a consolidation phase [38]. This also depends on the fact that every remediation is site-specific, and for each case involves numerous interdependent variables (soil and its characteristics, type, concentration and depth of the contaminant, plant species, etc.). However, this remediation approach is especially promising for addressing both PAHs and heavy metals, since plants are able to accumulate heavy metals and positively promote PAH-degrading microorganisms' proliferation in the rhizosphere.

Degradation rate of PAHs by phytoremediation mainly depends on the specific characteristics of the plants. In general, the rate of degradation of PAHs increased in presence of plants compared with non-vegetated soil. Several grasses and legumes have been found to promote the removal of PAHs from contaminated soil [76, 77]. *Medicago sativa* plants have led to an improvement of the physical–chemical properties of polluted soils restoring the initial values of pH and reducing salinity. These improvements have reduced the toxicity of the soil resulting in elongation of plant roots and positive impact on microbial activity in the rhizosphere. The presence of *Medicago sativa* has improved the degradation of benzo[*a*]pyrene, starting from a very high concentration [78]. In situ phytoremediation of PAH contaminated soil by intercropping alfalfa (*Medicago sativa* L.) with tall fescue (*Festuca arundinacea* Schreb.) has been positively tested. With the combination of *M. sativa*/*F. arundinacea*, high concentrations of PAHs were found in plants (270/284 μg kg^{-1} respectively). Intercropping of the two species led to percentages of removal of PAHs in soil up to 30 %, with an effect particularly relevant for hydrocarbons of high molecular weight, 30.9 % for 4 rings PAHs and 33.4 % for 5/6 rings PAHs. Intercropping also increased the number of PAH-degrading bacteria and microbial activity in soil [79].

The characteristics of the contamination play a very important role in the efficiency of phytoremediation; the action of plants is quite different in aged compared to freshly polluted soil. In a comparison of soils spiked with PAHs and soils with aged contamination, the efficiency of phytoremediation was quite different. In spiked soils after the growth of the plants, the PAHs concentration was reduced up to 80 %. In particular, the compounds with 2 or 3 rings underwent a greater reduction. In soils containing aged PAHs, the concentration of all hydrocarbons decreased up to 25 % [80]. Often in the presence of high amounts of contaminants, even the most resistant plants fail to grow. In many plants, the presence of high levels of metals induces the synthesis of ethylene from stress, which inhibits the elongation of the roots and causes a severe deprivation of iron, which in turn inhibits the synthesis of chlorophyll and chloroplasts [81].

A strategy that overcomes this limit involves the use of bacteria, which promote plant growth (PGPB). These bacteria are able to increase both the number of seeds that germinate and the amount of biomass produced from plants. With the addition of PGPB, a phytoremediation process is faster and more efficient. Plant growth-promoting bacteria can positively influence plant growth increasing the uptake of nutrients from the environment, reducing in the meantime the negative effects of phytopathogenic organism [82]. Until the early 1990s, field and laboratory studies have suggested that inoculation with non-pathogenic bacteria can have positive effects on the health of plants and their growth, and thus an increase in yield and their usage was widespread for applications in the agricultural field. Bacteria may supply atmospheric nitrogen to plants and produce siderophores, which can increase the available iron in soil and synthesize auxins and cytokinins, which promote various stages of plant growth. Bacteria employ any one, or more, of these mechanisms under different conditions; moreover, they produce enzymes that can promote plant development. The use of PGPR has been shown to positively influence the efficiency of phytoremediation both for PAHs and heavy metals [83].

In the case of inorganic contaminants, two main strategies can be used: phytostabilization and phytoextraction. Phytostabilization involves the ability of roots to immobilize the contaminants in the root zone while stabilizing the soil, thus reducing metal leaching and aerial dispersion of contaminated soil particles. Phytostabilization is particularly suitable in those cases when the concentration is so high, such as in the mining sites, that the processes of phytoextraction would require too long to achieve remediation goals; thus, it is essential to maintain the metals in nonmobile forms in soil. Moreover, the growing plants can control hydraulic fluxes and are able to improve the structural stability of soil decreasing the erosive processes and the consequent migration of contaminants providing an adequate immobilization. During phytostabilization, plants must not be removed. Phytostabilization can also be used as a transitory solution for those sites where the removal of metals seems to be unsustainable due to the long time required as well as high cost required. Of course, this technique does not imply the definitive removal of pollutants, which remain immobilized in the site [84].

The fundamental principle of phytoextraction is to use the plant as an extractant capable of absorbing metals from soil by means of the root system and transfer them through the transpiration flow in the aerial part. At end of growth plants can be harvested, removing the adsorbed metals from the soil. The efficiency of the absorption processes depends on the properties of the soil, on the physical–chemical properties of the contaminants, on their chemical form, and on the characteristics of the plant species. Phytoextraction was originally thought for the use of hyperaccumulator plants capable of absorbing metals from the soil and to concentrate them in the aerial part. Almost all hyperaccumulator species have been identified in soils with very high concentrations of heavy metals, in which selected varieties are able to grow in a particularly hostile environment [85].

Several species have been recognized as hyperaccumulators of different metals. Often, however, hyperaccumulators have a reduced biomass production that does not allow relevant removal of heavy metals. To overcome this limitation, it is possible to use plants commonly employed in agriculture that have a higher biomass production. In this case, the efficiency of metal removal may be increased modifying the bioavailability of contaminants by the use of suitable chemical additives in the soil: “assisted phytoextraction” [86, 87]. The efficiency of technology strictly depends on the pollutants’ bioavailability, which in turn is determined by the chemical and physical characteristics of the soil [38]. Therefore, it is very important to evaluate the bioavailability of heavy metals in soil since only the “bioavailable” amount can be absorbed by plants. When bioavailability is low, it may be increased by the use of metal mobilizing treatments, for example, by addition of chelating agents (EDTA, etc.) [88–91]. In the opposite case, however, if the quantity of heavy metals in the soil solution is too high, it is possible to reduce the soluble amounts, for example by changing the pH of the soil or by adding absorbent materials, which possess specific adsorption sites for the metals [44].

In the soil, a contaminant can interact with the surfaces of the solid phase, with reactions of adsorption—desorption and precipitation—dissolution. A fraction of the contaminant remains in the liquid phase, where it is transported with the soil solution. Contaminant's bioavailability for plants depends on all these reactions, and it is influenced by the chemical characteristics of the contaminants and by soil characteristics such as pH, organic matter, clay content, cation exchange capacity, and redox potential [39]. Heavy metal phytoextraction is a very attractive remediation strategy since it enables the use of a biological technique to remove nonbiodegradable contaminants from a contaminated site. Before phytoextraction could be effectively applied, the specific conditions of the contaminated site must be considered. In general, several preliminary aspects linked to the distribution of contamination must be verified:

- Whether the site is large enough to grow plants with an adequate opportunity to carry out normal agricultural practices
- Whether the treatment can be employed for a sufficiently long period of time
- Whether the concentration of pollutants is not too high to create problems of phytotoxicity to the used plants
- Whether the contaminants are in the soil depth explored by plant roots

In addition to the concentration of metals in plants, it is also essential to determine the total accumulation [92], resulting from the product of the concentration of the metals in the tissues of plants for the biomass produced. This quantity provides the amount of metal actually removed and thus of the efficiency of the technology. In this way, estimates can be made on the time needed to complete the remediation. Of course, to optimize the absorption of metals, it is essential that plants are able to grow and develop properly. It is shown that the appropriate use of fertilizers and phytohormones helps to overcome difficulties in plant growth due to phytotoxicity and to increase plant biomass production [93–98].

At the end of this brief review of technologies, it can be said that although several technologies are able to decontaminate soils polluted by metals and PAHs, it is not easy and often not possible to use the same technology at the same time for the two classes of contaminants. As an example, chemical soil washing of metals requires reagents that are quite different from those needed to solubilize PAHs. Similarly, electrokinetic is not easy to manage so that it can act simultaneously on metals and PAHs. Further studies must be carried out to apply a single technology in the presence of simultaneous contamination by metals and PAHs. A very promising technology choice seems to be the use of phytoremediation. As previously described, many plants species have the ability to take up high levels of metals and translocate them from roots to shoots. In the meantime, in the presence of growing plants, the processes of degradation of organic compounds are increased due to synergistic effects between plants and microbial communities in soil and the induced chemical changes in the rhizosphere. Some, among the others, plant species used in phytoremediation of heavy metals and PAHs are reported in Table 3.

Table 3 Some plant species used in phytoremediation of heavy metals and PAHs

Plants species	Metals	PAHs
Brassica juncea	Cu, Ni, Pb, As, Cd, Cr	Pyr, BkFL, AcPy, Ant
Pteris vittata	Cu, Ni, Zn, As	Phe
Thlaspi caerulescens	Zn, Cd, Ni, Pb, Hg	
Heliantus annuus	Pb, Cu, Ni, Zn, As	
Arobiodopsis thaliana	Zn, Cd, As, Hg	
Zea mays	Zn, Cu, Pb	Ant, Phe, BkFL
Medicago sativa	As, Cd, Cu	Ant, Pyr, Nap
Panicum virgatum		Ant, Pyr, BaP, BaA
Sorghastrum nutans	Zn, Cd, Ni, Pb	Chr, BaP, BaA, DBA
Festuca rubra	Pb, Zn, Cd	Nap
Festuca arundinacea	Pb	Nap, Chr, BbFL,BkFL, DBA
Echinacea purpurea	Cu, Cr, Pb, Zn	Flu, Pyr, BaA, Chr
Fire Phoenix		Chr, BbFL, BkFL, DBA
Trifolium pretense		Nap
Glycine max		Ant, BaA

Numerous researches have demonstrated the efficiency of the use of plants for the remediation of soils contaminated by organic compounds mainly by the process of rhizodegradation [16]. Many studies have highlighted the ability of the plants to facilitate the degradation of organic pollutants in soil. Plants and microorganisms have many symbiotic relationships making the rhizosphere a field of intense microbial activity with an increase in the number of microbial communities, able to improve the physical and chemical properties of the soil [99]. The efficiency of heavy metals phytoextraction can be greatly increased by modulating contaminants bioavailability in soil. If phytoextraction is planned to remove the bioavailable fractions of metals [39], it offers a sustainable approach to remediation since at the end of the treatment the quality of soil is increased. Even with the limitations related to the specific characteristics of the technology, phytoremediation appears as a versatile solution, cost-effective, and of high environmental quality for the remediation of soils simultaneously contaminated by heavy metals and PAHs.

4 Case Study

A phytoremediation feasibility test, at a microcosm scale, was carried out with the aim of evaluating the efficiency of two plant species, *Brassica juncea* and *Zea mays*, in the simultaneous remediation of a soil polluted by Pb and PAHs. These two plants were selected due to their ability to grow in the Mediterranean climate and in polluted soils. Moreover, the species seem particularly appropriate because their deep-rooted system can explore a large portion of soil.

4.1 Experimental Procedure

The soil used in this study was collected from a former industrial site in northern Italy where various industrial activities had been carried out since the beginning of the last century. The soil resulted simultaneously contaminated by PAHs (10000 mg kg^{-1}) and Pb (120 mg kg^{-1}).

Soil samples were air-dried and ground to pass through a 2 mm sieve before characterization analysis. Soil pH, cation-exchange capacity (CEC), and soil texture were determined according to Soil Science Society of America (SSSA) methods of soil analysis [100]. The contaminated soil was characterized by a pH value of 7.58, a CEC of 19.6 $c_{(+)}$mol kg^{-1}, and the following texture: sand 68.0 %, silt 19.8 %, and clay 12.2 %.

Experiments were carried out at microcosm scale using 300 g of the contaminated soil. A total of 1.0 g of *B. juncea* seeds or six seeds of *Z. mays* were used in five replicates for each species per microcosm with five unplanted controls run simultaneously. Experiments were carried out in a growth chamber in controlled conditions: 14 h of light with a temperature of 24 °C and 10 h in the dark at 19 °C. Relative humidity was maintained at 70 %. The growing period lasted 3 months, after which plants started to decay. The additive, Ethylenediamine-N,N'-disuccinic acid (EDDS) 10 mM, was added 7 days before harvesting. PAHs decrease in soil was tested by analyzing the concentrations in soil at the beginning and end of the experiments in vegetated and non-vegetated microcosms. At the end of the growth period, plants were collected and shoots were separated from roots and washed with deionized water. Pb uptake by plants was measured determining Pb concentration in roots and shoots of the two selected species. Aggregate stability, used as an index of soil quality, was determined by the single sieve method [101–104], using soil samples of 10 g of the 1–2 mm size air-dried samples. The soil material retained on the sieve was oven dried, weighed, and then corrected for sand content. The wet aggregate stability (WAS) was calculated as:

$$\text{WAS}\% = \left[\text{Retained Soil Material} - \text{Sand}\right] / \left[\text{Soil Sample} - \text{Sand}\right] \times 100$$

4.2 Analytical Methods

The soil samples in this study were extracted using EPA method 3550, with a mixture of acetone/hexane (1:1 v/v). Soil extracts were analyzed by GC/MS, according to US EPA method 8270C, using a Thermofinnigan “TRACE DSQ” GC-MS with a quadrupolar analyzer and PTV injector (DB 5 ms capillary column, 30 m × 0.25 mm ID, 0.25 μm stationary phase film thickness). All reagents were pesticide quality. The compounds determined by the analysis were the 16 PAHs in the US EPA list of priority pollutants: naphthalene, acenaphtylene, acenaphthene, fluorene, phenantrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, crysene, benzo[*b*]

	%
Nap	38.1
AcPy	49.8
AcP	33.7
Flu	41.5
Phe	48.8
Ant	50.9
FL	38.3
Pyr	40.3
BaA	28.2
Chr	40.8
BbFL	23.7
BkFL	69.0
BaP	38.5
InP	35.9
DBA	46.4
BghiP	22.7

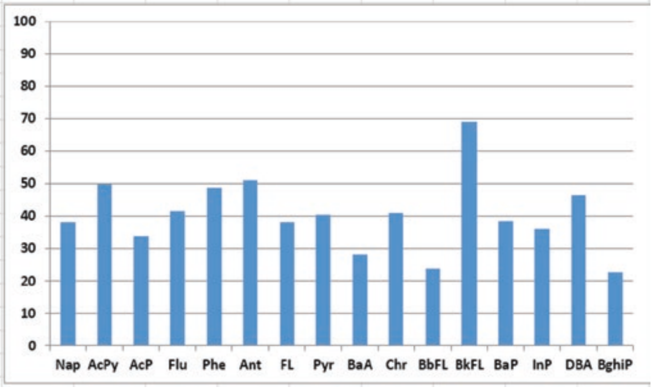

Fig. 1 Percentage degradation of each single PAH in vegetated soil with *B. juncea*. Percentages were calculated using mean concentration values

fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c*,*d*]pyrene, dibenzo[*a*,*h*]anthracene, and benzo[*g*,*h*,*i*]perilene.

Pb concentrations in soils and plants were determined by EPA method 3051 via atomic absorption spectrophotometry using flame AAS (Varian AA 240FS).

4.3 Results

4.3.1 Effect on PAH Remediation

At the end of the experiment, in the non-vegetated microcosms, PAH concentrations were the same as at the beginning of the experiment, 10150 ± 189 mg kg^{-1}. After the growing period in microcosms vegetated by *B. juncea* and *Z. mays*, the PAH concentration in soil decreased to 6146 ± 396 and 6293 ± 402 mg kg^{-1}, respectively, with a similar reduction of about 40 and 38 %. The degradation rate of PAHs with a large number of aromatic rings and high molecular weight increased more than those with a small number of aromatic rings, and the best results have generally been obtained for those PAHs with high molecular weight (5–6 rings). The addition of EDDS had no effects on PAH degradation, and no significant differences were found for each single PAH between soil treated or untreated with EDDS. This might be attributed to the fact that EDDS had no toxic effects on microbial communities [105]. Concentrations of each single compound tend to decrease in vegetated pots, both with *B. juncea* and *Z. mays*. To observe the efficiency of the two plant species in the degradation of the PAHs, percentage degradation values were calculated by considering the decrease in the concentration of a single PAH in the vegetated soil, with respect to that in non-vegetated soil (Figs. 1 and 2).

	%
Nap	46.1
AcPy	46.6
AcP	34.6
Flu	40.8
Phe	88.0
Ant	51.9
FL	29.0
Pyr	28.2
BaA	40.3
Chr	22.0
BbFL	13.6
BkFL	72.7
BaP	33.7
InP	21.7
DBA	24.2
BghiP	17.1

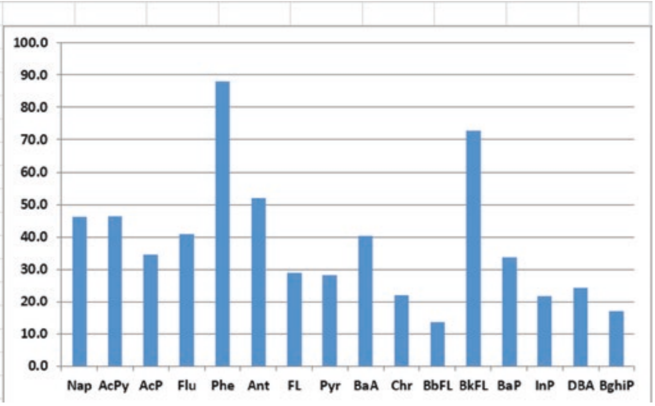

Fig. 2 Percentage degradation of each PAH in vegetated soil with *Z. mays*. Percentages were calculated using mean concentration values

In the case of soil vegetated with *B. juncea*, all the PAHs were degraded by at least more than 20 %, (benzo[*b*]fluoranthene, benzo[*g*,*h*,*i*]perylene) compared to degradation in non-vegetated soil. The highest percentage degradation was obtained for benzo[*k*]fluoranthene (69 %), while plant growing resulted in about 50 % of degradation for acenaphthylene, phenanthrene, anthracene, and dibenzo[*a*,*h*]anthracene. The remaining were degraded with an abatement percentage of about 30–40 %. *B. juncea* promoted degradation up to about 40 % of total PAH content. Similar results (about 38 %) of total PAH content were also obtained in microcosms planted with *Z. mays*, but the percentages of degradation of the single PAH were different (Fig. 2).

Most of the PAH degradation percentages increased by 20–40 % compared to those in non-vegetated soil. The lowest value of degradation was obtained for benzo[*b*]fluoranthene (10 %), while anthracene was degraded to 50 %. Benzo[*k*]fluoranthene and phenanthrene showed the highest degradations of 72 % and 88 % respectively. Thus, plants promoted the degradation of PAHs in the contaminated soil with different trends for the two investigated species. As is well known, plant growing stimulates the microbial biomass involved in PAH degradation [106, 107]. Microbial investigation (data not reported) showed that most (97 %) of the isolated bacterial strains belong to the phylum Proteobacteria in accordance with previous findings [108] and showed that growing plants were able to increase biodegradation of organics [109]. The process is highly complex, and the success of remediation depends on the specific site conditions. In this soil, the PAHs are derived from a long-time contamination, but although aged PAHs are considered to be of difficult degradation [110], the results can be considered highly positive. However, we must take into account that this high efficiency of phytoremediation is strictly linked to the specificity of microcosm experiments, where intimate contact between soil and roots exists, largely different from that in the field.

Table 4 Effect of addition of EDDS 10 mM increased Pb concentrations in shoots compared with the control

	B. juncea		*Z. mays*	
	Control	EDDS	Control	EDDS
Roots	7.5 ± 0.89	93.8 ± 2.1	6.8 ± 0.75	130 ± 4.3
Shoots	4.3 ± 0.11	51.2 ± 1.6	3.1 ± 0.10	40.3 ± 1.8

4.3.2 Plant Uptake of Lead

At the end of the growing cycle, the Pb concentrations in the tissues of *B. juncea* and *Z. mays* were determined. In the control microcosms (untreated soil), the concentration of Pb was very low in both the shoots and roots of *B. juncea* and *Z. mays*. The results highlighted the need to use assisted phytoextraction to remove Pb from the soil. Of the numerous additives reported in the literature for "assisted phytoextraction", EDTA is most commonly used, due to its high complexing capacity for most metals, such as Pb, Cu, Cd, and Zn, which generally leads to an increase in metal translocation from soil to plants [91, 111]. However, given the persistence in the soil of chelating agents such as EDTA, there is a greater risk that mobilized metals will leach into the ground or surface water. Hence, research is now aimed at new mobilizing agents that have no adverse effects on the environment while they promote the bioavailability of contaminants.

EDDS can be considered a valid alternative to EDTA for lead phytoextraction, and its efficiency is often greater than that of EDTA. These results could be due to the calcium present in the soil. In fact, the interaction of lead and EDTA can decrease due to the competition between lead and calcium for this complexing agent. Although the complex Ca-EDTA has a much lower stability constant ($\log K = 10.6$) than the Pb-EDTA complex ($\log K = 17.9$), the high solubility of calcium along with its high concentration in the soil makes this cation a powerful competitor of Pb. Regarding EDDS, the complexation constant ($\log K = 12.7$) with Pb is lower than that of the Pb-EDTA complex [112]. However, the low stability of the complex Ca-EDDS ($\log K = 4.3$) did not lead to a significant reduction in the concentration of Pb mobilized; in fact EDDS often has been reported to show an higher extractive efficiency than EDTA in the presence of significant amounts of Ca [88, 91]. In this experiment, the addition of the mobilizing agent (EDDS) led to a significant increase in Pb uptake by the plants (Table 4).

In both plant species, the amount of Pb was higher in the roots than in the shoots. The plants are able to uptake the metal but only partially translocate it to the aerial parts; in fact, as is well known, roots act with a defense mechanism against toxic elements. However, the addition of EDDS to the soil also promoted the translocation of Pb in the aerial parts of the plants. Pb concentration in the aerial parts reached 51.2 mg kg^{-1} for *B. juncea* and 40.3 mg kg^{-1} for *Z. mays*. Without addition of EDDS addition, the values in the controls were 4.3 and 3.1 mg kg^{-1} for *B. juncea* and *Z. mays*, respectively. The amount of contaminant extracted by the plants is a result of two dynamic processes, metal uptake, and biomass production and can be expressed

	B. juncea		Z. mais	
	Control	EDDS	Control	EDDS
shoots	3.0	35.8	2.1	27.4

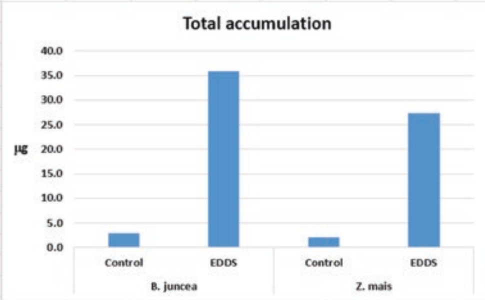

Fig. 3 Effect of EDDS 10 mM addition on Pb total accumulation in the aerial parts of plants

as “total accumulation” [92]. This is calculated as the product of the concentration of the metal in plant tissues and the respective dry biomass. The data are reported in Fig. 3. Total accumulation provides an estimation of the amount of Pb removed from the polluted soil and thus the phytoextraction efficiency. Results showed that the increase in metal bioavailability using EDDS promoted higher Pb accumulation in plants. In this experiment, *B. juncea* showed a greater efficiency than *Z. mays* and should be considered the best candidate for further phytoextraction tests at the field scale.

Effect of chelators on shoot biomass production was not significant, since EDDS was added only a few days before harvesting. This was necessary because the chelating agent could promote leaching of Pb in lower soil horizons. Plants absorbed and translocated a fraction of the metal mobilized by the treatments, and a certain amount could remain in the soil solution with an increased risk of percolation. Thus, the persistence of a high mobility of Pb after harvest should be avoided. Leaching can be countered by the degradation of the chelating agent with the consequent release of Pb, which tends to form stable precipitates due to the alkaline conditions of this soil. Therefore, the effects induced by the addition of chelating agents should be considered not only in relation to the increased Pb uptake by plants but also to the residual effects in the soil, including the metal’s release from decaying roots.

4.3.3 The Effect on Soil Quality

If one of the primary aims of the remediation process is to leave a good quality environment, evaluation of the physical properties of the soil is a very important issue [113]. Among the physical soil parameters, an important index to define the functionality of the soil is the stability of the structure. Aggregate stability influences a wide range of physical and biogeochemical processes in soil including the movement and storage of water and air in the pore system. The pore system provides zones rich of biological activity where plant roots can grow. At the end of the growth cycle, wet aggregate stability was determined using a wet-sieving methodology, in vegetated and non-vegetated microcosms. The results show how the growth of plants has improved soil structure stability. This positive effect can be ascribed to the high development of roots, which can release polysaccharide material through exudates; this may act as a binding agent promoting the increase of larger aggregates, reducing soil bulk density [114–116]. The wet aggregate stability increased from 27 to 61 % in the case of vegetation microcosms with *B. juncea* and 32–60 % in microcosm with *Z. mays*. The increased stability of the structure derived from the presence of plants highlights the improved soil quality following the green remediation approach. The aggregate stability of the soil is an important indicator of the quality of the soil. In fact, a good structural stability is able to counteract the process of compaction of the soil, typical of contaminated sites, and to increase water retention capacity, properties which in turn promote the growth of roots and improve physiological functions of the plant [117]. Good structural properties also provide better support for microbial communities; this effect can increase the efficiency of PAH degradation in subsequent cycles of growth.

4.3.4 Concluding Remarks

In the reported case study, a feasibility test was carried out to evaluate phytoremediation as a candidate technology for cleaning up a site contaminated by PAHs and Pb. Phytoremediation was shown to be a viable green remediation strategy for both Pb and PAHs. In particular, phytoremediation of PAHs appears to be a very promising technology for removing these contaminants from contaminated soils. Promoting an adequate substrate for microbial growth, plants greatly enhanced the degradation of PAHs, while in the meantime they were able to uptake, after addition of EDDS, a certain amount of the bioavailable fraction of Pb.

5 Conclusions

Understanding the mechanisms involved in the process of degradation of PAHs is important for promoting the use of green remediation strategies at contaminated sites. Soft technologies are certainly an advantageous alternative, being noninvasive and less expensive compared to traditional methods. Moreover, the application of bioremediation and phytoremediation improves the physical and biological properties of soils. In particular, phytoremediation promotes the activity of microbial species able to metabolize recalcitrant organic compounds and can speed up the process of natural attenuation very efficiently. Some contaminated sites can play a very important economic role after remediation. Sites located in areas of strategic importance from the point of view of production and trade can be transformed into high income activities, but also sites that can be devoted to service facilities and utilities can play an important role in protecting and promoting the local economy.

If remediation should ensure adequate levels of economic and financial sustainability, or generate economic benefits for the communities concerned, remediation technologies should also be directed toward recovering and preserving the local environment, starting from the function that soils can perform in the protection of the entire ecosystem. The opportunity to achieve a double purpose (social/environmental or economic/environmental) makes it essential to set up an integrated strategy of intervention based on the recovery of soil quality as an integral part of environmental restoration. This approach not only offers the advantage of stimulating economic development and employment in the areas subject to this work but also promotes the culture of "reuse", instead of that of excavation and landfilling. The positive effects associated with recovery strategies based on the principle of sustainability are very important: for the reduction of environmental risks and reclamation of degraded areas. In such contexts and conditions, when the aim of clean-up is the recovery of soil and environment quality, rather than the achievement of numerical values of pollutant concentrations, remediation of contaminated sites can become an important opportunity for local sustainable development and increased well-being.

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Phytoremediation of Polycyclic Aromatic Hydrocarbons (PAHs) in Urban Atmospheric Deposition Using Bio-retention Systems

Lakshika Weerasundara and Meththika Vithanage

Abstract Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds in urban environment and pose a great concern in environmental pollution due to their carcinogenicity. The inefficient fuel combustion is the major cause for the emission of PAHs in urban atmosphere. The emitted PAHs are either in particulate or in vapor phase however finally end up with deposition and directed into water reservoirs or groundwater table via storm water. Instead of conventional techniques such as solvent extraction, chemical oxidation, photocatalytic degradation, electrokinetic remediation, and thermal technologies, bio-retention systems can be used to remediate PAHs in storm water. However, bio-retention system does not facilitate the degradation or removal of PAHs, instead it facilitates the accumulation of PAHs in the soil. The use of phytoremediation in bio-retention systems is a hybrid technology that can provide efficient PAH removal by cutting down the biochemical cycling of PAHs. Although phytoremediation and bio-retentions systems are well-established technologies, their combination is rarely used. This chapter discusses the possibility of the use of phytoremediation in bio-retention systems, for remediation of deposited PAHs in the urban environment. Bio-retention systems with phytoremediation not only remediate PAHs but also reduce other pollutants such as heavy metals, nutrients, enhance the esthetic value, and create opportunities to produce biomass for bio-fuel production.

Keywords Polycyclic aromatic hydrocarbons • Phytoremediation • Bio-retention systems • Atmospheric deposition

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1 Polycyclic Aromatic Hydrocarbons in Atmosphere

Polycyclic Aromatic Hydrocarbons (PAHs) are semi-volatile, neutral, and nonpolar organic compounds that are found in fossil fuels and in tar deposits, and are produced by incomplete combustion [1]. Numerous PAHs are ubiquitous in the environment as a common pollutant [2, 3]. Atmosphere is an important pathway for PAHs for its global distribution [4]. The composition of PAHs in the atmosphere and its effects on the environment have been studied since early 1970s [1, 5]. Polycyclic aromatic hydrocarbons are prevalent in modern cities, and are considered as one of the frequently found compounds in the atmosphere [6]. The concentrations of PAHs in the air is usually present in few nanograms per cubic meters [7, 8] (Table 1). During the year 2007, the global total PAH emission is estimated as 504 Gg [9, 10]. Table 1 depicts the concentrations of PAHs reported in different studies.

Emission of PAHs depends on various factors, and hence the concentrations can be varied [10–12]. Studies have reported that the PAHs emission in urban areas is double the scale compared to the rural areas, globally [9, 10]. The toxicity, widespread presence, persistence, and difficulties in remediation are the concerns in the means of PAHs for human and ecosystem health [13, 14]. The multi-ringed High Molecular Weight (HMW) PAHs are relatively important because of their carcinogenic nature. Since, Lower Molecular Weight (LMW) PAHs are less carcinogenic, studies on such PAHs are lacking. However, LMW PAHs are the most abundant in urban atmosphere. Reactions of LMW PAHs with other compounds in the atmosphere be capable of forming more toxic derivatives is again a concern [15, 16]. The US Environmental Protection Agency (USEPA) has listed 16 PAHs as priority environmental pollutants and 7 of them have been identified as carcinogenic compounds [13]. Since PAHs are also listed as Persistent Organic Compounds (POPs) there is a developing concern about PAHs. The "Stockholm Convention" which has been signed in May 2001 includes instruments for total elimination of 12 POPs. Among 12 POPs, the most toxic compound is benzo[*a*]pyrene (BaP) which is a PAH compound [17]. The degradation of air quality by PAHs contributes for long-term impacts on water, soil, plant species and health defects by bioaccumulation of PAHs in wildlife and humans [18].

The PAHs are mainly produced by incomplete combustion of any type of fuel. Due to semi-volatile quality, PAHs are released into the atmosphere as gases and aerosols [19]. In general, PAHs contain a broad range of physical and chemical properties due to the availability in two different phases [19]. The distribution of PAHs in gas and aerosol phase is influencing scavenging of PAHs by particulates or storm water. The overall scavenging ratio has been defined as total PAH in rain water/total PAH in air [19]. A study has reported naphthalene, acenaphthylene, and acenaphthene as the most abundant PAHs in the gaseous phase while fluoranthene and pyrene in the particulate phase [20, 21]. The impacts also varied depending on the phase that is involved. Therefore it is important to consider the relative concentrations in different phases to total PAH loads [20, 22]. The types of PAHs available in the atmosphere are unique to the source. As an example, phenanthrene, fluoranthene, and pyrene are dominant from combustion of petroleum sources [23, 24]. Hence, the presence of different PAHs indicates the various sources of emission.

Table 1 Concentrations of PAHs found in different regions worldwide

Region		Type of deposition	Total PAH	Type of PAH (ng/m^3)															Year	Reference
				Ace	Acel	Ant	Flu	Phe	BaA	Chr	Fla	Pyr	BaP	BbF	BkF	DahA	BghiP	IndP		
Asia	Nepal, Kathmandu	Bulk	155	0.822	0.59	1.20	1.64	4.48	11.8	13.8	11.7	12.1	16.6	17.7	17.3	2.03	20.7	22.6	2013–2014	[89]
	Tainan, Taiwan	Gas and particulate				0.4			1.4	0.5		0.9		0.9	0.7	2.4	3.7		1996	[90]
	Seoul, Korea	Gas and particulate				2.7		16.4	2.6	3.6		12.5		4.8	2.5	3.2			1998–1999	[15]
	Singapore	Gas and particulate				2.0		7.2	0.5	0.9		7.3		1.2	0.2	0.07	1.0		2006	[91]
	Mount Taishan, China	Wet				3.64		33.31	1.76	3.11		6.07	2.26	2.62	1.97	2.02			2005–2007	[4]
	Guangzhou, China	Bulk	1910																2005	[92]
	Guangzhou, China	Bulk	170																2010	[93]

(continued)

Table 1 (continued)

Region		Type of deposition	Total PAH	Type of PAH (ng/m^3)															Year	Reference
				Ace	Acel	Ant	Flu	Phe	BaA	Chr	Fla	Pyr	BaP	BbF	BkF	DahA	BghiP	IndP		
Europe	Heraklion, Greece	Gas and particulate				3.3		20.0	1.1	3.1		6.6		1.5	1.8	0.1	3.4		2000–2002	[94]
	Greese	Wet	540																1996–1997	[95]
	Trier, Germany	Bulk						26	15	19		20	15	26					1999–2000	[96]
	Le Havre, France	Bulk				0.95		23.2	6.38	16.6		24.9	7.41	13.4	5.68	0.91	12.7	7.16	2001–2002	[97]
	Rouen, France	Bulk				0.60		12.7	4.60	11.0		12.1	5.04	7.10	3.62	0.11	1.22	4.32	2001–2002	[97]
	Strasbourg, France	Wet				10.26		1596.45	119.19	176.88		33.45	81.99	3.70	128.81	62.78	109.45	99.35	2002–2003	[98]
	Erstein, France	Wet				1.10		79.6	12.18	9.57		6.40	31.12	0.28	37.98	16.37	46.56	41.98	2002–2003	[98]
	Paris, France	Bulk	360																1999–2000	[19]
	West of Paris, France	Bulk	72																2001–2002	[99]
	Hungary	Wet				5.9		133.4	17.4	16.8		130.2	20.5	34.8	13.4	5.3			1995–1996	[100]
	Poznan, Poland	Wet	430																1996–1999	[101]
	Wielkopolski Park, Poland	Wet	280																1996–1999	[101]
	Brno, Czech	Bulk	93																2006–2008	[102]

Mediter-ranean	Bursa, Turkey	Gas and particulate				7.0		16.2	4.7	10.6		31.1		5.8	4.7	1.3	5.6		2004–2005	[103]
	Bursa, Turkey	Gas and particulate				6.4		73.7	4.2	4.2		19.3		3.5	2.8	0.3	2.2		2008–2009	[4]
	Ankara, Turkey	Wet, gas phase				75		112	23	46		60	67	65		5.9	26	31	2000–2002	[104]
	Ankara, Turkey	Wet, particulate phase				9.9		130	26	58		59	36	122		9.2	41	53	2000–2002	[104]
	Bursa, Turkey	Wet, gas phase				8.53		95.61	43.94	67.69		229.22	30.59	43.94	38.13	4.74	37.38	30.73	2008–2009	[4]
	Bursa, Turkey	Wet, particle phase				8.62		124.48	6.71	10.57		64.11	1.76	6.71	2.08	0.38	1.88	1.37	2008–2009	[4]
	Northern Italy	Bulk	110																2003–2004	[105]
USA	USA	Gas and particulate				0.5		15.7	0.05	0.5		2.4		0.1	0.02	0.01	0.1		2002	[106]
	Fortaleza, Brazil	Bulk	496.2					60.3				63.2		51.5	34.7		40.9	51.0	2005	[107]

2 Sources of PAHs

The urban atmosphere receives large amounts of PAHs from different anthropogenic and natural sources [2, 25].

2.1 Natural Sources

Polycyclic Aromatic Hydrocarbons tend to form naturally by reactions with in natural organic matters at low temperature and high pressure. As natural processes, forest fires and volcanic eruptions contribute PAHs in the atmosphere [26]. Oil spills, ship traffic, road traffic, urban runoff, emission from combustion, domestic activities such as tobacco smoke and residential coal burning, incineration, industrial boilers and kilns, aircraft, ships, locomotives, stoves and fireplaces, open fires, forest fires [27], agricultural production, farmland waste water irrigation, and industrial processes are considered as anthropogenic sources for PAHs in atmosphere [2, 28, 29]. Most of them are responsible as point sources for atmospheric PAHs while atmospheric deposition and surface runoff play a significant role as nonpoint sources [23].

2.2 Anthropogenic Sources

Among different anthropogenic sources power plants, industries, industrial waste incinerators, and residential heating are considered as stationary sources [25] while road traffics are considered as diffuse sources linked with unburnt lubricating oils, gasoline combustion [6, 25]. Sources play a prominent role in determining the presence of different PAHs. Phenanthrene, fluoranthene, and pyrene are the most abundant PAHs in deposited samples in France during 2002 where the source is the automobile sector [25]. In particular, phenanthrene, fluoranthene, and pyrene are unique PAHs in diesel engine emissions [20, 21, 30]. Naphthalene, acenaphthylene, acenaphthene, fluoranthene, and pyrene were the most abundant PAHs in Hong Kong during 2003, and it was found that diesel engines are the dominant sources for these PAHs [21]. The most abundant PAHs in Nanjing were naphthalene and acenaphthene during the Chinese spring festival in 2014, which were primarily due to firework. After the Chinese spring festival, the most abundant PAHs were rather different and reported as fluoranthene, chrysene, benzo[*b*]fluoranthene, and pyrene which were emitted by the traffic and industrial activities [31]. The automobiles and traffic incidences are the major contributors for PAHs in the atmosphere [32–34]. Among PAHs in the atmosphere most are benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, and their isomers which are basically from vehicular emissions [35]. The diesel exhaust emissions contain diesel particulate matter, elemental carbon as well as Organic Carbon (OC). The OC fraction contain PAHs and their methylated, nitrated, and oxygenated derivatives [20, 30].

The ratio between LMW PAH and HMW PAH indicates the origin of particular group of PAHs [25]. If the ratio is >1, that indicates the predominant of LMW in the total PAHs. The higher amounts of LMW in PAHs indicates its petrogenic origin [25, 36]. The ratio of

<1 indicates large amount of HMW PAHs denoting pyrolytic origin [25, 36]. The prolytic and petrogenic origin can be also determined by considering the ratio between phenanthrene and anthracene in the PAHs. If the phenanthrene/anthracene >10 the PAH indicates petrogenic origin and phenanthrene/anthracene <10 denotes pyrolytic origin [25, 37]. However, the phenanthrene/anthracene ratio depends on the temperature. The high temperatures are responsible for low phenanthrene/anthracene values [25, 37].

3 Deposition and Transport of PAHs

3.1 Deposition of PAHs

The PAHs that are released from different sources can be either deposited on earth surface or emitted into the atmosphere [38]. The emitted PAHs into the atmosphere may be retained as vapor or in particulate phase [25]. This phase will be determined by the chemical and physical properties of the aerosols. The vapor pressure, solubility of the compound, and Henry's low constant are the chemical properties while size and the composition of pollutants, temperature, size and surface area of suspended particulates act as physical properties [4, 25]. Wet and dry deposition as well as direct dissolution in water environment is the main process associated with fate of the PAHs [4]. In wet deposition, PAHs are associated with raindrops, snow, and fog or with particulates [25]. Dry deposition is gravitational settling of particulates [25, 39]. The ratio between wet and dry deposition may be determined by several factors such as emission source, distance from the emission source, the wind direction, wind speed, frequency, and the amount of precipitation [39]. The air-water exchange can be occurred when semi-volatile PAHs contacted with surfaces with large water environments [19, 25]. Deposition is the prominent mechanism for the removal of PAHs from the atmosphere [40]. While deposition, a loss of PAHs can occur through photodecomposition and reactions with nitrogen oxide, nitric acid, sulfur oxides, sulfuric acid, ozone, and hydroxyl radicals [23]. The deposited PAHs can be ended up on soil, impervious surfaces, or water environments [1, 41]. In urban cities, large area of surfaces has been covered with impervious surfaces. Therefore significant amount of deposition will be retained on these impervious surfaces.

3.2 Transport of PAHs

With the storm water runoff, most of deposited pollutants including PAHs tend to transport to urban water reservoirs [41]. The soil and sediment contamination with PAHs can be long lasting since the stability of PAHs is high in nature [42]. The initial oxidation of PAH is biologically slow and metabolically expensive which makes a strong stability on PAHs in atmosphere and water [42]. The partitioning of PAHs between water-air, water-sediment, and water-biota are the processes that play an important role on transportation of PAHs throughout the ecosystem [23]. Once PAHs bounded to water and sediments, it can circulate through the ecosystem and become more dangerous if they enter into the food chains [1, 43, 44]. Studies reported a total PAH in sediments in South China coastal region has a contribution of 30–40 % from the atmospheric deposition [1].

Particle size and meteorological conditions influence the transportation process of PAHs [45, 46]. Wind is a major factor for re-emission and transport over long distances even into high mountains. Therefore the atmospheric transport is an important pathway for the distribution of PAHs among various ecosystems [4, 17].

4 Different Remediation Methods

Several methods have been used in different studies to remove PAHs. Solvent extraction [47], biological remediation [48], phytoremediation [49], chemical oxidation [50], photocatalytic degradation [51], electrokinetic remediation [52], thermal technologies [53], and integrated remediation technologies [54, 55] have been tested [26]. The bioremediation methods are low cost, no secondary pollution and have the possibility of large area application [3]. In bioremediation perspectives, bioavailable PAHs are more important than the total PAHs load in particular ecosystem [56–58]. The bioavailability of PAHs in a particular environment can enhance the efficiency of the bioremediation process [8].

The studies on biodegradation of PAHs using microorganisms have been reported since 1970s. This biodegradation is efficient in remediating LMW PAHs in soil and water [8, 59, 60]. However the biodegradation of PAH is limited by lower capacity of microorganisms to degrade HMW PAHs [8]. Bio-augmentation is also another technique used in bioremediation of PAHs. It is a method that increases the biodegradation rates in PAHs [8]. Especially it is useful for the sites that are highly contaminated with HMW PAHs [8, 59, 61]. The phytoremediation is an in-situ method that can be used to extract, degrade, or sequester the pollutants in water and soil environments [13, 62].

5 Bio-retention Systems for PAH Removal

5.1 Bio-retention Systems

A bio-retention system is a collection of best management practices that can help to slow down the rate of release and increase the quality of storm water runoff to water reservoirs in a natural and esthetically pleasing manner [41]. The bio-retention systems are considered as cost effective and reliable control measure for nonpoint source pollution especially in urban environments. Unlike typical wetlands, the bio-retention systems are capable in improving the water quality and reducing the flow rates [63]. Bio-retention systems maximize pollutants removal via biological, chemical, and physical processes found in soil and plant communities [64]. Studies have found that the bio-retention systems are able to remove different types of pollutants that are available in water such as, nitrogen, phosphorous, oil, grease, heavy metals, suspended solids, PAHs, biological oxygen demand (BOD), and pathogens at the same time [63, 64]. The removal rates for total phosphorous, total nitrogen [65], oil and grease [41], copper (Cu), lead (Pb), zinc (Zn) [66, 67], cadmium (Cd), chromium (Cr), aluminum (Al), arsenic (Ar), iron (Fe) [68], total suspended solids [41], BOD [69], fecal coliform count [63], and PAH [70] have been reported as 70–80 %, 55–65 %, 96, 97, 95, 64, 66, 53, 17, 11, 53, 91, 63, 69, and 87 %, respectively (Table 2).

Recently, bio-retention systems have been experimented to remediate different pollutants such as PAHs and heavy metals in storm water [41]. The remediation processes in bio-retention systems include sedimentation, filtration, sorption, microbial decomposition and plant uptake and storage [64]. Generally bio-retention systems consist a porous media, vegetative layer, and the surface mulch [41, 64] (Fig. 1). The constructed pond areas may provide spaces as temporary water storage. It will provide an enough extra time to infiltration process [41]. To facilitate this, bio-retention systems consist of an excavated area backfilled with soil and organic matter mixture which has a high permeability [64]. Often in bio-retention systems, there is an inlet and overflow level that makes sure to keep constant flow rate in all the time to eliminate the effects that create from flooding [64]. The plants that are used in a bio-retention system can be different according to size of the bio-retention system (Fig. 1). Therefore the bio-retention systems have higher plant diversity and have an ecosystem like in terrestrial forest [64].

It has been proved that the particular bio-retention systems have an ability to remove PAHs in storm water via accumulation in the soil [64, 70]. The mean PAH removal was 2.08 to 0.22 μg L^{-1} with 87 % of mean PAHs reduction [64, 70]. Infiltrated and adsorbed PAHs remain in shallow depths of soil [64]. Phytoremediation may enhance the capacity of bio-retention systems to remediate PAHs and improve the pollutant removal efficiency.

5.2 Phytoremediation of PAH

The remediation of PAHs should be conducted in an effective way avoiding further cycling of PAHs within the environment. Since the stability of PAHs is high, there should be a mechanism to accelerate the remediation process [42, 62, 71]. Phytoremediation, the use of plants vegetative metabolism and simulation of associated rhizosphere microorganisms can be integrated to promote removal of PAHs in environment through biodegradation [13, 72]. It is a cost effective and sustainable method that can be used in eco-friendly manner [13, 28, 72]. The phytoremediation of organic pollutants is not via a hyper-accumulation process but it promotes mineralization or transformation of organic pollutants into less or nontoxic compounds [43]. Further it is an in situ method that can be used to extract, degrade, or sequester the pollutants in water and soil environments [13, 62].

Table 2 Identified pollutants that can be removed with bio-retention systems and reported removal rates

Type of pollutant	Removal rate (%)	Reference
Total phosphorus	70–80	[65]
Total phosphorus	65	[108]
Total phosphorus	77–79	[109]
Total phosphorus	42	[51]
Total phosphorus	85	[110]
Total phosphorus (high conductivity soil filtration media)	85	[111]
Total phosphorus (low conductivity soil filtration media)	65	
Total nitrogen	40	[108]
Nitrate	75	[108]
Nitrate	80	[112]
Nitrate	90–95	[109]
Total Kjeldahl nitrogen	55–65	[65]
Total Kjeldahl nitrogen	86	[108]
Ammonia	84.5	[113]
Ammonia	59	[114]
Organic carbon (with loam media)	58	[115]
Organic carbon (with sand and gravel media)	30	[115]
PAHs	87	[70]
Total suspended solids	97	[116]
TSS	91	[117]
TSS	41	[118]
BOD	63	[69]
Oil and grease	96	[41]
Oil and grease	83–97	[119]
Cu (with high infiltration capacity)	97	[66, 67]
Cu (with low infiltration capacity)	43	
Cu	81	[68]
Cu	60–75	[120]
Cu	81–99	[121]
Cu	90	[122]
Pb (with high infiltration capacity)	95	[66, 67]
Pb (with low infiltration capacity)	70	
Pb	75	[68]
Pb	83–89	[120]
Pb	81–99	[121]
Pb	90	[122]
Zn (with high infiltration capacity)	95	[66, 67]
Zn (with low infiltration capacity)	64	
Zn	79	[68]
Zn	90	[120]

(continued)

Table 2 (continued)

Type of pollutant	Removal rate (%)	Reference
Zn	81–99	[121]
Zn	90	[122]
Cd	66	[68]
Cd	81–99	[121]
Cr	53	[68]
Al	17	[68]
As	11	[68]
Fe	53	[68]
Fecal coliform counts	91.6	[63]
Fecal coliform counts	69	[69]
E. coli	71	[69]

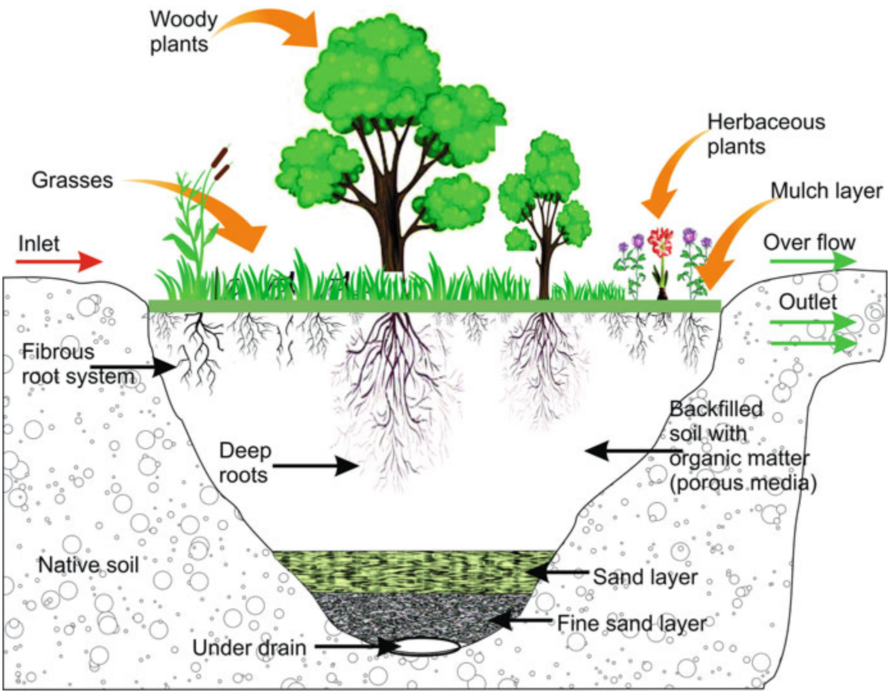

Fig. 1 A schematic diagram of a general bio-retention system

The use of plants to remediate PAHs provides natural alternatives which promotes green biotechnology [2]. There are number of plant species that have been identified as possible plants for phytoremediation and those are interfered with rhizosphere degradation of PAHs and other contaminants [73]. The use of native plants in a particular region that have phytoremediation ability provides an advantage of optimum growth rate with adoption to the environmental conditions and ensured long life span [74]. However, the efficiency of phytoremediation activity will be determined by the soil conditions, plant age, species, and the variety of plant used [75]. The mechanisms taken place in the phytoremediation process are, effect of the root system on physical and chemical soil conditions, overall increase of microbial population and diversity, supply of root exudates and litter for metabolic degradation, stimulation of humification, sorption and plant uptake and finally translocation within plant [2].

5.3 Phytoremediation of PAHs in Bio-retention Systems

The bio-retention system itself is capable in removing most of PAHs in runoff water. However, those PAHs will be accumulated in soil and finally will end up with infiltration into groundwater [64]. Hence, phytoremediation will be an efficient method to overcome above issue in bio-retention systems while increasing the PAH removal capacity [64].

5.4 Selection of Plants for Bio-retention Systems

Designing and selecting plants for a bio-retention system is an important step as the selection of wrong plants will be a waste of money as well as the time [76]. Hence, for PAH removal via phytoremediation, the plants must encompass characteristics of easy and low cost maintenance, ready availability of planting materials, fast growth rate, and root mass with a large surface area [2, 26]. Adaptation to new environmental conditions, higher resistivity to pollutants, and the ability to selective adsorption of pollutants also are considerable factors when selecting a phytoremediation plant for a bio-retention system [75]. The selected plants must be having a higher survival rate under stress conditions since the bio-retention system receives contaminants continuously. As an example, the Willow species, *Salix alba* and *Salix viminalis*, are type of phytoremediation plants that can remediate PAHs and many other organics and even heavy metals [75]. According to studies, the *Salix alba* and *Salix viminalis* Willow species have special characteristics for phytoremediation activities [75].

There are evidences from the past research where phytoremediation process has been failed due to the use of unsuitable plants [76, 77]. As an example, the use of cottonwood trees to remediate trichloroethylene has been failed although it had been chosen considering the indigenous availability. It remediated the trichloroethylene, but did not long last in the contaminated sites [76, 77]. The physiological stage of the plant is also an important factor which has an effect on the phytoremediation mechanism [74]. Until the flowering time of *Cyperus laxus* Lam., the efficiency of phytoremediation activity was higher and after flowering time the efficiency has been reduced [74]. Similarly, the plant density plays a significant role on phytoremediation efficiency [72, 78]. Plant growth rate, quality of plants, nutrient availability, plant reproduction, and the biomass accumulation can be affected by the plant density [72]. The higher plant density promotes more PAH removal from sediments. But in contrast, the low plant density increases plant growth rates and then the larger plants with higher root surface area also increase PAH intake, from polluted sediments [72]. And also in bio-retention systems the contaminants should be located in the root zone of the plant. Considerations must be given to the depth of the root zone, the nutrient requirements as well as the physical and chemical limitations [76].

The actual results from phytoremediation in a bio-retention system will not be seen as soon as planting and their establishment. The effective output of the cleanup process will be presenced after plant roots obtain their actual root depth in the system [76, 79]. When selecting plants for phytoremediation in bio-retention system, the esthetic value of plants also may be a minor consideration [76]. Instead of remediation, the prevention of dispersion and the contacting of human as well as animals also will be an additional output with bio-retention systems [76]. The selection of site is also an important factor in order to make an efficient and effective PAH remediation [76]. The site should have enough space that can perform necessary practices to manage plants and phytoremediation processes in the system [76].

5.5 Plants in Bio-retention System

5.5.1 Fibrous Rooted Plants

Since the top layer of a bio-retention system required plants for facilitating the better permeability and the filtering ability, the plants that have fibrous root system is better in the means of remediation of PAH and the water penetration through soil layers [41]. The plants in family *Graminaceae* that have phytoremediation ability are good to use since they have large surface area with their fibrous root system and the ability to penetrate deep into the soil [26]. Rye grass (*Lolium perenne*) has been proved as an effective phytoremediation plant with 15 of PAHs [26]. The concentrations of tested PAHs instead of HMW PAHs has been reduced initial concentrations by 50 % with Rye grass [26, 49]. The *Festuca arundinacea* (tall fescue) and *Panicum virgatum* (switch grass) have shown 40 % of PAH removal capability [26, 80]. Some native Korean grass species such as *Panicum bisulcatum* and *Echinochloa crus-galli* have been proved the 99 % degradation of phenanthrene and 77–94 % degradation of pyrene with phytoremediation activities [26, 81]. Studies have revealed that the use of *Medicago sativa* (alfalfa), *Panicum virgatum* (Switch grass), and *Schizachyrium scoparium* (Bluestem grass) for primary remediation and final polishing in the phytoremediation process has reduced 57 % of PAHs from contaminated soils during six months of period [76].

Cucurbita pepo spp. *pepo* (Zucchini), *Cucumis sativus* (Cucumber), and *Cucurbita pepo* spp. *ovifera* (Squash) are plant species that have been studied to remediate soil contaminated with PAHs in means of phytoremediation [13]. The Zucchini plant is reported as more effective for phytoremediation of PAHs in soil compared to Cucumber and Squash. Along with the increasing growth cycles the efficiency of accumulation of PAHs in Zucchini plant increased by 85 % but in Cucumber and Squash remained constant [13]. But still Zucchini, Cucumber, and Squash have possibility to be used for phytoremediation of PAHs in soils that are contaminated from atmospheric PAH deposition [13]. *Cyperus laxus* Lam., a native plant in swamps in Mexico, exhibited potential factors for phytoremediation of PAHs contaminated soils [74].

5.5.2 Deep Rooted Plants

Trees play an important role in bio-retention systems since it has a deep and large root system. Studies have reported that the *Morus rubra* (red mulberry), *Salix nigra marsh*. (black willow), rooted hybrid poplar, *Ficus sycomorus* (sycamore), and *Robinia pseudoacacia* (black locust) are capable in phytoremediation of PAHs. *Pinus banksiana* (Jack pine), *Pinus resinosa* (red pine), and *Pinus strobus* (white pine) are also effective in phytoremediation of PAHs with about 74 % removal capability [26]. *Populus tremula* (Poplar) is a plant species that can be successfully used for phytoremediation of PAHs with its fast growth, tolerability to organic pollutants, and ability to uptake large amounts of PAHs from growing soil [2]. According to the type of the PAHs that are available in soil or sediment, different phytoremediation plants act in different ways. As an example in benzo[*a*]pyrene, 4.1 % have been mineralized and 0.28 % have been utilized by plants [82]. From 3-6 ring PAHs, 65 % have been removed from planted soils and 39 % of removal from unplanted soils [36]. Not only vascular plants, there are some other organisms that showed phytoremediation abilities. The algae are such an example [43, 83]. Benthic microalgae have been successfully used for the phytoremediation process to remove the PAHs in sediment environment [43, 83].

5.5.3 Aquatic Plants

Aquatic phytoremediation plants can also be used in bio-retention systems. The submerged hydrophytes, dominant in the shallow water bodies, are identified as the phytoremediation plants that can remove the contaminants accumulated in sediments [72, 84]. The PAHs are identified as susceptible for aerobic degradation and therefore the aerobic conditions can increase the PAH removal rate [72, 84]. The submerged hydrophytes have ability to release oxygen to root environment and create aerobic environment [72]. *Vallisneria spiralis* is such a submerged hydrophyte which can be used as a phytoremediation plant [72]. As the *Vallisneria spiralis* can be easily grown in most of the environmental conditions it can be used in bio-retention systems in a successful way [72]. The accumulation of PAHs in roots is more promoted than translocation into the shoots in *Vallisneria spiralis* [28, 72]. Since the PAHs content in shoots are low and PAHs contains hydrophobic properties, after plant degradation releasing of PAHs into water environment is low [72].

5.6 Microorganism Associations in Bio-retention Systems

The associated microorganisms with the phytoremediation plants play an interactive role on phytoremediation process [74]. The availability of microorganisms increases the efficiency of the phytoremediation and the degradation of PAH in contaminated soils and sediments. And also the efficiency of microbial activities on degrading PAHs is high in planted soils than in unplanted soils [74]. With a study on pyrene phytoremediation in soil the pyrene have been disappeared 74 % in planted soil while the unplanted soil removing only 40 % [85]. With microorganisms, native species showed higher efficiency than that are inoculated from outsides [74]. Also since the PAHs in contaminated soils are toxic to even phytoremediation plants the growth retardation easily can be occurred in a bio-retention system. Therefore the growth promoting strategies are important in phytoremediation process [26]. Combination of plants with PAH degrading microorganisms provides advantages that promote plant growth by reducing the toxic effects of PAHs to plants [36]. The use of rhizobacteria can be able to promote plant growth and the biomass production [26].

It is possible to remove HMW PAHs from soil or sediments by rhizobacteria [26]. This multi-remediation strategy has been a success with *Festuca arundinacea* plant and the plant growth promoting rhizobacteria while increasing PAH removal by 23 % [26, 80]. *Bacillus cereus*, *Pseudomonas* sp., *Gordonia rubripertincta*, *Kocuria rosea*, *Arthrobacter oxydans*, *Bacillus subtilis* A, *Bacillus subtilis* B, and *Micrococcus luteus* are bacterial strains that have associated with the *Cyperus laxus* Lam. and *Penicillium janthinellum*, *Aspergillus carneus*, and *Aspergillus terreus* are fungal strains with *Cyperus laxus* Lam. plant [74]. The monocotyledons plants have higher root surface, provide optimum conditions to higher active microbial populations. Therefore, the efficiency of the phytoremediation mechanism also increases [74]. Interestingly, with the increase of plant density in the sediment environment, the population of PAHs degrading bacteria are also increasing [72]. Therefore the plants are not only for the phytoremediation but also for promoting other PAHs degradation ways [72].

As an example, with the use of alfalfa plant the PAH degradation rate by microorganisms has been increased by 0.3–1.1 % than in non-planted soils and the pyrene degradation rate was increased by 2.4–53.8 % [3]. The composting of contaminated soils before of at the time of phytoremediation is an another strategy to promote the phytoremediation through enhancing the plant growth [26, 86]. In most of PAH contaminated soils and sediments the major limiting factor is the nonavailability of plants and associated microorganisms due to hydrophobicity nature of PAHs [14].

In the phytoremediation studies there are evidences of use of surfactants to overcome this issue [14]. Tween 80 has been used as surfactants to enhance the PAH intake by plants. With mixing 6.6 mg/L of Tween 80 to PAH contaminated soil the plant uptake of PAHs was enhanced by 18–115 % [14].

6 Possible Other Plants and Materials for PAH Removal in Bio-retention Systems

There are several other compounds that can be used in bio-retention systems for the removal of deposited PAHs in soil and water environments. The use of naturally occurring microbes to enhance the efficiency of remediation activity can be found in bio-retention systems that are called as natural attenuation [2, 28, 72]. *Burkholderia fungorum* can be used to degrade the PAHs in bio-retention systems [2]. The DBT1 strain of *Burkholderia fungorum* has been isolated from a waste water drain located in an oil refinery. The *Burkholderia fungorum* has capability to degrade several PAHs such as dibenzothiophene, phenanthrene, fluorine, and naphthalene. And also this *Burkholderia fungorum* have compounds that can promote plant growth such as 1-aminocyclopropane-1-carboxylic acid (ACC) that are responsible for deaminase production and phosphate solubilization [2]. *Mycobacterium parafortuitum* and *Sphingobium yanoikuyae* from mangrove environments are some other bacteria which can be used for PAHs degradation [28]. Table 3 depicts the several identified microbial species to degrade particular PAHs.

Enzymatic degradation also can be incorporated into bio-retentions systems to degrade PAHs instead of phytoremediation. Here oxygenase, dehydrogenase, and lignolytic enzymes are involved with PAH degradation [87]. Incorporating of lignolytic fungi species will provide these PAH degrading enzymes into the bio-retention systems [87]. There are fungal species such as *Nematoloma forwardii* that can produce Mn-dependent peroxidase enzyme. The Mn-dependent peroxidase is also a type of enzyme that has ability to degrade a broad range of PAHs in contaminated environments [87].

7 Summary

The emission control of PAH into the atmosphere is a matter to consider in the context of human and the ecosystem health. The use of efficient energy sources such as natural gases and nuclear power will play significant role on reduction of PAH emission into the atmosphere [6]. However the remediation of PAHs available in environment is a great concern since the pollution conditions are crucial worldwide. The selection of natural mechanisms instead of conventional methods will be sustainable with remediation process.

Table 3 Identified microorganisms that have abilities to degrade PAHs, incorporated with phytoremediation process

PAH	Organism	Reference
Naphthalene	*Alcaligenes denitrificans*	[2, 29, 87, 123, 124]
	Mycobacterium sp.	
	Pseudomonas putida	
	P. fluorescens	
	P. paucimobilis	
	P. vesicularis	
	P. cepacia	
	P. testosterone	
	Rhodococcus sp.	
	Corynebacterium venale	
	Bacillus cereus	
	Moraxella sp.	
	Streptomyces sp.	
	Vibrio sp.	
	Cyclotrophicus sp.	
	Burkholderia fungorum	
	Paenibacillus sp.	
Phenanthrene	*Aeromonas* sp.	[2, 29, 87, 125, 126]
	Alcaligenes faecalis	
	A. denitrificans	
	Arthrobacter polychromogenes	
	Beijerinckia sp.	
	Micrococcus sp.	
	Mycobacterium sp.	
	Pseudomonas putida	
	P. paucimobilis	
	Rhodococcus sp.	
	Vibrio sp.	
	Nocardia sp.	
	Flavobacterium sp.	
	Streptomyces sp.	
	Bacillus sp.	
	Burkholderia fungorum	
	Pseudomonas aeruginosa	
	Pseudomonas fluorescens	
	Haemophilus sp.	
	Paenibacillus sp.	
Dibenzothiophene	*Burkholderia fungorum*	[2]

(continued)

Table 3 (continued)

PAH	Organism	Reference
Benzo[*a*]pyrene	*Sphingomonas paucimobilis*	[87]
	Apseudomonas sp.	
	Agrobacterium sp.	
	Bacillus sp.	
	Burkholderia sp.	
	Sphingomonas sp.	
	Rhodococcus sp.	
	Mycobacterium sp.	
Pyrene	*Mycobacterium* sp.	[87]
	Pseudomonas fluorescens	
	Haemophilus sp.	
	Mycobacterium flavescens	
Acenaphthene	*Pseudomonas fluorescens*	[87]
	Haemophilus sp.	
Fluorene	*Pseudomonas fluorescens*	[87]
	Haemophilus sp.	
Anthracene	*Pseudomonas fluorescens*	[87]
	Haemophilus sp.	
	Rhodococcus sp.	

Even though the bio-retention systems are capable to remove PAHs in runoff water, the removed PAHs will be accumulated within the system. With the increasing concentrations the PAHs can be infiltrated and flow towards the groundwater table. The phytoremediation of PAH will be a better solution to degrade and remove PAHs from bio-retention systems. These hybrid technologies will be more effective in means of environmental protection and well-being of human lives [88]. The phytoremediation, an in situ remediation practice, always act in environmental friendly and cost effective manner. However the hydrophobic property can limit the availability of PAHs to plants in phytoremediation process [42]. It can limit the absorption and the degradation of the PAHs in soil or sediments [42]. With incorporating microbial activities and some of agents like Tween 80 the bioavailability of PAH can be increased in bio-retention systems. Therefore with combining microbial activities and some other possible methods the phytoremediation capacity can be increased in bio-retention systems.

The use of phytoremediation in bio-retention systems will create a pathway for many other opportunities as well. As the plants that are used for phytoremediation are not for food purposes and hence it is open to use genetically modified crops (GMO). The GMO crops that have phytoremediation capability and the ability to use as energy production will provide opportunities to produce bio-fuels after completing the phytoremediation process [88]. The bio-retention systems are used not only for PAH remediation but also for variety of other environmental pollutants [88].

However, there are some disadvantages with phytoremediation of PAHs in bio-retention systems in the means of remediating atmospheric PAHs [76]. The growth of vegetation can be limited with environmental toxicity. The accumulated PAHs can be released into the environment with litter-fall and with litter degradation the mechanism of phytoremediation will be taking much longer time than other conventional methods [76]. Anyhow, the phytoremediation is a famous and growing remediation method in global scale. But the use of phytoremediation in bio-retention systems is yet infancy for all over the world. This is a good pathway to conduct research for improving efficiency of PAH remediation as well as the field applications [88]. Research on developing innovative methods to overcome limitations of phytoremediation in bio-retention systems such as nonavailability of plants and associated microorganisms due to hydrophobicity nature of PAHs, the nonavailability of bio-available PAHs, etc., may provide successful mechanisms in future. Conducting research with field applications and by providing risk assessments of use of phytoremediation in bio-retention systems will be helpful to popularize the technology for betterment of the environment as well as the human beings. The low cost, easiness, environmental friendly, and effectiveness are major important factors together with phytoremediation in bio-retention systems. Therefore, phytoremediation in bio-retention systems is a successful story for PAHs degradation and removal.

Since considerable amounts of nutrients receive bio-retention systems future research should be explored to promote nutrient cycling within bio-retention systems such as nitrification and denitrification processes. This will help to overcome the issues created with overaccumulation of nutrients. The available forms of nutrients will enhance the growth of phytoremediation plants as well as incorporated microbes. Then, the bio-retention system can develop as a self-sufficient system rather than depend on additional fertilizer applications. The sustainability and the cost effectiveness also will be promoted. The risk of pollutant leaching into groundwater is always together with bio-retention systems. It is better to identify plants that have an ability to remove a range of pollutants. Identification of the optimum stage of a plant to remediate PAHs is another factor to look into when it is experimented or selected for phytoremediation in bio-retention systems. Plant replacement should have to practice after reach to ineffective stage. Most importantly there are number of studies in temperate region but not in the tropics. Therefore the tropical plants do not have received much attention in this regard. Hence, more research is needed in tropical plant species as well as for the tropical environment.

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Part II
Wastewater Engineering and Technology

Plant Growth-Promoting Bacteria: A Good Source for Phytoremediation of Metal-Contaminated Soil

Iqra Munir and Muhammad Faisal

Abstract Phytoremediation is a sustainable technique for the removal of contaminants from the polluted environments, but the removal of contaminants by plants and microorganisms together is more effective than phytoremediation alone. Phytoremediation is enhanced with the involvement of microorganisms in soil as well as in water. Rhizosphere microorganisms develop beneficial interactions with plants which ultimately results in increased plant growth and improved phytoremediation of heavy metals. Microorganisms play a vital role in mobilization and immobilization of metal contaminants from the environment for availability to different plants. Different microorganisms produce different metabolites which interact and make complexes with the contaminants and decrease their levels of toxicity by transforming them to less toxic state.

Keywords Phytoremediation • Plants • Microorganisms • Contaminants • Rhizobacteria • Metals

1 Introduction

Industrialization is increasing with increased global economy during past century, due to which a dramatically increased level of anthropogenic chemical release is observed into the atmosphere. Predominant pollutant includes halogenated hydrocarbons, aromatic hydrocarbons, petroleum hydrocarbons, salts, solvents, heavy metals, and pesticides. These pollutants are causing stress to environment as well as to human health [1–3]. Phytoremediation is a plant-mediated viable technique for the removal of contaminants from the environment. Phytoremediation of polluted environment are usually occurred by phytodegradation, phytoextraction, phytovolatilization, phytostabilization, rhizodegradation, and rhizofiltration [4, 5]. The process of phytoremediation depends on the capability of plants for accumulating or metabolizing the metal contaminants to less toxic state. The accumulation, degradation, or uptake of pollutants differs from species to species.

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The selection of plants for phytoremediation process is usually based on growth and yield of plant, tolerance towards contaminants, rate of accumulation, root formation, and transpiration [4]. Phytoremediation has several advantages as compared to other remediation techniques, such as fewer costs for installation and maintenance, less environmental disruption, and some valuable side effects including biofuel production and sequestration of carbon [6, 7]. Soil polluted with combination of contaminants is difficult to treat; different strategies are required to remediate different contaminants [8, 9]. Mixed contamination of different toxic materials is causing huge number problems all over the word in soil, sediments, and water [9, 10]. The process of degradation depends on microbial population present at polluted site [11].

2 Plant Growth-Promoting Bacteria as Tool for Phytoremediation

Microorganisms are present ubiquitously in environment. They can grow in and bear the extreme environmental conditions. Rhizosphere is an important environment for different microbes including protozoa, algae, fungi, and bacteria [12]. Plants develop advantageous relationships with microorganisms which can help in improving growth and yield of plants. Endophytic microorganisms live inside the tissues of plants without causing damage to host. Their application can result in increased availability of nutrients and metals to plants, cause reduction in their level of toxicity, and promote plant growth for better accumulation of heavy metal contaminants [13, 14]. The uses of plants in combination with contaminant tolerant/resistant plant growth-promoting bacteria are helpful in possible cleanup of polluted soils [14]. Effectiveness of bioremediation is influenced by different factors which affect each other in complex ways; these factors include environmental conditions and characteristics of contaminants [9]. Different evidences suggest that roots of plants stimulate the microenvironment by releasing secretions, which influence the microbial pool in rhizosphere soil [15, 16].

In soil rhizosphere, phytoremediation efficacy is enhanced by the activities of different microorganisms by the following two methods: (1) by direct enhancement of phytoremediation, microbes associated with plants improve translocation of contaminants/metal or decrease their availability/mobility in rhizosphere, (2) by indirect enhancement of phytoremediation, microorganisms enhance tolerance towards metals in plants or by improving biomass production in plant to arrest or remove the contaminants. Beneficial microorganisms play an important role to mobilize metal contaminants, and the metabolites produced by these microorganisms are less toxic, biodegradable, and their in situ production is possible in rhizosphere soils. Plant growth stimulating components include fixation of atmospheric nitrogen, phosphate solubilization, production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, growth hormones, and siderophore by microorganisms associated with plants for better plant growth in polluted soils [13, 14, 17–19]. Nodule forming bacteria also affect plants by enhancing plant growth and development of roots, which improves tolerance/resistance towards a number of ecological stresses [19–22]. Some examples of microorganisms involved in enhanced phytoremediation of metals are summarized in Table 1.

Table 1 Phytoremediation of some heavy metals by microorganisms associated with plants

Microorganism	Metal uptake	Plant	Reference
Bacillus subtilis	Ni	*Brassica juncea*	[61–67]
Klebsiella sp. and *Enterobacter* sp.	Zn, Pb and Cd	*Brassica napus*	[68]
Burkholderia sp.	Zn and Cd	*S. alfredii*	[5, 69]
P. tolaasii, *P. fluorescens*, and *Mycobacterium* sp.	Cd	*Brassica napus*	[70]
G. diazotrophicus	Zn	–	[71]
Pseudomonas sp.	Cd	*Solanum nigrum*	[72]
Psychrobacter sp.	Ni	*Helianthus annuus* and *Ricinus communis*	[13]
Arthrobacter nitroguajacolicus	Ni	*A. serpyllifolium*	[63]
P. fluorescens and *Chromobacterium violaceum*	Cu and Ni	–	[53]
Pseudomonas aeruginosa	Cr and Pb	*Zea mays*	[26]
Eleocharis acicularis	Zn, Cu, Pb and As	–	[73]
Sanguibacter sp.	Cd	*Nicotiana tabacum*	[74]
Stenotrophomonas sp., *Comamonas* sp., and *Pseudomonas* sp.	As	*Pterisvittata*	[64]
Pseudomonas putida	Cd	*Helianthus annuus*	[75]

3 Activities of Microorganisms for Phytoremediation

3.1 Siderophore Production

Microorganisms associated with plants are able to produce iron chelators also termed as siderophores, due to iron deficiency in rhizosphere. These compounds have low molecular weights ranging from 400 to 1000 Da, having high affinity for making complexes with iron as well as with other metals including cadmium, copper, aluminium, zinc, gallium, lead, and indium [19, 23–25]. They also contain some functional groups, which can be generally divided into three major groups on the basis of chemical nature of oxygen ligands donating moieties to coordinate with iron. These functional groups include carboxylates, hydroxamates, and catecholates. Siderophores dissolve inaccessible forms of minerals containing heavy metals by forming complexes with them. Therefore, these microorganisms are known to play significant role in phytoextraction of heavy metals [26–29]. Microbial siderophore production depends on different factors such as availability of iron, nutrients, pH, and type and level of heavy metals present in soil.

For example, *Pseudomonas aeruginosa* is a good example of rhizosphere bacteria which produces pyochelin and pyoverdine, and as a result it increases the bioavailability of Pb and Cr in soil [26, 30]. Similarly, Siderophore producing *Streptomyces tendae*-F4 enhanced the cadmium uptake in sunflower plant. Siderophore production by mycorrhizal fungi has also been reported [31, 32].

Braud et al. [33] reported increased production of pyoverdine in the presence of Ni, Cr, and Cu. Ectomycorrhizal fungi isolated from *Pinus radiata* including *Rhizopogon luteolus*, *Suillus luteus*, and *Scleroderma verrucosum* are reported for producing hydroxamate and catecholate siderophores under iron deficiency in environment. Such reports suggested that siderophore producing microorganisms can be inoculated to plants for improved uptake of heavy metal by plants.

There are some reports which oppose the idea of microbe-assisted increased uptake of metals by plants. For example with siderophores producing *Pseudomonas aeruginosa*-KUCd1 inoculation, decrease in uptake of cadmium was observed in shoots and roots of *Brassica juncea* and *Cucurbita pepo* [34]. Similar results were reported [35] when siderophores producing nickel-resistant *Pseudomonas* were inoculated to chickpea plants; decreased nickel uptake but increased growth of plant was observed. Siderophore positive bacterial strains are not always involved in enhanced uptake of metal by plants [36, 37]. The possible reason behind this conflict in reported results might be the plants capability to uptake heavy metals, which depends on several other factors such as availability of metal, type of plant, and its capacity of transporting metals towards shoot. In plants, root activities play an important role in metal uptake by releasing root exudates, which is influenced by the properties of soil, and also affects the nutrients, pH, and diversity of microorganisms associated with plants [38]. The synthesis of siderophores production by microbes and their importance in transport and tolerance towards metals have been studied well [24], but their interaction with plants in metal-contaminated soil still needs more attention. Siderophore production can also increase or decrease the harmful effects of metals in microbes [18]. Yasin et al. [39] also reported increased uptake of iron and selenium through bio-fortification of wheat when inoculated with *Bacillus pichinotyi* strain.

3.2 Organic Acid Production

Microorganisms associated with plants produce organic acids having low molecular mass. These compounds contain CHO and carboxyl groups having molecular mass of up to 300 Da. They play an important role in solubilization and mobility of heavy metals and other minerals in soil [40, 41]. Organic acid bind with metals by making complexes with them, and their stability depends on various factors which include the nature of acid, binding form of heavy metal, and pH of soil [19, 40, 42]. Organic acid production by microorganisms associated to plants play a significant role in improving the mobility of essential ions and nutrients to be taken up by plants. Mobilization of heavy metals or ions can be correlated with improved release of organic acids including acetic acid, oxalic acid, succinic acid, tartaric acid, and formic acid [43]. Among the microbially produced organic acids, oxalic acid, citric acid, and gluconic acid have gained more importance because they increase the bioavailability of heavy metals.

According to a recent study, zinc solubilizing *Gluconacetobacter diazotrophicus* produced a derivative of gluconic acid (5-ketogluconic acid) which helps in zinc solubilization under in vitro conditions by solubilizing $Zn_3(PO_4)_2$, ZnO, and $ZnCO_3$. In a study related to *Sedum alfredii* (Zn/Cd hyperaccumulating plant), inoculation with Zn/Cd-resistant rhizosphere bacteria significantly improved the concentration of water soluble zinc and cadmium as compared to untreated control. Mycorrhizal fungi also produce organic acids in rhizosphere and play an important role in mobilization of heavy metals. *Oidiodendron maius* has also been studied for the production of citric and malic acid for improved production of Zn [44]. Similarly, *Beauveria caledonica* and *Aspergillus niger* also facilitate the release of zinc and lead from pyromorphodite through the production of acids for increased uptake by plants [45, 46]. These reports highlight the importance of organic acid producing microorganisms to promote phytoextraction in polluted environments. The formation of complexes between organic acid and metal is influenced by type and concentration of organic acid, physical and chemical properties of soil, and presence of minerals in the environment [19]. Properties of rhizosphere soil including buffering capacity, sorption, metal complexation, and biodegradation may change organic acid profile and behavior [18, 47].

3.3 Production of Biosurfactant

Petroleum hydrocarbons are most common amongst the persistent organic contaminants found in shoreline and estuaries, which is causing great concerns [48]. Another essential metabolite having potential to improve mobility of metal and phytoremediation is biosurfactant production by microorganisms. It is an amphiphilic molecule having hydrophobic (nonpolar) tail and a hydrophilic (ionic/polar) head. Hydrophilic moiety contains proteins or peptides, and hydrophobic group comprises of hydroxylated, saturated, or unsaturated alcohols and fatty acids. Biosurfactant production by microorganisms increases the solubility and bioavailability of heavy metals in soil by making complexes with them on soil interface to desorb from the environment. These surfactant producing microbes play an important role in mobilization of heavy metals in contaminated soils [19, 49]. An experiment was conducted to check the petroleum degradation by tall fescue, and for this purpose three bacterial strains were used including *Pseudomonas* sp. (SB), *Klebsiella* sp. (D5A), and *Streptomyces* sp. (KT) having potential of biosurfactant production, plant growth enhancement, and petroleum degradation. The results showed that palmitic acid production by microorganisms was most critical for petroleum removal by phytoremediation [50]. A study revealed that *Enterobacter ludwigii* can efficiently colonize the endosphere and rhizosphere of alfalfa, birdsfoot trefoil, and Italian ryegrass. It also contained alkane hydroxylase due to which they actively degrade hydrocarbons in estuaries [51].

3.4 Glycoprotein and Polymeric Substances Production

Microorganisms associated with plants play a significant role in decreased mobility of toxic contaminants in soil by producing extracellular polymeric substances (EPS), muco-polysaccarides, and proteins [19]. According to a study conducted on plants inoculated with EPS releasing *Azotobacter*s pp, inoculation resulted in the immobilization of Cr and Cd by 21.9 mg g^{-1} and 15.2 mg g^{-1}, respectively [52]. *Azotobacter* inoculation has also been reported for reduced uptake of metal by wheat plant. The production of glomalin (insoluble glycoprotein) by arbuscular mycorrhizal fungi can bind heavy metals in metal-contaminated soils. The concentration of immobilized heavy metals depends on the quantity of glomalin produced by mycorrhizal fungi, more the production of glomalin the strain become more appropriate for phytostabilization. Although arbuscular mycorrhizal fungi can immobilize the heavy metals by the releasing glomalin, but its complete structure and mechanism which lead to decreased uptake of metals by plants is still poorly understood [18].

3.5 Oxidation and Reduction Reaction

A number of microorganisms associated to plants can change the mobilization of toxic metals by reduction or oxidation reactions. From the phytoextraction view point, oxidization of metals by microorganisms is getting more importance. For example, sulfur oxidation by microbes present in rhizosphere soil showed increased mobility and uptake of Cu by plants tissues in polluted soils [53]. Sulfur and iron oxidizing bacteria have been reported for increased availability of heavy metals in soil by the production of acids [54]. Plant-associated chromium tolerating *Cellulosimicrobium cellulans* strain inoculation in green chilly resulted in decreased uptake of chromium in root as well as shoot by 56 and 37 %, respectively, in Cr(VI) polluted soil. Abou-Shanab et al. [55] reported lower Cr accumulation in water hyacinth shoots by chromium reducing bacteria [18]. *Streptomyces* sp. M-7 has also been reported to remediate lindane (pesticide) and Cr(VI) in contaminated places [56].

3.6 Biosorption

Plant-associated microorganisms also have the potential to enhance mobilization of metal by means of biosorption mechanism. Biosorption is the process of metal adsorption by microorganisms either by dependent or independent metabolisms [13, 19]. A number of studies have been reported for decreased metal uptake by plants due to microbial biosorption processes. For example, according to a study conducted to assess the mobilization of metal by bacterial inoculation, it was found that *Burkholderia* sp. and *Magnaporthe oryzae* reduced the concentration of Cd and Ni accumulation in tomato plant [57]. Likewise, Vivas et al. [58] reported that *Brevibacillus* sp. inoculation in *Trifolium repens* resulted in reduced Zn accumulation in shoots as compared to untreated control plants due to enhanced biosorption of Zn. From these reports, it can be concluded that metal binding microbes are able to restrict or reduce the bioavailability of metals in plants. Similarly, mycorrhizal fungi has also been studied for biosorption activities; they act as a barrier for translocation of metals in plants. Investigation of pine seedling showed that treatment with *Lactarius rufus*, *Amanita muscaria*, and *Scleroderma citrinum* resulted in reduced translocation of Pb, Cd, or Zn in shoots in comparison with untreated plants [59]. Large surface area of mycorrhizal fungi helps in increased adsorption capacity for metals in polluted soils. Extracellular and intracellular components of fungal cell wall may also arrest/immobilize toxic ions inside the plant roots [18, 60].

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Biotechnological Approaches to Remediate Soil and Water Using Plant–Microbe Interactions

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Abstract A fast increase in the industrialization causes environmental pollution and contaminates water and soil throughout the world. The plants can also accumulate and store the toxic compounds/pollutants in their tissues and ultimately enter into the food chain. Hazardous pollutants in soil affect not only both animal and human health but also plant's growth. The symbiotic association between plants and microbe in the rhizosphere of plants or endophytic association between them is the suitable solution to improve remediation of soil and water pollution from the environment. Plant–microbe interactions enhance the plant health but also help them in well acclimatization in environment. Phytoremediation is a slow process in comparison to other remediation technologies. In plant–microbial interactions, plant roots help microorganisms to reach deeper in soil and improve aeration and nutrients supply, and the endophytic microorganisms allow degradation of pollutants within the plants. Genetic engineering can be used to improve the remediation efficiency of microorganisms and plants and also to increase the plant–microbial interactions to degrade those toxic substances which were impossible to degrade by naturally occurring bacteria and plants.

Keywords Plant–microbe interactions • Bioremediation • Phytoremediation • Heavy metal pollution

1 Introduction

Rapid industrialization leads to make the water and soil contaminated throughout the world by means of releasing pollutants like heavy metals, organic contaminants, toxic wastes, smoke, and fumes in the environment [1, 2]. Not only the industrialization but agricultural practices like use of chemical fertilizers, pesticides, etc. also

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make the water and soil contaminated [3]. Gradual deposition of these pollutants accumulated in the environment and in excess has severe negative impact on human, animal, and plant health. The removal of contaminants from the environment has been a great concern today and need an efficient and inexpensive solution. In fact, several techniques have been developed to decontaminate soil and water from pollutants such as excavation, landfilling, and incineration but they are expensive and cause other type of pollution. These methods may severely affect soil properties i.e., biological, physical, and chemical properties of soil. Nowadays, for the protection of environment from the various pollutants, biotechnology is considered as an emerging science. The technology realizes upon the use of living organism i.e., bacteria, fungi, yeast, or plants for biological treatment of elements of environment. The less energy is required in biotechnological treatment in comparison to conventional physicochemical treatment and is also cost-effective process [4].

As in classical approach, excavation and off-site treatments are costly and cause other type of pollution; in situ bioremediation is inexpensive and eco-friendly technology. In situ bioremediation techniques like bio-augmentation, biostimulation, phytoremediation and rhizoremediation become an attractive techniques to revive contaminated sites [5]. Our environment is contaminated with various kinds of pollutants and no single remediation technology is appropriate for handling these contaminants; to deal with them effectively more than one remediation technology is required [6].

1.1 Bioremediation

The bioremediation of the environmental pollutants can be done by the natural organisms which may be either indigenous or extraneous. They are diverse and depending on the chemical nature of pollutants. During selection of biological agent, type of contamination and site-specific conditions always keep in mind and biological agent meticulously selected [7]. Several microorganisms like, bacteria, fungi, and yeasts are equipped with the enzymes that permit them to degrade chemicals that are toxic for other species, or even utilize them as energy source. The bacteria *Dehalococcoides* sp. completely detoxifies vinyl chloride (VC), a proven human carcinogen in groundwater [8]. In bioremediation process, we can use several species of microorganisms like *Pseudomonas*, *Penicillium*, *Aspergillus*, *Pseudomonas*, *Ochrobactrum*, *Bacillus*, *Joostella*, *Sphingobium*, *Enterobacter*, *Aeromonas*, *Enterobacter*, *Exiguobacterium*, *Alcaligenes*, *Escherichia coli*, etc. for cleanup of our environment from hazardous chemicals. Microorganisms can tolerate highly toxic environment especially those indigenous are well adapted to polluted soil. This adaptive behavior of microorganisms pointed to that they might have some mechanism to grow under such highly toxic conditions. A brief summary of some microorganisms that can tolerate pollutants in the environment has been given in Table 1.

Table 1 Microorganism and their pollutant tolerance level in the environment

Microorganism	Pollutant	Tolerance level	References
Pseudomonas aeruginosa Strain JN102340	Lead nitrate	600 mg L^{-1}	Kumar et al. [57]
Penicillium citrinum and *Aspergillus niger*	Pb	30 mg L^{-1}	Wu et al. [58]
Pseudomonas, *Ochrobactrum*	Copper	900 mg L^{-1}	Heck et al. [59]
Bacillus malikii sp. nov.	Cr, Pb, and Cu	1200, 1800, and 1200 ppm, respectively	Abbas et al. [60]
Joostella sp.	Cd, Cu, Zn	–	Rizzo et al. [61]
Sphingobium sp.	Hg	44.15 mg L^{-1}	Mahbub et al. [62]
Enterobacter cloacae	Pb, Cd, Ni	Pd 1100 ppm, Cd 900 ppm, Ni 700 ppm	Banerjee et al. [63]
Bacillus cereus	Ni	434 ppm	Nagarajan et al. [64]
Aeromonas sp. *Enterobacter* sp., *Bacillus* sp. *Exiguobacterium* sp.	Cu, Co, Pb, Fe	Cu <2 mM, Zn <3 mM, Co <3.5 mM, Pb <12 mM, Fe 25 mM	Prabha et al. [65]
Alcaligenes faecalis	Pb, Cd, Al, Cu, Ag, and Sn	800–1400 μg mL^{-1}	Abo-Amer et al. [66]
Pseudomonas sp.	Phenanthrene, Cd	Phenanthrene 200 mg L^{-1}, Cd 5 mg L^{-1}	Thavamani et al. [67]
Bacillus dabaoshanensis sp.	Cr(VI)	600 mg L^{-1}	Cui et al. [68]
Pseudomonas putida, *Bacillus safensis*	Pb, Cd	Pb 110 mg L^{-1}, Cd 50 mg L^{-1}	Singh et al. [69]
Escherichia coli (P4)	Cd, Zn, Pb, Cu, Cr, As, Hg	10.6, 4.4, 17, 3.5, 4.4, 10.6, and 0.53 mM, respectively	Khan et al. [70]
Stenotrophomonas sp. G1	Organophosphorus pesticides (OPs)	Degraded 100 % methyl parathion, methyl paraoxon, diazinon, and phoxim, 95 % parathion, 63 % chlorpyrifos, 38 % profenofos, and 34 % triazophos in 24 h at initial concentration of 50 mg L^{-1}	Deng et al. [71]

1.2 Phytoremediation

The thought of using plants for remediation of environment is not new. In early 1960s, extensive research was conducted in Russia for treating the radionuclide-contaminated water using semiaquatic plants [9]. It is the remediation of the environment by using green plants. It is used to remove environmental deleterious waste to make them eco-friendly [10]. A wide range of plants can be used for soothing the environment from various contaminants, and it is a cost-effective eco-friendly approach that utilizes the ability of plants to accumulate, metabolize, and remove the contaminants from ambient contaminated site. Uptake, translocation, transformation, compartmentalization, and sometimes mineralization are the common steps involved during phytoremediation [11]. It is the most acknowledged technology by community and has many advantages over conventional techniques such as low cost, eco-friendly, and ability to remediate a range of pollutants. A brief summary of the plants used for phytoremediation is enlisted in Table 2.

2 Plant–Microbe Interactions for the Remediation of Environmental Pollution

2.1 Rhizospheric Microbial Association and Bioremediation

Hazardous pollutants in soil affects not only human health but also plant growth. The soil loaded with high concentration of metals drastically reduces the growth of plants; these metals can disrupt membranes, disintegrate cell organelles, and disrupt physiological and metabolic processes. Some microorganisms can degrade a range of pollutants in environment in the presence of sufficient nutrients for their growth but stop when they are ravenous of nutrients. Root exudates of plants are the best available food and nutrient source in soil for bacteria and promote their colonization. Combining the root colonization and pollutant degrading properties of microorganism with root exudation capacity of plant helps to clean up the environment from toxic substances/pollutants.

2.2 Endophytic Microbial Association and Bioremediation

Endophytes are nonpathogenic microorganisms (bacteria or fungi) that colonize in internal tissues of plants and observed maximum density in the roots and gradually decrease from the stem to the leaves [12]. It shows symbiotic association with plants and enhances the plant health and growth and also helps them in well

Table 2 Plants used for phytoremediation of contaminants

Plant	Pollutants	Remarks	Reference
Agrostis capillaris L.	Pb	Pb accumulation in roots ranged from 9.82 to 1107.42 mg kg^{-1} and in shoots from 6.43 to 135.23 mg kg^{-1}	Rodríguez-Seijo et al. [72]
Ricinus communis L.	Organochlorine pesticides	Remediate 25–70 %	Rissato et al. [73]
Mimosa, *Zinnia*, *Gazania*, and *Cypress vine*	Oil-contaminated soil	Oil concentration of the soils decreased by 45–49 %	Ikeura et al. [74]
Chenopodium album L., *Sedum emarginatum* Migo, and *S. lineare* Thunb	Sb	High accumulation of Sb levels above 1000 mg kg^{-1}	Ning et al. [75]
Brassica juncea	Hg, As	As and Hg phytoaccumulation increased up to 85 % and 45 %, respectively.	Franchi et al. [76]
Zea mays, Vicia faba	Cu, Zn, Pb, and Cd	Accumulation of Cu and Zn increased by *V. faba*; Cd accumulation increased in *Z. mays*	Lemtiri et al. [77]
Trifolium alexandrinum	Cd, Pb, Cu, and Zn	Heavy metal phytoextraction increased	Ali et al. [78]
Tithonia diversifolia and *Helianthus annuus*	Pb and Zn	Accumulated substantial Pb and Zn in their shoots	Adesodun et al. [79]
Alyssum murale subsp. *pichleri* and *Thlaspi* sp.	Ni	Hyperaccumulation of nickel above 1000 mg kg^{-1}	Bani et al. [80]
Aspalathus linearis	Al	Grown in high aluminum concentration ranging from 110 to 275 μg Al g^{-1}	Kanu et al. [81]
Helianthus annuus	Zn, Cd	Phytostabilization increase in the inoculated plants	Marques et al. [82]
Pelargonium roseum	Ni, Cd, Pb	high concentrations of Ni, Pb, and Cd accumulated in shoots	Mahdieh et al. [83]
Solanum nigrum	Cd, Zn, Cu	Significantly increased total Cd (46.6 %), Zn (16.4 %), and Cu (16.0 %) in the aerial parts	Chen et al. [23]
Arundo donax L.	Cu	Cu uptake increased by 45 % of total content	Elhawat et al. [84]

acclimatization in environment [13, 14]. These microorganisms are very valuable tool in bioremediation process; it has wide range of metabolic pathways to degrade, assimilate, and biotransformation of range of inorganic and organic pollutants in environment [15].

3 Plant–Microbial Interactions for the Removal of the Heavy Metal Pollutants

A large number of plants and microbes individually can tolerate high concentration of heavy metal pollutants as discussed above. But the contaminated soil and water remediation using plants and microbes separately is not very much effective and sufficient. The plants can also accumulate and store the toxic compounds/pollutants in their tissues and ultimately enter into the food chain [16]. These are also inadequate in site contaminated with a mixture of pollutants. The remediation by plants is a slow processing in comparison to other remediation technologies [14]. Whereas, the microorganisms are limited to soil surface only and they are unable to reach deeper in soil and also stop the process when lacking food. Symbiotic association between plants and microbe in the rhizosphere of plants [17] or endophytic association between them is the suitable solution to overcome these problems. Plant roots can help microorganisms to reach deeper in soil and improve aeration and nutrients supply [18], and endophytic microorganisms allow degradation of pollutants within the plants [19].

Rhizospheric or endophytic association of microbes and plants increased the remediation potential many folds for heavy metals, organic, and xenobiotic compounds [20]. In plant–microbe associated remediation process, plants are considered as a solar driven biological pump which draw pollutants into their rhizosphere via transpiration stream [21]; subsequently, degradation of pollutants occurs within plant, in rhizosphere or in both [12].

Many microbial cultures have been used to decrease the heavy metal contamination, synthetic plastic pollutants and agrochemicals such as herbicides, pesticides etc. from the contaminated sites, when used in conjunction with plants (Table 3). These microbes sustain high contaminant levels and have performed well in exerting bioremediation showing synergistic effects of plants and their root exudates. Level of dioxins in soil was decreased with 35 % when barley growing with white rot fungi [22]. *Solanum nigrum* when inoculated with *Pseudomonas* sp. Lk9 the bioaccumulation of Cd has been increased by 28.9 % [23]. Plants like *Eruca sativa* when inoculated with *Pseudomonas putida* enhance the uptake of Ni by up to 46 % from soil and reduced Ni level in the soil [24]. Introduction of *Brevibacterium casei* MH8a into soil with *Sinapis alba* significantly increased Cd accumulation by 208 %, Zn accumulation by 86 %, and Cu accumulation by 39 % in the plant shoots [25]. The plant growth-promoting bacteria *Pseudomonas* species isolated from different rhizospheric soils of wheat and

Table 3 Plant and associated microorganism and target contaminants

Plant	Associated microorganism	Target pollutants	Remarks	Reference
Lentil (*Lens culinaris*)	*Providencia vermicola*	Cu	Leaf chlorophyll content, root nodulation, number of pods, seed weight were increased	Islam et al. [85]
Plant associated bacterium	*Enterobacter* sp. strain EG16	Cd	Tolerate MIC >250 mg L^{-1} Cd^{2+}	Chen et al. [44, 45]
Eucalyptus tereticornis	*Pisolithus albus*	Cu and Cd	High *PaMT1* expression levels in the presence of Cu and Cd	Reddy et al. [86]
Eruca sativa	*Pseudomonas putida*	Ni	Enhanced the uptake of Ni by up to 46 %	Kamran et al. [24]
Oryza sativa (L.) Var. Sarju 52	*Brevundimonas diminuta*	As	High As tolerance, promote plant growth, and phytostabilization of As	Singh et. al. [87]
Oryza sativa L.	*Bacillus flexus* ASO-6	As	Significantly improved As uptake in root and shoot	Das et al. [88]
Zea mays L.	*Micrococcus* sp. TISTR2221,	Cd	Enhanced cadmium phytoextraction in *Z. mays* L.	Sangthong, et al. [89]
Vigna radiate	*Acinetobacter* sp. AVLB2	4-Nitroaniline (4-NA)	V_{max} for biodegradation of 4-NA was 0.541 mg L^{-1} h^{-1} and 0.551 mg L^{-1} h^{-1}	Silambarasan and Vangnai [90]
B. juncea and *L. albus*	Plant growth-promoting bacteria	As, Hg	As and Hg phytoaccumulation increased up to 85 % and 45 %, respectively	Franchi et al. [76]
	Providencia sp. *2D*	*Di-n-butylphthalate*	*Strain 2D can degrade Di-n-butylphthalate completely, 89.0 %, or 84.9 % within 72 h when initial concentrations were ≤200, 500, and 1000 mg* L^{-1}*, respectively*	Zhao et al. [91]

(continued)

Table 3 (continued)

Plant	Associated microorganism	Target pollutants	Remarks	Reference
Solanum nigrum	*Pseudomonas* sp. Lk9	Cd	Elevated the bioaccumulation factor of Cd by 28.9 %	Chen et al. [23]
Brassica napus	*Enterobacter* sp. and *Klebsiella* sp.	Cd, Pb, and Zn	Cd, Pb, and Zn uptake significantly increased	Jing et al. [92]
Brassica juncea	*Enterobacter* sp.	Cd and Pb	*gcsgs* gene introduced into *Enterobacter* sp. CBSB1 upgraded the phytoremediation efficacy of *B. juncea*	Qiu et al. [93]
Cicer arietinum L.	*Kocuria flava*	Cr	*K. flava* assists in growth and Cr accumulation and increases phytoremediation potential of the plant	Singh et al. [4]
Alyssum pintodasilvae	*Arthrobacter nicotinovorans* SA40	Ni	Microbial association enhanced plant growth and Ni uptake and accumulation in plant	Cabello-Conejo et al. [94]
Soybean	*Bradyrhizobium*	Cu	Association improves root and shoots growth	Sánchez-Pardo and Zornoza [95]
Pisum sativum	*Rhizobium, Microbacterium* sp.	Cr	Enhanced root and shoot length, increase dry root and shoot biomass	Soni et al. [96]
Brassica napus	*Enterobacter* sp. and *Klebsiella* sp.	Cd, Pb, and Zn	Cd, Pb, and Zn uptake enhanced in both shoot and root tissues	Jing et al. [92]
Solanum nigrum L.	*Pseudomonas* sp.	Cd, Zn, and Cu	Enhanced shoot dry biomass (14 %) and accumulation of Cd (46.6 %), Zn (16.4 %), and Cu (16.0 %) in aerial parts	Chen et al. [23]
Helianthus annuus L.	*Bacillus safensis* + *Kocuria rosea*	Ni	Increase Ni uptake, pigments of photosynthesis, and plant growth	Mohammadzadeh et al. [97]

Helianthus annuus L.	*Bacillus safensis*	Fe	Fe level increased significantly in shoot	Mohammadzadeh et al. [97]
Cicuta virosa L.	*Pseudomonas putida* and *Rhodopseudomonas* sp.	Zn	Enhance accumulation of Zn in root and increase the seedling growth	Nagata et al. [27]
Maize	*Burkholderia cepacia* SCAUK0330	P	Enhanced phosphate solubilization and promote plant growth	Zhao et al. [98]
Zea mays L.	*Ralstonia eutropha and Chryseobacterium humi*	Cd	Inoculation of bacteria increases *Z. mays* biomass up to 63 %, level of Cd decreases up to 81 % in shoot levels, and increase Cd accumulation in root of up to 186 %	Moreira et al. [99]
Solanum nigrum L.	*Pseudomonas* sp. Lk9	Cd, Zn, Cu	Significantly increased total Cd (46.6 %), Zn (16.4 %), and Cu (16.0 %) in the aerial parts	Chen et al. [23]
Helianthus annuus	*Ralstonia eutropha, Chryseobacterium humi*	Zn, Cd	Phytostabilization of Zn and Cd increase in the inoculated plants	Marques et al. [82]

pigeon pea not only exhibited the plant growth but also enhanced the heavy metal tolerance activities [26].

Several studies have been indicated that the bioremediation efficiency can be enhanced by plant–microbe interaction. Association between plant *Cicuta virosa* L. and root endophytic bacteria *Pseudomonas putida* and *Rhodopseudomonas* sp. can increase the accumulation of Zn in plant root by solubilizing the Zn in pond sediment, detoxify the metals in the plant tissues, and promote plant growth also [27]. However, endophytic bacterium *Burkholderia* sp. GL12 and *Bacillus megaterium* JL35 increases the Cu uptake by 132 % and 48.2 %, respectively in *Elsholtzia splendens* and *Brassica napus* from the Cu-contaminated soil [28]. The bacteria *Acidovorax* sp. (U3), *Ralstonia* sp. (U36), *Pseudomonas* sp. (R16), and *Ochrobactrum* sp. (R24) in endophytic association with *Juncus acutus* L. plant have been found to reduce Cr(VI) to Cr(III) and provide a very good option for removal of Cr from contaminated groundwater and later use in crop irrigation [29]. A long list of the remediation of the heavy metal pollutants by using plant–microbe interaction is summarized in Table 3.

4 Plant–Microbial Interactions for the Removal of Other Pollutants

In addition to the heavy metal contaminants, a wide range of other pollutants also contaminate the soil and water which ultimately affects the land and aquatic life. Plant–microbe interactions can be used in the degradation and removal of such pollutants from water and soil. A brief summary of the plant–microbe interaction used for the remediation of some other pollutants is given in Table 4.

4.1 Bioremediation of Plastic

Plastic is durable, water resistant, flexible, lightweight, and relatively low-priced material extensively used in production of numerous products. However, rapid accumulation of plastic in environment causes a serious problem to the ecosystem due to its slow degradation and long sustainability in the environment. It includes polythene, propylene, polystyrene, polyurethane (PUR), nylon, polyethylene either LDPE (low density polyethylene) or HDPE (high density polyethylene) etc. [30]. Since, the chemical bonds in several plastics are rather similar to the bonds of natural plant polymers [31]; researchers give their attention to use the ability of endophytes in plastic degradation. The endophytic fungi *Paecilomyces lilacinus* and *Lasiodiplodia theobromae* from plant *Humboldtia brunonis* and *Psychotria flavida* respectively are reported to be capable in plastic (polyethylene and polypropylene) degradation [32]. Endophytic bacteria

Table 4 Bioremediation of other pollutants by using plant–microbe associations

Plant	Microorganism	Pollutants	Reference
Astragalus sinicus L.	*Mesorhizobium* sp.	PCB	Teng et al. [49]
Seduce alfredii	*Microbacterium* sp. and *Candida tropicalis*	Polycyclic aromatic hydrocarbons (PAHs)	Chen et al. [44, 45]
Lolium multiflorum	*Mesorhizobium* sp. *HN3*	Chlorpyrifos	Jabeen et al. [41]
Orychophragmus violaceus	*Gammaproteobacteria* and *Flavobacteria*	Hexachlorocyclohexanes (HCHs), dichlorodiphenyltrichloroethanes (DDTs)	Sun et al. [28]
Poplar	*Enterobacter* sp. strain *PDN3*	Trichloroethylene (TCE)	Kang et al. [100]
Lotus corniculatus var. *Leo, Lolium multiflorum* var. *Taurus* and *Medicago sativa* var. *Harpe*	*Enterobacter ludwigii*	Diesel	Yousaf et al. [101]
Zea mays	*Streptomyces*	Pesticides like Lindane	Abhilash et al. [40]
Medicago sativa (alfalfa)	*Rhizobium meliloti*	PAHs	Teng et al. [43]
Zea mays and *Triticum aestivum*	*Burkholderia cepacia*	Toluene	Wang et al. [102]
Poplar	*Pseudomonas putida W619-TCE*	Trichloroethylene (TCE)	Weyens et al. [103]
Lolium multiflorum var. *Taurus birdsfoot trefoil*	*Pantoea* sp.	Diesel	Yousaf et al. [104]
Lolium multiflorum L.	*Pseudomonas* sp. strain *ITRI53*	Diesel	Andria et al. [105]
	Rhodococcus sp. strain *ITRH43*		
Corbicula fluminea, Rice	*Rhizobium* sp.	Atrazine, Carbaryl, Carbofuran, Glycophosphate, Parathion, Coumaphos, Diazinon, Propham	Roche et al. [106]
Sugar beet	*Pseudomonas fluorescens*	4-Chlorobiphenyl	Ionescu et al. [107], Narasimhan et al. [108]
Hordeum vulgare	Marine microalga	2,4-Dichlorobiphenyl	Petroutsos et al. [109], Macková et al. [110]
	Tetraselmis marina		
	Burkholderia cepacia		
Astragalus chrysopteru	*Rhizobium* sp.	Pentachlorophenol	Wei et al. [111]
Lolium multiflorum	*Pseudomonas putida*-PCL1444	Naphthalene	Kuiper et al. [112]

Nocardiopsis sp. mrinalini9 from *Hibiscus rosasinensis* can degrade 22 % of polythene and 10 % of plastic in 2 months [33].

4.2 Bioremediation of Explosives

Explosives are very toxic compound recalcitrant to remediation. These compounds cause severe environmental pollution at the site of ammunitions manufacturing and military action (testing, training, or battlefield) [34]. Three major classes of explosive are nitrate esters, nitroaromatics, and nitramines [35]. These compounds pollute the soil and rapidly seep into the groundwater resulted into the bioaccumulation and biomagnifications in terrestrial and aquatic organisms [35, 36]. Several studies reported that synergistic approach of plant and microbes in successful remediation of explosive-contaminated soil and water. The bacterial spp. of *Acer pseudoplatanus* when inoculated with *Agrostis capillaries* (bent grass) shows Trinitrotoluene (TNT) detoxification ability from the soil as well as promotes plant growth [37]. The nitramine hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is one of the major explosives used in the military operations. As per the USEPA (United States Environmental Protection Agency) it is toxic to the environment and categorized as a carcinogen and also nondegradable in the environment [38] and its accumulation in the soil and groundwater increases in such areas [39]. The XplA gene from the bacterium *Rhodococcus rhodochrous* 11Y when introduced into *Arabidopsis* root colonizing bacteria *Pseudomonas fluorescens* F113 showed degradation of the RDX [36].

4.3 Bioremediation of Organochlorine Pesticides (OCPs)

Pesticides are insect repellent or insect killer that manage pests which considered being harmful to crops. These compounds are toxic and not easily degraded by natural processes. These compounds contaminate soil and water. That ultimately entered into the food chain and accumulated at different tropic levels. Due to environmental concern organochlorine pesticides have been banned in past, but abandoned plants and dump sites are still contaminating the environs [28]. In several researches, plant–microbial interactions have been found the most effective in remediation of these organochlorine pesticides. The plant–microbe association like *Withania somnifera* and *Staphylococcus cohnii* can enhance the Lindane uptake by 76 % from soil in 90 days [40]. According to Sun et al. [28], bacteria *Gammaproteobacteria* and *Flavobacteria* can degrade hexachlorocyclohexanes (HCHs) 81.18 % and dichlorodiphenyltrichloroethanes (DDTs) 85.40 % when soil was bioaugmented with plant *Orychophragmus violaceus*. However, the association of root endophytic bacteria *Mesorhizobium* sp. with *Lolium* *multiflorum* (ryegrass) successfully degrades the Chlorpyrifos and its metabolite 3,5,6 trichloro-2-pyridinol inside the root and rhizosphere [41].

4.4 Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are omnipresent organic pollutant in environment. These are generally produced by combustion of fossil fuels in industries, automobiles, and by natural process like volcanic eruption or by forest fire [42]. PAHs have immense negative impact on environment as these compounds are highly toxic and continue for long period in environment. It impairs the human health as well as ecosystem. The plant–microbe interactions can also be used to degrade or remediate the PAHs. Inoculation of *Medicago sativa* (alfalfa) plant with *Rhizobium meliloti* can decrease the PAHs level from soil [43]. When the plant *Seduce alfredii* was inoculated with *Microbacterium* sp. and *Candida tropicalis*, 96.4 % decrease in PAHs level was reported by Chen et al. [44, 45].

4.5 Bioremediation of Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are synthetic organic chemicals consisting group of 209 congeners of similar structure. PCBs are array of oily liquids to waxy solids and are colorless to light yellow compounds [46, 47]. These compounds are highly toxic and not easily degraded by natural processes and hence stayed accumulated in the environment for a long duration. PCBs can pollute almost all the components of the environment like air, water, and soil and cause serious problem to human health [47, 48]. Researchers use the potential of plant–microbe interaction in bioremediation of PCBs. Synergistic association between PCBs degrading bacteria *Mesorhizobium* sp. ZY1 with the plant *Astragalus sinicus* L. can boost the phytoextraction of PCBs and degradation by microflora of rhizosphere. Up to 53.1 % of the PCBs from soil has been degraded in 100 days [49].

4.6 Bioremediation of Phenolic Allelochemicals

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a highly toxic substance produced by agro-industrial operations and has negative effect on the growth of organisms even at a low concentration [50]. Endophytic fungus *Phomopsis* *liquidambari* from *Bischofia polycarpam* stem can degrade 97 % of ferulic acid in 48 h by using it as sole carbon source [51]. Cinnamic acid is also a phenolic allelochemical which has been widely found in continuous cropping soils and its accumulation leads to affect the plant productivity. The plant–microbe interactions like peanut with the endophytic fungus *P. liquidambari* degrade cinnamic acid and also improved seedling growth [52]. In another study, the endophytic fungus *P. liquidambari* B3 with the peanut showed increase in the peanut pod yield and number of root nodules. This interaction shows the degradation of the allelochemicals from the soil environment [53].

4.7 Bioremediation of Pharmaceutical Drug Pollutants

Today increased consumption of wide range of pharmaceutical drugs resulted in the pollution of surface water, groundwater and seawater and even drinking water [54]. E.g., the pharmaceutical drugs like carbamazepine, a synthetic compound of the benzodiazepine class, used as an antiepileptic and mood-stabilizing drug. Due to its persistency in environment and potential toxicity, overuse of it causes accumulation in municipal wastewater and a serious problem to ecosystem [55]. Bioremediation of such contaminants can be done by using plant–microbe interactions. In one of the study, the endophytic association of *Phragmites australis* plant and *Chryseobacterium taeanense* successfully removes the carbamazepine from the wastewater [55].

5 Improvement of the Biodegradation Efficiency Using Genetic Engineering

With progression in better understanding of environmental biotechnology and role of genes and enzymes, some microorganisms and plants are genetically engineered to degrade those toxic substances which were impossible to degrade by naturally occurring bacteria and plants. Genetic engineering can provide an effective cleaning process of environment at low cost. In 1974, *Pseudomonas putida* was the first stain patented as biological agent for degradation of petroleum [7]. Later on many microbes and plants are genetically modified. These genetically engineered microorganisms and plants have higher degradative capacity. The genetically modified microbes and plants can sustain high concentration of pollutants and showing great potential in bioremediation. The genetically engineered yeast with a gene *WaarsM* isolated from a soil fungi *Westerdykella aurantiaca* in association with rice plant shows arsenic bioremediation [56]. A list of the genes and the genetically modified plants and microbes used for the improvement of the bioremediation efficiency is listed in Table 5.

Table 5 Genetic engineering of plant and/or microbes for the improvement of bioremediation

	Gene	Gene source	Host plant/microbe	Pollutant	Remarks	Reference
1.	*WaarsM*	*Westerdykella aurantiaca* (*Soil fungi*)	*Yeast*	As	Arsenic tolerance in rice after co-culture with genetically engineered yeast suggested its potential role in arsenic bioremediation	Verma et. al. [56]
	PaMT1	*Pisolithus albus*	*Eucalyptus tereticornis*	Cu and Cd	High PaMT1 expression levels increased in the presence Cu and Cd	Reddy et al. [86]
4.	merRTPAB	pDU1358-*Serratia marcescens*	*E. coli* K12	Hg	Expression of mer operon enables transport of Hg^{2+} into the cell, cleavage of organic C–Hg bonds, and reduction of Hg^{2+} into Hg(0)	Kane et al. [113]
5.	*gcsgs*	*Enterobacter* sp. CBSB1	*Brassica juncea*	Cd and Pb	gcsgs gene introduced into *Enterobacter* sp. CBSB1 upgraded the phytoremediation efficacy of *B. juncea*	Qiu et al. [93]
6.	*ScMTII*	*Saccharomyces cerevisiae*	*N. tabacum*	Cd and Zn	Transgenic tobacco accumulated 457 mg Cd kg^{-1} DW, for 80 mg Cd kg^{-1} soil treatments, 3.5–4.5-fold higher than threshold value of Cd hyperaccumulation	Daghan et al. [114]
7.	*ScYCF1*	*Saccharomyces cerevisiae*	*Populus alba*	Cd, Zn, and Pb	Increased Cd accumulation in the aerial tissues and increased Cd, Zn, and Pb accumulation in the roots	Shim et al. [115]
10.	*YCF1* and AsPCS1	Garlic and baker's yeast	*Arabidopsis thaliana*	As and Cd	2–10 folds cadmium/arsenite accumulation by dual-gene transgenics	Guo et al. [116]

6 Conclusions

Remediation of water and soil pollution from the environment by using plant–microbe interactions is a highly efficient cost-effective technique. By using various biotechnological approaches researchers are not only trying to improve the remediation efficiency of the plants and microbes but also trying to draw some novel plant–microbial interactions for the remediation and degradation of the soil and water pollutants from the environment.

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Current and Future Opportunities for Forest Land Application Systems of Wastewater

Elizabeth Guthrie Nichols

Abstract Global human population growth will continue to threaten loss of forested lands, which, in turn, will negatively impact water quality as well as global and local environmental processes that regulate climate and biogeochemical cycling. Forests are integral to drinking water supplies for 30 % of the major cities in the world, and our existing managed and natural forests provide cleaner and more stable water supplies for surface water and groundwater than any other land use. One opportunity to minimize loss of forested landscapes, improve water quality, and regulate water availability is to consider coupling natural and managed forests with other environmental services such as wastewater treatment and management. These opportunities exist globally in wild lands, urban, suburban, and rural contexts at various scales across municipal, industrial, and agricultural systems. Wastewater treatment via land application has occurred since the 1500s in Europe and was established both in England and the United States in the late 1800s. Greater pressures exist today to manage water, wastewater, and forest systems in a sustainable manner. Advances in wastewater management provide new opportunities for forest-wastewater system design and use. The land application of wastewaters to forest ecosystems has historically focused on wastewater treatment and recycling. In the future, provisioning the world's forests with adequate water may become as important as managing wastewaters with forested landscapes.

Keywords Wastewater • Forests • Land application • Ecosystem services

1 Introduction

The world's forests will continue to decline in quality and quantity if current human population and urbanization trends continue [1, 2]. The loss of forested lands negatively impacts water quality as well as global and local environmental processes that regulate climate and biogeochemical cycling [3, 4]. Forests are integral to drinking

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water supplies for 30 % of the major cities in the world [4]. Currently, managed and natural forests provide cleaner and more stable water supplies for surface water and groundwater than any other land use with nearly 60 % of all water flows from forests [5, 6]. Forests regulate water availability, and young forests or new forest plantations can reduce surface water availability [4]. One opportunity to minimize loss of forested landscapes, improve water quality, and regulate water availability is to consider coupling natural and managed forests with other environmental services such as wastewater treatment and management [7]. These opportunities exist globally in wild lands, urban, suburban, and rural contexts at various scales across municipal, industrial, and agricultural systems [8–11].

Climate change will cause greater variability and intensity of water abundance and shortages for temperate climates such as the southeastern USA [12]. Water security will require sustainable systems to moderate these more frequent and greater intensity extremes [13]. In more-arid regions of the world, water shortages continue to advance our understanding of water reclamation challenges, best management practices, and the dynamics of community acceptance [8, 14, 15]. However, temperate climates are also experiencing water availability pressures and competition for surface and groundwater allocation between agriculture, industry, and municipal stakeholders [6]. Water–forest systems can help mitigate climate change across a diversity of spatial and economic scales. This chapter focuses specifically on municipal and agricultural wastewater–forest land application systems (forest LAS), but other opportunities and applications exist for forest–water systems in urban (storm water, recreational areas) and rural contexts (mining reclamation, agro-forestry, and rangelands) [11, 13].

2 Forest Land Application Systems

Wastewater treatment via land application has occurred since the 1500s in Europe and was established both in England and the United States in the late 1800s [11]. The application of wastewaters to forested lands has been an active component of wastewater management in the United States since the middle 1900s with a great deal of research in the 1970s and 1980s by academic researchers, the United States Environmental Protection Agency (USEPA), and the United States Army Corps of Engineers (USACE) [11, 16–18]. This rich and informative literature is very germane to appropriate technology design, regulatory policy, and best management practices for wastewater application to forests today [9, 11]. The basic principles for effective and sustainable forest land application systems (LAS) will be summarized herein, but the reader is encouraged to utilize the primary literature when considering forests systems for wastewater management and recycling. These prior studies document fundamental aspects of appropriate design, forest ecology and silviculture, hydrology, and wastewater management that is still relevant today. Greater pressures exist today to manage water, wastewater, and forest systems in a sustainable manner [7], and advances in wastewater management provide new opportunities for forest–wastewater system design and use [9, 13]. To date, forest LAS have focused on wastewater treatment and recycling, but, in the future, provisioning the world's forests with adequate water may become as important as managing wastewaters with forested landscapes.

3 Forest Structure Considerations for Forest LAS

Forest lands may be attractive for land application due to their proximity to wastewater sources, their lower land values, and aesthetic habitat for wildlife and recreational use [19]. Forests have long term capacity to store wastewater nutrients, and they accept intermittent excesses of wastewater volume without adverse consequences to their biological integrity and water treatment processes. The structure of forest soils provides higher percolation and infiltration and less opportunity for surface runoff compared to agricultural and livestock lands which experience more soil compaction over time [20]. Greater infiltration allows for longer periods of application even during winter months [11, 16, 19]. Infiltration in forest systems improves with forest stand development which is often not the case for agricultural lands under livestock and crop management.

Forest LAS are slow-rate infiltration operations where intermittent hydraulic loading allows for adequate soil aeration and limited soil saturation. Appropriate and intermittent hydraulic loadings should avoid surface ponding and runoff while maintaining the drainage capacity of the forested area. Forest LAS favors uneven topography and hill slopes, and sites with significant slopes (15–30 %) are often only appropriate for forested LAS [11, 16, 20]. Over-application of wastewater can cause groundwater to remain too close to the soil surface thus encouraging shallow root development of trees and potential loss of trees due to winds (wind throw). Chronic soil saturation will also impair tree growth, nutrient uptake, and transpiration [20].

Forest systems naturally change according to precipitation, soil quality, nutrient availability, and hydrology. Because tree species have different tolerances for saturated or flooded soils, land application of wastewater can alter the existing forest structure over time with increased soil saturation and higher water tables [20, 21]. Slow-rate infiltration systems must integrate soil quality, forest structure, evapotranspiration potential (ET_p), and hydrology into the site design if the existing forest structure is to be maintained, and the distribution of water must be appropriately matched to tree structure and characteristics. Improper LAS design and management can cause forest structure failure on existing forest lands and slow tree establishment and growth on newly established forests.

Much of the prior literature on forest LAS has focused on the appropriate design for hydraulic and nutrient loadings of forest LAS. Most forest LAS have utilized natural forest stands which require careful management of hydraulic loading in order to maintain current forest structure and function. Doubling the annual hydraulic loading of an area will alter forest hydrology and foster a different natural forest structure over time; in fact, excessive hydraulic loading can remove the forest system entirely [20]. Prior studies (1970–1990) have reported on tree survival, nitrogen removal, and biomass growth in the first 5–10 years for a variety of deciduous and softwood trees such as Douglas fir, poplar, red pine, spruce, and mixed hardwood forests in the northwestern, northeastern, and southeastern USA [17, 20]. More recent studies have examined long-term metrics for salt accumulation, nutrients, metals, and biomass growth in semi-arid and arid regions (Table 1). More long-term data are needed to assess forest structure, integrity, and productivity in temperate areas. These data are particularly important to advance innovative forest LAS and to quantify other ecosystem services of forest LAS in addition to wastewater treatment.

Figure 1 shows selected forest stands at 18–20 years age at forest LAS in the temperate, southeastern USA (North Carolina, Fig. 1). One facility has land -applied municipal wastewater onto a 109 ha of managed hardwood stands and natural mixed hardwood/pine forests for 22 years. During this period, more flood-tolerant trees, such as bald cypress (*Taxodium distichum*) or green ash (*Fraxinus pennsylvanica*), have had better survival and productivity than American sycamore (*Platanus occidentalis*), sweet gum (*Liquidambar styraciflua*), and cottonwoods (*Populus deltoides*) [22] (Fig. 1). Bald cypress and green ash are not typically found in these Piedmont physiographic soils, but hydraulic loading favored their survival and growth over a 20-year period of irrigation. The estimated revenue generated for bald cypress was more profitable than the faster, growing sweet gum, cottonwood, and sycamore [22].

Similar results were observed at a smaller municipal LAS with more sandy soils that was planted with *Populus*, green ash, *Eucalyptus*, and bald cypress in 2012 [23]. Prior mismanagement of wastewater application resulted in loss of initial sycamore stands. Today, survival and growth of bald cypress and green ash are excellent compared to poorer performances for *Populus* and *Eucalyptus*. In a freshwater wetland in Louisiana, USA, tree core analyses showed that bald cypress stands receiving wastewater had significantly greater total and annual growth over 35 years compared to bald cypress in the reference stands without wastewater exposure. The average tree age was 86 years in the 1400 ha swamp which has received primary-treated wastewater since 1948 and secondary-treated wastewater since 1953 [21]. Use of native trees for forest LAS should be considered as they are often more ecologically and economically beneficial than nonnative species. They are often less expensive as seedlings, require less pest control, and can be more weed tolerant to irrigated conditions thus requiring less site maintenance and weed control [22].

Tree biomass, wood quality, and wood value are important considerations when designing forest LAS for existing forests or new managed plantations for wastewater land application. Biomass, wood quality, and wood value depend upon management practices, site establishment and maintenance costs, and harvest rotations. Initial site design should give careful consideration to specific tree species selection, productivity over a 10–25 year periods, and available or evolving wood markets around the LAS site. Obviously, the primary regulating service for water purification must be achieved and maintained at forest LAS, but careful tree species selection can meet this obligation and yield greater biomass productivity and profitability.

Table 1 Examples of Forest LAS

Citrus paradise Macf	Grapefruit	USA	Maurer et al. [42]
Citrus paradise Macf	Grapefruit	USA	Pedrero et al. [43]
Citrus	Lemon	Spain	Pedrero and Alarcon [44]
Citrus	Lemon	Spain	Pedrero et al. [45]
Citrus clementina	Mandarin	Spain	Mounzer et al. [46]
Citrus paradise	Grapefruit	Israel	Bardhan et al. [25]
Jatropha curcas L.	Jatropha	Spain	Dorta-Santos et al. [47]
Robinia pseudoacacia (L.)	Black locust	Iran	Tabari and Salehi [48]
Swietenia mahagoni (L.)	Mahogany	Egypt	Hayssam et al. [49]
Olea europaea	Olive	Israel	Segal et al. [50]
Olea sp.	Olive	Tunisia	Petousi et al. [51]
Olea sp.	Olive	Greece	Bedbabis et al. [52]
Olea sp.	Olive	Greece	Bourazanis et al. [53]
Rhizophora mucronata	Mangrove	Mayotte (France)	Herteman et al. [54]
Ceriops tagal	Mangrove	Mayotte (France)	Herteman et al. [54]
Eucalyptus sp.	Eucalyptus	USA	Minogue et al. [55]
Eucalyptus sp.	Eucalyptus	India	Minhas et al. [32]
Populus deltoides Bartr.	Eastern cottonwood	USA	Minogue et al. [55]
Populus	Poplar, hybrid	USA	Ghezehei et al. [23]
Plantanus occidentalis L.	Sycamore	USA	Ghezehei et al. [23]
Pinus taeda L.	Loblolly pine	USA	Ghezehei et al. [23]
Taxodium distchum L.	Bald cypress	USA	Ghezehei et al. [23]
Fraxinus pennsylvanica L.	Green ash	USA	Ghezehei et al. [23]
Liquidamabar styraciflua L.	Sweetgum	USA	Ghezehei et al. [23]
Cupressus sempervirens L.	Italian cypress	Egypt	Farahat and Linderholm [27]
Salix spp.	Willow	Estonia	Holm and Heinsoo [31]
Populus alba	Poplar	Spain	Miguel et al. [56]
Pinus spp.	Pine	Egypt	Zalesny et al. [10], Evett [57]
Eucalyptus spp.	Eucalyptus	Egypt	Zalesny et al. [10], Evett [57]
Populus spp.	Poplar	Egypt	Zalesny et al. [10], Evett [57]
Khaya ivorensis	Mahogany	Egypt	Zalesny et al. [10], Evett [57]
Tectona grandis	Teak	Egypt	Zalesny et al. [10], Evett [57]
Gmelina arborea Roxb.	Beechwood	Egypt	Zalesny et al. [10], Evett [57]
Populus spp.	Poplar	USA	Smesrud et al. [24]
Eucalyptus camaldulensis	Eucalpytus	USA	Smesrud et al. [24]
Causarina spp.	Sheoak	Egypt	Farahat and Linderholm [27]
Taxodium distchum L.	Bald cypress	USA	Hesse et al. [21]

Fig. 1 Municipal forest LAS in North Carolina (USA) at 18–20 years of irrigation. *Top left*: bald cypress—*Taxodium distichum*, *right:* green ash—*Fraxinus pennsylvanica*, *middle left:* coppiced American sycamore *Platanus occidentalis*, *right:* sweet gum *Liquidambar styraciflua*, *bottom left:* cottonwoods *Populus deltoides* and green ash, and *bottom right:* green ash

4 Appropriate Forest and Site Structure for LAS

Potential sites for wastewater land application may meet specifications for either forest or non-forest vegetative crops [11, 15, 18]. Some specific site conditions favor forest LAS such as land with existing forests and lands with significant topographical slope (15–35 %). Sites that require year-around wastewater application also require forested stands [18]. Determining the minimum amount of area to meet wastewater treatment needs will depend on what factors limit wastewater application, such as soil permeability for hydraulic loading or loading rates for a particular wastewater parameter such as nitrogen. Primary treatment of wastewater is the minimum requirement for forest LAS which requires settling and removing solids, oil,

Table 2 Suitable features for forest slow-rate infiltration systems

Forest type	Hardwood > mixed hardwood/softwood > softwood
Stand age	0–30 years hardwood and 0–20 years softwood
Slope	0–35 %
Soil depth	>1 m
Expected area	23–300 ha
Application	Sprinkler, surface, or drip year around for most climates
Hydraulic loading	0.5–6 m per year or 1.9–6.5 cm per week
Depth to groundwater	>0.6 m
Soil permeability	>0.15 cm per hour of most restrictive soil layer
Hydraulic soil conductivity	5–10 cm per hour
Surface infiltration rate	>5 cm per hour
Soil cation exchange	>10 mEq/100 g soil
Rock outcrops	10 % or less of total surface
Pretreatment	Primary and secondary

Sources: Adapted from USEPA [18] and Crites et al. [11]

and grease to avoid problems in effluent irrigation (plugging), odor problems, and capacity loss [11]. Secondary treatment will ensure protection of groundwater resources by removing many wastewater constituents via biological treatment.

Table 2 provides metrics that are appropriate for forest LAS. These metrics favor hardwood or mixed softwood/hardwood stands over softwood stands and younger trees (<25 years) to older trees (>30 years) for nutrient and water uptake. Particular site features require that irrigated areas be adequate distances from surface waters (>30–60 m) with sloped topography between 7 and 30 % and moderate depths to groundwater (at least 1–3 m) [18].

Sufficient soil depth (>1 m) and permeability (>0.50 cm/h) is crucial as more shallow soils and lower soil permeability will restrict wastewater loading rates or require more area for wastewater distribution [11]. Soils with higher cation exchange capacity (CEC) such as loams, clays, or humus have greater capacity to treat and remove wastewater constituents than sandy soils, but, these soils are less permeable to water and require lower hydraulic loading. Water infiltration or nitrogen removal are often the limiting design factors for forest LS, and the treatment area is determined by balancing hydraulic loading with wastewater constituent removal. A detailed ranking system that incorporates climate, soils, geology, and vegetation can help provide preliminary estimations for hydraulic loading [18].

5 Appropriate Wastewater Characteristics and Loading Rates for Forest LAS

Forest systems have limitations in terms of managing particular wastewater characteristics relative to other LAS crops or LAS systems. A forest LAS design must balance the capacity of water management (hydraulic loading) with the adverse impact of physical, biological, and chemical characteristics of wastewater for several decades if not indefinitely. In addition to tolerating high hydraulic loading rates and most soil conditions, forest LAS must also have high nutrient uptake capacity and a tolerance to wastewater constituents such as total dissolved solids (TDS), chloride, and boron [11, 24]. Table 3 provides general wastewater characteristics that are appropriate for forest LAS. To manage particularly high parameters of any given wastewater requires additional pretreatment before application, lower hydraulic loading rates for a given area, intermittent irrigation with freshwater, or application of a given hydraulic rate over a larger forested area.

For forested LAS, typical loading rates are 25 mm per week [18]. If irrigation is allowed year round, loading rates may be lower during winter for areas that receive significant winter precipitation. Storage reservoirs help manage wastewater irrigation when conditions do not favor application such as cold weather, precipitation, site maintenance, and crop or forest management. Biodegradable organics (BOD_5) are efficiently removed at the soil and near-soil surface where microbial activity is high in forest LAS systems. These materials reduce the availability and mobility of trace metals, salts, and organic contaminants in the forest LAS. Intermittent hydraulic loading is necessary to allow forest soils to sufficiently aerate, promote degradation, and maintain infiltration capacity. Likewise, total suspended solids (TSS) are efficiently removed via soil filtration and infiltration. Higher loading rates for BOD_5 and TSS are possible but require more careful attention to avoid odor and soil clogging [13], particularly for long-term irrigation with wastewater [25]. Forest LAS soils should have adequate buffering capacity for extreme acidic or caustic pH. However, in temperate climates, wastewater storage for forest LAS can produce effluents with high pH due to photosynthesis activity in the reservoirs. Industrial wastewaters can often lower pH levels which would favor softwoods. More alkaline wastewater will require consideration of hardwoods over rotation periods of 20–25 years.

Most municipal effluents contain sodium at acceptable ratios (Table 3) to magnesium and calcium, and intermittent application and appropriate hydraulic loading can avoid salinization of soils. An excess of sodium in soils will reduce soil permeability and hydraulic capacity over time thus impacting the fundamental functioning of the LAS system [15, 18]. Sodium and salinity can be problematic for industrial and some food processing effluents due to high total dissolved solids (TDS).

The elements listed in Table 3 are particularly phytotoxic to plants, but are often not the limiting factor for hydraulic loading at a forest LAS. In general, trees tolerate trace metals better than other agricultural crops [15, 16, 18, 20]. Concentrations of these elements can be much higher in industrial wastewaters and should be managed in concert with soil cation exchange capacity (CEC) because soils with greater CEC can adsorb more cations [15, 19]. Wastewaters with significant metal content should utilize pretreatment, lowered hydraulic loading, or application to greater acreage to avoid adverse impacts on forest health, soil accumulation, and groundwater contamination. For semi-arid and arid regions, long-term irrigation can increase heavy metal concentrations at soil surfaces and with depth over time due to soil texture (sandy) and transport from abnormal hydraulic loading [26].

Table 3 Municipal wastewater characteristics and annual loading rates suitable for forest land application systems

Parameter	Wastewater	Loading rates/concentrations
Organics		
Biological Oxygen Demand (BOD_5)	110–350 mg/L	<300 kg/ha-day
Total Suspended Solids (TSS)	120–400 mg/L	<200 kg/ha-day
pH	5–9	6.4–8.4
Salinity as Total Dissolved Solids (TDS)	270–860 mg/L	<450 mg/L
Sodium Adsorption Ratio (SAR)	5–8	<10 for clay soils
Phytotoxic elements to trees[a]		
Boron	<0.7 mg/L	0.75 mg/L continuous
Cadmium	0.005 mg/L	1.9 kg/ha
		0.01 mg/L continuous
Chloride	30–90 mg/L	<140 mg/L
Copper	11 mg/L	75 kg/ha
		0.20 mg/L continuous
Nickel	0.02	21 kg/ha
		0.20 mg/L continuous
Sodium		<70 mg/L
Sulfur	20–50 mg/L	200–600 mg/L
Zinc	0.15 mg/L	140 kg/ha
		2 mg/L for all soil
Chlorine		1.0–5.0 mg/L
Nutrients		
Phosphorus (P)	2–12 mg/L	150–300 kg/ha-day
Total Nitrogen (N)	10–80 mg/L	100–500 kg/ha-day
Nitrate	<1.0 mg/L	
Ammonia	10–100 mg/L	

[a]Most likely to be phytotoxic to plants and averaged values for urban stormwaters and dependent on soil cation exchange capacity. *Sources*: USEPA [29], Loehr et al. [19], USEPA [18], Isosaari et al. [13], Levy et al. [15]

For forest LS, metal accumulation occurs in biomass at the soil surface close to application entry and because some trees accumulate metals in leaves which are returned to soil at senescence [27]. For temperate climates, long-term application (>20 years) has been shown to be an effective practice to meet regulatory requirements for metals and trace elements in well-designed and managed systems [17, 18, 28].

There can be major differences in composition between industrial, municipal, and agricultural wastewaters, and, over the life of a forested LAS, the wastewater composition may change with increased urbanization and industrialization in an area. Wastewaters must be characterized to determine if specific physical or chemical parameters will be a limiting design parameter [18]. Municipal and food processing wastewaters are more consistent in composition, but food processing wastes often have much higher concentrations of BOD_5 (10,000 mg/L), total suspended solids (3000 mg/L), total nitrogen (100 mg/L N), and pH which may require secondary treatment prior to land application [18]. In combined sewer systems, storm water runoff will contribute priority pollutants such as copper, zinc, lead, and polycyclic aromatic hydrocarbons to municipal wastewaters [15].

Industrial wastewaters should be more closely evaluated and may require additional pretreatment prior to application for forest LAS. These wastewaters often contain higher total nitrogen and other trace elements that may be toxic to plants [15, 17, 24]. Secondary treatment of wastewater prior to land application will reduce the presence of these analytes but also lower the fertigation quality of the wastewater [18, 20].

6 Nutrient Removal in Forest LAS

Various studies have shown that trees irrigated with wastewaters grow more than reference trees without irrigation [20, 29, 30]. Beneficial components of wastewater include nutrients and micronutrients and low-to-moderate biodegradable organic carbon [15] (Table 2). As shown in Fig. 2, phosphorus and nitrogen content are different for different wastewaters. Nutrient management is often the limiting design factor for forest LAS because trees do not remove nitrogen as efficiently as other agricultural crops [20, 30]. The form of nitrogen in wastewater and the nitrogen uptake capacity of the forest system factor significantly into determining wastewater loading rates for forest LAS [13, 17].

Forest LAS primarily remove phosphorus using adsorption/precipitation reactions with clays and iron/aluminum oxides in their soils; plant uptake is secondary to this process [17]. Phosphorus removal by trees can be significant (34 %) but soils have sufficient capacity to remove the phosphorus added by wastewater irrigation under proper management and application rates [16]. In soils, greater clay content and longer contact times contribute to phosphorus removal. For a forested LAS, about 0.3 m of soil becomes saturated with phosphorus every ten years. Thus, phosphorus leaching will not occur until the entire soil profile is utilized [18]. Soil type, depth, and loading rates factor into the life time of a forested LAS with deeper with fine textured, clay soils providing a longer life span than coarse-textured, shallow sandy soils.

Nitrogen loading rates for forest LAS will depend on site conditions as well as forest type, age, and structure. General loadings rates average 150–680 kg/ha-year for published values of forest LAS sites across the USA. Wastewaters containing primarily ammonia and organic nitrogen are more amenable to forest LAS than oxidized, mineral nitrogen (nitrate) wastewaters [11, 13, 16]. The latter wastewater is more likely to leach nitrate to groundwater during dormant season of the forest, particularly for hardwood and mixed hardwood stands. As shown in Fig. 2, most wastewaters are comprised of organic N, ammonia, and phosphorus; a few wastewaters contain nitrate. Most municipal, livestock, and food processing wastewaters primarily contain organic nitrogen (40 %) and ammonia (60 %) while industrial

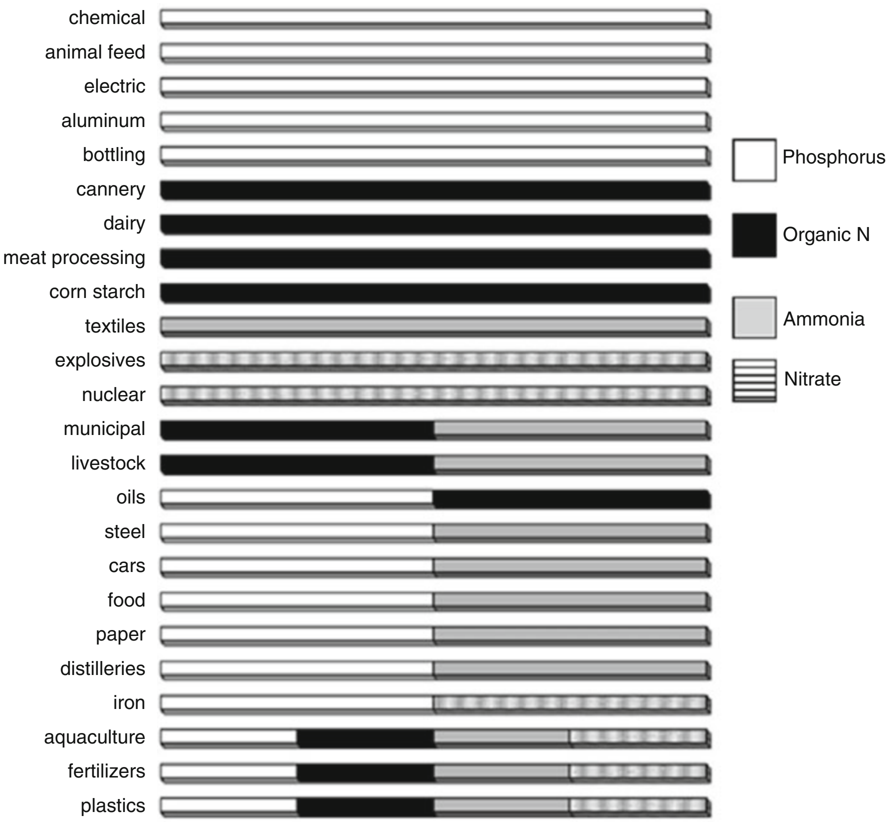

Fig. 2 General phosphorus and nitrogen composition of different wastewaters [15, 18, 23]

wastewaters favor mostly one or two of these nitrogen species (Fig. 2). In general, land treatment systems, particularly forests, more effectively retain and remove nitrogen when nitrogen is less oxidized [18]. Figure 3 shows typical nitrogen removal (kilogram-nitrogen/ha-year, kg-N/ha-year) for different forest types, select grasses, and select agricultural crops irrigated with municipal wastewater.

Natural forests are nitrogen efficient and have adapted to nitrogen-poor soils. Trees reabsorb nitrogen back into woody tissue prior to leaf fall and then release nitrogen to support new leaf development [20]. Overall, in mature forest stands, nitrogen exists mainly in the mineral soil with less nitrogen retained in forest floor litter and tree; thus, the main removal of nitrogen is via soil denitrification, soil volatilization of ammonia, and soil storage with some loss to tree uptake. After application of primarily organic nitrogen and ammonia, soils need aerobic conditions to foster nitrification and to release ammonia adsorption sites. At the next wastewater application, anoxic conditions develop and nitrate is denitrified to nitrogen and nitrous oxide gases while soils re-saturate with effluent ammonium [18]. Appropriate carbon to nitrogen ratios (five or lower) and anoxic conditions provide the primary

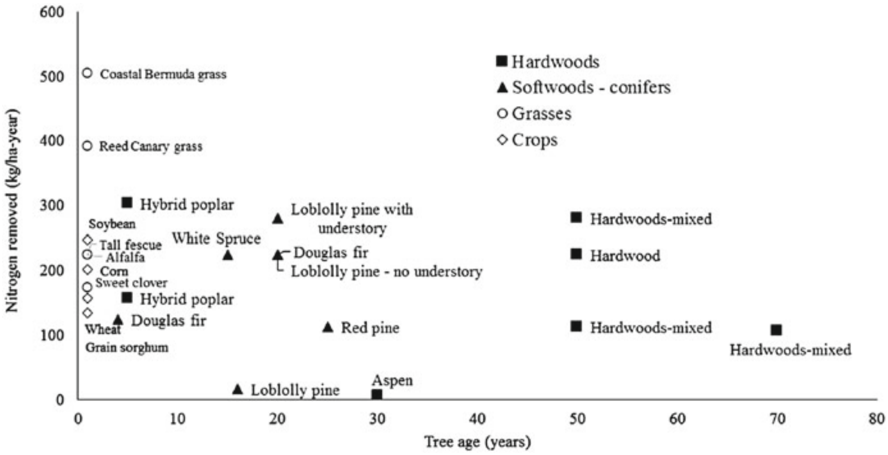

Fig. 3 Typical nitrogen removal (kilogram-nitrogen/ha-year, kg-N/ha-year) for different forest types, select grasses, and select agricultural crops irrigated with municipal wastewater [11, 16, 18, 20, 29]

mechanisms to mitigate nitrogen and avoid nitrate contamination of groundwater in forest LAS [15].

Observed removal of total nitrogen applied to forests varies with climate, tree species, and tree age [16]. Younger tree stands take up more nitrogen than mature stands which impact silvicultural practices and favors harvest rotations of 25 years or less (Table 1). Greater nitrogen removal can occur when tree stands are maintained with understory or grass cover crops [18, 20]. The presence of grasses or other herbaceous cover crops is particularly important for nitrogen removal in the first few years of forest LAS establishment [16]. Otherwise, nitrogen removal may not be sufficient to avoid nitrate leaching to groundwater above regulatory levels (10 mg/L) [18].

There are varying reports on total nitrogen removal by forest LAS based on whole tree harvests. Figure 3 shows that forest LAS nitrogen removal range between 224 and 300 kg/ha-year for different aged stands of hybrid poplars (5 years), loblolly pine (20 year), white spruce (15 years), Douglas fir (20 year), and mixed hardwoods stands (50 year) [16, 18]. Silvicultural practices can be adjusted for established forests to optimize nitrogen removal by selective harvesting for mixed-aged forests and intermediate thinning for even-aged stands. Maintaining lower tree densities to foster understory vegetation and thinning every decade to avoid soil and site damage are appropriate strategies for established forests.

Monoculture forests may provide more flexibility for biomass productivity by increasing initial planting densities and thinning more frequently to maintain greater water and nitrogen removal. Higher productivity should contribute to greater nitrogen removal that can be maintained and improved upon in subsequent harvests of coppiced trees. Emerging bio-energy markets [22] and established wood product markets [30] may favor more intensively managed forests if site and harvesting costs can be revenue neutral or profitable [22]. Candidate species for these systems are trees such as cottonwoods, hybrid poplar, sycamore, eucalyptus, and other coppicing hardwoods [23, 30–32]. High intensity pine is also a possibility although nitrogen removal rates may be lower than mixed hardwood/pine forests or hardwoods. Intensively managed forest LAS is encouraging, but these systems may limit the extent of other supporting ecosystem services and wood quality value that more natural forest stands can provide.

7 Future Opportunities for Forest LAS

Increasing pressures on natural resources favor expanded use of forest LAS for urban, suburban, and rural landscapes to preserve forest habitat and gain additional ecosystem services from forest LAS in addition to water regulation. Opportunities exist to bridge forest LAS with bio-energy production in urban and rural lands to manage a variety of municipal, storm water, industrial, and agricultural wastewaters. Coupling new forest LAS to other industrial, municipal, and agricultural systems will improve existing support services and can enhance provisioning, cultural, and regulating services.

As an example, opportunities exist to couple high-intensity aquaculture effluent management with forest LAS. High-intensity aquaculture, like other industrial agriculture systems, can have an inverse relationship between provisioning (greater fish production) and supporting ecosystem services (biodiversity) or regulatory services (water quality) due to monoculture management practices [33]. In the southeastern USA, many commercial aquaculture farms overlay the largest and most productive aquifers. Aquaculture ponds are filled from these aquifers which are ultimately discharged to receiving streams during harvesting, pond maintenance, and disease control operations. Effluents from pond aquaculture operations contain significant amounts of nitrogen, phosphorus, and total suspended solids (primarily phytoplankton, chlorophyll *a*) that are difficult to remove and can exceed federal regulatory standards for ambient water quality, especially in nutrient sensitive waters. Current alternatives to effluent discharge to receiving streams have negative impacts on production economics [34]. Land application of pond effluents to forest systems is a viable alternative because most aquaculture farms are adjacent or near to forest systems, and if a forest system is not present, managed plantations could be established on adjacent lands. This alternative has the advantage of creating a longer-term use of nutrients to promote tree growth, encourage water disposal through evapotranspiration, and allow excess water to percolate into the soil and recharge superficial aquifers.

Current water regulating services provided by forests is at risk with climate change, particularly for the southeastern USA [6], which is a significant producer of forest products nationally and globally. A water supply stress index for this region predicts that water deficits will increase during summer and fall seasons for the southeastern USA due to warming temperatures over the next 50 years. Warming

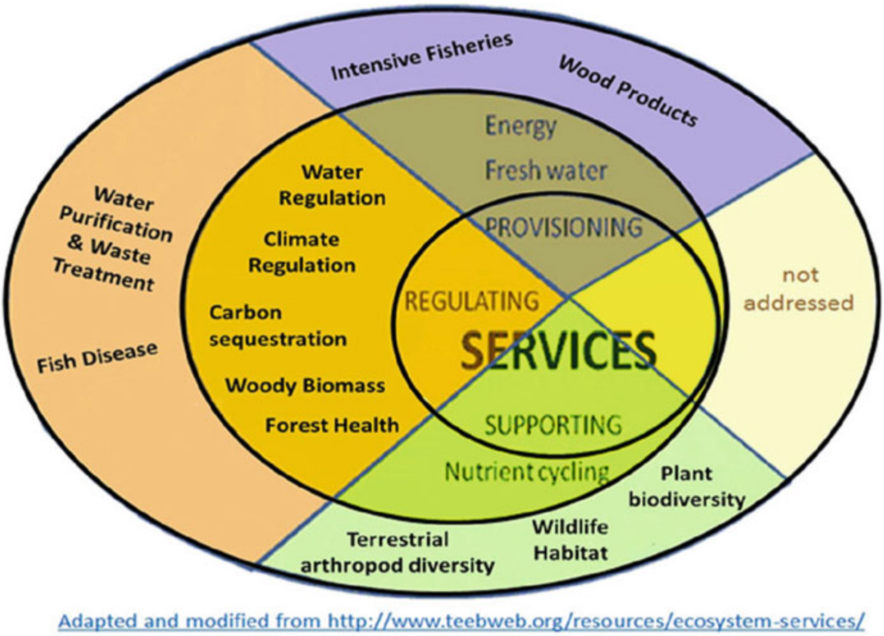

Fig. 4 Ecosystem services from coupled forest and aquaculture agroecosystem

temperatures will cause greater evapotranspiration from soils and reduce surface water stream flows, and these changes could foster conversion of forests to open woodlands due to hotter and drier conditions [12]. Coupling industrial and agricultural systems to forest LAS provides new opportunities to enhance weak ecosystem services with more robust ecosystem services from each entity (Fig. 4).

8 Improved Understanding of Existing Forest LAS

Forest LAS continue to grow in use globally to manage landfill leachate, produce specialty tree crops, such as citrus and olives, and provide bio-energy and wood products using various industrial, municipal, and agricultural wastewaters (Table 1). Since their installations in the 1970s through 1990s in the USA, the processes and mechanisms by which forested LAS mitigate trace organic chemicals in municipal wastewaters has not been characterized beyond required monitoring for priority organic pollutants or regulated contaminants [35, 36]. While forested LAS filter nutrients and elements from municipal wastewater to acceptable criteria levels before reaching groundwater and surface water, the potential human exposure pathways and risks of nonregulated organic chemicals and candidate organic contaminants (CCL4) are largely unknown [35, 36]. The USEPA does provide a literature review database for organic contaminants of concern in wastewater treatment systems, but LAS are not part of that review as a waste treatment system [35].

For forest LAS, removal rates for priority pollutants are greater than 99.95 % with dissolved concentration reductions from µg/L in effluent to ng/L concentrations in percolate [18, 37]. However, in the mid-2000s, national and international researchers began to report the presence of various pharmaceuticals and personal care products (PPCPs) in surface waters, particularly in waters near outfalls of conventional wastewater treatment plants [38, 39]. These compounds, such as estrogens, antibiotics, antidepressants, and nonsteroidal anti-inflammatory drugs, can elicit detrimental effects on aquatic wildlife. Other trace organic chemicals that could also be present include household chemicals, food additives, flame retardants, plasticizers, biocides, and industrial chemicals if significant industry is present in the municipality. Perfluorooctance sulfonate (PFOS) was detected above ecological threshold levels in surface waters downstream of large forested LAS in Dalton, GA (9220 acres), the carpet capitol of the world [40], but human health risks were not assessed.

Understanding major industrial, agricultural, and residential chemical sources to a given locality is important to understanding human health risks to wastewater reuse. Preliminary findings suggest that human health risks for quantified PPCPs in LAS surface waters and ground waters are equivalent to or lower than those in current drinking water supplies [36, 41]. Recent findings show that concentrations of targeted PPCPs in forest LAS surface waters and ground waters were similar to concentrations detected in receiving streams for conventional wastewater treatment plants. Thus, forested LAS can provide trace organic contaminant protection comparable to public exposure from most current drinking water supplies [36].

Non-targeted screening now provides even more information for not only regulated organic contaminants but nonregulated organic chemicals. Table 4 shows non-targeted analyses of forest LAS effluent, groundwater, and surface waters. The total number of chemicals detected among all water samples in the forest LAS was 300, but Table 4 shows only those chemicals found in one or more water type. The plethora of chemicals in natural and anthropogenic waters is a concern for ecological and human health risk and exposure. More work is needed to evaluate how forest LAS mitigate, remove, or mobilize unregulated organic chemicals and how forest LAS can be further utilized to improve water quality for various municipal, agricultural, and industrial uses.

9 Conclusion

New data and assessments of forest LAS are needed to advance prior research from the mid-1900s in order to utilize forest LAS for emerging issues such as climate change and water security not just for arid environments but for temperate environments as well. Opportunities exist to provide important ecosystem services for wastewater treatment and protect forested landscapes in urban, suburban, and rural

Table 4 Non-targeted analyses of forest LAS waters using liquid chromatography-time of flight-mass spectrometry

Chemical use	Chemicals in wastewater in irrigation system	Irrigated shallow groundwater	Irrigated groundwater riparian	Nearby stream	LAS sub-watershedoutlet
Negative mode LC TOFMS					
Anti-Inflammatory (veterinary use)	Prednisolone succinate		x	x	x
Cosmetic	Hexyl dodecanoate	x	x	x	x
Cosmetic/Food Additive/Industrial	Palmitic acid glycerol ester			x	x
Food Additive	Trethocanic acid		x		
Food Additive/Sweetner	Sucralose		x	x	x
Fragrance	Citronellal hydrate		x		
Fragrance	DPMI/Cashmeran			x	x
Fragrance	Phenylacetic acid	x	x		
Herbicide	Butroxydim	x			
Industrial Chemical/Dispersing agent	Stearamide		x		
Pharmaceutical	Docusate	x	x	x	
Pharmaceutical (Antibiotic)	Netilmicin	x			
Pharmaceutical (Anticholinergic)	Dicycloverine (Dicyclomine)	x			x
Pharmaceutical (Anticonvulsant)	Topiramate			x	
Pharmaceutical (Antihypertensive)	Metyrosine	x		x	
Pharmaceutical (Anti-Inflammatory)	Palmitamide	x	x	x	x
Pharmaceutical (Sedative)	Valerenic acid		x	x	x
Pharmaceutical/Cosmetic	Undecylenic acid		x		
Teniacide (tapeworm)	Embelin	x		x	x
Positive mode LC TOFMS					
Cosmetic	Isopropyl palmitate	x	x	x	

Pharmaceutical (Anabolic Steroid)	Norethandrolone	x	x		
Pharmaceutical (Antibiotic)	Netilmicin	x			
Pharmaceutical (Antiulcerative)	Rosaprostol	x	x		
Pharmaceutical (Vasodilator)	Cyclandelate 17-beta-Dihydroandrosterone	x	x		
Pharmacutical (Endogenous Steroid Hormone)	(3alpha Androstanediol)	x		x	
Surfactant (Spermicide)	Nonoxinol 9	x		x	

landscapes. Since the 1970s, forest LAS have demonstrated efficient nutrient and hydraulic management of wastewaters, particularly municipal wastewaters. These "working" forests are productive systems for wastewater treatment and woody biomass production. Forest LAS merit additional quantification for regulating emerging contaminants and nonregulated chemicals as well as supporting ecosystem services for water resources, soil quality, wildlife habitat and diversity, food production, wood products, carbon storage, and biogeochemical cycling. Increased pressures on water, forest, agriculture, and wildlife resources will provide unique opportunities for innovative forest LAS for the future.

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Bio-retention Systems for Storm Water Treatment and Management in Urban Systems

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Abstract Among different anthropogenic activities, urbanization has greatly influenced the hydrological cycle. Due to increased impervious surfaces, the amount of infiltration has been reduced, thereby increasing the runoff volume leading to flood conditions even for low rainfall events. Storm water flow along these impermeable surfaces finally ends up in surface water reservoirs. Urban systems are fundamentally responsible for a lot of pollutants by different sources: vehicle, industries, atmospheric deposition, soil erosion, etc., which may release various types of pollutants such as metals, organics, nutrients, oil and grease, detergents, surfactants, etc., into the atmosphere. With the storm water runoff, these pollutants may end up in surface waters. This indicates the importance of storm water treatment. Although there are several storm water treatment methods available, low-cost environmental-friendly methods (e.g., bio-retention systems) will be more sustainable with urban systems. Bio-retention systems can manage storm water and improve water quality through containment and remediation of pollutants within the urban system. However, the limitation of these systems is its finite capacity to hold contaminants. Hence, suitable plants grown along the bio-retention systems will be an effective phytoremediation option to address the challenges encountered in these remedial systems.

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1 Introduction

Storm water generally originates during precipitation processes and melting of snow or ice [1]. It can soak into the soil by infiltration or runoff and end up in nearby water bodies or be held on the surface and evaporate. If a man-made construction occurs in a watershed, the area of impervious surfaces typically increases and, therefore, a corresponding decrease in the area of naturally porous surfaces could result in an increase in storm water runoff volumes with a degradation of runoff quality. The degradation of runoff quality can be observed in increased concentrations and total mass loads of nutrients, organics, metals, chlorides, hydrocarbons, bacteria, viruses, etc. [2]. Conventional storm water management systems collect storm water runoff and drain it from the city or into surface waters. These systems have been improved over time and cities are being heavily relied on them. In return, it reduces groundwater infiltration and lowers groundwater recharge rates.

Landscape architects, environmental scientists, engineers, urban designers, planners as well as local government play a vital role in managing the urban water cycle as it is supported by key sustainability principles of water recycling, consumption, environmental protection, and waste minimization [3]. In order to support these issues, sustainable storm water practices in the world such as Low Impact Development (LID), Sustainable Urban Drainage Systems (SUDS), Water Sensitive Urban Design (WSUD), Best Management Practices (BMP), Integrated Urban Resource Water Management (IURWM), Decentralized Rainwater Management (DRWM), and Green Infrastructure (GI) have been demonstrated by employing the ecosystem processes to offer numerous water quantity and quality benefits to soil and vegetation [4, 5]. They also require concept designs and strategic planning that are underpinned by sound engineering practices to carry out multiple treatment processes of storm water. Hence, bio-retention systems have been introduced as a mitigation management practice to promote infiltration and absorption of storm water runoff around the world [6, 7].

Bio-retention system which can also be named as ‘rain garden’ is used to control water at its sources, and it is the most adaptable method applied throughout many regions [8]. Bio-retention system can retain and treat urban runoff using vegetation before it flows into the main storm drain system. It is commonly made up of an excavated basin or landscape depression consisting of plants, ponding area, a mulch layer, several layers of planting soil, and an optional underdrain [9]. These systems are capable of removing pollutants from the runoff via physical, chemical, and biological processes, including sedimentation, filtration, and sorption on mulch and soil layers, plant uptake, and biodegradation by soil microorganisms [10].

Since the bio-retention systems enhance the storm water infiltration capabilities, the groundwater can be polluted over a period of time. Therefore, it is important to remediate dissolved pollutants in storm water within the bio-retention system to avoid further cycling within the ecosystem. In that case, phytoremediation using suitable plants or plant–microorganism combination can be used to minimize pollution in an environment-friendly manner in contaminated soils, sediments, water, and air [11, 12]. The phytoremediation process includes mitigation, transformation, stabilization, and/or degradation of undesirable substances [13]. In this process, it is possible to remove and/or mineralize heavy metals (HMs), organic compounds, nutrients, and even radioactive elements [14]. Hence, phytoremediation can serve as an integrated process in the bio-retention systems for storm water treatment.

In this chapter, a focused attempt has been made to discuss the relevance, feasibility, and effectiveness of bio-retention systems for storm water treatment and the use of phytoremediation technology to remediate different types of pollutants in storm water.

2 Pollutants in Storm Water

As the urban areas release various pollutants from its vast sources of pollution, the storm water that wash-off impervious surfaces has been considered as a primary pollutant source and a reason for the degradation of waterways [6, 15]. It is composed of all sediments, metals, organic compounds, microorganisms, oils, surfactants, and nutrients that finally end up with surface water bodies [6, 15]. These pollutants can easily deteriorate the water quality and disturb the biodiversity [15]. Major sources of these pollutants are motor vehicles, construction activities, soil erosion, industrial pollutants, spills and leachates, atmospheric deposition, and domestic pollutants [16].

The sediments carried by the storm water mainly consist of materials from soil erosion, particles by construction sites, vegetation debris, particulates that release from vehicles, and atmospheric deposition. It is a fact to be considered that most of the microorganisms, viruses, bacteria, and protozoa are transported along with the sediments. Among HMs, Cd, Cr, Zn, Cu, and Pb found in storm water are significant toward human and ecosystem health. The sources of these HMs could be vehicle emissions, vehicle ware, tire wears, industrial wastes, and atmospheric deposition [15, 17]. Oil, grease, and surfactant are one of the groups of contaminants that can be easily found in storm water. Since they are accumulated in roadways, parking lots, and service stations, it can largely be accumulated in urban storm water and finally surface and ground water systems [15]. The nutrients especially nitrogen and phosphorous can be found in storm water at great concentrations [16]. Organic matter, soil, fertilizers, vehicle exhausts, domestic organic wastes, detergents, animal waste, and leachates are the sources for nutrients [15, 16]. Fig. 1 illustrates general sources of pollutants in storm water. Due to their ready availability, they may create eutrophic impacts in water streams [15]. Especially phosphorous as a most prioritized nutrient for increased eutrophication, controlling of phosphorous accumulation is a

Fig. 1 Major sources for pollutants in storm water

key factor in a bio-retention system [13]. These may create eutrophication in surface water bodies. Also, the nitrogen may result in excessive growth of algae and some other aquatic weeds. The build-up of nitrates in drinking water creates health hazards to human and animals [13, 18]. The WSUDs are focusing on reuse and treatment of storm water, to meet the water quality measures and toxicity limits [15].

In the past, the biggest contributor of nitrogen and phosphorus in urban waters has been the wastewater effluent [19, 20]. During the last decade, strict regulations and improvements in wastewater management technologies have been applied to reduce the input. Attention has shifted to nutrient loading from untreated non-point sources of nutrients such as urban runoffs as concentrations of nutrients in wastewater discharges have been reduced [21]. Up to one-third of total phosphorous loading has been attributed to the urban runoff in some lakes [22]. Often pollutant concentrations found within storm water runoff exceed levels that are considered both acutely toxic and chronic to aquatic biota [23]. The economic cost of eutrophication on freshwater bodies alone within the United States is estimated to exceed 2.2 billion dollars a year [24]. Table 1 summarizes the different pollutants and their concentrations that have been reported in storm water.

3 Different Storm Water Management Methods in Urban Systems

Urban storm water management is not a new concern. However, conventional storm water management still raises many associated issues. At the beginning, priority has been given to maintain runoff volume, but with the negative impacts on the

Table 1 Different pollutants and their concentrations in storm water in the world

Location	TSS (mg/L)	TN (mg/L)	NH_4-N (mg/L)	NO_3^-N (mg/L)	TP (mg/L)	PO_4-P (mg/L)	Zn (mg/L)	Pb (mg/L)	Cu	Reference
Charlotte, NC	49.5	1.68			0.19		0.072			[103]
College Park, MD	34				0.61		0.107			[104]
Haddam, CT		1.2			0.012–0.019					[105]
Greensboro, NC		1.35			0.11					[60]
Louisburg, NC		1.70			0.29					[106]
	375						0.659	0.212	37 μg/L	[107]
									2.12 g m^{-2} yr^{-1}	[108]
									1.0–3.9 g m^{-2} yr^{-1}	[109]
									8.6 g m^{-2} yr^{-1}	[110]
Into Sed Basin, Brisbane, Australia		1.27	0.1	0.83	0.11	0.09				[111]
Wetland 1, Brisbane, Australia		1.01	0.12	0.47	0.11	0.08				
Wet land 2, Brisbane, Australia		0.92	0.09	0.25	0.09	0.03				
Bypass, Ecosol Brisbane, Australia		1.75	0.09	0.53	0.15	0.07				
Urban areas, New Hampshire					0.15					[112]
Residential areas, New Hampshire					0.4					

environment, efforts have been taken for improving water quality as well [6, 15]. Although there is a reduction in groundwater infiltration and lowering of the groundwater recharge rates, conventional storm water systems are beneficial as they rapidly drain storm water from surfaces. Moreover, it can limit available drinking water in cities due to decreasing groundwater recharge rates [25]. Present conventional storm water management systems to a large extent are neither adaptable nor sustainable to developing conditions or changing climates. As infiltration and evaporation are reduced, conventional systems have negative effects on local climate. The climate of the cities becomes warmer and dryer compared to the surrounding areas where this phenomenon is also known as the 'Heat Island Effect' [26].

During uncertain conditions as a result of increased city development and resultant climate change, conventional systems cannot adapt to unmanageable storm water runoff. Adaptation to these changes requires higher running costs and investments in return [25]. It is important to consider about the hydraulic loading capacity and the pollutant size range in storm water and the available space [15]. In addition, there should be a widespread collective responsibility toward the water with increased awareness of water resources. If visible water systems can promote intelligent use and change of attitudes, inhabitants are likely to appreciate and understand storm water management. It has now become a necessity to reform storm water management while initiating a paradigm shift in urban water management [16, 27].

3.1 Combined Sewage and Storm Water Management System

To avoid flooding of storm water from paved areas, many cities have implemented sewage systems to drain water as well as to regulate domestic and industrial wastewater. Here, the storm water and wastewater are collected in one pipeline of the network. This mixed water is taken to the wastewater treatment plant, cleaned and then it is discharged into the water bodies [28].

3.2 Sustainable Storm Water Management Systems

In conventional urban development, storm water management has been driven by a view which reflects that storm water runoff has no value as a useful resource. Also, it is environmentally benign and adds little to the amenity of an urban environment. As a result, conventional storm water management systems are used to collect storm water runoff rapidly and drain it with a focus on highly efficient drainage systems. These systems kept storm water runoff 'out of sight' and consequently 'out of mind' [29]. Hence, this practice is considered out of touch with the environmental values of the society while it impedes the broader pursuit of advancing comparatively sustainable urban environments [30–32].

Yet, there has been significant development of new management approaches and techniques to improve the sustainability of urban storm water management since 1980s [33, 34] and advanced legislation has also been introduced. The storm water runoff treatment is no longer considered in isolation to the urban designing and planning of a particular area, as it is a part of an emerging new paradigm in urban management. Management of storm water is considered at all stages of the urban planning and design process by ensuring that architecture, site planning, landscape architecture, and engineering infrastructure are provided in such a way that supports the management of storm water as a valuable resource and the improvement of storm water quality, however not in the case of developing world [29].

However, in some cases, storm water management can be seen as unusual and messy when it is not properly designed or poorly maintained. In return, people may not use sustainable storm water management practices as they do not vividly see an added value for the extra cost [35]. In addition, there are identified impediments to sustainable urban storm water management, uncertainties in performance and cost, insufficient engineering standards and guidelines, fragmented responsibilities, lack of institutional capacity, lack of legislative mandate, lack of funding and effective market incentives, and resistance to change [6].

3.2.1 Storm Water Management Through Best Management Practices

As there was a perceived conflict generated between the environment and the existing drainage systems, new concepts and proposals came on to the surface with both considerations on public health and the environment. The main idea behind the proposals presented for urban storm water management is the use of structures to mimic some of the processes of the hydrological cycle while maintaining natural water flow mechanisms. Both structural and non-structural BMPs [36] gained popularity as a method to treat nonpoint sources of pollution such as urban runoff and as a part of an international trend driven by a public demand for integrated water management and sustainable development [33]. Best management practices are agronomically sound practices that protect or enhance water quality and are at least as profitable as existing practices [37].

The term BMP usually refers to structures that mimic natural hydrological processes of a stream network but it can include educational programs and policy changes. The choice depends on the land use, public perceptions, available space, funding, and intended function. Some structural BMPs range from ponds to surfaces for infiltration. Even, they can be designed to be multifunctional by providing green spaces for wildlife and recreation at the same time by improving storm water quality and reducing flood risks. Consequently, storm water has truly become a liquid asset in the suburbs [38].

3.2.2 Integrated Urban Storm Water Management

In response to the knowledge that rapid conveyance of storm water has led to environmental degradation in receiving water bodies, Integrated Urban Storm water Management (IUSM) is another management concept that has evolved over the last three decades [32]. However, the significance of IUSM varies between places while getting more attention in places like New Zealand, Australia, and many parts of United States as the storm water drainage system network is typically a separate system from the wastewater network unlike many places across Europe. Overall, it is concerned with enabling more sustainable management of urban storm water environments [39]. Reducing storm water pollution for protecting the urban environment in addition to reusing and harvesting rainwater and storm water locally have become equally integral parts in the flood protection focus of IUSM initiatives. However, due to separate administration of water quality management, flood management, environmental protection, and urban design, these aspects are not always synergistic [40]. Entrenched implementation processes, intergovernmental relations, the current institutional framework, and historical low political profile of urban storm water have been revealed as barriers to IUSM [39].

3.2.3 Sustainable Urban Drainage Systems

Another supportive stance by United Kingdom which has been referred to as Sustainable Urban Drainage System (SUDS) is designed in such a way by allowing water to either by retaining in devices to imitate the natural disposal of surface water or infiltrating into the ground to manage the environmental risks from urban runoff [41, 42]. Therefore, SUDS objectives are to maximize biodiversity and amenity opportunities and to minimize the impacts from the development due to the quality and quantity of the runoff [43]. As preferred solutions of storm water management, SUDS have been constantly in the usage. For example, the Town and Country Planning Assessment of Environmental Effects Regulations [44] determine that in mitigating negative impacts on the environment, SUDS should be used. Uncertainties about operational factors and long-term maintenance have slowed down the widespread adoption of SUDS. However, as an addition to traditional systems, many local authorities, developers, and environmental regulators are keen to implement SUDS [45].

3.2.4 Water-Sensitive Urban Design

To provide a broader framework for sustainable urban water management, WSUD in Australia has evolved from its early association with storm water management. It provides a unified and common method for integrating the interactions between the urban water cycle and the urban built form including urban landscapes. Four major inter-related issues that have been identified as essential elements in advancing the concept of WSUD include: Regulatory Framework, Assessment & Costing, Technology & Design, and Community Acceptance and Governance [29].

In other words, WSUD is the interdisciplinary cooperation of urban design, landscape planning, and water management. With principles of urban design, it combines the functionality of water management. WSUD develops integrative strategies for economical, social, ecological, and cultural sustainability [25]. Storm water acts as a key element, both as a resource and for the protection of receiving water bodies though WSUD considers all parts of the urban water cycle [46].

3.2.5 Low Impact Development for Storm Water Management

The Department of Environmental Resources in Prince George's County, Maryland, introduced a comprehensive approach to sustainable storm water management called LID and described in detail [47]. It opened up a new way of approaching storm water management as a potential resource as its main goal is to replicate or maintain the predevelopment hydrological regime using evapotranspiration and enhanced infiltration to reduce off-site runoff and ensure adequate groundwater recharge by minimizing the impact of development, especially for impervious surfaces [47]. Multiple purposes can be seen in LID practices such as improving habitat, enhancing management of runoff, improving groundwater recharge, improving surface water quality, and enhancing the aesthetics of the community [47].

In recent years, one structural LID practice that has gained attention is the bio-retention system. Research on bio-retention systems is an active field, particularly in terms of treatment and mix design despite its widespread usage [48]. Since the introduction of the first bio-retention manual in 1993 by Prince George's County, it has rapidly become one of the most widely used storm water BMPs throughout the world [8]. Bio-retention systems have been also referred to as rain gardens and these BMPs use the chemical, biological, and physical properties of plants, microbes, and soils to improve water quality. Bio-retention system contains a shallow vegetated depression to detain or retain storm water [49]. In addition, it provides canopy interception, water quality control, evapotranspiration, runoff volume and peak flow discharge control, and groundwater recharge [50]. Yet, there are many aspects in design and implementation of which active research challenges remain to the widespread adoption of this practice.

4 Bio-retention Systems for Storm Water Treatment and Management

Bio-retention systems are important since it requires low-tech and low-cost. In a typical bio-retention system there are several processes to improve the storm water quality and to reduce the runoff volume; evaporation, evapotranspiration,

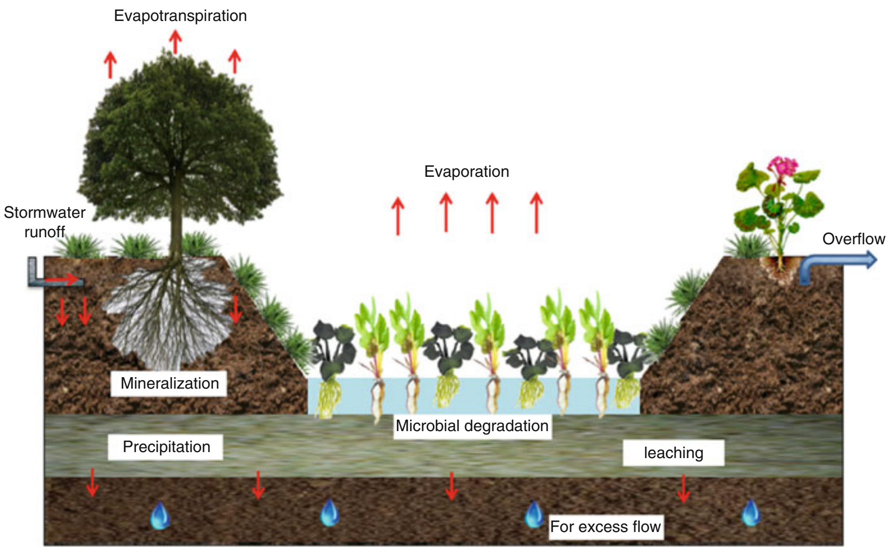

Fig. 2 Typical bio-retention system and general pollutant removal mechanisms

sedimentation, filtration, sorption, and enhanced denitrification as well [8, 15, 51]. The remaining water will be absorbed by subsoil or collected by subsoil pipes. Further water quality treatments and reuse of storm water will be facilitated thereafter [15, 52]. Rain gardens, swales, and porous pavement can also be incorporated into the bio-retention system to increase the infiltration [53]. The major objective of bio-retention system is the reduction of runoff volume by enhancing infiltration and evapotranspiration as well as for increasing the urban biodiversity [53]. Since the storm water bearing quite a number of pollutants in considerable quantities, it should have a clear way to improve water quality as well. Fig. 2 illustrates a typical bio-retention system and major mechanisms within the system.

5 Design of Bio-retention System

In recent years, there have been many engineering manuals with design recommendations for a bio-retention system. Some manuals originate in Maryland [54], North Carolina [55], and Washington [56] of the United States, North Shore City's Bio-retention Guidelines from New Zealand [49], and The Toronto Region Conservation Authority's Low Impact Development Storm Water Design Guide from Canada [57]. The basic design is same though there may be specific recommendations for design on different regional levels. Yet, depending on the site characterizations, design variations could be observed. For instance, an underdrain to allow water to drain from the system in a certain period of time is needed to areas where very low permeability is associated with native soils. Also, an overflow or bypass to a sewer drain is needed to accommodate a large flow of water in areas where flooding is not acceptable [57].

A typical design of a bio-retention system includes a sloped grass buffer strip, a ponding area with vegetation, a three-foot deep soil planting layer, and a one-foot deep sand layer. Some systems contain gravel and underdrain piping below the sand layer when soils are not appropriate for groundwater recharge (Fig. 2). The soil planting layer acts as a primary filter to attenuate pollutants. Also, it provides rapid infiltration of storm water runoff, complete infiltration within 72 h to avoid mosquito breeding. It sustains healthy vegetation at the surface too. To achieve infiltration requirements, the soil planting bed consists of a high sand content. The sand layer acts as a secondary filter and transition between the soil planting bed and underdrain system or the underlying soil. A thin mulch layer can be applied to the top of the planting substrate to retain moisture. The underdrain system can be connected to a storm water sewer system, which eventually discharges into surface waters. For systems without an underdrain, ground water is recharged through infiltration [58]. Simply a bio-retention system can be viewed as a landscaped depression which consists of vegetation, several filter media layers, an overflow weir, optional under drain and receives runoff from upgradient impervious surfaces [48].

6 How Do Bio-retention Systems Work?

Bio-retention process starts by routing storm water runoff into such landscaped depressions where they are designed to remove pollutants in a similar manner to the ecosystems. Larger storm runoffs are diverted to the storm drain system. The remaining runoff filters through the soil mix. It can either be collected in an under drain or can be designed to enhance groundwater infiltration and later be discharged according to local storm water management requirements [48]. Though runoff is filtered through each layer, the soil media layer does the main filtration [10]. The vegetation layer traps sediment and slows down the runoff velocity [59]. In this system, the pollutant removal treatment from urban runoff is performed by a variety of unit processes which make use of the biological, chemical, and physical properties of soils, plants, and microbes [8].

Bio-retention facilities are used to capture and infiltrate rainwater runoff from the ‘first flush’ of a particular rain event. Storm water that is infiltrating may recharge groundwater or can be collected in subsurface perforated pipes and then conveyed to traditional storm drains [10]. To control the initial volume of runoff by implementing adequate bio-retention gardens may have the potential to remove the majority of mobilized pollutants during a precipitation event [54]. In addition by storing, detaining and infiltrating storm water, bio-retention gardens are able to reduce runoff volumes as well as peak flows [60]. The usage of bio-retention facilities can also increase runoff time of concentration. A typical time of concentration value would be in the range of 5–10 min for a parking lot 0.2–0.4 ha in size draining directly to a storm drain.

In contrast, the placement of a bio-retention facility in front of the drainage outlet will increase the time of concentration, or time for the runoff to discharge, from a quarter hour to several hours [61], depending on the flow rates through the treatment media. Up to 31 % of runoff entering the bio-retention cells was lost through this exfiltration, and up to 19 % was lost to evapotranspiration [62]. Apart from that, there have been numerous studies of low nitrogen and/or phosphorous removal rates and even leaching of these nutrients in bio-retention systems [60, 63, 64]. Some studies have also reported on factors influencing bio-retention system treatment performance, such as the presence of vegetation [65, 66], the filter depth [10, 65] or the type of filter media [66–68]. Hence, use of phytoremediation integrated into bio-retention systems may enhance the treatment of storm water toward minimizing the pollutants.

7 Phytoremediation Integration into Bio-retention Systems

Bio-retention systems are designed in such a way to facilitate the chemical, physical, and biological processes, which naturally occurs in a terrestrial ecosystem. Some of the natural processes contributing to water quality improvement are sedimentation, adsorption, filtration, volatilization, ion exchange, decomposition, phytoremediation, and bioremediation [69]. Among different pollution removal methods, the urban systems require remediation method that has low cost, environmentally friendly as well as the easy maintenance. Phytoremediation is such a concept that can be easily adopted to the bio-retention systems. Briefly, the phytoremediation is a method of exploiting plants to extract contaminants from soil [70].

In the process of phytoremediation, there are few different ways that activate the plants and microorganisms: phytoextraction, phytodegradation, rhizofiltration, phytostabilization, and phytovolatalization. The phytoextraction acts to remove metals or organics from the soil by allowing them to concentrate on harvestable parts. The phytodegradation is there to degrade pollutants in association with microorganisms. During rhizofiltration process, the plant roots may absorb pollutants from water and aqueous water streams. The process that uses the plants to reduce the mobility of pollutants in the environment is called as phytostabilization. Phytovolatilization involves in uptake of the pollutants by plants and release later as volatile substance through transpiration process [15].

8 Selection of Plants for Phytoremediation in Bio-retention Systems

Plants in a bio-retention system often consist of native grasses, shrubs, and trees that are intended to adapt well to the soil and climate of the region. They must also tolerate pollutants and varied depths of water. The plants are intended to uptake water contaminated with excess nutrients. However, plant roots may also provide pore spaces that will provide a habitat for microorganisms, thus promoting biological degradation of some pollutants and predation of other bacteria [8]. Bio-retention systems are intended to remove the typical pollutants found in storm water such as suspended solids, nutrients, metals, hydrocarbons, and indicator bacteria [15].

Therefore, plant selection for a bio-retention system should be conducted in a careful manner. The selected plants should have a great scale of tolerability to various pollutants. Also, the water requirements and water tolerability are there for consideration [71]. In a bio-retention system, there are three layers; lower elevation, middle elevation, and the outer edge [71]. For the lower elevation, the selected plants should have tolerability for higher water level fluctuations. For middle elevation, the plants can be selected based on their ability to grow on normal soil media and also have tolerated flood stress up to some extent. The plants selected for the outer edge should be adopted for drier conditions [71].

It will be more efficient if plant species of different root systems are selected. The roots spread in different soil depths, will filter and absorb pollutants in an efficient manner. The overall root density should be higher to ensure an efficient filtration and absorption processes [71]. Also, it will suppress the weed growth and increase the evapotranspiration ability. Since the bio-retention system also employs large canopy trees, the plants selected for the ground cover should have the capability to thrive under low sunlight [71]. Table 2 depicts the different pollutants and phytoremediation plants that can be used in an urban bio-retention system.

9 Phytoremediation of Pollutants in Bio-retention Systems

9.1 Potential Plants for Phytoremediation of Organic Pollutants

Various plants have been tested for phytoremediation of different organic pollutants. Poplar plant is one such and has been identified as a plant that has the ability to remediate halogenated organic pollutants such as trichloroethylene [14, 72]. Not only in the soil, there are evidences that the poplar tree has the ability to remediate pollutants even in ground water. The *Myriophylum aquaticum* (parrot-feather) has been successfully tested for remediation of perchlorate, 2,4,6-trinitrotoluene, trichloroethylene, chlorinated pesticides, and Atrazine [73]. Bermuda grass, rye grass, white clover, and fall fescue have the ability to remediate total petroleum hydrocarbon [74]. *Juncus subsecundus* is a plant that has an ability to remove polycyclic aromatic hydrocarbons (PAHs) from the contaminated soils [75].

Hence, it can be easily used in bio-retention systems to remediate PAHs in urban storm water. Incorporating PAH degradation bacteria into the system will enhance the process of PAH removal with higher efficiency [75]. Removal of endosulfan, a persistent and toxic organochlorine compound, has been successfully tested with tomato, sunflower, soybean, and alfalfa plants; however, sunflower showed significant phytoremediation capabilities [76]. *Medicago sativa* (alfalfa), *Panicum*

Table 2 Different plant species that can be used for phytoremediation of pollutants in bio-retention systems

Pollutant type		Plant species	Common name	Reference
Nutrients (N, P, NO_3^-, NH_4^+)		*Acalypha wilkesiana*	Copperleaf	[71]
		Arundo donax	Carrizo	
		Sakura variegata	Bougainvillea	
		Bulbine frutescens	Orange bulbine	
		Chrysopogon zizanioides	Vetiver grass	
		Codiaeum variegatum	Croton	
		Complaya trilobata	Yellow creeping daisy	
		Cymbopogon citratus	Serai	
		Dracaenaceae reflexa	Song of India	
		Ficus microcarpa	Indian laurel fig	
		Galphimia glauca	Shower of gold	
		Ipomoea pes-caprae	Beach morning glory	
		Leucophyllum frutescens	Barometer bush	
		Loropetalum chinense	Chinese loropetalum	
		Melastoma malabathricum	Indian rhododendron	
		Nerium oleander	Oleander	
		Ophiopogon jaburan	Lilyturf	
		Osmoxylon lineare	Green araliya	
		Pennisetum alopecuroides	Swamp foxtail	
		Pennisetum advena	Rose fountain grass	
		Phyllanthus myrtifolius	Ceylon myrtle	
		Sanchezia oblonga	Zebra plant	
		Serissa japonica	Japanese	
		Carex rostrata	Bottle sedge	[113]
		Carex appressa	Tall sedge	[114]
			Creeping juniper	[10]
		Aronia prunifolia	Chokeberry	[105]
		Ilex vertiallata	Winterberry	
		Ilex compacta	Compact inkberry	
	N	*Eichhornia crassipes*	Water hyacinth	[115]
	NH_4^+	*Eichhornia crassipes*	Water hyacinth	[116]
	NO_3^-	*Eichhornia crassipes*	Water hyacinth	
	P	*Eichhornia crassipes*	Water hyacinth	
	PO_4^{-3}	*Eichhornia crassipes*	Water hyacinth	
Toxic metals		*Carex appressa*	Tall sedge	[114]
			Creeping juniper	[10]

(continued)

Table 2 (continued)

Pollutant type		Plant species	Common name	Reference
		Betula nigra	River birch	[60]
		Juncus effuses	Common rush	
		Iris pseudacorus	Yellow flag iris	
		Magnolia virginiana	Sweetbay	
		Iris virginica	Blue flag iris	
		Labelia cardinalis	Cardinal flower	
		Juncus effuses	Common rush	
		Hibiscus spp.	Hibiscus	
		Acer rubrum	Red maple	
		Clethra alnifolia	Sweet peperbush	
		Itea virginica	Virginia sweet-spire	
		Chasmanthium latifolium	Wild oat grass	
		Lythrum salicaria	Purple loosestrife	[92]
		Iris pseudacorus	Yellow flag iris	
		Vinca minor	Periwinkle	
		Hippophae rhamnoides	Sea-buckthron	
	Hg	*Jatropha curcas*		[82]
		Eichhornia crassipes	Water hyacinth	[117]
	Pb	*Avena sativa*	Oat	[118]
		Helianthus annuus	Sunflower	
		Elodea canadensis	Canadian Waterweed	[85]
		Potamogeton natans		
		Carex panacea		[119]
		Juncus conglomeratus		
		Phalaris arundinacea		
		Eichhornia crassipes	Water hyacinth	[120]
	Cd	*Avena sativa*	Oat	[118]
		Helianthus annuus	Sunflower	
		Juncus subsecundus		[75]
		Elodea canadensis		[85]
		Potamogeton natans		
		Potamogeton pectinatus		[121]
		Lemna polyrhiza		[122]
		Carex panacea		[119]
		Juncus conglomeratus		
		Phalaris arundinacea		
		Eichhornia	Water hyacinth	[120]
	Cr	*Avena sativa*	Oat	[118]
		Helianthus annuus	Sunflower	
		Eichhornia crassipes	Water hyacinth	[123]

(continued)

Table 2 (continued)

Pollutant type		Plant species	Common name	Reference
	Cu	*Elodea canadensis*		[85]
		Potamogeton natans		
		Dunaliella tertilecta (algae)		[124]
		Carex panacea		[119]
		Juncus conglomeratus		
		Phalaris arundinacea		
		Eichhornia crassipes	Water hyacinth	[125], [120]
	Zn	*Elodea canadensis*		[85]
		Potamogeton natans		
		Fucus vesiculosus		[126]
		Carex panacea		[119]
		Juncus conglomeratus		
		Phalaris arundinacea		
		Eichhornia crassipes	Water hyacinth	[127], [120]
	Ni	*Eichhornia crassipes*	Water hyacinth	
	Fe	*Eichhornia crassipes*	Water hyacinth	[128]
	Mn	*Eichhornia crassipes*	Water hyacinth	
	As	*Eichhornia crassipes*	Water hyacinth	[129]
Organic pollutants	PAHs	*Juncus subsecundus*		[75]
	Naphthalene (PAH)	*Avena sativa*	Oat	[118]
		Helianthus annuus	Sunflower	
	Phenanthrene (PAH)	*Avena sativa*	Oat	
		Helianthus annuus	Sunflower	

virgatum (switch grass), and *Schizachyrium scoparium* have been tested successfully for the removal of PAHs [77]. Hence, these plants may incorporate together with other plants which may improve the efficacy of the bio-retention system for phytoremediation of organic pollutants.

9.2 *Phytoremediation of Nutrients*

Regarding nutrient removal, the priority has been made for the phosphorus and nitrogen since they are high in concentrations. The plant species used for nutrient removal should have a great ability to uptake higher amounts of dissolved nutrients [78]. Because of the biofiltration process, the salinity conditions can be increased within the bio-retention systems. Therefore, it is important to consider about the salt tolerability of plants before establishing the plants. It is an important factor because salinity can result in growth retardation in affected plants and it can play a negative effect on the whole bio-retention system [78]. Most importantly, halophytes (salt-tolerant plants) have the ability to maintain a great nitrogen and phosphorus removal efficiency, even at salt concentrations similar to sea water [78]. Therefore, it is important to incorporate halophytes into the bio-retention system [78]. *Canna x. generalis* could be an effective plant for phytoremediation of nitrogen and phosphorus and it has a promising ability to remove phenolic compounds. Not only the particular plant, but also the *Canna x.* genera have the ability to improve physical characteristics: color, turbidity, and odor of the water [13]. Table 2 depicts several plant species that can be used for phytoremediation of nutrients in bio-retention systems.

9.3 Phytoremediation of Toxic Metals

Chemical, physical, and biological methods are there to remove different toxic metals from storm water and contaminated soils. Mainly in urban areas, the major source for metals is vehicles and vehicle-related sources [79]. Vehicle emission, vehicle leakage, tire ware, and discharges from service stations are responsible for that. Runoff that generated by roads, parking lots, and service stations bear a number of heavy metal types as well as higher heavy metal concentrations. Vanadium (V), Ni, Fe, Mg, Ca, Cu, Zn, Pb, Cr, Ni, and Cd are mostly found metals with road and parking lot runoff [80, 81]. Due to the presence of various metals, the plant selection should be conducted carefully [46]. For different metals, there are different plant species. Most of the plant species have the ability to remediate several metals. Table 2 depicts the different plant species that can be used for phytoremediation of different toxic metals.

However regarding cost and the environmental impact, the phytoremediation is considered as far more effective in terms of bio-retention [82]. The metal accumulating plants have the ability to remove metals from the soil up to 100 times higher than non-accumulator plants. Studies show that the use of hyper-accumulating plants may enhance the removal rates of metals as 10 mg kg^{-1} for Hg, 100 mg kg^{-1} for Cd, 1000 mg kg^{-1} for Co, Cr, Cu, and Pb, and 10,000 mg kg^{-1} for Zn and Ni [83]. *Jatropha curcas* plant which commonly known as a physic nut has been successfully tested for removal of Hg from contaminated soils [82]. *Jatropha curcas* roots have higher phytoremediation ability than all other plant tissues and the plant has low translocation factor and higher bio-concentration factor. Therefore, it has been recommended as a remediation material for Hg-contaminated soils and water [82]. Although it can be used as a fuel source [84], it may be harmful to use as a fuel source after it has been used for Hg removal.

Juncus subsecundus is a plant that has been used for the removal of Cd from the contaminated soils [75] so that it has a potential to be used in bio-retention systems to remove HMs. *Elodea canadensis* and *Potamogeton natans* are two submerged plant species that have the ability to uptake Cu, Zn, Cd, and Pb [85]. The submerged plants are important in bio-retention systems considering storm water management aspects. An area with overflow water to be stagnated as a modification to the bio-retention system may allow a place for the submerged plants, however this must be managed in a way prohibiting mosquito breeding. Submerged plants are far important due to their ability to uptake metals directly from storm water [85]. This may increase the aesthetic value of the bio-retention system as well. Yet, the management of such water retaining area needs quite a good attention and management.

9.4 Phytoremediation of Other Pollutants

Rather than toxic metals and nutrients, there are many other pollutants present in storm water, however most probably in low concentrations. Textile dyes, surfactants, and detergents are some of them [86]. *Alternanthera philoxeroides* plant has been successfully tested for removal of highly sulfonated textile dye called as Remazol Red. The removal rate is significantly high with *Alternanthera philoxeroides*. There are some identified wild plants: *Phragmites australis, Blumea malcolmii, Typhonium fagelliforme*, and *Ipomea hederifolia* for removal of textile dye from water [87]. Also some common ornamental plants: *Aster amellus, Glandularia pulchella, Portulaca grandifora, Petunia grandifora, Zinnia angustifolia*, and *Tagetes patula* have potential to remove textile dye from contaminated soil [86]. Aquatic macrophytes also reported for their capability to remove dyes and other pollutants. Because of their habits and stress tolerance characteristics, the phytoremediation capabilities are strong [86].

10 Advantages and Limitations of Phytoremediation in Bio-retention Systems

Bio-retention systems are proving to be a promising technology as it relies on the ecological interactions to provide storm water retention and removal of pollutants in a natural system. One of the major advantages in integrating phytoremediation into bio-retention systems is, it is low cost than other remediation methods [14]. It should be noted that the cost on phytoremediation is less than even half of any other remediation method [14]. Also do not need specific dump sites to dispose of these plants. Some are long-term plants while others are mineralizing the pollutants. This mineralization has the ability to cut down the cycling of pollutants. Since the plants enhance the biodiversity, the public acceptance is also high. Therefore, do not need extra awareness programs. Due to its applicability on a far range of pollutants, this approach does not need several remediation methods to remove all the pollutants in storm water [14]. However, a limited number of studies in the tropics, arid and semi-arid regional plants on phytoremediation may be a restraint when a phytoremediation integrated bio-retention system is to be used in such areas.

Due to the high tolerance toward changing hydrological regimes of bio-retention vegetation, these flexible and adaptive systems have the potential to be used in a wide variety of environments and it has been viewed as an attempt to maximize every available physical, biological, and chemical removal processes found in the plant and soil complex of a terrestrial forested community [69]. They can be integrated with urban development while providing at-source treatment.

Bio-retention systems are most often used as an initial runoff treatment system as they detain runoff while contributing to pollutant removal during short pulses associated with precipitation. This may be considered as a limitation as plants may react slowly. Biotechnological advances may provide input to increase the potential of plants to react fast during such pulses. Apart from the storm water quantity and quality benefits, bio-retention systems with phytoremediation integration host other benefits such as improved air quality, reduced noise, increased real estate values, shade and wind cover, as well as the creation of habitat for native wildlife and plant species by improving site aesthetics and the pride of the community [57, 88].

Some identified obstructions to the implementation of sustainable practices are inadequate engineering standards and guidelines; a lack of legislative mandate and institutional capacity; uncertainties in performance and cost and inadequate funding and effective market incentives [6]. As most contractors are not familiar with bio-retention system construction with an integration of phytoremediation, it has led to poor vegetation establishment and improper soil mixture selection or placement [89, 90]. Hence poor construction practices have also been an implementation concern. Though current bio-retention design guidelines require storm water drain within 72 h to minimize mosquito breeding [54], there are certain risks to public health regarding the breeding of mosquitos and other vector diseases as well.

Also, there is a lack of knowledge in the performance of bio-retention systems and the process of phytoremediation in tropical, arid, and semi-arid climates compared to the studies carried out in cold climates [91–93]. Sufficient studies are needed to be performed to generalize the observations under various climates. In comparison to conventional practices, bio-retention systems experience lower marginal costs as these systems promote proactive maintenance [94]. Due to characteristics of a given site and design objectives, construction costs of bio-retention system vary significantly. The costs can even vary, depending on the conducted activities as maintenance requirements are still being established [8]. In addition, the opportunity costs of the space occupied by a particular bio-retention system are substantial but is an often overlooked component [95, 96]. Unless implementation is targeted on a small watershed scale, measuring of the performance enhancements will be very difficult. The inseparable relationship of cost and performance was highlighted through watershed scale implementation. As a result, further research is needed to identify specific cost drivers and proper tools for cost prediction in the long run with an aim to gain extra knowledge on the life cycle costs for bio-retention and phytoremediation.

In recent years, well-developed computer models have provided to develop appropriate guidance by modeling various aspects of bio-retention gardens. Some of the introduced mainstream storm water models are Model for Urban Storm water Improvement Conceptualization (MUSIC) [97] and Storm water Management Model (SWMM) [98, 99]. The used model inputs may often not be suitable as there is a lack of detailed bio-retention performance information for many regions other than the limitations of the models themselves [8]. The United States Environmental Protection Agency (US EPA) developed a decision-support system called SUSTAIN in 2003 for the selection and placement of BMPs at strategic locations in urban watersheds as they have recognized that there was no comprehensive modeling system available to systematically evaluate the location, cost, and type of storm water BMPs [100]. Yet, there is still a need for additional modeling tools to verify the suitability of current guidelines and accurately predict the hydrologic and water quality performance of bio-retention system designs integrated with phytoremediation [88].

11 Summary

Bio-retention systems are one of the most recognized methods at source structural BMP under LID practice that has been utilized to improve the quality of water and mitigate hydrologic impacts due to urbanization. This was first developed in the early 1990s by Prince George County, Maryland, United States. It provided as a mean for treating the 'first flush' runoff from a particular urban area. Over the years, extensive research had been carried out to assess its performance and applicability in the urban storm water treatment and management. Quite a number of field scale studies have been carried out to provide a light in design architecture with an emphasis on water quality goals and hydrological performance [60, 101, 102]. Considering water quality goals and environmental quality, phytoremediation is an important consideration to remediation of pollutants. As the phytoremediation is a low-cost method, it can easily implement into bio-retention systems.

One drawback has been the current design guidelines which are inconsistent across various demographical regions. It is quite evident that geographical factors and the climate influence the performance of phytoremediation in bio-retention system in addition to treatment objectives which also vary with jurisdictions of a particular location. Although there is a wide usage of computational models for simulating the functions of phytoremediation in bio-retention, there has been a noted short coming in each case while aiming for perfection. Hence, there is a growing need for advanced modeling tools to verify the applicability of current guidelines, accurately predict hydrologic performance, and provide suggestions to water quality improvements with an emphasis on pollutant removal. Identification of alternative and favorable conditions for nitrification, denitrification, and phosphorus sorption is also needed. Even there should be an attempt in the bio-retention systems to the optimization of nutrient removal processes beyond field monitoring.

In fact, bio-retention systems are complex structures where there is a replication of natural ecological processes within the system. It has been proven to be applicable as a sustainable and cost-effective treatment practice among the urban storm water treatment and management techniques around the world. As improved performance and design specifications are evolving with continuous research, bio-retention systems and phytoremediation within bio-retention systems should enable learning culture that values integrated urban storm water management while acting as a guidepost for improving urban management practices.

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Fungal Laccase Enzyme Applications in Bioremediation of Polluted Wastewater

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Abstract Environmental pollution had emerged by the beginning of urban life and increased parallel to the industrial development. Chemicals are being produced and used largely in the branches of mainly textile industry with today's technology such as leather tanning, paper industry, food technologies, agricultural investigations, hair dyes, and many other branches, mainly the field of cosmetics. Various amounts of pollutants found in the wastewaters are the chemicals that cause color pollution in waters. In addition, they threaten the photosynthetic activity of the life in water and are also hardly decomposed. The classical methods used in the treatment (refinement) of wastewater (classical precipitation, ion exchange, ozone treatment, coagulation, flocculation, adsorption, etc.) are far from being practical and economical because of their investment and management costs and also reemergence of new pollutants after a certain period. The ability of laccase enzyme to oxidize many different forms of substrates made them to be used in different industrial and biotechnological applications as biocatalysts. Laccase activity and occurrence of laccases in fungus species were demonstrated in these studies. In addition, determination of the expression levels of the gene coding for laccase enzyme which is thought be very important in defense against oxidative stress will give information about the mechanism of the enzyme and will illuminate the development of the production of laccase-based methods. This result is going to form a major step for the studies that will provide the fungus species to be used as biosorption agents for the detoxification purposes of the wastes mainly of textile and petrochemical industries.

Keywords Wastewater • Laccase • Gene expression

1 Introduction

The increase in urbanization and industrial activity has led to harmful ecological impacts in recent years. All industry sectors compared with the textile industry which volume and composition of waste has the capacity to produce the most

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pollutant source. In particular, the accumulation of wastewater resulting from industrial activity has led to toxic and persistent pollutants produced in large quantities. Textile waste is significant amount of substances such as dyes, additives, salts, and detergents, and it is quite a threat to primarily human and all biological organisms' health. The provide of clean freshwater is serious to the future of man and biosphere [1, 2]. Nowadays, public awareness and unconscious situation to this subject have influenced all industry sectors and governments to take actions to control the situation [2, 3]. There has been various research in industrial wastewater treatments to propose more effective technologies and to reduce the release of toxic and polluting substances in water courses [2].

Traditional technologies that include different physical and chemical methods for wastewater treatments are not preferred to use due to expensive, inefficient, and often do not reduce the toxic effect. But in recent years, innovative physical, chemical, and biological methods by using treatment process are obtained to higher effective results. Especially, innovative biological methods have enabled low cost, less energy intensive, easy handling, environmentally safe, and rapid degradation thus they could be possible for wastewater treatment and provide enough information on the serious effects of pollutants on the wastewater. Biological species show different sensitivity to vary sources of pollution. Many different biological organisms that including bacteria and fungi have been standardized for ecotoxicity studies in recent years. In particular, fungi species are always proposed for toxicity monitoring of wastewaters [2, 4, 5].

Fungi, mainly white rot fungi, have long been recommended for their ability to degrade a synthetic dye, through the use of relatively nonspecific, extracellular oxidative enzymes [6, 7]. This enzymatic system is involved in lignin degradation, consists mainly of oxidative enzymes like laccases (Lac), lignin peroxidases (LiP), and manganese peroxidases (MnP) that have been known as effective against an industrial dyes [8]. White rot fungi has able to the low efficiency of dye removal by mixed bacterial communities and the high rates of dye decolorization. In this respect, many researchers suggest a combination of both processes as an option of treatment of textile wastewater containing dyes and high concentrations of organic compounds [2, 9].

2 Laccase Enzymes and Its Applications in Industrial Areas

Fungal ligninolytic enzymes have broad biotechnological applications. Especially, laccase enzyme has been developed up to pilot scale for degradation of pollutants in water in recent years. Laccase (E.C.1.10.3.2, p-benzenediol:oxygen oxidoreductase) is a copper-protein belonging to a small group of enzymes denominated blue oxidase [10]. Copper, which is located in the active center of the enzyme, plays an significant role during the catalyzed reaction [11, 12]. The catalytic core of the enzyme involve to the cluster of four copper atoms. It carries out four single electron oxidations of the substrate to a four electron reductive cleavage of the dioxygen bond. The laccase molecule could be formed four copper atoms distributed to three sites and four type copper ions [13]. Laccase is a crucial role for oxidoreductase able to catalyze the oxidation of various aromatic compounds with the concomitant reduction of oxygen to water [14]. Additionally, in the presence of primary substrates [2,20-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) or 1-hydroxybenzotriazole (1-HBT)] which act as electron transfer mediators, the substrate range can be extended to non-phenolic compounds [12].

The first laccase was identified in the latex of the lacquer tree *Rhus vernicifera* 130 years ago [15]. To date, laccases have been identified in plants [15], insects [16], some prokaryotes [17], and a few bacteria [18, 21]. Moreover, most known laccases are from fungi, especially from the white rot fungi. Among fungi species, the basidiomycetes, especially *Agaricus bisporus, Pleurotus ostreatus, Trametes versicolor, Phanerochaete chrysosporium*, and *Coprinus cinereus* produce various laccase isoforms [19, 20]. Finally, mention that although most laccases have been characterized from white rot basidiomycetes, other groups of fungi-producing laccases but they have been studied to a much lesser extent [13, 20]. Lichens are fungi often belonging to division Ascomycota or rarely Basidiomycota that together with live in green algae and cyanabacteria [21].

Lichen species can tolerate the environmental extremities, hence they are well known for tremendous abilities to adapt and survive under extreme conditions and for a rapid restoration of their metabolic activity [22]. A few reports exist for laccases in an important group of fungi, the lichenized ascomycetes [23, 24] demonstrated the presence of strong extracellular redox activity in some species of lichens. According to the examined study demonstrate that in lichenized Ascomycota, was occur high laccase activity especially species of in Peltigerineae family [25]. Recent studies support the view that the laccase activity was recently discovered in lichens of varies taxonomic and substrate groups [25, 26]. Lisov et al. purified the two main laccases from two different lichens species, which were *Solorina crocea* and *Peltigera aphthosa* after four sequential purification steps. Comparison of the molecular weight of these two laccases using SDS-PAGE and gel filtration chromatography demonstrated two lichen species were dimeric laccases [27].

Potentially important new application of lichen laccases are increasingly used in a growing number of industrial areas. Lichen laccases are shown to be promising alternative to their fungal counter partners for commercial applications in especially biotechnological areas. When compared to fungi and plant, bacteria, lichen enzymes, the high redox potential of copper Type 1 makes fungal laccases preferred for commercial application [28]. On the other hand, fungi are slow growers and therefore they make low production rate and contain low enzymes.

Due to the presence of copper, laccases are also named "blue enzymes" and defined as blue multi-copper oxidases (MCOs) [29]. By the reason of the efficient and low cost degradation of the pollutants properties, laccases have obtained great attention and largely used in various industry area [30–33]. Firstly, in the food industry, laccases are used for the selective removal of phenol derivatives to stabilize beverages like mainly beer, wine, and juices. Secondly, in the pulp and paper industries, laccases are extensively usage for bleaching process, olive oil, dye and printing area of delignification of woody fibers [30]. Thirdly, common use of laccases is described biosensor, hair dyes for cosmetic industry and skin lightening [30]. Another interesting usage of laccases is also performed for decolorization of dyes, such as bleaching coupled with stone washing with cellulase of indigo dyed jeans [34].

3 Molecular Mechanisms of Wastewater Treatment

Molecular mechanism of oxidation by laccase enzymes was shown by Forootanfar and Faramarzi [35]. It was explained that the reaction catalyzed by laccase is based on the transfer of four electrons from a suitable substrate to the final acceptor molecular oxygen to form the corresponding reactive radical and water as a by-product [35–37]. The free radical may undergo additional enzymatic or spontaneous reactions to produce the final products [38]. A cluster of three copper sites containing T1 copper (blue), T2 copper (normal), and T3 copper (coupled binuclear coppers) in the catalytic core of the enzyme assists in the electron transition [35, 37, 39]. However, not all laccases have four copper ions in their active site.

4 Alternative Laccase Production Procedures

Laccase is produced by various organisms which mainly fungus species. The great potential and value in industrial and biotechnological applications have demonstrated strong interest in obtaining a large amount of laccase for practical use. However, these fungi produce laccase enzyme in small amounts and cannot meet the demand of practical applications in industry and biotechnology areas [40]. However, these fungi produce this enzyme in small amounts under normal conditions. Cheap and abundant production of laccase enzyme is very important for related areas. Thus, the main problem is to obtain sufficient laccase enzyme. Its production is dependent on various factors such as species and inducers, cultivation method [41–43]. For this purpose, many studies have been concentrated on expansion of the laccase production by inducing the laccase gene expression in fungi species. Study on the regulation of laccase gene expression may greatly contribute to the improvement of native laccase productivity in white rot fungi [19, 44].

Previous research has demonstrated that expression of laccase gene can be stimulated by some different external factors, for example, metal ions [42, 45–47], aromatic compounds structurally related to lignin or lignin derivatives [48–52], nutrient nitrogen [45, 53], and carbon [46, 54]. It was explained that the regulation of laccase gene expression by these factors previously occurs at the level of transcription [19, 44]. The effect of the same factor on the transcription of different laccase genes encoding various isoenzymes is also very different, with some being constitutively expressed and others being inducible [46]. Yang et al. show that the putative cis-acting-responsive elements present in the promoter of laccase gene, like metal-responsive elements, xenobiotic-responsive elements could be involved in the transcriptional regulation of laccase gene [53–56].

Use of laccase need to induce both its expression and productivity through up-regulation of the enzyme-encoding genes. Contrary to an effective but complex and expensive tools of bioengineering, increasing the enzyme yield by adding inducers is perceived as simple and cost-effective [57, 58]. There are many different inducers for laccase production [59], but the most common of the effect of copper [60, 61]. Although research on the production, isolation, and expression optimization of laccases has brought many promising results in laboratories scale in the last 15 years, much more work to find the best and general conditions for the high level of heterologous expression of any laccase in yeast hosts is still needed [61].

Another promising approach; further research could be practice in nonsterile wastewater and scale-up in a bioreactor and to determine the metabolites produced during the dye decolorization process [62]. A more effective wastewater treatment of industrial scale was demonstrated by fungus species [62], and it can maintain the metabolic activity of the organism in very difficult conditions. The use of several bioreactors has been demonstrated for dye decolorization by white rot fungi [9]. However, for the establishment of a practical treatment process of textile effluents, several problems have to be overcome. Maintaining fungal growth under nonsterile operation of bioreactors represents important limitations of long-term biodegradative processes in immobilized fungal cultures that have to be overcome [63–65]. Moreover, despite the fact that the fungal process of decolorization of synthetic dyes has been too much studied, little attention has been paid to the possibility of its cooperation with the traditional biological wastewater treatment technology [66]. Another important and often underestimated aspect in related areas, laccase immobilization method were used to reduce the production cost of laccases in order to make their application more economical. Among such approaches laccase immobilization allows its reuse and improves its stability [67].

5 Conclusion

Laccases have a great importance for a wide range of industrial and biotechnological areas. Fungi and surprisingly lichen species nowadays seem to be operations such as easy handling, cheap cultivation media, and the possibilities of well-described genetic manipulations for improving the quantity and/or properties of the secreted enzyme for the industrial production of laccases [68]. Therefore, future studies will very likely focus more on in silico approaches for laccase engineering and subsequent construction of mutated and chimeric versions of laccase enzymes to improve their yields and properties [68]. A successful design of the specific heterologous production system and optimization of cultivation/fermentation conditions are fundamental for all kinds of industrial and biotechnological applications since it is necessary to provide large-scale production and commercialization for related areas [21].

The design of improved this is omit laccase more appropriate to temperature and pH value, less dependent on metal ions, and less susceptible to inhibitory agents and aggressive hard environmental conditions [21, 67, 69]. As conventional bioremediation methods are costly with low efficiency, laccase enzymes could be good candidates to detoxify these compounds. Novel and engineered laccases are being developed to "green" biotechnological applications [31, 70] suggesting that this improved laccase is an environment-friendly candidate for use in the treatment of wastewaters from industrial area [31].

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Part III
Natural and Constructed Wetlands for Phytoremediation

Phytoremediation Applications for Waste Water and Improved Water Quality

Klaudia Borowiak and Jolanta Kanclerz

Summary Macrophytes play an important role in natural and constructed wetlands (CWs). Their most important function is removal of excessive levels of some substances, such as nutrients, total suspended solids, trace elements, etc. CWs are widely used all around the world to treat many types of wastewater, with relatively high removal efficiency (5-day biochemical oxygen demand [BOD_5]—around 80 %, total nutrients—approx. 40 % in the case of domestic sewage). Considering the purpose of CWs application, a few types were created with several variants in certain environmental conditions and for many effluent types with various loads of many substances. Two main types of flow through CWs are considered—surface and subsurface flow. The latter is further divided into horizontal and vertical flow. The most popular use of CWs is for domestic and municipal wastewater as secondary and tertiary treatment stages. Among macrophytes applied for phytoremediation, great diversity of plant species has been observed, especially native species and a wide range of ubiquitous species, such as *Phragmites australis* and *Typha* spp. Most macrophyte species also play an important role in natural ecosystems in improvement of surface water quality. Many species are utilized as indicators of water quality, even when low pollutant levels occur, while others are important for phytoextraction or phytostabilization.

Keywords Macrophyte • Constructed wetland • Natural water ecosystems • Nutrient and heavy metals removal

1 Constructed Wetlands

Water plants can contribute to removal/absorption of many substances and significantly improve water quality, both in constructed wetlands (CWs) and natural water ecosystems (NWEs). The list of substances removed from the ecosystem is quite

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long, and includes excess concentrations of nutrients (such nitrogen and phosphorus), organic compounds, suspended solids, various elements (including heavy and noble metals), and pathogens [1]. CWs have been becoming more and more common due to their high removal efficiency and relatively low costs of construction and maintenance [2, 3]. Therefore, in rural areas CWs can be used as an alternative to treat wastewater. Moreover, the growing interest in using CWs can be related to growing recognition of the natural treatment functions performed by wetlands and organisms living in these ecosystems, as well as to increasing costs of conventional treatment systems and to some additional benefits provided by CWs [4]. It has also been reported that CWs are still not widely used in tropical climates due to a lack of knowledge and design criteria that are inappropriate for the local weather conditions. These authors also noted that the climate and local conditions strongly affect the removal efficiencies in constructed wetlands [5]. Hence, there is a great necessity to investigate possibilities of wider use of CWs.

Constructed wetlands initially were mostly used for domestic or municipal sewage from separate and combined sewerage [6]. Presently, they are widely used as small wastewater treatment plants, for purification of storm water runoff [7] or municipal wastewater [2, 8]. Many investigations have been performed for many other types of wastewater, such as cadmium-polluted water [9], pulp and paper industry wastewater [10], highway runoff treatment [11], different land structure conditions such as mountainous areas [4], petrochemical industries wastewater [12], an airport-runoff treatment system [13], dairy effluent [14], pig farm effluent [15], fish-farm effluent [16, 17], horticultural plant nursery runoff [18], agricultural runoff [19], textile industry [20], chemical industry [21], tannery industry [22], landfill leachate [23], and laundry wastewater [24].

Another application of constructed wetlands concerns salt-enriched soils and water. It is a global level problem due to the assessment that 20 % of agricultural land and 50 % of cropland in the world are salt-stressed. Around 1 % of all plant species are halophytes that can complete their life cycle in relatively high saline environments, as much as 200 mM NaCl or more [25]. For the purpose of water phyto-desalination, salt includers are more suitable if they are able to accumulate sodium in their tissues and reduce the media's sodium content and overall salinity [26]. Shelef et al. [27] found that *Bassia indica* can accumulate sodium in the amount up to 10 % of its dry weight, significantly improving water quality.

In the literature there is a discussion on the proper nomenclature for constructed wetlands. Their other proposed name is "treatment wetlands" [28], a term that is also used in scientific papers. Founder and Headley [29] proposed to use the term treatment wetlands (TWs) for wetland systems constructed specifically for water quality improvement for the first time. Moreover, these authors pointed out those also natural or restored wetlands can provide treatment functions. However, they proposed in this case to use the name "natural treatment wetlands" to avoid misunderstanding. The definition of CWs or TWs can be formulated as a human-made system to increase natural water system possibilities to improve or balance physical and/or biochemical processes for further removal of unwanted substances from polluted water [29]. Additionally, Zhang et al. [30] also proposed the term "engineered wetlands" (EWs), which can be used for semi-CWs in which operating conditions are more actively monitored, manipulated and controlled, which allows optimization of operating conditions. All EWs are CWs, but not all CWs can be EWs.

2 Types of Constructed Wetlands

There are several types of CWs designed to work in different conditions using various plant species. The main classification of CWs is related to hydrology and vegetation characteristics. Furthermore, such features as water position, flow direction, media saturation, surface flooding, vegetation traits, vegetative growth form, and emergent vegetation variants can also be taken into account. Considering hydraulic regimes and the life-form of dominating macrophytes, the following types of constructed wetlands can be distinguished:

1. Surface flow wetlands with an exposed free water surface: free-floating macrophyte-based systems; submerged macrophyte-based systems, and rooted emergent macrophyte-based systems.
2. Subsurface flow emergent macrophyte-based systems: with horizontal subsurface flow; with vertical subsurface flow (percolation)—up or down flow direction; fill and drain CWs with mixed flow directions.
3. Complex multi-stage systems—a combination of the above-mentioned and other types of low-technology systems [29, 31].

Based on the literature it is possible to identify many CWs variants (Table 1) depending on hydrology, vegetation, and flow direction. It should be emphasized that the majority of plant species applied in CWs can grow under water-logged (saturation) conditions, whereas some others (e.g., *Salix sp.*) can grow under unsaturated conditions, in which oxygen diffusion from the atmosphere plays an important role in the purification processes.

2.1 Surface Flow Systems

This system is quite similar to natural wetlands due to the occurrence of an open water surface, floating vegetation, and emergent plants. It is also the most common CWs type. There is a horizontal flow direction. Quite high efficiency of removed substances has been demonstrated. It was reported that for total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), and pathogens, primarily bacteria and viruses, removal efficiency reaches the level of 70 % [32], while for N and P the level is 40–50 % and 40–90 %, respectively [33]. The real removal efficiency is of course dependent on many factors such as pollutant loading, hydrologic conditions, and vegetation type [32]. The removal of organic compounds is connected with microbiological properties and processes.

Table 1 Scheme of constructed wetland types [29]

Constructed wetlands				
Hydraulics		Vegetation		
Water position	Flow direction	Sessility	Growth form	CWs types
Surface flow		Sessile	Emergent	Surface flow
			Submerged	Surface flow
			Floating leaved	Surface flow
		Floating	Free-floating	Free-floating macrophytes
			Emergent	Floating emergent macrophyte
Subsurface flow	Horizontal			Horizontal flow
	Vertical mixed			Fill and drain
	Vertical Down flow			Down flow
				Stormwater retention
				Evapotranspirative down flow
				Saturated down flow
				Anaerobic down flow
	Vertical Up flow			Up flow
				Non-flooded up flow

Nitrogen removal is connected with anaerobic water conditions through de-nitrification. The phosphorus removal mechanism is peat accumulation by which phosphorus is stored in organic matter and buried through sedimentation [34, 35]. However, chemical precipitation and adsorption of this element to binding sites of sediments plays an important function in removal of P from wastewater [2]. Constructed wetlands also play an important role in elimination of trace elements from effluents. However, the process of heavy metal removal is affected by many environmental factors, such as redox potential, pH, and the availability of several anions (e.g., sulfide and carboxyl groups of organic matter) in wetlands [36, 37]. These factors influence how heavy metals transform and interact with other elements in the environment.

Plants play an important role in nutrient removal, as well as in heavy metal absorption. During the growing season, high accumulation of nutrients is noted in above-ground plant organs. At the senescence stage, most of them are translocated to the below-ground parts. However, these parts are characterized by lower tissue decomposition than shoots, and their nutrients can be stored through burial by litter in a low oxygen environment, where the decomposition rate is relatively slow [38]. Surface flow CWs for phosphorus elimination from wastewater are mainly constructed to be kept flooded for a whole year, and anaerobic conditions result in higher possibility of P storage in sediments. To ensure high efficiency of nitrogen removal, it is necessary to maintain 50 % plant coverage in CWs [39]. There is a huge application variability of surface flow CWs in the world. It has also been emphasized that this type of CWs for municipal wastewater should follow a primary or secondary pre-treatment [2, 40].

Moreover, the post-treatment for disinfection may also be needed for pathogen removal. The primary applications of surface flow CWs are municipal and domestic wastewater, animal wastewater, agricultural, and urban runoff [32, 41]. However, several applications have been investigated for this type of CWs, such as dairy wastewater, pharmaceutical (including antibiotics) and personal care removals, improvement of surface water quality, highly polluted rivers, etc. (Table 2). The important role of surface flow CWs as a polishing step in municipal wastewater reclamation and its reuse is also emphasized. Investigations revealed that tertiary free-water CWs have a potential for efficient removal of fecal coliforms. However, as the authors indicated, the efficiency varied between systems, and further analyses are required to definitely indicate the possibilities of surface flow CWs in this process. Anyway, in some cases the water was suitable to reuse after the treatment in the wetland [2, 59].

The main role of surface flow CWs is removal of excessive amounts of some compounds and substances. There are however some additional applications, such as biodiversity conservation in the ecosystem for this wetland as well as for surrounding areas. Esthetic values and biotic regulation are very important aspects of landscape and nature conservation [60]. Moreover, an occupied area for CWs can be an important avian area for many important and endangered bird species [61]. Additionally, the removed above-ground biomass with high nitrogen nutrient load may also be used for composting or energy generation [62], and can be used as biogas production through fermentation [63].

Several macrophyte species are used in surface flow CWs. The most popular in many countries is common reed (*Phragmites australis* Trix. ex Steudel), which is characterized by very intensive biomass production, absorption of compounds and substances, as well as by the environmental range of occurrence in natural ecosystems. The usefulness in surface flow CWs of this species was found for such types of wastewater as municipal, domestic, industrial, pharmaceutical and personal care product removal, improvement of surface water quality, and highly polluted river. The second most common plant is the cattail group (*Typha* spp.), including *T. latifolia*, *T. orientalis*, and *T. angustifolia*, which were successfully used in wetlands for removal of excess substances from municipal and domestic wastewater, pharmaceutical and personal care products, urban sewage, agricultural runoff, polluted river, and storm water. Also very common is *Lemna* spp., used for various types of effluents. Finally, many geographically specific and native macrophyte species are utilized in surface flow CWs in various countries with high possibilities for removal of unwanted substances and relatively high biomass production (Table 2).

2.2 Subsurface Constructed Wetlands

Subsurface CWs are also widely used in the world. Most of the flow occurs through the porous media, and most treatment processes take place in this part. In some systems ephemeral or permanent flooding of the surface of the media can also occur.

Table 2 Plant species used in surface flow constructed wetlands in various countries and types of wastewater

Plant species	Type of wastewater	Country	Author/s
Alisma lanceolatum, Carex cuprina, Epilobium hirsutum, Iris pseudacorus, Juncus inflexus	Industrial effluents and road runoffs constitute	France, Marignane	Guittonny-Philippe et al. [42]
Phragmites australis	Highly polluted river water	Xi'an, northwestern China	Zheng et al. [43]
Typha latifolia	Stormwater	Canada, Ontario	Goulet and Pick [44]
Lemna minor	Dairy wastewater	East Lansing, USA	Adhikari et al. [45]
Phragmites australis, Typha spp.	Improvement of water quality	Bahr El Baqar, Egypt	El-Sheikh et al. [46]
Brachiaria mutica, Ludwigia taiwanensis, Eichhornia crassipes, Lemna aequinoctialis, Typha orientalis, Phragmites australis, Cyperus imbricatus	Municipal wastewater	New Taipei City, Taiwan	Hsueh et al. [47]
Cyperus flabelliformis, Hymenocallis littoralis, Phragmites australis, Vetiveria zizanioides	Domestic wastewater	Guangzhou, China	Kong et al. [48]
Phragmites australis	Improvement of water quality	Castelnovo Bariano, Italy	Bragato et al. [49]
Phragmites australis, Bolboschoenus maritimus	Improvement of water quality	Venice, Italy	Bragato et al. [50]
Ipomoea aquatic, Typha latifolia Scirpus fluviatilis, Zizania caduciflora, Najas marina, Hydrilla verticillata, Myriophyllum spicatum, Elodea canadensis, Potamogeton crispus, Nelumbo nucifera, Canna indica	Polluted river	Jialu River, China	Wang et al. [51]
Typha sp., *Scirpus* sp.	Agricultural runoff	Washington, USA	Beutel et al. [52]
Zizania latifolia	Secondary effluent from a dormitory	Mito, Japan	Abe et al. [53]
Phalaris arundinacea, Typha latifolia	Antibiotic removal	Quebec, Canada	Hussain et al. [54]
Typha sp., *Phragmites australis*	Industrial and domestic sewage	Granollers, Spain	Regueiro et al. [55]
Typha sp., *Phragmites australis*	Pharmaceutical and personal care removals	León, Spain	Hijosa-Valsero et al. [56]
Typha latifolia	Urban sewage	India	Badhe et al. [57]
Typha latifolia	Domestic wastewater	Garip village, Turkey	Gunes et al. [58]

This type of CWs is subdivided into horizontal and vertical concerning the flow direction [64]. Horizontal flow subsurface CWs are the most widely used type in European countries [6] and are characterized by an inlet and outlet which are horizontally opposed. The trench or bed contains a medium which supports growth of emergent vegetation. There are several media used in this type of CWs, such as different soils, sand, gravel, and crushed rocks, alone or in combinations. There are also some investigations concerning the usefulness of other media, such as light-expanded clay aggregates (LECA), zeolite, shale, and industrial wastes, and the investigators found them to be efficient filter materials [65–67]. The wastewater comes through the rhizosphere part of plants, and these systems are usually small, less than 0.5 ha, and characterized by higher hydraulic loading rates than surface flow CWs. The anaerobic conditions mostly occur low in the media, but the subsurface zone is saturated through the root system supporting aerobic micro sites adjacent to roots and rhizomes [29, 31].

The primary pretreated wastewater slowly passes through the media and when it reaches the outlet is collected before discharge via level control management at the outlet. A common horizontal flow subsurface CWs is planned with a filtration depth of 0.6–0.8 m to give an opportunity for plants to grow roots inside the media and properly penetrate the whole bed and ensure oxygenation through oxygen release from roots. The amount of oxygen should be sufficient to achieve aerobic degradation of oxygen-consuming substances in the wastewater, and for nitrification of the ammonium. However, many studies have shown that this type of CWs has quite a low possibility for nutrient removal due to the system's inability to oxidize ammonium, the predominant form of nitrogen in domestic and municipal wastewater, as well as the low sorption capacity of the filtration medium for phosphorus. Also harvesting of aboveground plant organs is optional; hence there is quite a small amount of nitrogen sequestered [68]. However, this type of CWs is sufficient for removal of organics and suspended solids and fulfils the criteria for small sources of pollution. The efficiency of horizontal flow subsurface CWs is approximately at the level of 40 % for nutrients, and around 80 % for total suspended substances, as well as BOD_5 and COD [41]. There is a very important role of soil microbes in removing many substances. As well as soil, enzymatic activity is responsive to the intensity and direction of biological activities in CWs. The mineralization of organic matter is mainly carried out by microbes both under aerobic and anaerobic conditions. Microbes play an important role in nitrogen and phosphorus removal. Hence, their role and activities have been more and more thoroughly investigated in CWs [69].

The most common species for this type of constructed wetland is *P. australis*. However, it is also found that quite often species from the genera *Schoenoplectus*, *Cyperus*, *Typha*, *Baumea*, and *Juncus* are used [29]. *P. australis* is used very often in combination with *Typha* spp. or *Phalaris arundinacea*. The range of wastewater types embraces mainly municipal and domestic sewage. There are however some other uses of these species in horizontal flow subsurface CWs, such as purification of heavy metal rich wastewater, sulfate rich groundwater, or highway runoff. Moreover, some other species were also investigated in this type of CWs for possible removal of excessive substances in pharmaceutical wastewater, urban runoff, dairy effluent, etc. There are several species used in horizontal flow CWs whose range of occurrence is small; hence they are typical only in some countries (Table 3). It has also been reported that mixed vegetation is more effective in pollutant removal as compared to stands of single species [84, 85]. However, this subject is still being discussed and investigated.

Table 3 Plant species used in subsurface flow constructed wetlands in various countries, types of wastewater, and type of flow direction

Plant species	Type of wastewater	Country	Author/s	Flow direction
Cyperus alternifolius, Cynodon dactylon	Refining and petro-chemical company effluent	Kaduna, Nigeria	Mustapha et al. [70]	Vertical
Phragmites australis, Typha spps., *Canna indica*	Municipal	Turkey, Edirne Province	Çakir et al. [71]	Horizontal
Phalaris arundinacea, Phragmites australis	Mechanically pre-treated municipal sewage	Czech Republic, Morina and Cicenice	Brezinová and Vymazal [72]	Horizontal
Typha latifolia, Phragmites australis, Colocasia esculenta	Urban wastewater	Haridwar, India	Rai et al. [73]	Horizontal
Phragmites australis, Typha angustifolia, E. arundinaceus	Pulp and paper industry	India, Karur	Arivoli et al. [10]	Vertical
Acorus calamus	Domestic wastewater	China	Chen et al. [1]	Vertical
Phragmites spp.	Highway runoff treatment	Nanjing city, China	Singh et al. [11]	Vertical
Cyperus alternifolius Typha latifolia	Urban wastewater	Sicily, Italy	Leto et al. [74]	Horizontal
Phragmites australis	Sulfate-rich groundwater	Germany	Chen et al. [59]	Horizontal
Phragmites australis	Domestic wastewater	France	Silveira et al. [75]	Vertical
Typha angustifolia	Pharmaceutical compounds	Singapore	Zhang et al. [76]	Horizontal
Schoenoplectus, Tabernaemontani, Bidens comosa	Dairy wastewater	East Lansing, USA	Adhikari et al. [45]	Horizontal
Phragmites australis	Domestic wastewater	Ain, France	Morvannou et al. [77]	Vertical
Phragmites australis	Heavy metal-rich wastewaters	Belgium	Lesage et al. [78]	Vertical, horizontal
Phalaris arundinacea, Phragmites australis	Municipal sewage	Morina, Czech Republic	Vymazal et al. [8]	Horizontal

(continued)

Table 3 (continued)

Plant species	Type of wastewater	Country	Author/s	Flow direction
Bassia indica	Salt phytoremediation	Midreshet Ben Gurion, Israel	Shelef et al. [26]	Vertical
Arundo donax, Acorus calamus	Micro-polluted river water	Chongqing, China	Huang et al. [69]	Horizontal
Panicum maximum	Domestic wastewater	Côte d'Ivoire	Pétémanagnan Ouattara et al. [79]	Vertical
Typha domingensis	Mercury enriched wastewater	Tręs Marias, Brasil	Teles Gomes et al. [80]	
Phragmites australis, Typha latifolia	Municipal wastewater	Greece	Akratos et al. [81]	Horizontal
Phalaris arundinacea Phragmites australis	Municipal wastewater	Ceske Budˇejovice, Czech Republic	Vymazal et al. [82]	Horizontal
Typha orientalis, Arundo donax, Canna Indica, Pontederia cordata	Domestic wastewater	Wuhan City, China	Chang et al. [83]	Vertical

Vertical flow subsurface constructed wetlands were first designed as pre-treatment units before wastewater treatment in horizontal flow beds [82]. There are several types of vertical flow subsurface CWs, which are categorized according to downward flow, upward flow, and combinations of these, which are called mixed flow. The vegetation is always emergent [29]. The system consists of vertical flow through several beds and discharge via a drain [31]. The structure of vertical flow CWs usually comprises a flat bed of graded gravel topped with sand planted with macrophytes. The size fraction decreases to the top of the bed (from ca. 30–60 mm to ca. 6 mm) to facilitate the uniform distribution of applied sewage [29]. Vertical flow constructed wetlands (VFCWs) are popular when the nitrogen forms contained in wastewater have to be nitrified.

In the down flow the system remains unsaturated for most of the time. Pipes distribute the flow across the surface of the bed. Surface flooding should be avoided. The bottom layers with coarse media usually consist of a network of perorated drainage pipes, which promote ventilation for passive aeration of the substrate. The second type of vertical CWs is up flow with a constantly saturated medium which is permanently flooded over the surface. Wastewater is distributed from the bottom of the bed through the series of pipes and afterward is moved slowly to the surface of bed. The last type of vertical flow CWs is fill and drain, which is a mixture of upward and downward flow directions. The flow can sometimes be close to a diagonal direction. The sequences of filling and draining are the reason for the occurrence of saturation and instauration periods of the bed. The upper surface is usually not flooded. The system is a very good solution to complete nitrogen removal in one reactor, through ammonia adsorption on the medium during the filling stage, nitrification in aerobic conditions during the draining phase, and de-nitrification with anaerobic condition in the next filling stage [86].

Concerning possibilities of application, vertical flow CWs are mostly applied for municipal and domestic wastewater treatment. There are, however, many other applications, such as salt phytoremediation, highway runoff, pulp and paper industry, refinery, and petrochemical company effluent (Table 3). Several applications of vertical flow subsurface CWs can be observed, which are especially common in the USA, Australia, and New Zealand with down flow direction. This system in European countries is especially useful for achieving the secondary treatment of pre-treated sewage. This system is also more common for removal of higher concentrations of ammonium, due to higher oxygen transfer rates. The up flow vertical CWs are applied to provide anaerobic conditions. They can be sufficient for removal of total suspended solids and organic compounds. Hence, their applications include mining and industrial wastewater. The fill and drain systems can be applied for wastewater with high oxygen demands or high nitrogen removal. Moreover, due to lower loss of evapotranspiration they are more suitable in arid regions [29].

The vertical flow CWs can provide complete nitrification and promote the mineralization of organic matter [87], but do not provide de-nitrification. It would be sufficient to use a combined vertical and horizontal CWs system [88]. However, it requires space and can be costly. There is variation of macrophyte species used for vertical flow CWs, beginning with *P. australis* and *Typha* spp. and including various native wetland species, as well as those whose range of countries is wide (Table 3).

3 Macrophyte Function in Surface Water Quality Improvement

Natural water ecosystems are a type of sink for surrounding areas; hence elevated amounts of some elements and substances can be noted. Almost three-quarters of water in rivers, lakes, and wetlands are threatened by excessive levels of organic pollutants and trace elements, which furthermore are also a threat to macrophytes and phytoplankton [89]. The wetland systems may play a role of natural filters for the abatement of heavy metals [49]. There is a well-known role of macrophytes in removal of excess levels of nutrients [90–92]. Plants can also survive some concentrations of heavy metals. Some mechanisms have already been described. It is known that plant rhizospheric secretion of various organic acids, aided by plant-producing chelating agents, pH changes, and redox reactions, are able to solubilize and accumulate trace elements at low levels, even from nearly insoluble precipitates [93]. It is also known that plants tolerant of metal contamination are able to segregate toxic elements in the root cortical tissue outside the endodermis, thereby preventing or reducing translocation to other parts of the plants [94]. Using vegetation to remove, detoxify, or re-stabilize polluted sites has been a widely accepted tool in developed countries for cleaning such polluted water as it regenerates the original water permanently [95].

Heavy metal accumulation varies between plant species and even among morphologically similar species growing in the same area [96]. Most of them have a toxic effect on the plant life cycle and biochemical processes. There is however a group of trace elements which are necessary for proper plant functioning. The dual role elements include zinc, copper, and nowadays nickel, which are necessary for many metabolic/biochemical processes, including enzyme activity. Hence, some amount in the environment is necessary, while an excess can result in a negative plant response, including faster senescence and lower growth. Other heavy metals, such as cadmium, chromium and lead, are non-essential and extremely toxic to plants even at low concentrations. Moreover, there has also been observed a synergistic effect of several trace elements on plants, such as Cd and Pb [97]. It is important to recognize macrophyte species with higher efficiency to tolerate or even resistant to elevated concentrations of heavy metals in the water and sediment. A plant which accumulates higher levels of the contaminant in its harvestable sections (leaves and stems) is considered as a good candidate for phytoextraction, while a species which restricts the accumulation to its roots will be useful for stabilization of the contaminated environment, reducing human health and environmental hazards by a different and protective strategy, which is called phytostabilization [98].

Several investigations have proved that many species of macrophytes revealed features of phytostabilization in their natural habitat, which is very important from a practical point of view, due to possibilities of their usefulness while avoiding depletion of a specific plant population [99]. Moreover, phytoextraction can be very worthwhile, because some species have been proved to remove and translocate to above-ground plant parts some precious metals, such as gold, under certain circumstances [99]. Knowledge about the accumulation properties of wetland plant species is useful in choosing appropriate plants for wetland phytoremediation systems. There have also been conducted investigations confirming the water cleaning abilities shown by littoral plants, which can keep heavy metals away from bank zones and can protect water against human pressure on the bank zone. Littoral plants can be used as heavy metal bioindicators and/or as buffers against the spread of heavy metals over large areas in a freshwater environment. Besides the important role of macrophytes as accumulators and cleaning functions in the case of high trace element concentrations, they can also indicate the level of water contamination even when low concentrations occur [100, 101].

Investigations concerning possibilities for use of macrophytes in removal of trace elements from the environment are widely conducted, using plants naturally grown in water ecosystems as well in constructed wetlands. There are however many doubts concerning translocation of elements in plant bodies. Studying the range of macrophyte species revealed the high possibilities of accumulation and wide range of trace element translocation among plant species. Some investigators also suggest that mobility of elements in a plant is closely related to concentration ratios between certain trace elements (Table 4). Regarding uptake and translocation issues of trace elements, it is also important to remember that this depends on physicochemical processes, such as metal solubility, water temperature, and pH. Temperature and pH may change in both a spatial and a temporal manner. Seasonal changes increase the pH and decrease the metal solubility [116].

Table 4 Plant species and their accumulation potential in plant organs for trace elements in various countries and types of wetlands

Plant species	Country	Trace elements	Organs with higher accumulation, translocation or elements mobility	Type of ecosystem	Author/s
Typha angustata L.	India	Mn, Cu, Zn, Cr, Ni, Pb	Roots	Constructed wetland	Bose et al. [102]
Phragmites australis	Belgium	Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Belowground parts, except Mn	Constructed wetlands	Lesage et al. [78]
Spartina alterniflora, *Phragmites australis*	USA	Hg, Cu, Zn, Cr, Pb	Hg, Cr higher in leaves of *S. alterniflora* Cu, ZN higher in leaves of *P. australis*	Contaminated low marsh	Windham et al. [103]
Rubus ulmifolius, *Phragmites australis*	Portugal	Zn	Roots, poor translocation	Contaminated soil	Marques et al. [98]
Schoenoplectus lacustris, *Phragmites australis*	Turkey	Pb, Cr, Cu, Mn, Ni, Zn, Cd	Root accumulation except Cr	Lake Sapanca	Duman et al. [96]
Phragmites australis	Italy	Cu, Zn, Ni, Cr	Similar or higher accumulation in leaves	Constructed wetland	Bragato et al. [49]
Phragmites australis	Italy	Cr, Cu, Fe, Mn, Ni, Pb, Zn	Except Zn, higher levels in roots	Volcanic lake	Baldantoni et al. [104]
Potamogeton pectinatus, *Myriophyllum verticllatum*	Poland	Cd, Pb, Zn	Transport to leaves	River Przemsza	Lewander et al. [105]
Phragmites australis, *Phalaris arundinacea*	Czech Republic	Cu, Cr,	Roots as a filter for trace elements	Natural and constructed wetlands	Vymazal et al. [8]
Typha latifolia, *Phragmites australis*	China	Pb, Zn	Translocation from roots to shoots	Six wetlands	Deng et al. [106]
Phragmites australis, *Potamogeton natans*, *Iris pseudoacorus*, *Phalaris arundinacea*, *Carex remota*, *Calamagrostis epigeios*	Poland	Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, Zn	Only leaves were measured. Elevated level of Cd, Co, Cr, Cu, Mn	Anthropopgenic lakes	Samecka-Cymerman and Kempers [107]

Phragmites australis	USA	Cu, Zn, Pb, Cd, Fe	Transport of Pb and Zn to aboveground parts	Constructed wetland for landfill leachate treatment	Peverly et al. [108]
Salix spp., *Carex rostrata*, *Eriophorum* sp., *Phragmites australis*	Sweden	Cd, Cu, Zn, Pb, As	Most plants keep elements in roots, except *Salix sp.*	Submerged tailings impoundment	Stoltz and Greger [109]
Typha angustifolia, *Potamogeton pectinatus*	Turkey	Cd, Pb, Cr, Ni, Zn, Cu	High translocation of all elements, however *P. pectinatus* transported much higher Zn to leaves	Natural wetland	Demirezen and Aksoy [110]
Najas marina, *Potamogeton lucens*, *Nuphar lutea*, *Potamogeton nodosus*	Slovenia	As, Ni, Pb, Cr	Pb stays in roots, Ni relatively high mobility to upper parts of N. lutea	Artificial lake	Mazej and Germ [111]
Phragmites australis	Czech Republic	Al, Fe, Mn, Ba, Zn, Cd, Hg	Small mobility of Cd, while very high mobility of Zn	Constructed wetlands for treatment of municipal wastewater	Vymazal et al. [112]
Typha latifolia	Turkey	Zn, Mn, Cr	High Zn mobility to above parts of plants	Steam carrying secondary effluent	Sasmaz et al. [113]
Phragmites australis	Italy	Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn	High accumulation in all plant organs	Mouth area of the river	Bonanno and L Giudice [114]
Phragmites australis, *Typha angustifolia*	Poland	Cd, Pb, Zn, Cu	*T. angustifolia* revealed higher Cd translocation potential than *P. australis*, Pb and Cd stayed in rhizomes, Zn mobility	Natural and artificial lakes	Drzewiecka et al. [100], Drzewiecka et al. [101], Borowiak et al. [115]

An important issue in using macrophytes in CWs is to remember about the period of acclimation to certain loads of treated wastewater, such as a feeding day with a new type of wastewater prior to starting a real dose. Low strength wastewater was provided [117]. It is important to use native plants of the contaminated site for phytoremediation because these plants adapt better in terms of survival, growth, and reproduction under environmental stresses than those introduced from another environment. There has been continuing interest in searching for native plants that are tolerant of heavy metals. *P. australis* is the most widely distributed wetland plant species throughout the world. Moreover, it is known that this species grows very well in unpolluted ecosystems, as well as in polluted ones, e.g., by heavy metals. As mentioned before, this species is widely used as a main species for constructed wetlands. Moreover, several investigations have revealed the capacity of this species for removal of many trace elements from natural water ecosystems. *P. australis* is not a hyperaccumulator; however, due its high growth ratio and high biomass production, deep root system and tolerance to higher trace element concentrations in the environment, it can be treated as a plant for reduction of metal concentration in soils, sediments, and waters in natural and constructed wetlands [49].

Several investigations confirm its role as a great accumulator and bioindicator and its removal potential for both natural water ecosystems and constructed wetlands all around the world. However, various results were obtained concerning mobility/translocation of heavy metals from below- to above-ground plant organs. This discussion concerns especially the mobility potential of Cd and Pb, while in the case of zinc most investigations indicated a high translocation possibility. Possibly this is also connected with the dual role of this element and association with the concentration of other elements in the environment, such as Cu (Table 4). Other common species in natural water ecosystems are *Typha angustifolia* and *Typha latifolia*. Both species are already also well known as successful plants used in constructed wetlands as removal plants for heavy metals, such as Pb/Zn mine tailings. These species are resistant to stress factors in the polluted environment and have the capability to accumulate heavy metals in their tissue from contaminated wastewater [118]. *Typha angustifolia* is a perennial macrophyte that has an ability to produce large amounts of biomass and can grow rapidly [119]. The investigations in natural ecosystems revealed that *Typha* spp. has the ability to extract Pb, Cd, Cr, Mn, and Fe from their water surroundings [120]. Recent investigations based on calculation of the accumulation factor and translocation factor led to the conclusion that this species would be most appropriate for use in phytostabilization [121].

The above-mentioned macrophyte species are the most widely used and investigated. However, several other species are widely used, and their capacity for removal of excessive levels of many compounds and substances is also highly evaluated. Hence, it is extremely important to keep natural water ecosystems in a good condition in order to maintain the state of our environment and health, as well as for their esthetic values.

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Plants for Constructed Wetlands as an Ecological Engineering Alternative to Road Runoff Desalination

Ana de Santiago-Martín, Gaëlle Guesdon, and Rosa Galvez

Abstract De-icing salt and snow pollution in urban and road areas is a growing threat seriously menacing the ecosystem goods and services provided by soils, rivers, wetlands, and lakes in the world. Up to 90 % of de-icing salt used for winter road maintenance (salt spreading and storage sites) can be transported, together with co-pollutants, e.g. metals, from tens to hundreds of metres from roads reaching soils, and both surface and ground water. Within ecological engineering, there are several strategies to reduce the impact of road de-icing salts once they are in the environment. Among them, constructed wetlands (CWs) have proved to be techno-economically feasible, energy efficient, and a green strategy. This chapter provides extensive information on the use of macrophytes in CWs for de-icing salt removal and presents: (a) an overview of phytoremediation in CWs and a summary of the full-scale facilities specifically conducted to road runoff treatment; (b) a compendium of studies focused on salt removal with macrophytes in greenhouses and those aimed at assessing macrophyte response to salinity in combination with other stressors (waterlogging, water depth, storm events, temperature, competitive interactions, nutrients, pollutants, and so on); and (c) a case study on treatment of runoff from an urban snow disposal site with *Scirpus maritimus* and *Spartina pectinata*.

Keywords De-icing salts • Road runoff water • Snow disposal site • Phytoremediation • Macrophytes • Constructed wetland • Ecological engineering

Abbreviations

ABA	Abscisic acid
ANOVA	One-way analysis of variance
BOD	Biochemical oxygen demand

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chl	Chlorophyll
COD	Chemical oxygen demand
CW	Constructed wetland
DO	Dissolved oxygen
EC	Electrical conductivity
FB	Filter bed
FEAS	Flame emission atomic spectroscopy
HPLC	High performance liquid chromatography
NRE	Negative removal efficiency
OASTL	O-acetylserine (thiol) lyase activity
OM	Organic matter
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
ppt	Parts per thousand (‰)
SLA	Specific leaf area
SRP	Soluble reactive phosphorus
SS	Suspended solids
TI	Tolerance index

1 Introduction

Anthropogenic salinity and urban (and peri-urban) snow pollution are growing threats seriously menacing the ecosystem goods and services provided by soils, rivers, wetlands, and lakes in the world. The application of NaCl as de-icer agent during winter road maintenance has become a regular practice in northern countries. De-icing salts are used to manage road networks by governments, authorities, and/or municipalities for keeping winter road conditions safe. The amount of de-icing salts used in roads varies largely around the world, but is at a minimum several hundred thousands of tons per year [1]. Table 1 shows some examples of the amounts of de-icing salts spread on roads. The countries with cold climates advertise a clear concern of their environmental impact. For example, Canada in 1999

Table 1 Amount of de-icing salts spread in roads

Amount of de-icing salts	Area	References
5,000,000 tonnes per year	Canada	[16]
100 000 tonnes (corresponding to 200 g/m^2)	Greater Toronto Area, Canada	[41]
24 000 tonnes in 2009–2010	Central part of Shenyang, China	[23]
10–14 tonnes/km	Southern and central Sweden, Sweden	[25]
2,000,000 tonnes in 2010 (0,75 tonne/km)	France	[1]
270,000 tonnes (corresponding to 3060 g/m^2)	Moscow, Russia	[13]

included road de-icing salts in the list of priority substances whose toxicity needs evaluation: “road salts that contain inorganic chloride salts with or without ferrocyanide salts are ‘toxic’ as defined in Section 64 of the *Canadian Environmental Protection Act, 1999*” [2]. In Europe, several countries, e.g. Finland, Norway, Sweden, Switzerland, and Germany have included the regulation of de-icing salts in their environmental law.

Urban and peri-urban snow pollution is known in various countries with cold climates, e.g. Northern and Eastern Europe, Canada, and Russia [3–5], and has been studied as road pollution for the past several years. Urban snow is essentially composed of a package of pollutants including suspended solids, most heavy metals, phosphorous (P), polycyclic aromatic hydrocarbons (PAHs), and de-icing salts containing chloride (Cl) and sodium (Na) [5–9]. The spread of de-icing salts degrades the urban snow quality and enhances the release of pollutants (such as metals) which increases the pollution of soil, and ground and surface water. In fact, the increase on soil metal concentrations is often correlated to electrical conductivity and Cl amounts in roadside soils [10, 11].

Overall, de-icing salts and urban snow represent three types of pollution sources, including: (1) spreading on roads, (2) salt storage sites, and (3) snow disposal sites. First, as mentioned above, the road environment is directly impacted by the use of de-icing salts. Up to 90 % of de-icing salt used for winter road maintenance may be transported by air and deposited from tens to hundreds of metres from roads [12]. Moreover, in spring the snowmelt causes runoff pollution reaching soils, surface water, and groundwater [13]. Second, de-icing salt storage sites are also likely to contribute significantly [14]; the main concerns relate to the pollution of groundwater and discharges to surface waters [15]. The loss of salts is essentially produced during salt handling (loading and unloading trucks) but also from salt piles—*Environment Canada* recommends protecting the piles from weathering and improving the management of washing water [16]. Third, in order to manage road pollution of dirty snow, and to respect legislation, urban snow disposal sites have been created to store dirty snow (Fig. 1). Unfortunately, snow disposal sites become also pollution sources. A study showed that 50 % of most pollutants in the snow (including metals) are dissolved and removed with runoff from urban snow disposal sites [17]. The higher concentrations of metals in the melt water often correspond to the peak of Cl concentrations [18].

The impact of de-icing salts in soils has been studied by several authors. Soil Na and Cl concentrations linked with de-icing salt operations have been measured, values ranging from 16 to 513 mg kg^{-1} (Na) and from 8 to 2353 mg kg^{-1} (Cl) [19–23]. De-icing salts in soils affect: (a) important biogeochemical cycles (C, N, and P); (b) soil properties (aggregate stabilization, organic matter dispersion, and infiltration); (c) soil ecology, favouring pedofaune and floremore salt tolerant; and (d) sorption–desorption processes, increasing Cd, Cr, Cu, Fe, Ni, and Pb concentrations in leachates by a number of mechanisms such as ion ex-change, lowering the pH, and Cl complexation [10, 23–27]. In the urban environment, de-icing salt soil pollution directly impacts roadside vegetation. In a study of *Tilia x vulgaris*, along a main street in Latvia [28], the authors observed a decrease in the Na and Cl concentrations

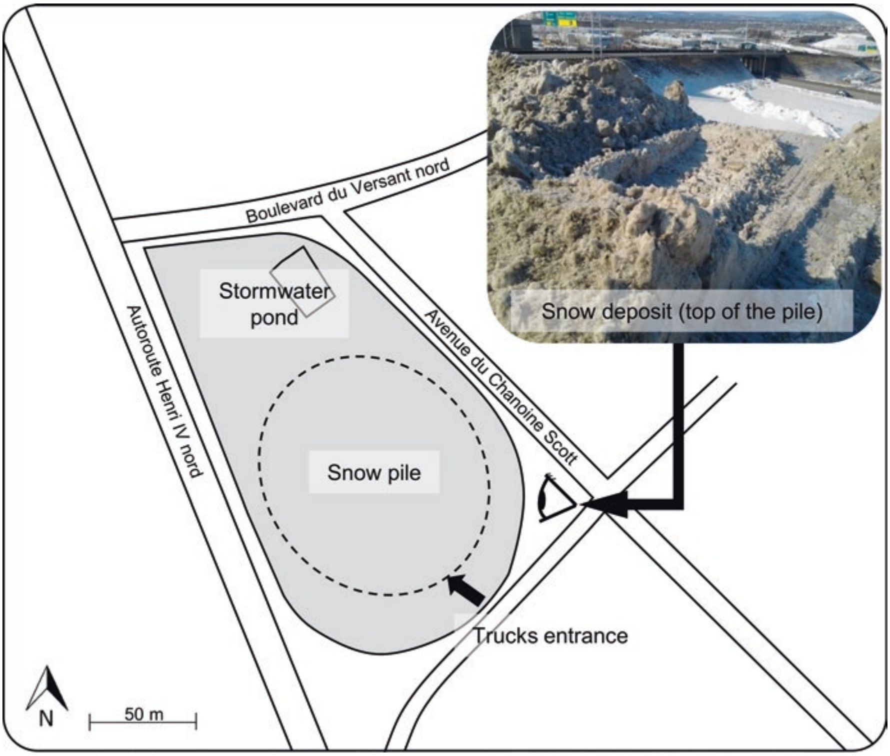

Fig. 1 Scheme and picture of a typical snow disposal site, Quebec City

in soils, in Spring, due to leaching and percolation processes as well as to plant uptake. The study shows that in summer there was a highly significant and positive correlation between the extent of leaf necrosis and the Na and Cl concentrations in leaves. In a similar way, the negative effect of de-icing salts on roadside trees was also reported by Czerniawska-Kusza et al. [21]. At levels of 13.2 (Na) and 3.9 (Cl) mg per 100 g of soil, the authors observed: (a) increasing Na and Cl amounts in tree leaves, (b) salt injury symptoms (chlorosis and necrosis of the edge of leaf blades), and (c) nutrient imbalance (reduction on K, Ca, and Mg in leaves).

De-icing salts can reach both surface and ground waters [22, 29, 30]. Thus, Cl concentrations can reach up to 2 g L^{-1} in highway runoff [31]. Kaushal et al. [32] demonstrated that Cl concentrations in freshwater increase as a function of imperme-ability of surface and can exceed tolerance for freshwater life in suburban and urban watersheds. Similar conclusions were obtained by Novotny et al. [33]: Cl concentra-tions in individual lakes were positively correlated with the percent of impervious surfaces in the watershed and inversely with lake volume. Some studies have shown the continuing degradation of the water quality by the increased human settlements due to the application of de-icing salts. Such is the case of St. Augustin Lake (Quebec, Canada) which has registered an increase on its electrical conductivity from 250 to

1385 μS cm^{-1} over the past 40 years, coincident with the use of road de-icing salts on Félix-Leclerc highway that passes through the lake catchment area [34]. De-icing salts can affect urban lake stratification with significant consequences for the lake's water quality and ecology, as stressed by Novotny and Stefan [35]. These authors showed that: (a) NaCl concentration in several lakes in Minnesota (USA) was correlated with the use of road de-icing salts; (b) the chemocline eroded quickly after ice-out and the saline benthic layer completely mixed with the water column; (c) the saline layer eliminated and delayed the transport of oxygen to the benthic lake waters during the spring and fall turnover periods. According to Perera et al. [36], 40 % of the Cl spread during winter road management enters in ground water although percentages can vary as a function of the watershed and the land use. In urban area, Howard and Janet [37] and Howard and Beck [38] reported that up to 45 % of the spread Cl reached groundwater in Toronto, with concentrations up to 2.8 g Cl L^{-1} in spring. High positive correlations between urbanization and groundwater contamination by Cl contained in road salts were also reported in the south of Toronto [39]. This increase in Cl concentrations in groundwater intensifies the chronic toxicity effect to aquatic ecosystem and to human health through drinking water [40]. As described by Howard and Maier [41], urban development contributes to the possible deterioration of groundwater quality in shallow aquifers with Cl concentrations approaching the drinking water standard of Canadian law, 250 mg L^{-1} [42].

There are several strategies aiming to reduce the impact of road de-icing salts in the environment [43]. Among them, constructed wetlands (CWs) have proved to be techno-economically feasible, energy efficient, and a green strategy. The main goal of this chapter is to provide recent advances on the use of macrophytes in CWs for de-icing salt removal from road and/or urban snow disposal runoff. The chapter presents: (a) an overview of phytoremediation in CWs and a summary of the works specific to salted road runoff treatment; (b) a compendium of studies focused on salt removal with macrophytes and those aimed at assessing the macrophyte response to salinity in combination with other stressors; and (c) a case study on treatment of road runoff from an urban snow disposal site.

2 Ecological Engineering Facilities for Road Runoff Phytoremediation

2.1 General Aspects of Constructed Wetlands

Ecological engineering is defined as the design of sustainable ecosystems that integrate human society with its natural environment for the benefit of both [44]. Ecosystems are used, imitated, copied, or modified to solve or reduce a pollution problem in an ecological manner, to manage ecosystems, and to develop eco-technologies [45, 46]. Within ecological engineering, CWs have proven to be a green, low cost, and efficient means of treating polluted water. Innovative water management strategies should be aimed at promoting ecosystem services in line with sustainability development [47–49]. In this way, the ecosystem services provided by wetlands are manifold including: (a) provisioning (water, timber, genetic resources); (b) regulating (air and water quality regulation and purification, pollination); (c) habitat conditioning (nutrient cycling, primary productivity); and (d) cultural services (aesthetics, ecotourism) [50].

Overall, CWs can be classified as surface flow (free water surface) or subsurface flow (vertical downflow or upflow, horizontal, or hybrid), both built with a natural or constructed underground barrier to limit leakage [51]. Pollution in CWs is removed by the same processes which are common in natural wetlands but under organized conditions [52]. The physiological action of plants will lead to different processes [53]: (1) Phytoextraction by metal-tolerant plants or hyperaccumulators; (2) Phytodegradation by plants capable of enzymatic breakdown of compounds; (3) Phytostabilization by plants immobilizing pollutants in the soil; (4) Rhizofiltration by plant root systems that intercept or degrade pollutants; and (5) Phytovolatilization by plants releasing pollutants into the atmosphere. Several plant species can be used [54]: (1) Emergent species (*Typha* spp., *Phragmites* spp., *Schoenoplectus* spp., *Carex* spp., *Hydrocotyle* spp., *Scirpus* spp., *Cyperus* spp.); (2) Submerged species (*Ceratophyllum demersum*, *Elodea* spp., *Potamogeton* spp., *Myriophyllum* spp., *Valisneria americana*); and/or (3) Floating species (*Lemna* spp., *Spirodela* spp., *Echhornia* spp., *Pistia stratiotes*, *Salvinia* spp.). Plants should ideally have one or more of the following characteristics: tolerant to high levels of the target pollutant, accumulate reasonably high levels of the target pollutant, rapid growth rate, produce reasonably high biomass in the field, and profuse root system [55].

Despite CW's numerous benefits like small ecological footprint, simple technology, and aesthetic value [56], the disadvantages cannot be neglected: (a) plants can oxidize sediments making pollutants, such as metals, more (bio)available; and (b) pollutants translocated to aboveground tissues can be excreted, with decaying litter acting as a source pollutant rather than a sink [57]. To address these limitations, further studies with different candidate plants for each specific pollution context are required. The most common macrophytes studied in CWs are: *Juncus effusus*, *Phalaris arundinacea*, *Phragmites australis*, *Phragmites communis*, *Scirpus sylvaticus*, *Scirpus validus*, *Typha angustifolia*, and *Typha latifolia* [58–68]. Most studies focused on wastewater, aquaculture, or storm water treatment by a number of mechanisms to remove N (volatilization, ammonification, (de-)nitrification, N_2 fixation, plant/microbial uptake, etc.) and P (soil accretion, adsorption, precipitation, plant/microbial uptake, etc.) [69], with less work focused on road runoff.

2.2 Road Runoff Phytoremediation in Constructed Wetlands

Studies aimed at road de-icing salt removal are remarkably scarce [70–73] and those targeted on road runoff are generally focused on metals and PAHs [63, 74–82]. Table 2 summarizes some studies carried out in full-scale CWs, as discussed below.

Table 2 Summary of field studies on constructed wetlands with macrophytes for road runoff treatment

Country	Vegetal specie(s)	Full-scale system characteristics	Main parameters monitored	Reference
Canada	*Atriplex patula, Echinochloa crus-galli, Eleocharis palustris, Sagittaria latifolia, Salicornia europaea, Spergularia canadensis, Typha angustifolia*	Free water surface-flow CW (filled with black earth soil, compost, and sand) in parallel to an active underground filter bed (calcite as reactive media), and preceded by a water detention basin	(1) Influent and effluent water: Na, Cl, Ca, trace metals (Cd, Cr, Cu, Ni, Pb, and Zn), pH, EC, turbidity, SS, redox potential, SRP, COD, OD, and total and faecal coliforms	[70–73]
			(2) Substrate (0–15 cm—depth cores): pseudototal Na, Ca, and trace metals, Cl and pH in pore water, OM, and P Olsen	
			(3) Plants: Na, Cl, Ca, and trace metals in leaves, shoots, and underground tissues	
England	*Glyceria maxima*	Oil separator and sediment trap with grass slope leading to a CW (filled with chalky clay with flints or river gravels and fen peats) with plants on benches of different depths	Influent and effluent water and in sediment trap: Cu, Fe, and Zn (size-fractions), SS, BOD, COD, redox potential, DO, and pH	[75, 77, 78]
England	*Phragmites australis, Typha latifolia*	Horizontal subsurface-flow CW containing a gravel substrate and preceded by a small settling pond, an oil separator, a silt trap, and a grass filter	Influent and effluent water: Cd, Cr, Cu, Ni, Pb, Zn, SS, BOD, DO, pH, EC, NO_3–N, SO_4–S, and PO_4–P	[76, 79, 80]

(continued)

Table 2 (continued)

Country	Vegetal specie(s)	Full-scale system characteristics	Main parameters monitored	Reference
England	*Phragmites australis, Typha latifolia*	Horizontal subsurface flow system under normal conditions and surface flow system in storm conditions within a CW. Also an adjacent area of natural wetland surface flow system	(1) Influent and effluent water and in sediments: Cd, Cr, Cu, Ni, Pb, and Zn concentrations	[74]
			(2) Plants: Cd, Cr, Cu, Ni, Pb, and Zn in leaf, stems, rhizome, and root tissues	
Greece	*Arundo donax, Phragmites australis*	Two free water surface and two subsurface-flow pilot-size CWs filled with different gravel media	Influent and effluent water: COD, SS, pH, EC, N, NO_3–N, P, Cu, Ni, Pb, Zn, PAHs (16 priority)	[81]
Scotland	*Phragmites australis*	Vertical-flow CW filters located outdoors and filled with large gravel and stones, Filtralite, Frogmat, small and medium gravel, sand, and/or water	(1) Influent and effluent water: Cu, Ni, SS, BOD, NO_3–N, NH_4–N, PO_4–P, and pH	[60]
			(2) Plants: Cu and Ni in aboveground tissues	
The Netherlands	*Phragmites australis*	Vertical-flow CW filled with gravel, root cloth and sand, and preceded by a water detention basin	(1) Runoff and influent water:Cl	[82]
			(2) Runoff, influent, and effluent water: Cd, Cr, Cu, Ni, Pb, Zn (particulate and dissolved), and PAHs (16 priority)	

CW = constructed wetland; SS = suspended solids; SRP = soluble reactive phosphorus; BOD = biochemical oxygen demand; COD = chemical oxygen demand; DO = dissolved oxygen; EC = electrical conductivity; OM = organic matter; PAHs = polycyclic aromatic hydrocarbons

In Quebec, with the aim of treating de-icing salted runoff from Félix-Leclerc highway that is intensely polluting St. Augustin Lake [34, 83], a free water surface-flow CW in parallel to an active underground filter bed (FB) (with calcite as reactive media) and preceded by a water detention basin was constructed (Table 2) [70–73]. The authors demonstrated that: (1) the system can successfully remove high NaCl concentrations from the influent road runoff (up to 85 % NaCl during the first 2 years of operation, besides several metals); and (2) both the CW (substrate and macrophytes disposed in multi-culture planting) and the FB played an important role. As previously reported, lower removal rates were observed in storm events; the order of Na uptake by plants from the highest to the lowest was as follows: *Echinochloa crus-galli* > *T. angustifolia* > *Eleocharis palustris* ≫ *Sagittaria latifolia* ≈ *Atriplex patula* ≫ *Spergularia canadensis* = *Salicornia europaea*.

Overall, studies show that a range of PAHs and metals (Cd, Cr, Cu, Ni, Pb, and Zn) can be successfully removed from road runoff by wetland processes. However, some limiting factors still need to be addressed: de-icing salts (the case at hand), storm events, and the controversial role of plants, among others. Thus, Tromp et al. [82], studying PAHs and metal removal from road runoff in a facility consisting of a detention basin, a vertical-flow reed bed (*P. australis*), and a final groundwater infiltration bed, reported that most metals (Cd, Cu, Ni, and Zn) showed a high increase in effluents during applications of de-icing salts. Noteworthy is that the authors reported PAHs retention efficiencies of 90–95 %. In the same way, higher metal (Cu, Pb, and Zn) concentrations in effluents were also observed by Revitt et al. [80] during April storms in a horizontal subsurface-flow CW planted with *P. australis* and *T. latifolia*. The authors associated this metal increase with the elevated Cl concentrations accumulated in the CW during winter de-icing activities being released by the increased flows of storms. However, despite the proven ability of de-icing salts to displace metals [23], there are dramatic lacks of studies considering de-icing salt removal.

Another major challenge in the design of CWs for road runoff treatment is the variability in the water quality and quantity during storm events [77] as well as between dry and wet weather seasons [74, 76]. That is why most of the full-scale versions of these systems are equipped with mechanical pre-treatments including: water detention basin, sediment trap, settling ponds, oil separator, and/or grass filter. Terzakis et al. [81] reported a 2-year ΣPAHs removal efficiency of 59 % and observed a better performance in subsurface flow than in free water surface CWs. The authors also showed that Cu, Ni, and Pb were not efficiently removed by the studied systems; this was attributed to the flushing of sediments following a storm event. Some authors have shown the controversial role of macrophytes [84]. As seen in Table 2, the bulk of the studies have been carried out with *P. australis*, followed by *T. latifolia.* However, Lee and Scholz [63] reported higher effluent Ni concentrations from the planted filters with *P. australis* than from unplanted ones which was attributed to the decrease in pH produced by plants (respiration, litter decomposition, nitrification), providing undesirable conditions for Ni precipitation. So, further studies with a larger variety of candidate plants are required to optimize the CW performance.

3 Plants for Salinity Phytoremediation

3.1 Salt Removal Efficiency by Macrophytes

Plant species differ in their sensitivity and behaviour (accumulative or excluder) to pollution and may be directly helpful in removing pollutants [85]. In order to improve the efficiency in removing de-icing salts from road and urban snow runoff, studies involving NaCl removal with a variety of candidate macrophytes in a range of salt concentrations should be explored. Table 3 summarizes some studies focused on Na and/or Cl removal in pilot-scale CWs or pots under greenhouse conditions, as discussed below.

With the aim of de-icing salt removal from road runoff, Morteau et al. [86, 87] exposed some pot cultured plants to a range of NaCl concentrations. Based on Cl uptake, the rank of plants from the highest to the lowest was as follows: *T. latifolia* ≫ *S.europaea* > *A.patula* > *S. canadensis*. Nonetheless, the authors reported that *T. latifolia* and *A. patula* were the most interesting plants due to their higher biomass and therefore total Cl removal. Thereafter, Morteau et al. [88] showed that the performance of NaCl removal by *A. patula* and *T. angustifolia* under hydroponic culture increased up to fourfold (case of *T. angustifolia*) when a nutrient supply was added (Hoagland's solution). The authors also studied a complementary halophyte, *Ligusticum scothicum*, for potential further planting in the full-scale CW located in Quebec [89]. According to previous works [90], plants were cultured under greenhouse conditions (monoculture) and exposed to a level of NaCl corresponding to that measured in the runoff near Félix-Leclerc highway (3.2 dS m^{-1}). After 32 days of greenhouse experience, the aboveground parts of the plants were harvested and Cl and Na concentrations were quantified. Results showed, on average, 191 mg Cl g^{-1} and 118 mg Na g^{-1}. Recently, Guesdon et al. [91] performed a study in pilot-scale subsurface horizontal-flow CWs for treating a range of de-icing salt solutions (from 0.2 to 13 dS m^{-1}) provided by Ministry of Transport of Quebec. Based on the NaCl removal rate, the authors concluded that *T. angustifolia* and *Juncus maritimus* were the plants that performed best (removing 31–60 % and 22–36 %, respectively). *E. palustris* was not recommended because plant growth was significantly affected at high de-icing salt levels (>8 dS m^{-1}).

Other studies have been conducted with macrophytes with the aim of Na and/or Cl removal though not in the context of treatment of road runoff (Table 3). Thus, Lymbery et al. [92], studying the treatment of salt-enriched aquaculture effluents in pilot-scale subsurface horizontal-flow CWs with *Juncus kraussii*, recorded up to 54.8 % of NaCl removal efficiency. Nonetheless, plants were adversely affected by salinity concentration. Rozema [93] assessed the viability of macrophytes to remove Na and Cl from a recycled greenhouse nutrient solution. The authors showed that *Juncus torreyi*, *Schoenoplectus tabernaemontani*, *T. angustifolia*, and *T. latifolia* are good candidates for phytodesalinization, based on the Na and Cl uptake. Additionally, they observed that the Na and Cl uptake by *J. torreyi* and *T. latifolia* was increased up to two times after harvesting of plants due to biomass production increase. This effect was not observed in *S. tabernaemontani*.

Table 3 Summary of greenhouse studies on Na and/or Cl removal with macrophytes

Objective	Vegetal specie(s)	Experimental greenhouse conditions	Main parameters monitored	Reference
Treatment of salted road runoff	*Atriplex patula, Salicornia europaea, Spergularia canadensis*	Pot culture (filled with natural soils)	Plants: Na and Cl, height, biomass, and water consumption in above- and underground tissues, and plant survival	[86]
		Salt solutions added: 0, 10, 100, or 1000 mg L^{-1} Cl		
Treatment of salted road runoff	*Atriplex patula, Typha latifolia, Spergularia canadensis*	Germination in incubator and pot culture (filled with silica sand)	Plants: germination rates, Cl uptake, and biomass	[87]
		Salt solutions added: 0, 150, 1500, 10,000, or 20,000 mg L^{-1} NaCl with Hoagland's solution		
Treatment of salted road runoff	*Atriplex patula, Typha angustifolia*	Hydroponic culture	Plants: Na and Cl uptake, biomass, stem and root elongation. Removal kinetics (0 to 180 h) were calculated	[88]
		Salt solutions added: 0, 150, or 1500 mg L^{-1} NaCl with or without Hoagland's solution		
Treatment of salted road runoff	*Ligusticum scothicum*	Pilot-scale subsurface horizontal-flow CWs (filled with organic substrate)	(1) Influent and effluent water: pH, EC, Cl, and Na	[89]
		NaCl solution added: 3.2 dS m^{-1}	(2) Plants: Na and Cl in above- and underground tissues, and biomass	
Treatment of salted road runoff	*Eleocharis palustris, Juncus maritimus, Typha angustifolia*	Pilot-scale subsurface horizontal-flow CWs (filled with black earth, composted manure, sand, and lime)	(1) Influent and effluent water: pH, EC, Cl, and Na	[91]
		De-icing salt solutions added: 0.2, 0.7, 4, 8, or 13 dS m^{-1}	(2) Plants: Na and Cl in above- and underground tissues, height, biomass, and water consumption	
Treatment of saline aquaculture effluents	*Juncus kraussii*	Pilot-scale subsurface horizontal-flow CWs (filled with soil)	(1) Influent and effluent water: NaCl, N, and P	[92]
		Rainbow trout aquaculture effluents added: 24,030, 24,800, 6600, or 7530 mg L^{-1} NaCl (+ N and P)	(2) Plants: length, and number of fronds	

(continued)

Table 3 (continued)

Objective	Vegetal specie(s)	Experimental greenhouse conditions	Main parameters monitored	Reference
Treatment of greenhouse nutrient solution	*Atriplex prostrata, Distichlis spicata, Juncus torreyi, Phragmites australis, Schoenoplectus tabernaemontani, Spartina alterniflora, Typha angustifolia, Typha latifolia*	Exp. (1) Removal and harvesting: Pot culture (filled with granite stone) Salt solution added: ~8 mmol L^{-1} NaCl (+NPK) Exp. (2) Concentration effect: Hydroponic culture in greenhouse Salt solutions added: 2, 4, 8, or 16 mmol L^{-1} NaCl (+NPK solution)	Plants: Na and Cl uptake, and biomass in aboveground tissues	[93]
Treatment of saline synthetic reactive dye wastewater	*Typha angustifolia*	Synthetic reactive dye wastewater added: 0, 10, 20, 30, 100, 200, or 300 mg L^{-1}	Plants: Na uptake, colour removal, survival, symptom appearance, and growth (height, biomass, number of leaves, shoots, and roots)	[94]
Treatment of saline winery wastewater	*Juncus acutus, Pennisetum clandestinum, Scirpus maritimus, Typha latifolia, Vetiveria zizanioides*	Pot culture (filled with coarse sand) Artificial winery wastewater added: 0, 16, 40, 162, or 400 μM L^{-1} Na with 0, 5000, 10,000, 15,000, or 20,000 mg L^{-1} COD	Plants: Na, Ca, Mg, N, P, and K uptake, and growth in above- and underground tissues, and survival	[95]
Treatment of saline wastewater	*Bassia indica*	Exp. (1) Hydroponic culture with 1, 5, 8, or 16 dS m^{-1} with Ling Ashton nutrient solution	(1) Influent and effluent water: EC, Na, K, Ca, Mg, Fe, P, and S	[96]
		Exp. (2) (Recirculating) vertical flow CW with domestic wastewater	(2) Plants: Na, Ca, and K in branch, leaf, and root tissues, biomass, basal stem diameter, shoot height	

COD = chemical oxygen demand; EC = electrical conductivity

Nilratnisakorn et al. [94] reported that the maximum Na removal by *T. angustifolia* was ~44 % in a study aiming to treat saline reactive dye wastewater. The accumulation of Na observed in roots suggested that plants have an avoidance mechanism to prevent damage to the stem, and to maintain a balance of water potential and osmotic pressure. Another study on treatment of saline wastewater was performed by Zingelwa and Wooldridge [95] with *Juncus acutus*, *Pennisetum clandestinum*, *Scirpus maritimus*, *T. latifolia*, and *Vetiveria zizanioides*. Results indicated that *J. acutus* (767 mg Na uptake per m^2) is potentially a better alternative to *T. latifolia* for use in wetlands, and even more suitable than *S. maritimus*. The authors concluded that in order to maximize Na uptake in harvestable plant components, *J. acutus* should be planted in wetlands, and *P. clandestinum* in pastures (removing 1427 mg Na per plant). Also with the aim of treating saline wastewater, Shelef et al. [96] tested the phytodesalinization capacity of *Bassia indica* in a pilot-scale vertical flow CW which showed toleration within a wide range of salinities and removal of up to 60 % of salt.

3.2 Macrophyte Response to Salinity

Salt effect (including osmotic, ion-specific, influx rate, and duration factors) on plant growth and survival of macrophytes can be very different when is combined with other stressors usually found in the field: water logging, water depth, hypoxia, storm events, temperature, light, intra and interspecific competitive interactions, nutrients, and pollutants (as metals), among others. These stressors should be taken into account when selecting macrophytes and designing the CW. Table 4 summarizes some studies focused on macrophyte toxicity and/or tolerance to salinity when considering most of these stressors.

Most studies have been conducted with *Juncus* species. Boscaiu et al. [97, 98] studied the response to salt stress (0–500 mM NaCl) of three halophytes (*J. acutus, J. maritimus*, and *Plantago crassifolia*). Seed germination of the two Juncus species was optimal under non-saline conditions, reduced by moderate salt concentrations (200 mM NaCl, <50 % germination), and completely inhibited above 300 mM NaCl (10 % germination). As for plant growth NaCl inhibited growth in a concentration-dependent manner. Both species survived at 400–500 mM NaCl, but optimum was registered in the absence of salt. Increasing NaCl concentrations increased Na accumulation in leaves with respect to controls (20-fold increase in *J. acutus* at 500 mM NaCl). The maximum Na accumulation in *J. maritimus* was at 300 mM NaCl. A recovery test was also performed in which seeds that did not germinate in previous salt tests were then germinated in distilled water.

Results showed that Juncus seeds previously exposed to NaCl germinated even better than control seeds with water, being more accentuated in *J. maritimus* than in *J. acutus*. *P. crassifolia* seeds were the most sensitive, germination being highly or completely inhibited at 100 and ≥200 mM NaCl, respectively. In subsequent studies, Boscaiu et al. [99] observed that salt treatments led to proline accumulation in

Table 4 Summary of greenhouse or growth chamber studies on macrophyte toxicity and/or tolerance to salinity

Vegetal specie(s)	Used concentrations and growth medium	Main parameters monitored	Reference
Juncus acutus, Juncus maritimus, Plantago crassifolia	0, 100, 200, 300, 400, or 500 mM NaCl. Medium: peat, coconut fibre, and sand for pots	Plants: Na, K, Ca, and Mg in leaf tissues, plant growth, and seed germination capacity	[97, 98]
Juncus acutus, Juncus maritimus	0, 75, 150, or 300 mM NaCl. Medium: peat and vermiculite	(1) Plants: length, biomass, and proline content	[99]
		(2) Medium: Na, Cl, EC, pH, and particle size	
Juncus kraussii	0, 10, 20, 40, 60, 80, 100, or 110 % seawater for germination; 0.2, 10, 30, 50, or 70 % seawater for pots at two levels of soil moisture (drained or flooded). Medium: sand and potting soil for pots	Plants: Na, Cl, K, Ca, Mg, proline in root, rhizome, and shoots tissues, water potential, leaf gas exchange, chl fluorescence, seed germination rate	[100]
Juncus acutus, Juncus kraussii, Phragmites australis	0, 5, 10, 15, 20, 25, or 30 ppt artificial seawater at 10–25 °C or 15–30 °C	Plants: seed germination %, recovery, and speed index	[101]
Juncus acutus, Juncus maritimus	0, 100, 200, 300, or 400 mM NaCl at various light (10–14 or 12–12 photoperiod) and temperatures (10/20 °C, 15/25 °C, or 20/30 °C)	Plants: seed germination %, time, speed, and rate	[102]
Juncus acutus, Juncus kraussii	0, 5, 10, or 20 ppt salinity + NPK. Medium: sand, loam soil, and coconut fibre with: (a) 1, 2, 3, or 6 plants (intraspecific competition); or (b) mono or mixed culture (interspecific competition)	Plants: height, and root and shoot biomass	[103]
Alisma triviale, Glyceria grandis, Scirpus validus, Sium suave, Typha angustifolia	0, 100, 250, 500, or 1000 mg L^{-1} NaCl. Medium: steam-sterilized clay loam soil to kill all weed seeds (interspecific competition)	(1) Plants: total biomass	[104]
		(2) Soil water: Na, Al, Si, P, K, Fe, Sr, Ba, Ca, Mg, Mn, Cl, F, NO_2–N, NO_3–N, Br, PO_4– P, and SO_4– S	

(continued)

Table 4 (continued)

Vegetal specie(s)	Used concentrations and growth medium	Main parameters monitored	Reference
Distichlis spicata, Juncus gerardii, Limonium nashii, Potentilla anserina, Salicornia europaea, Scirpus robustus, Solidago sempervirens, Spartina patens, Typha angustifolia	0, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 ppt salt. Medium: soil (interspecific competition)	Plants: aboveground biomass	[105]
Arthrocnemum macrostachyum, Juncus acutus, Schoenus nigricans	0, 1, 2, 3, 4, or 5 % of NaCl, $MgCl_2$, $MgSO_4$, or Na_2SO_4	Plants: seed germination % and time	[106]
Phragmites australis	0, 5, 12, 20 g L^{-1} NaCl and/or 0, 8.1, 19.4, 32.4 g L^{-1} Na_2SO_4 + NPK + $FeSO_4$ at 4 levels of water potential (−0.09, −0.50, −1.09, or −1.74 MPa). Medium: commercial peat substrate	Plants: Na, K, Ca, Mg, and S in leaf and root tissues, CO_2 exchange, stomatal conductance, transpiration, osmolality, chlorinity, and proline in leaves, above- and underground biomass	[107]
Phragmites australis	0, 50, 100, or 200 mM NaCl (hypoxia). Hydroponic culture	Plants: Na, Cl, K, Ca, and Mg in leaf, shoot, and root tissues, biomass, chlorophyll content, photosynthetic measurements, and water relations	[108]
Phragmites australis, Typha latifolia	0–300 mM Cd + 100 mM NaCl + exogenous ABA (0.1–10 mM) with Hoagland's solution. Medium: vermiculite	Plants: protein and ABA content, O-acetylserine (thiol) lyase activity	[109]
Eleocharis palustris, Panicum hemitomon, Sagittaria lancifolia, Scirpus americanus	0, 6, or 12 g L^{-1} salinity; 3 days or 3 weeks of influx rate; and 1, 2, or 3 months of duration of salinity exposure. Medium: commercial potting soil	Plants: above- and underground biomass, number and height of stems and leaves, mortality, and plant height	[110, 111]
Distichlis spicata, Phragmites australis, Schoenoplectus californicus, Schoenoplectus robustus	0, 10, or 25 g L^{-1} salinity at 1 or 10 cm depth water (*D. spicata, S. robustus*); 0, 4, or 10 g L^{-1} salinity at 1 or 20 cm depth water (*P. australis*, *S. californicus*). Medium: potting soil + sand	(1) Plants: mortality, steam number and height, and above- and underground biomass (2) Medium: salinity, pH, and interstitial water extraction (S^{2-}, NH_4− N, PO_4− P)	[112]

ppt = parts per thousand (‰); ABA = abscisic acid; chl = chlorophyll; SLA = specific leaf area both *Juncus* species, especially in *J. maritimus*. No direct correlation between salt concentration and proline levels was established, but seasonal variations were detected (increased proline contents under water deficit conditions). Similarly, Naidoo and Kift [100] studying the effect of salinity (0–110 % seawater) and waterlogging stresses on morphological and physiological adaptations of *J. kraussii*, observed that proline concentration in roots and culms increased with salinity and was considerably higher under drained than flooded conditions. The authors also reported that total biomass, as well as the number and height of culms, decreased with increasing salinity, while Cl and Na contents in culms increased. The study demonstrated that *J. kraussii* can tolerate and grow at salinities up to 70 % under both flooded and drained conditions but that higher salinities reduce seed germination, photosynthetic performance, and biomass development.

Temperature can be an important stressor. Greenwood and MacFarlane [101] studied the interaction between salinity (0–30 ppt) and temperature (10–25 °C or 15–30 °C) on germination characteristics of *J. acutus, J. kraussii*, and *P. australis*. Results showed that increased salinity decreases germination in all species but that only *P. australis* was highly influenced by temperature (~2 % in the highest salinity treatment and temperature regime). In a similar way, Mesleard et al. [102] evaluated the effect of salt (0–400 mM NaCl), temperature (10/20 °C, 15/25 °C, or 20/30 °C), and photoperiod (10–14 or 12–12 h) on *J. acutus* and *J. maritimus* seed germination capacity. Salt treatments slowed down and decreased the germination process but spring temperatures had a positive effect, attributed to a capacity of plants to withstand salty conditions which could be decisive in the ability of both species to colonize saline environments. The effect of competitive interactions on growth of Juncus species (*J. acutus* and *J. kraussii*) under a range of salinity (0–20 ppt) was tested by Greenwood and MacFarlane [103]. The authors observed that interspecific interactions were dependent on relative salinity tolerance of each species as follows: (a) in freshwater the co-presence of *J. kraussii* facilitated the growth of *J. acutus*; (b) at 5 ppt *J. acutus* reduced biomass of *J. kraussii*; and (c) at 10 ppt *J. kraussii* adversely affected biomass of *J. acutus*. Results indicated that in areas receiving regular freshwater inputs, thereby reducing salinity stress, *J. acutus* has the potential to displace *J. kraussii*.

Competitive interactions in wetlands were also studied by other authors. Miklovic and Galatowitsch [104] evaluated, under greenhouse conditions, how an established freshwater native wetland community (*Alisma triviale*, *Glyceria grandis*, *Scirpus validus,* and *Sium suave*) would respond to NaCl with regard to both direct (0–1000 mg L^{-1} NaCl) and indirect effects (competition with *T. angustifolia*). Direct effects on biomass of the native wetland community were observed at ≥500 mg · L^{-1} NaCl. Indirect effects on biomass were observed at 1000 mg · L^{-1} NaCl. In fact, diversity and species richness decreased slightly with increasing NaCl concentration. The authors concluded that both road salt runoff and the presence of *T. angustifolia* reduced wetland diversity. In a similar manner, Crain et al. [105] studied the effect of salinity (0–100 ppt) on the distribution patterns of salt-tolerant species (*Distichlis spicata, Limonium nashii*, *S. europaea*, and *Spartina patens*) and salt-sensitive species (*Juncus gerardii*, *Potentilla anserina*, *Scirpus robustus*, *Solidago* *sempervirens*, and *T. angustifolia*). Results showed that: (a) when salt-sensitive species were transplanted to salt marshes, they grew poorly and generally died with or without neighbours present; and (b) when salt-tolerant species were transplanted to freshwater marshes, they thrived in the absence of neighbours (growing better than in salt marshes) but they were strongly suppressed when neighbours were present. The authors concluded that plant spatial segregation across salinity gradients is driven by competitively superior salt-sensitive species displacing salt-tolerant ones, whereas salt-sensitive species are limited from living in salt marshes.

Some authors have showed the different ion-specific effect on macrophytes. For instance, Vicente et al. [106] studied the germination responses of *Arthrocnemum macrostachyum*, *J. acutus*, and *Schoenus nigricans* to saline stress caused by chloride and sulphate salts (0–5 % NaCl, $MgCl_2$, $MgSO_4$, and Na_2SO_4). Plants were ranked from most to least germination capacity in the presence of salt as follows: *A. macrostachyum* > *J. acutus* > S. *nigricans*. Overall, sulphates showed to have less inhibitory effect than the equivalent chloride concentrations. In the same way, Pagter et al. [107] evaluated the osmotic and ion-specific effects of NaCl and Na_2SO_4 on *P. australis* morphological and physiological processes. With increasing salt levels, Na and S or Cl root concentrations increased but exclusion mechanisms of Na and S, and partly Cl, from leaves were recorded. The authors concluded that *P. australis* is tolerant to both NaCl and Na_2SO_4 stress, probably due to the restricted uptake and translocation of Na, S, and Cl. The incomplete Cl exclusion may affect aboveground biomass development, which was significantly more reduced by NaCl than Na_2SO_4.

Conversely, leaf proline concentration increased equally in NaCl and Na_2SO_4-treated plants. Similar efficient mechanisms of Na exclusion from leaves were observed by Gorai et al. [108] studying the effect of both salinity and hypoxia on physiological attributes of *P. australis*. The authors also reported that plants grew well under hypoxia at ≤100 mM NaCl. However, changes in leaf turgor occurred with the combined effect (salinity and hypoxia) suggesting an adjustment of the plant water status. Fediuc et al. [109] studied the combined effect of Cd and NaCl on *P. australis* and *T. latifolia*. Results showed that O-acetylserine (thiol) lyase activity (OASTL) increased under stress (25–300 mM Cd, 100 mM NaCl, 1 mM abscisic acid—ABA) in both *P. australis* and *T. latifolia,* mainly in roots, contributing substantially to satisfy the higher demand of cysteine for adaptation and protection. Also, Cd treatments led to increased ABA levels in roots (higher in *P. australis* than in *T. latifolia*), which indicates its involvement in early stress responses. The stimulation of OASTL following the ABA application suggested that ABA has a role in an OASTL activation pathway.

The influx rate and the duration of exposure to salinity may play an important effect on macrophytes. These factors were evaluated by Howard and Mendelssohn [110, 111] on *E. palustris*, *Panicum hemitomon, Sagittaria lancifolia*, and *Scirpus americanus* exposed to salinity (0–12 g L^{-1}) during 1, 2, or 3 months at an influx rate of 3 days or 3 weeks. *S. lancifolia* was the first species to show visible signs of stress while the salt effect was delayed for 6–8 weeks in *P. hemitomon*. Overall, the magnitude of growth suppression in response to salinity increased for all species as the duration of exposure increased. Species were ranked from least to most salt tolerant as follows: *P. hemitomon* < *S. lancifolia* < *E. palustris* < *S. americanus*. A recovery test was also performed in which after each exposure period plants were placed into freshwater until the end of the 120-day experiment. The four species varied in their ability to recover from the salinity pulses: (a) *S. americanus* was able to recover even under the most extreme conditions (12 g L^{-1} salinity, 3 months); but (b) *P. hemitomon*, *S. lancifolia*, and *E. palustris* recovery ability decreased with increased salinity and duration of exposure, and to a lesser extent with salinity influx rate.

The authors concluded that the different recovery patterns displayed by plants may lead to changes in species dominance following short-term salinity pulses that can occur during storm events, which in turn may affect community composition and structure. Subsequently, Howard and Rafferty [112] studied the effect of both salinity and water depth tolerance to clones from a brackish marsh (*P. australis* and *Schoenoplectus californicus*) or a salt marsh (*D. spicata* and *Schoenoplectus robustus*). Results showed that: (a) *P. australis* was more tolerant than *S. californicus* to increased salinity; and (b) *D. spicata* was more tolerant to increased salinity but less tolerant to increased water depth than was *S. robustus*.

4 Case Study: Treatment of Runoff from an Urban Snow Disposal Site

4.1 Context and Selection of Plants

In urban areas, winter leads to the accumulation of large amounts of snow and ice on roadways. The dirty road snow contains a range of contaminants including metals and de-icing salts used to ensure safe driving. The high impact of these contaminants on the natural environment justifies the importance of good road management and maintenance, as well as the set-up of snow disposal sites. In Quebec, *Regulation respecting snow elimination sites* requires that those snow disposal sites having a discharge into the natural environment ensure a quality monitoring of releases in order to comply with environmental regulations [113]. In this context, a greenhouse study was conducted in collaboration with Gatineau City (Quebec) to further implement a free water surface-flow CW for the treatment of the salted runoff discharged from the city snow disposal site.

Two candidate plants were selected to target NaCl removal (*S. maritimus* and *Spartina pectinata*) based on five main criteria: (1) origin of the species, (2) capacity to remove NaCl and co-pollutants, (3) biomass production capacity, (4) behaviour in relation to mowing, and (5) availability for sale or culture. *S. maritimus* is a halophyte circumboreal species also present in the temperate zones of the northern hemisphere. In Quebec, it's present from the St. Lawrence estuary to Île-aux-Oies (Montmagny) and Saint-Jean-Port-Joli. It is also present in the Montreal region (Varenne) near salt sources. *S. maritimus* has showed to be effective on Cl, metal, and petroleum hydrocarbon removal [114, 115]. A study realized by Almeida et al. in 2006 [116] showed that *S. maritimus* was a potentially interesting species to phytopurification. In this study, the plant was able to absorb and accumulate metals like Cd and Pb in different plants tissues. So, *S. maritimus* would be able to remove a range of pollutants likely to be present in snow disposal site runoff. Regarding *S. pectinata*, it is a species found in North America. In Quebec region, it is abundant on the shores of Saint-Laurent and Saint-Jean Lakes. Several studies have showed that *S. pectinata* has an interesting potential in phytoremediation [117–120]. These works, mainly focused on road runoff treatment, showed that *S. pectinata* has a high absorption capacity of metals (Cu, Cd, Pb, and Zn) but also of P and Cl ions and oils. Moreover, this plant has also capabilities for the treatment of polychlorinated biphenyls (PCBs) in soils and sediments.

4.2 Materials and Methods

4.2.1 Experimental Design

Six pilot-scale subsurface horizontal-flow CWs, in duplicate ($n = 12$), were designed in an environmentally controlled greenhouse (Fig. 2). Each CW was built in a high density polyethylene container (length: 0.56 m, width: 0.40 m, height: 0.38 m) and filled with a growing media composed by a mixture of substrates purchased from a local gardening company (Fafard, Quebec): 50 % "*Organic gardening topsoil*"—mixture of black earth, composted manure, sand and lime—(pH6.1; 21 % organic matter, OM; particle-size distribution 99 % sand/1 % silt and clay); 25 % compost with peat and shrimp (pH7.1; 33 % OM; 2.5:0.6:1.7 % N:P:K); and 25 % sand (0.2–1.2 mm particle size). Gravel was placed at the bottom (~0.625 cm particle size) in order to favour an adequate hydraulic conductivity in the CWs. Then, six CW were planted with *S. maritimus* (named CW_{Sm}, Fig. 3), and six with *S. pectinata* (named CW_{Sp}, Fig. 4). The environmental greenhouse conditions correspond to weather observed in southern Quebec. To take into account the different growth periods of the two plants species, climatic conditions matched the spring-summer period between April and September. Greenhouse parameters were adjusted as follows: 25 °C/15 °C (day/night), 55 % relative humidity (maximum), photoperiod 15 h/9 h (day/night), and minimum illumination at 25,200 lx at day periods.

A peristaltic pump delivered, from tanks to each CW, a solution prepared with de-icing salt in tap water (called influent) for a period of 1 month and a half (45 days) at a flow rate of 4 L per day, which resulted in a hydraulic retention time of approximately 7 days. The water level in the CW remained at about 4 cm below the surface of the substrate. Three different de-icing salt solutions were prepared to feed the CWs: tap water (~0.2 dS m^{-1}), a mild saline solution (~3 dS m^{-1}), and an intermediate saline solution (~8 dS m^{-1}), named CW0, CW1, and CW2, respectively. Treatment applications were performed in duplicate. De-icing salts were obtained from Ministry of Transport of Quebec [121].

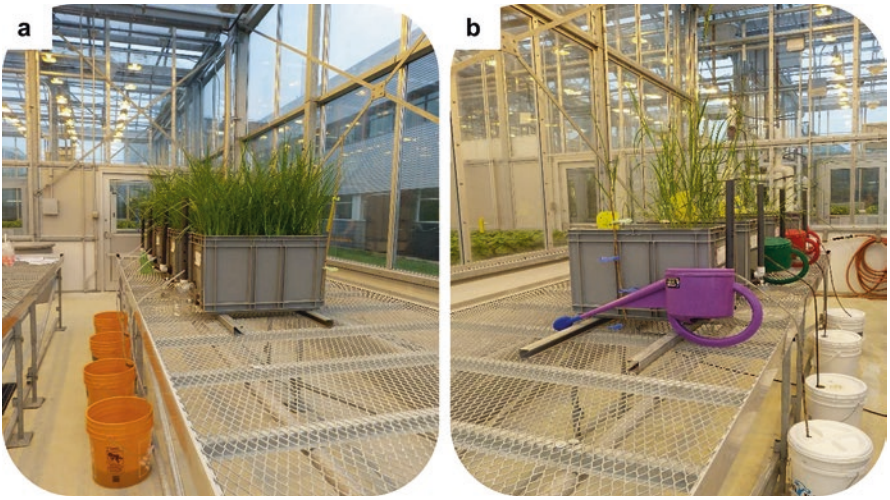

Fig. 2 Photograph showing the greenhouse experimental set-up with *Scirpus maritimus* (**a**) and *Spartina pectinata* (**b**) XX days after planting (from Galvez-cloutier and Guesdon [124])

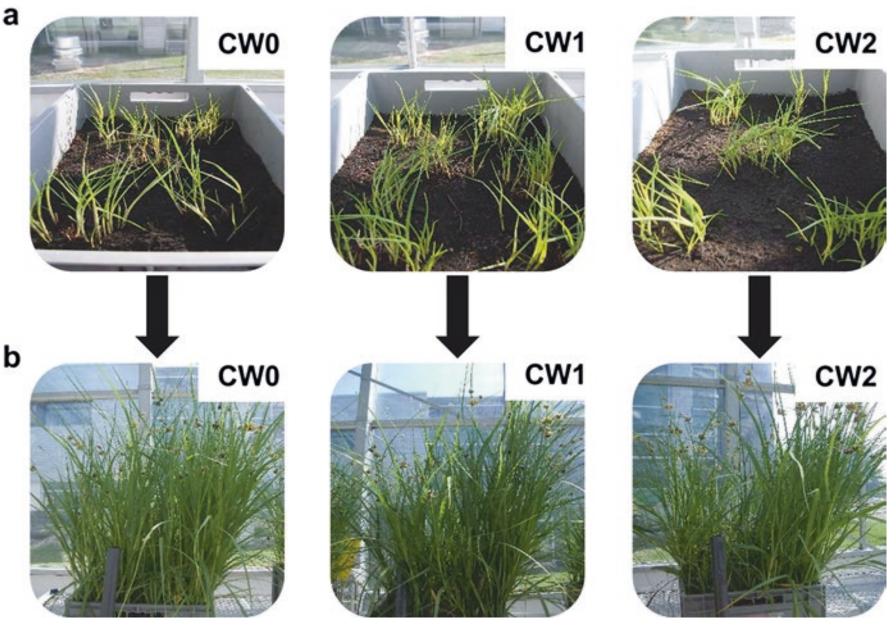

Fig. 3 Illustration of *Scirpus maritimus* growth after 0 days (**a**) and 45 days (**b**) of exposure to different levels of de-icing salts: 0.2, 3, and 8 dS m^{-1} (CW0, CW1, and CW2, respectively) (from Galvez-cloutier and Guesdon [124])

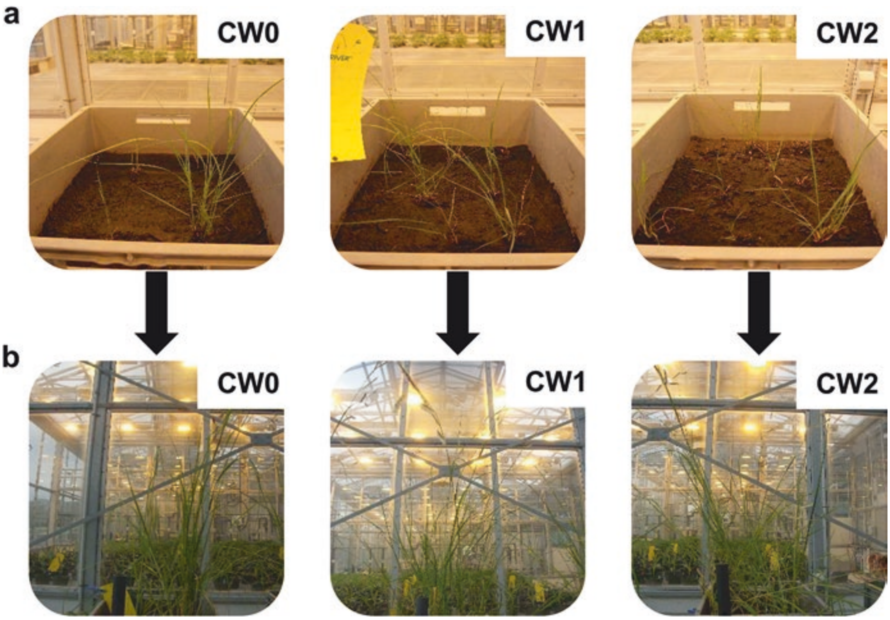

Fig. 4 Illustration of *Spartina pectinata* growth after 0 days (**a**) and 45 days (**b**) of exposure to different levels of de-icing salts: 0.2, 3, and 8 dS m^{-1} (CW0, CW1, and CW2, respectively) (from Galvez-cloutier and Guesdon [124])

4.2.2 Sampling and Analysis

All chemicals and reagents were from analytic grade from Fisher Scientific, Inc. (Canada) and EMD Chemicals, Inc. (USA). All glassware used was rinsed with nanopure water (Ultrapure Water System, Barnstead Nanopure).

During the experiment, plant height was measured once a week in six randomly selected plants per CW. Temperature, volume, electrical conductivity (EC), and pH in both influent and effluent water samples from each CW were monitored thrice weekly with a multi-probe system (Model YSI 556). Effluent volume was measured at each time and water outflow calculated. Water samples were taken three times per week from both CW influent and effluents for Cl and Na quantification. The water samples were filtered under vacuum through a 0.45-μm-pore-size cellulose ester membrane filter (Advantec, Inc.). Filtrate was frozen (for Cl quantification) or acidified to pH2 by adding the necessary volume of concentrated HNO_3 and kept at 4 ºC (for Na quantification) until analysis. The concentration of Cl was quantified by high performance liquid chromatography (HPLC) (1525 Binary HPLC Pump System, Waters, Inc.) and the concentration of Na by flame emission atomic spectroscopy (FEAS) (AA240FS, Varian, Inc.). Quantification limit in mg L^{-1} was: Cl = 0.1 and Na = 0.01. The Cl and Na amount in both water influent and effluent was calculated by multiplying the Cl or Na concentrations in water by the volume measured at each time and, thereafter, the total amount of Cl and Na recovered per

CW over the 45 days of greenhouse experiment. Total Cl and Na removal rate per CW was calculated in percentages with the following equation [122]:

$$\text{Removal rate }(\%) = \frac{(Q_{\text{in}} \times C_{\text{in}}) - (Q_{\text{out}} \times C_{\text{out}})}{(Q_{\text{in}} \times C_{\text{in}})} \times 100$$

where Q_{in} is the average inflow rate (L d^{-1}); C_{in} is the average inflow Cl or Na concentration (mg L^{-1}), Q_{out} is the average outflow rate (L d^{-1}), and C_{out} is the average outflow Cl or Na concentration (mg L^{-1}).

At the end of the experiment, all plants were removed from each CW, separating the above- and underground parts, washed in abundant tap water followed by de-ionized water to remove particles, and weighed to obtain fresh biomass weight. Tolerance index (TI) was calculated as a percentage of aboveground fresh biomass of plants grown in each CW where de-icing salt was added (CW1 and CW2) with respect to those without salt addition (CW0). Dried biomass was measured after oven drying the plant material to constant weight at 60 °C for 48 h. Significance of differences of the means of above- and underground biomass in plants at the end of the experiment was investigated by means of one-way analysis of variance (ANOVA) using a post-hoc test (Tukey). Before performing the ANOVA test, a Levene's test was performed in order to analyse the homogeneity of variance. These analyses were made using the Statistical Package for the Social Sciences v.17 (SPSS, Inc.) software.

4.3 Results and Discussion

Several physicochemical parameters were monitored in the influent and effluent water from the CWs where *S. maritimus* and *S. pectinata* were grown (named CW_{Sm} and CW_{Sp}, respectively) throughout the 45 days of the greenhouse experience (Table 5). Water effluent monitoring showed that plants affected physicochemical parameters differently. Thus, pH values decreased from water influents to effluents between 0.4 and 1.4 pH units after 1 day of contact time with the plant system and then increased during the following 45 days of experience.

The calcareous nature of the substrate may account for this result. At the end of the experiment, higher pH values were recorded in the effluents from CW_{Sm} than from CW_{Sp}. A similar pattern was observed in EC values in the sense that higher values were recorded at day 45 in the effluents from CW_{Sm} than from CW_{Sp}. As shown in Table 5, values decreased with time in the case of control systems (CW0) for both plants but increased when de-icing salt were added (CW1 and CW2). As previously reported by Guesdon et al. [91], the decreased effluent volume usually recorded from CWs (relative to influent) may account for the higher levels of pH and EC. Thus in the present study, the flow rate considerably decreased over the

Table 5 Mean of flow rate, pH, and EC in water influent and effluents (days 1 and 45) from constructed wetlands where *Scirpus maritimus* (CW_{Sm}) and *Spartina pectinata* (CW_{Sp}) were grown at different levels of de-icing salts added (CW0, CW1, and CW2)

			Water influent			Water effluent					
						Day 1			Day 45		
			Flow	pH	EC	Flow	pH	EC	Flow	pH	EC
Plant	CW CODE	Treatment	L day^{-1}		dS m^{-1}	L day^{-1}		dS m^{-1}	L day^{-1}		dS m^{-1}
Scirpus maritimus	CW_{Sm}	CW0	4	7.8	0.24	2.0	6.6	4.26	0.9	8.6	0.81
		CW1	4	7.1	3.10	1.5	6.7	6.27	1.3	8.1	8.87
		CW2	4	7.1	7.83	1.5	6.5	10.93	1.0	7.9	19.00
Spartina pectinata	CW_{Sp}	CW0	4	7.8	0.24	1.9	6.4	5.26	2.8	7.8	0.49
		CW1	4	7.1	3.10	1.8	6.4	7.30	2.9	7.8	3.86
		CW2	4	7.1	7.83	1.9	6.2	9.85	2.8	7.3	9.33

EC = electrical conductivity

experiment, being more evident in the case of CW_{Sm} which explains our results. Table 6 shows the total amount of Cl and Na in water influent and effluents, as well as the total removal rate at each CW and de-icing salt level. Chloride amounts in water effluents from controls (CW0) were slightly higher than influent amounts, resulting in no Cl accumulation and therefore in negative removal efficiencies, as usually reported [123].

When de-icing salts were added (CW1 and CW2) the NaCl removal rate was very high. Based on the NaCl removal rate, *S. maritimus* showed the greatest desalinization potential (77–88 % Cl, 87 % Na), followed closely by *S. pectinata* (76 % Cl, 77–83 % Na). No linear relation was observed between the amount of de-icing salts added and the removal rate. The salt removal rate showed by these plants is much higher than that usually reported with other macrophytes, e.g. up to 44 % *T. angustifolia* [94], up to 36 % *J. maritimus* [91], ~55 % *J. kraussii* [92], ~60 % *B. indica* [96]. Results therefore indicate that both species, *S. maritimus* and *S. pectinata*, present a great phytodesalinization ability.

Plant height was evaluated over the 45 days (Fig. 5). In controls (CW0) plant height wason average: 27 and 58 cm (*S. maritimus*) and 3.6 and 74 cm (*S. pectinata*), at the planting time (day 0) and at the end of the experiment (day 45), respectively. As shown in Fig. 5 *S. maritimus* height was not hindered by de-icing salts at level CW1 but slightly at level CW2 (~13 % plant height inhibition). In the case of *S. pectinata*, plant height was favoured at the highest de-icing salt level (CW2) and no differences were recorded between CW0 and CW1. Noteworthy is that *S. maritimus* growth height stabilized from day 20 while *S. pectinata* height showed a trend to increase throughout the experiment. Biomass of plants was also quantified (Fig. 6).

At the end of the experiment (day 45), the aboveground dry biomass per m^2 in CW0 was on average: 253 g m^{-2} (*S. maritimus*) and 689 g m^{-2} (*S. pectinata*). In agreement with plant height, *S. maritimus* biomass was not negatively affected in CW1, and an increase of 1.6-fold with respect to CW0 was recorded. At the highest de-icing salt level added (CW2) biomass was not affected, showing similar values to the controls (CW0). Regarding *S. pectinata*, biomass was decreased with de-icing salts, being significant in CW2. This result differs from that observed in the plant height. The tolerance index (TI) was calculated considering the aboveground biomass: 161 and 104 % (*S. maritimus*), and 89 and 76 % (*S. pectinata*), in CW1 and CW2, respectively. As can be seen, tolerance to salt is very high in the case of *S. maritimus* and somewhat low in the case of *S. pectinata*. Total water amount in plants was calculated by measuring the difference between fresh and dry biomass weight to evaluate the water status in vegetal tissues. Relative water content in percentage (water content vs fresh biomass) was calculated to overcome differences in biomass production. Factually, higher percentages of water were quantified in *S. maritimus* than in *S. pectinata* biomass, attributable to a more efficient mechanism of *S. maritimus* to counter the vacuolar ion content. A lower accumulation of water in *S. pectinata* tissues could explain its lower tolerance and the dissimilar patterns observed between biomass and height. Overall, *S. maritimus* accumulated 83, 68, and 86 % of water in its aboveground tissues; while *S. pectinata* 60, 55, and 56 %;

Table 6 Mean total amount of Cl and Na (g) in water influent, water effluent, and removed from influent (accumulated loading), and percentage of removal rate over the 45 day greenhouse experience from constructed wetlands where *Scirpus maritimus* (CW_{Sm}) and *Spartina pectinata* (CW_{Sp}) were grown at different levels of de-icing salts added (CW0, CW1, and CW2)

Plant	CW CODE	Treatment	Cl Water influent	Water effluent	Accumulated loading	Removal rate	Na Water influent	Water effluent	Accumulated loading	Removal rate
Scirpus maritimus	CW_{Sm}	CW0	2.6	3.5	0	NRE	5.3	1.6	3.7	69.3
		CW1	446.6	51.3	395.3	88.5	278.8	36.0	242.8	87.1
		CW2	159.0	36.2	122.8	77.2	110.5	13.9	96.6	87.4
Spartina pectinata	CW_{Sp}	CW0	2.6	4.1	0	NRE	5.3	4.5	0.8	15.4
		CW1	446.6	106.2	340.4	76.2	278.8	47.3	231.4	83.0
		CW2	159.0	38.4	120.7	75.9	110.5	25.9	84.6	76.6

NRE = negative removal efficiency

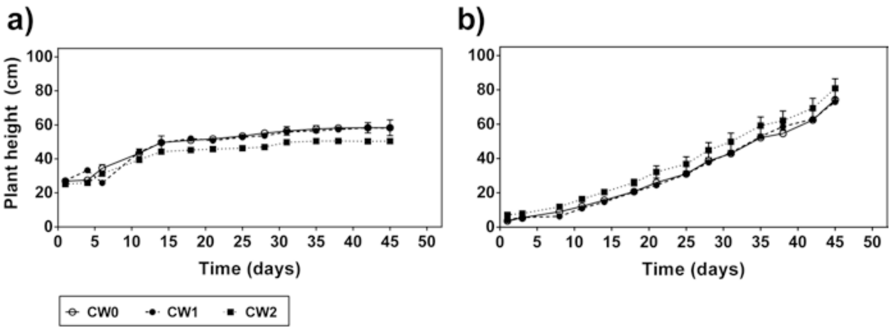

Fig. 5 Above- and underground biomass (g wet weight per CW) of *Scirpus maritimus* (**a**) and *Spartina pectinata* (**b**) after 45 days of exposure to different levels of de-icing salts: 0.2, 3, and 8 dS m^{-1} (CW0, CW1, and CW2, respectively). Vertical bars are standard errors. Different letters indicate significant differences among levels within above- or underground biomass for each plant at $p < 0.05$ after one-way ANOVA

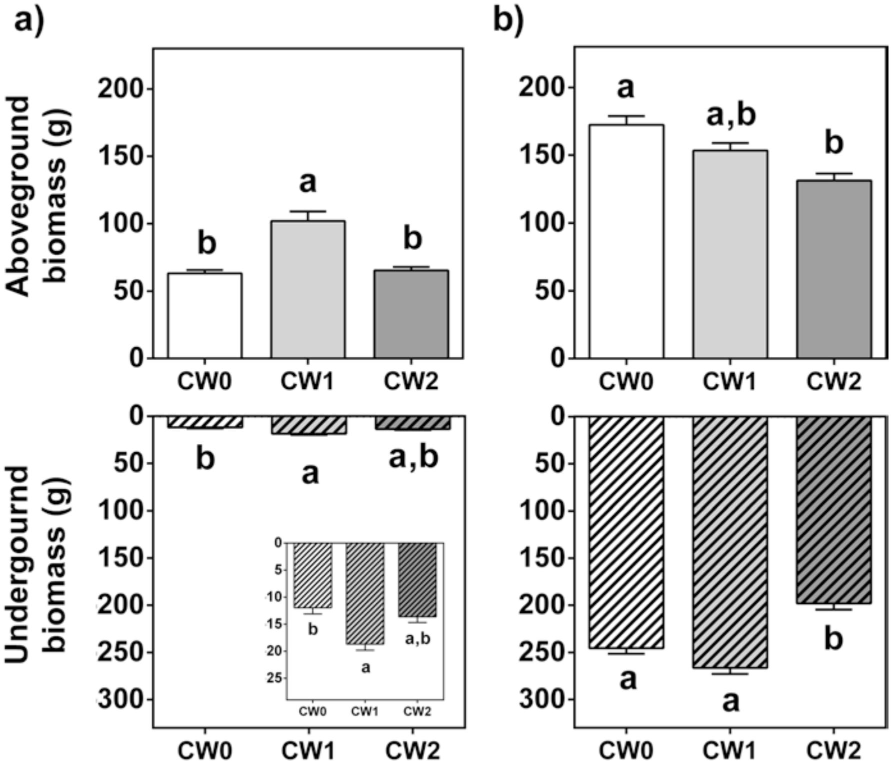

Fig. 6 Plant height (cm) of *Scirpus maritimus* (**a**) and *Spartina pectinata* (**b**) after exposure to different levels of de-icing salts: 0.2, 3, and 8 dS m^{-1} (CW0, CW1, and CW2, respectively) over the 45 days of the greenhouse experience. Vertical bars are standard errors

in CW0, CW1, and CW2, respectively. The largest water consumption of *S. maritimus* also highlights its highest capacity for treating a larger volume of water. Differences in water uptake may also account for the EC patterns observed in water effluents, as discussed above.

5 Conclusions

Studies aimed at de-icing salt removal from road runoff using macrophytes in CWs are dramatically scarce. In terms of full-scale experiments, most of the studies are intended to remove PAHs and metals. Although high quantities of these pollutants can be successfully removed from road runoff in an ecological manner by wetland processes, some limiting factors have been highlighted and still need to be addressed:

(a) The Cl concentrations accumulated in CWs during winter de-icing activities may be released by the increased flows in spring which also increases metal release
(b) The variability in the water quality and quantity during storm events makes mechanical pre-treatments necessary
(c) Macrophyte plants could decrease pH providing undesirable conditions for pollutant accumulation, e.g. by metal precipitation

Further studies with a larger variety of candidate macrophytes are required to optimize the CW performance, especially if the emphasis is placed on de-icing salt removal. In fact, several factors can affect the phytodesalinization ability of a given CW and therefore should be taken into account:

(a) CW maintenance: plant harvesting, intra- and interspecific competitive interactions
(b) Salt effect: osmotic and ion-specific effects, influx rate, time
(c) Environmental stressors: waterlogging, water depth, hypoxia, storm events, temperature, light, nutrients, pollutants, and so on

From the literature review performed and from our own findings, several macrophytes have emerged as good candidates for salt removal that deserve to be studied in depth with the aim of treating de-icing salts from road runoff: *A. patula*, *B. indica*, *E. crus-galli*, *J. acutus*, *J. Kraussii*, *J. maritimus*, *J. torreyi*, *L. scothicum*, *S. latifolia*, *S. tabernaemontani*, *S. maritimus*, *S. pectinata*, *T. angustifolia*, and *T. latifolia*. Ideally, plants should have one or more of the following characteristics: tolerant to high levels of the target pollutant, remove reasonably high levels of NaCl and co-pollutants (hydrocarbons and metals), rapid growth rate, produce reasonably high biomass in the field, profuse root system, be endemic from the area or region where the CW will be installed, behaviour in relation to mowing, and availability for sale or culture.

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Constructed Wetlands for Livestock Wastewater Treatment: Antibiotics Removal and Effects on CWs Performance

C. Marisa R. Almeida, Pedro N. Carvalho, Joana P. Fernandes, M. Clara P. Basto, and Ana Paula Mucha

Abstract Constructed wetlands (CWs) are defined as engineered wetlands that utilize natural processes involving soil, wetland vegetation and their associated microbial assemblages to assist the treatment of wastewaters or other polluted water sources. Although CWs have been applied to different types of wastewaters, CWs application to livestock wastewaters is more complicated do to the characteristics of these waters and only a few studies have reported CWs application in these cases. Livestock wastewater can contain diverse veterinary drugs, including antibiotics, which are normally not removed in wastewater treatment plants. Consequently, veterinary antibiotics or their active compounds can enter directly in the water system through effluent discharges, which can lead to serious toxic effects in organisms and promote antibiotic resistance. Therefore, efficient wastewater treatments are needed. Considering the problematic of antibiotics release in the environment, the need for methodologies to efficiently remove these compounds from wastewater effluents, namely from livestock wastewater effluents, and the scarcity of studies on the application of CWs to deal with this problem, authors have been developing a series of studies to evaluated CWs applicability for livestock wastewater treatment. Studies have been assessing not only antibiotics removal but also antibiotic possible effects on CWs performance. These studies will be presented in this chapter.

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1 Constructed Wetlands

"A wetland is an area where water is above, at, or just below, the ground's surface during part, or all, of the year, so producing water-logged soil conditions" [1]. There are two types of wetlands, the natural occurring ones and those that have been purposely constructed, namely treatment wetlands, man-made systems that have been designed to emphasize specific characteristics of wetland ecosystems for improved treatment capacity [2]. Historically, natural wetlands have been used (often unintentionally) to clean liquid effluents and also to provide a mode of conveyance for water-based transportation, sewer for wastes, supplier of nutrients to agricultural land (via flooding), coastal defence and as a buffer against flooding, habitat for wildlife, as recreational areas and as aquaculture resources ([3] and references therein). Moreover, wetlands have a high rate of biological activity compared with other ecosystems, and they have the potential to transform several common pollutants, some released by wastewater treatment plants (WWTPs), in harmless by-products or essential nutrients that can be used for additional biological productivity ([4] and references therein).

Constructed wetlands (CWs) are defined as engineered wetlands that utilize natural processes involving soil, wetland vegetation and their associated microbial assemblages to assist the treatment of wastewaters or other polluted water sources [5].

Normally, CWs are land-based wastewater treatment systems that consist of shallow ponds, beds or trenches that contain floating, submerged or emergent rooted wetland vegetation, being classified as a low-cost technology [6]. These systems are designed to take advantage of the many processes that occur in natural wetlands, but with a more controlled approach, offering relatively low investment and operation costs while producing high-quality effluent with less dissipation of energy [7]. Constructed wetlands are recognized as a reliable wastewater treatment technology, representing a suitable solution for the treatment of many types of wastewaters [8]. In fact, these wetlands can be used for primary, secondary and tertiary treatment of municipal or domestic wastewaters, stormwater, agricultural wastewaters (such as landfill leachate) and industrial wastewaters (such as petrochemicals, pulp and paper, food wastes and mining industries), and are usually combined with an adequate pre-treatment ([3] and references therein).

Performance efficiencies of constructed or natural wetlands depend on several variables, such as the quality and quantity of the effluent to be treated and of the biological (biological degradation, plants uptake), chemical (chemisorption, photodecomposition and degradation) and physical (volatilization and sorption) processes in that particular wetland system [9–11]. Constructed wetlands can reduce/remove pollutants present in wastewaters, such as organic matter, nutrients and metals. In fact, through a combined action among plants, microorganisms and matrix components, the concentration of the compounds can decrease to levels that are safe for the aquatic biota or eventual production of reusable water. The removal of "common" wastewater contaminants (organic matter, suspended solids, nutrients and metals) in CWs has been object of extensive research (e.g. [8, 12, 13]).

In the recent years, CWs have been also used to remove organic pollutants, for instance, pesticides [14] and hydrocarbons [15], as well as the so-called emerging pollutants or, as defined by US EPA, contaminants of emerging concern. This class of emerging contaminants includes among others, pharmaceutical compounds that have been detected in urban and industrial wastewaters as well as in surface waters (e.g. [16–18]). In fact, these pharmaceutical compounds represent a variety of organic compounds, sometimes also referred as pharmaceuticals and personal care products (PPCPs). The application of pharmaceuticals in human and veterinary medicine is an essential part of modern life. Due to their normally low removal in wastewater facilities, these compounds end up being discharged in the environment. In fact, pharmaceuticals in their native form or as metabolites are continually being introduced into sewage waters, mainly indirectly in excreta [19].

Wastewater treatment plants were designed to remove organic pollutants, mainly estimated as dissolved organic matter, solids and nutrients but not pharmaceutical compounds. For the majority of drugs, removal by conventional biological treatments seems inefficient, being these drugs found in significant amounts in WWTPs effluents and surface waters receiving these effluents. In fact, at present, urban wastewaters are considered the most important source of pharmaceutical compounds in the aquatic environment ([3] and references therein). Concern about pollution by pharmaceuticals has grown after confirmation of their presence and the ability to pseudo-persist in the environment [20, 21], namely in fresh water resources. Occurrence of medical substances in ground water, river water, oceans, sediments and soils was already reported two decades ago [22]. More recently, Zucatto and co-workers [23] showed that illicit drugs are also common contaminants of the aquatic environment of populated areas.

The increasing demand for food and fibres has pushed agricultural industry toward using more and more organic and inorganic chemicals, namely application of veterinary pharmaceuticals in the growing animal food industry [24]. In fact, veterinary medicines are used worldwide to protect animal health, prevent economic loss and help ensure a safe food supply [24]. Within the drugs approved for agriculture, antibiotics are among the most widely administered for animal health and management. However, about 75 % of the antibiotics can be excreted as active metabolites being this a major source of antibiotic input into the environment [25]. Livestock effluents can contain diverse veterinary drugs that, due to their use in confined space for a high amount of animals, can result in high loads in the wastewaters.

Several livestock industries and slaughterhouses have now WWTPs to deal with their effluents to treat organic, nitrogen and phosphorus loads. But, similar to municipal WWTPS, most of these WWTPs are also not designed to remove pharmaceutical compounds. Although some of these effluents are directed to municipal

WWTPs, most of them are directly discharged into the environment. Consequently, veterinary antibiotics or their active compounds can enter directly in the water system through livestock WWTPs effluent discharges ([3] and references therein). Therefore, these WWTPs effluents must also be considered as a potential source of veterinary drugs and should be taken into consideration.

Although CWs have been applied to different types of wastewaters, CWs use to treat livestock wastewaters is more complicated to do the characteristics of these waters. In fact, very high organic matter and suspended solids contents and very high levels of nutrients can be found in these wastewaters. A few studies have reported CWs successful application to these types of wastewaters for the removal of the "common" pollutants, organic matter and nutrients [11, 26]. Moreover, CWs have been also applied for the removal of metals from livestock wastewaters [27]. But pharmaceuticals removal from livestock industry wastewaters in CWs has been only recently reported [28, 29]. Particularly for antibiotics, studies are still scarce. Even though antibiotics are normally found at low concentrations in the environment (ng L^{-1} to μg L^{-1}) [30], these compounds can cause serious toxic effects in organisms and promote antibiotic resistance [22, 31]. In fact, antibiotics are the group of most concern due to the risk of spread of antibiotic resistance in the environment. Their presence in the environment leads to a repeated low-dose exposure of bacteria to sub-lethal dosage, which can cause the development of resistance.

Considering the problematic of antibiotics release in the environment, there is a need for methodologies to efficiently remove these compounds from wastewater effluents, namely from livestock wastewater effluents. Due to the scarcity of studies on the application of CWs to deal with this problem, authors have been developing a series of studies to evaluate the applicability of CWs for livestock wastewater treatment, assessing not only antibiotics removal but also antibiotic possible effects on CWs performance [3, 4, 32–34]. These studies will be presented in this chapter to highlight CWs potentialities to address the stated problem, in order to reduce the environmental impact of livestock industry. Studies developed by authors have been devoted mainly to three antibiotics, enrofloxacin (ENR), tetracycline (TET) and ceftiofur (CEF) (Fig. 1), as models of three different pharmaceutical families, fluoroquinolones, tetracyclines and cephalosporins, respectively.

The antibiotic TET belongs to tetracyclines family, which are broad-spectrum agents, showing activity against a varied range of Gram-positive and Gram-negative

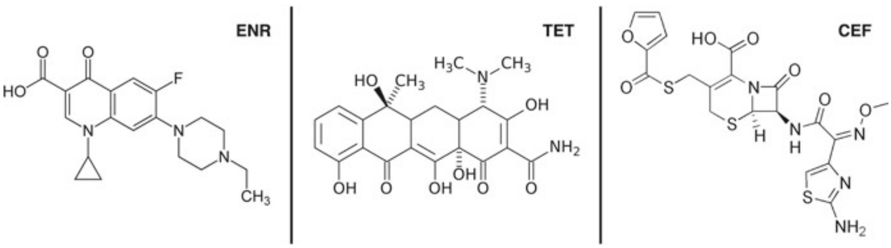

Fig. 1 Enrofloxacin (ENR), tetracycline (TET) and ceftiofur (CEF) structural formulas

bacteria, atypical organisms and protozoan parasites [35]. These antibiotics are commonly used in pig farms [36]. The antibiotic ENR belongs to fluoroquinolones family, a class of synthetic antibacterial compounds that has broad-spectrum antibiotic properties [37]. Nowadays, fluoroquinolones represent the third largest group of antibiotics with an increased use in hospitals, households and veterinary [38]. ENR is the most important antibiotic for veterinary use in this class [39] and has a high environmental interest not only because of its intensive use in livestock industry but also because one of its primary degradation products is ciprofloxacin, another relevant antibiotic [37]. The antibiotic CEF belongs to the family of semi-synthetic antibiotics cephalosporins, a third-generation broad-spectrum cephalosporin used exclusively in veterinary [40]. The World Health Organization (WHO) has ranked antimicrobials according to the importance that they have for human and veterinary therapy. Enrofloxacin and CEF were characterized as "critical important antimicrobials" and TET as "highly important antimicrobials" [41].

2 Potential of Constructed Wetlands Microcosms for Antibiotics Removal from Livestock Wastewater

Due to the scarcity of studies regarding the application of CWs to the treatment of livestock wastewaters contaminated with veterinary antibiotics, present authors started their research investigating the potential of CWs for the removal of these problematic compounds from this type of wastewaters [3, 33]. Studies were carried out in microcosms simulating sub-surface flow CWs. In this type of CWs, wastewater flows under the surface of the planted bed, while water percolates through the substrate. Normally, the substrate is made of different layers, a drainage layer with coarse gravel, a main filter layer of sand and a plant root bed soil on top [42].

Identification and quantification of the important removal processes of emerging organic pollutants in CWs is complicated by the complexity of interactive processes present in wetlands. Although full-scale wetland studies are valuable for assessing overall removal efficiencies, microcosm scale studies are crucial to differentiate processes, particularly with regard to those associated with plants and microorganisms [43]. The microcosm approach has shown to suitably represent interactions among plants, microorganisms, substrates and contaminants within a complex rhizosphere system [5] and was the approach selected in present studies.

The choice of plants is an important issue in CWs once they mediate important processes. For example, plant metabolic activity releases oxygen into the rhizosphere, promoting the activity of bacteria involved in carbon and nitrogen cycles. On the other hand, the access and availability of nutrients affects plant growth response and resource allocation, which influence removal efficiency in wetlands. For example, nutrient removal can be optimized by selecting suitable species with higher capacities for absorption of inorganic nitrogen and phosphorus and conversion into plant biomass [10]. However, plants must also survive the potential toxic effects of the wastewater and its variability [44]. The most widely used plants in

CWs in North America are cattails (*Typha* spp.), bulrushes (*Schoenoplectus* spp.) and reeds (such as *Phragmites australis*) [10].

At Europe, the most common plants are the reeds *P. australis* although other plant species, such as cattails bulrushes (*Scirpus* spp.) and reed canary grass (*Phalaris arundinacea*) have also been used for both domestic and industrial wastewater treatment [1]. In Portugal, the main macrophyte species used in CWs are *P. australis*, *Iris pseudacorus* (yellow iris) and *Cyperus* spp. In some systems, *Juncus effuses* (soft rush), other *Juncus* spp. and *Scirpus* spp. are also found to establish spontaneously [44]. Macrophytes have shown an overall good tolerance to the exposure to contaminants and are capable of contributing to the removal of many of these substances [45], including pharmaceuticals. In fact, due to their characteristics macrophytes are frequently chosen for the application of phytoremediation technologies to treat waters contaminated with organic pollutants. Actually, previous studies have shown both *Typha* spp. [46, 47] and *P. australis* [48, 49] have a high capacity to tolerate and remove some pharmaceuticals from contaminated waters.

For the present studies, the macrophyte plant *P. australis* was chosen. This plant was collected in Lima River margins (NW Portugal) with the soil attached to its roots (rhizospheric soil). The soil was removed in the lab and mixed with sand collected at the same site [3]. Microcosms were set up in plastic containers ($0.4\ m \times 0.3\ m \times 0.3\ m$) filled with a first layer of gravel (4 cm depth), a second layer of lava rock (2 cm depth) and finally the roots' bed substrate (a mixture (1:1) of sand and rhizospheric soil to where plants were transplanted) (10 cm depth) reaching a total depth of 16 cm (Fig. 2). The systems were designed to operate in a batch mode, i.e., with the initial load of water and without any running flow during the assays, having only a tap at the base for sample collection [3].

All microcosms were wrapped in aluminium foil to simulate a real system (where there is no light penetration at substrate level) and prevent the occurrence of photodegradation of the compounds under study. The set of microcosms was kept under greenhouse conditions, subject to environmental temperature variations and environmental light (day/night) exposure [3]. Livestock wastewater, collected at a livestock facility, was doped with two veterinary antibiotics individually, ENR and TET. For each drug, a $100\ \mu g\ L^{-1}$ level of concentration was tested, a concentration already found in the environment [50]. The wastewater was kept in the microcosms for 1 week (a hydraulic retention time normally used in full-scale CWs) [3]. Water level was always maintained just above the substrate surface to ensure a constant flooding rate of ca. 100 %.

The experiment was prolonged for twelve 1-week cycles, completely draining and refilling the microcosms with new-doped wastewater each week, to simulate the cumulative effect of introducing contaminated wastewater in the system. Obtained results showed removal efficiencies up to 94 and 98 % for TET and ENR, respectively [3]. Removal of compounds in CWs depends on the interactions among plants, microorganisms and substrates, the contaminants being uptake by plants, degraded by microorganisms, or removed by the synergisms between plant and microorganisms, and by adsorption to substrate. In a previous work carried out by the authors [51], significant ($p < 0.05$) difference in ENR concentrations in solution

Fig. 2 Experimental design (adapted from [3])

in the presence and in the absence of plants was observed. When *P. australis* was exposed to a solution doped with ENR, ENR concentration in solution decreased between 67 and 91 %, which denotes a potential uptake/degradation of ENR by *P. australis*. A similar study using TET [51] showed also a positive effect of the plant on TET degradation/removal from the solution. But in the CWs microcosms, the plants' role was not so evident, being probably adsorption and/or microbial degradation in the microcosms' substrate the main mechanism for TET removal [33].

In fact, CWs microcosms with and without plants showed similar removal rates. However, planted microcosms did not show clogging problems whereas those unplanted clogged after the eighth week [33]. So, despite plants did not have an evident role in drugs removal they were very important for the entire system functionality. The selection of a substrate with a high sorption capacity can be an important step in the optimization of CWs performance [9]. It has been indicated soil plays the main role in antibiotics removal from wastewater in vertical flow CWs [52]. However, in the root bed substrate (the top layer of the microcosm with an expected affinity for drug adsorption due to soil high fine fraction and high organic matter content) none of the drugs was detected either in planted or unplanted systems along time [33]. The generally high microbial biomass and activity in wetland soils may also promote degradation of pharmaceuticals [53]. The coexistence of several micro-environments in CWs allows for a variety of microbiological communities that might be able to offer different metabolic pathways leading to pharmaceuticals degradation. However, these pathways are still unclear [45]. So, in the present work a significant microbial degradation of the drugs cannot be discarded [33].

Overall results from the described study show a successful application of CWs for the removal of veterinary antibiotics from livestock wastewater. However, one should be aware that in the present work a subsurface flow CW was simulated and each type of CW may present different efficiencies. But application of a CW of a different design, a constructed macrophyte floating bed system microcosm with three varieties of Italian ryegrass (*Lolium multiflorum Lam.—Dryan, Waseyutaka* and *Tachimasari*), has provided also high removal efficiencies (between 73 and 99.5 %) of another type of antibiotic (sulphonamide antimicrobials) [29]. As mentioned above, the application of CWs to treat livestock wastewater contaminated with veterinary drugs is still scarce. In fact, when the presently described study was published [33] only two recent studies describing the application of CWs to treat livestock wastewaters contaminated with veterinary drugs could be found [28, 29]. One of the studies focused on the removal of the veterinary antibiotics sulphonamides from swine wastewater by a mesocosm constructed macrophyte floating bed systems (FWS CWs) [29]. Another study investigated ionophores (pharmaceuticals used exclusively for veterinary application considered as high-risk compounds) removal by a mesocosm scale FWS CWs [28].

In the meantime, a few more studies were published on this topic: Dordio and Carvalho [54] applied CW mesocosms for the treatment of swine wastewater contaminated with oxytetracycline; Liu et al. [52] investigated the efficiency of two vertical flow CWs for the removal of three common antibiotics (ciprofloxacin, oxytetracycline and sulfamethazine) from swine wastewater; and Hsieh et al. [55] investigated the removal of 13 antibiotics of five different families (tetracycline, sulphonamide, quinolone, nitrofuran and chloramphenicol) in an FWS system treating highly polluted wastewater from livestock operations and aquaculture farms. In general, all studies showed good removal rates. So, results available so far show that CWs, independently of their configuration, have the potential to remove veterinary antibiotics from livestock wastewaters reducing the impact these compounds might have in the environment.

3 Effects of Antibiotics on CWs Microbial Communities and Plants

Depuration in CWs is achieved by the concerted action between plant roots and rhizomes, microorganisms and the solid media (substrate) components. The CWs treatment systems provide different micro-environments where various removal processes can take place: physical (retention, adsorption on the substrate, adsorption on the biofilm, photodegradation, volatilization), chemical (degradation), vegetal (uptake, phytovolatilization, release of exudates, oxygen pumping to the rhizosphere, providing an adequate surface for biofilm growth) or microbiological (metabolization) ([3] and references therein). Plants play an important role in CWs, having in general positive effects on water clean-up. Plants can influence soil enzyme activity by excreting exogenous enzymes and can affect microbial communities' composition, diversity and structure [34]. This process is normally due to the release of exudates and oxygen into the rhizosphere that indirectly affect enzyme activity [56]. In fact, correlations between soil enzyme activity and root activity, as well as, correlations between contaminants (organic matter, ammonium and phosphate) removal efficiency and enzyme activity have been reported [56]. For instance, active plant processes have been reported to affect depletion of pollutants due to enhancement of microbial degradation of ibuprofen, uptake of fluoxetine and uptake of degradation products of triclosan and 2,4-dichlorophenoxyacetic acid [43].

Plants can promote degradation or sequester many toxic compounds although they can be sensitive to those compounds. In fact, the stress response of plants to the presence of pollutants can influence plants capacity to control the uptake of those pollutants, increasing the uptake and sometimes causing serious problems to the viability of the plant ([57] and references therein). For instance, enrofloxacin phytotoxicity to several crop plants (*Cucumis sativus, Lactuca sativa, Phaseolus vulgaris and Raphanus sativus*) is known to be related to plant drug uptake [20]. However, pharmaceutical phytotoxicity can be also due to pharmaceutical toxicity to soil microorganisms which affects plant-microorganism symbiosis [58, 59]. Moreover, not only pharmaceutical compounds but also the wastewater by itself can be toxic to the plants. For instance, in the study carried by the authors [34], toxicity of the wastewater (evaluated by ToxScreen test, a test based on the highly sensitive variant of the luminescent bacteria *Photobacterium leiognathi*) was observed.

Phytotoxicity can, therefore, represent a problem for CWs. However, phytotoxicity can depend on the plant species, on the pharmaceutical compound and on the type of wastewater, as well as on the substrate. For instance, a previous study from the authors [51], in which *P. australis* was exposed to solution doped with ENR, pointed to some plant stress due to ENR presence. Nevertheless, in CWs microcosms in the study describe in Sect. 2, there were no induced stress or phytotoxicity signs indicating that the tested plant was able to cope both with the selected wastewater (which showed toxicity) and with the antibiotics tested [33]. An important factor to be taken into consideration regarding plant phytotoxicity is the compound bioavailability for uptake, usually readily available from solution but less available due to subtract interactions in subsurface flow CWs [58]. So, one should be aware that previous tests should be conducted to determine which plants are more suitable for each particular type of wastewater, as well as which contaminants are present to properly select the CWs type/configuration.

In CWs, biodegradation in plant root substrate is a potentially significant removal process for organic compounds, in which the microbial communities play a key role ([32] and references therein). Few studies have been reported about biodegradation of pharmaceuticals in CWs. Mineralization or transformation into more hydrophobic or more hydrophilic compounds that remain in the liquid phase can occur during pharmaceuticals biodegradation [16, 31]. These processes can occur in CWs under aerobic and anaerobic conditions, being the aerobic degradation faster than anaerobic degradation [16]. However, it is unlikely that antibiotics present in wastewater can be effectively degraded by biodegradation alone. The low concentrations of antibiotics, comparing with other pollutants present in wastewater, may be insufficient to induce enzymatic degradation processes. Moreover, the bioactivity of antibiotics can inhibit the growth or the metabolism of microorganisms [31].

Microbial communities, in general, as well as bacterial diversity present in the environment are susceptible to antibiotics effects [60, 61]. In fact, in general, antibiotics have selective effects on various groups of microbes, especially antibiotics designed to be broad-spectrum drugs [62]. Effects of antibiotics in microbial communities depend on existing microbial groups, antibiotics concentrations and soil properties ([62] and references therein). In fact, selected pressure on soil microbial communities by antibiotics at trace concentrations has been reported [63]. There is an increasing body of evidence documenting a reduction of bacterial diversity in soils contaminated with antibiotics [61, 64]. Moreover, veterinary antibiotics effects on structure and functionality of soil microbial communities have been reported ([64] and references therein). For instance, changes in microbial community structure after application of manure containing sulfadiazine in soils have been observed, an effect that increased over time [65]. Effects of slurry from sulfadiazine and difloxacin medicated pigs on soil microbial communities were also detected [66].

Since microbial communities present in CWs substrate have an important role in water quality improvement, the presence of antibiotics in livestock wastewaters can affect CWs functionality and capacity for the removal of "conventional" and emergent pollutants [67]. In fact, several biological processes that occur in CWs, like nitrification, denitrification and nitrogen fixation, are mediated by different types of bacteria that can be affected by antibiotics presence. So, evaluating if antibiotics can affect CWs microbial communities is necessary to fully validate the application of this technology [32]. Therefore, authors investigated the microbial community dynamics associated with veterinary antibiotics removal in CW microcosms in the presence and absence of ENR and TET. This study was complementary of the one described in Sect. 2 in which plant roots substrate (from the CW microcosms assembled to test CWs potential to remove veterinary antibiotics from livestock wastewater) was collected over time. Microbial communities present in plant root substrate were assessed to evaluate their response in terms of microbial abundance and bacterial richness, diversity and community structure [34].

Obtained results indicated CWs microbial communities were able to adapt to drugs presence without significant changes in bacterial abundance, richness and diversity

[34]. But changes in bacterial community structure were observed. In fact, one of the important factors for bacterial community structure definition was the type of treatment, i.e., the absence or the presence of one of the tested antibiotics (control, TET or ENR) [34]. So, present results indicated the two tested antibiotics can affect bacterial communities' structure although not affecting overall abundance, richness or diversity. Despite that, CWs microcosms maintained the depuration capacity and functionality, significantly removing drugs from doped livestock wastewater [34].

4 Effects of Antibiotics on CWs Performance

As mentioned before, CWs can reduce/remove pollutants present in livestock wastewater, such as organic matter, nutrients and metals (e.g. [11, 27]). Depending on the concentration levels, pharmaceuticals, including antibiotics, can be harmful to microorganisms and plants, the key players in CWs removal processes. In fact, plants and rhizospheric bacteria have an important role in the system functionality, being involved in the carbon and nitrogen cycles and in the biodegradation of the different organic substances [16]. As discussed previously (Sect. 2 and 3) in the particular conditions used by the authors to study the potential of CWs for antibiotic removal from livestock wastewater, plants did not seem to be significantly affected by the presence of two of the selected antibiotic, ENR and TET [3, 33]. Despite the observed change in the microbial communities' structure, CWs microcosms showed capacity to remove the two selected drugs [4, 34].

Nevertheless, despite these results it is utterly important to guaranty that all the "common" pollutants (e.g. organic matter, nutrients and metals) are also removed in CWs and that antibiotic presence does not affect their removal. The influence of emergent pollutants, namely antibiotics, on the removal of pollutants from livestock wastewater was then assessed [32]. Experiments in CW microcosms similar to those described in Sect. 2 were prepared. Microcosms with the same plant, *P. australis*, and the same livestock wastewater were used. Wastewater was doped with 100 μL^{-1} of ENR or CEF, individually or mixed [32]. The experiment lasted fourteen 1-week cycles, and each week freshly spiked wastewater was introduced in the microcosms as before. Throughout time drugs removal rates from wastewater were evaluated as well as the removal rates of organic matter (through biological oxygen demand (BOD) measurements), nutrients (ammonium and phosphate) and metals (Cu, Fe, Mn and Zn).

Obtained results showed BOD removal rates of ca. 90 % and ammonium removal rates between 80 and 98 % after treating the wastewater in the CW microcosms [32]. For phosphate removal rates reached 90 % [32]. Removals of Cu were higher that 85 % and those of Zn were ca. 89 % [32]. For Mn and Fe removals were up to 75 % and 99 %, respectively [32]. Both antibiotics were also significantly removed from the wastewater (removals >90 %). So, high removal rates (up to 90 % for most pollutants) were observed, removal rates that were independent of the presence of the veterinary antibiotics [32]. In general, no significant differences were observed throughout time. So, in the present tested conditions the presence of either veterinary antibiotic, ENR or CEF, alone or in a mixture, did not affect significantly the biogeochemical processes occurring in CW microcosms, the systems maintaining their performance for the removal of "common" and emergent pollutants over time.

5 Outlook

Research carried out so far indicates CWs are a valuable alternative to remove conventional pollutants (nutrients, metals and organic matter), as well as antibiotics, from livestock wastewater and attenuate the environmental impact of the livestock industry. In fact, although more studies are needed to understand the complex reactions/mechanisms occurring in antibiotics removal, through full-scale data as well as long-term evaluations, the authors' early research in this topic highlight CWs importance as a viable ecotechnology for removal of veterinary antibiotics from livestock wastewaters, reducing the risk of antibiotic release in the environment. The main disadvantage of CWs is the large surface area per inhabitant needed, but on the other hand, the low operational and maintenance costs and easy exploitation make this technology very attractive [14], for instance, for rural areas where most of livestock facilities can be found. Moreover, when properly planned, these treatment wetlands offer opportunities to regain some of the natural functions of wetlands and offset some of the significant losses in wetland areas that have been occurring throughout the world [68]. In addition, besides water quality improvement and energy savings, CWs have other features related to the environmental protection such as promoting biodiversity, providing habitat for wetland organisms and wildlife (e.g. birds and reptiles in large systems) and serving climatic (e.g. less CO_2 production) and hydrological functions [69]. So, CWs use should be potentiated.

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Phytoremediation Potential of Selected Mangrove Plants for Trace Metal Contamination in Indian Sundarban Wetland

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Abstract Uptake, accumulation and distribution pattern of trace metals in mangrove plants organs along with rhizosediment were studied in Indian Sundarban Mangrove Wetland. The mean concentration of metals in rhizosediments was as follows (expressed in mg kg^{-1}) 36.03 ± 24.88 for Cu, 11,097.10 ± 12,880.67 for Fe, 709.04 ± 274.25 for Mn, 14.10 ± 10.88 for Pb, 76.63 ± 77.20 for Cr and 40.42 ± 5.74 for Zn. In the context of geochemical characteristics of the sediment, values of geo-accumulation index (I_{geo}) and pollution load index (PLI) suggest no metal pollution, but enrichment factor (EF) ensures their anthropogenic sources. Concentrations of Cr and Cu were higher than sediment quality guidelines at some sampling sites, implying potential adverse impacts of these metals. In mangrove organs, the concentration of metals showed the following descending order (expressed in mg kg^{-1}): Mn (2298.77) > Fe (1796.47) > Cr (61.30) > Cu (36.51) > Zn (33.13) > Pb (2.55). *Sonneratia apetala* displays a high bioconcentration factor for Fe (10.7) and Mn (5.99) as well as high translocation factor for Mn (31.99), Pb (18.01) and Zn (9.95) and therefore may be employed as a biological indicator to protect this productive environment as the species showed its potential in accumulating metals in its tissues. Pearson's correlation coefficient indicated that a significant positive correlation existed amongst the metals. One-way ANOVA shows that there are significant differences between metal concentrations of mangrove organs in monitored sites.

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Keywords Metals • Phytoremediation • *Sonneratia apetala* • Biological indicator • Indian Sundarban Wetland

1 Introduction

Mangrove forests are diverse communities that commonly thrive in the intertidal zones of tropical and subtropical coastal rivers, estuaries and bays. As one of the most productive ecosystems in the world, mangrove forests provide multiple ecosystem services such as food sources and diverse habitats for large numbers of organisms provide erosion mitigation and stabilization for adjacent coastal landforms. Similar to other estuarine environments, mangrove ecosystems receive large contaminant inputs from catchments derived from run-off, as well as atmospheric and marine inputs. Consequently many of these environments have become important sinks for nutrients, organic and inorganic contaminants including heavy metals. Mangrove sediments have a high capacity to retain heavy metals from tidal water, freshwater rivers and storm water runoff, and they often act as sinks for heavy metals [1, 2–3]. Heavy metals are not biodegradable and persistent in the environment and thus received significant attention due to their long-term effects on the environment especially coastal regions. Therefore, understanding the distribution of heavy metals including the toxic one, and monitoring their potential bioavailability to mangrove plants have become increasingly important [4]. Phytoremediation is described as a natural process carried out by plants and trees in the cleaning up and stabilization of contaminated soils and ground water. It is actually a generic term for several ways in which plants can be used for these purposes. It is characterized by the use of vegetative species for in situ treatment of land areas polluted by a variety of hazardous substances [5]. It is a novel, cost-effective, efficient, environment- and eco-friendly, in situ applicable and solar-driven remediation strategy [6]. Plants are especially useful in the process of bioremediation because they prevent erosion and leaching that can spread the toxic substances to surrounding areas [7]. Plants generally handle the contaminants without affecting topsoil, thus conserving its utility and fertility. They may improve soil fertility with inputs of organic matter [8]. It is suitable for application at very large field sites where other remediation methods are not cost-effective or practicable [9]. 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, and when they interact with microorganisms in the rhizosphere. 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 [10].

Presently, there are several types of phytoremediation in practice. One is phytoextraction, which relies on a plant's natural ability to take up certain substances (such as heavy metals) from the environment and sequester them in their cells until the plant can be harvested. Another is phytodegradation in which plants convert organic pollutants into a nontoxic form. Next is phytostabilization, which makes plants release certain chemicals that bind with the contaminant to make it less bioavailable and less mobile in the surrounding environment. Last is phytovolitization, a process through which plants extract pollutants from the soil and then convert them into a gas that can be safely released into the atmosphere [11]. Mangroves are highly productive intertidal forests that interface between marine and terrestrial environments in the tropics and subtropics. These ecosystems generally occur in estuaries, bays and harbours which are areas of rapid urban development. Mangroves include approximately 16 families and 40–50 species (depending on classification). According to Tomlinson [12], the following criteria are required for a species to be designated a "true or strict mangrove": Complete fidelity to the mangrove environment, major role in the structure of the community and has the ability to form pure stands. These plants possess morphological and physiological adaptation to their habitat. They should be isolated taxonomically from terrestrial relatives.

Thus, mangrove is a non-taxonomic term used to describe a diverse group of plants that are all adapted to a wet, saline habitat. Mangrove may typically refer to an individual species. Terms such as mangrove community, mangrove ecosystem, mangrove forest, mangrove swamp and mangal are used interchangeably to describe the entire mangrove community [13]. Anthropogenic impacts from urban growth include metal contamination from sources such as industrial wastes and effluents, mining, sewage treatment plants and runoff [14]. Mangrove forests protect coastal landforms from erosion and act as sediment traps simply by reducing tidal flows and inducing sedimentation at low tides [15]. Mangroves are one of the most productive ecosystems that enrich coastal waters, yield commercial forest products, protect coastlines and even support coastal fisheries and storehouse of numerous endangered faunas. They act as a fragile link between marine and fresh water ecosystems, pollution sink and source of nutrient flux into marine ecosystem.

But, it is surprising to know that such a natural fighter against pollution is constantly being affected by the rising level of pollution [16]. Mangrove plants' special capability of surviving in high-salt and anoxic conditions and high tolerance to trace metal stress [17] contribute to their potential use in preventing dispersion of anthropogenic pollutants into aquatic ecosystems [18]. In spite of their importance, mangrove ecosystems have suffered significant anthropogenic contaminant inputs due to their location close to urban development [19], amongst which the majority are trace metal pollutants [20]. Mangrove plants absorb and store trace metals mainly in roots and still transport a part upward into sensitive tissues: Metal concentrations in shoots appear to be half that of roots or lower [19, 21]. Previous cultivation experiments have proved that excessive essential metals and non-essential metals could affect the growth metabolism activities and cell structure [22] of plants.

The present investigation is an effort to assess the phytoremedial potential of selective mangrove plants growing on metal enriched sediments of Indian Sundarban Wetland. It deals with the absorption, accumulation and dynamics of six trace metals in Indian Sundarban. The aim is to reveal the potential of mangrove plants to accumulate and tolerate the above-mentioned metals, and to find out a potential species for bioindication and phytoremediation.

2 Materials and Methods

2.1 Study Sites

The Indian Sundarban Mangrove Wetland (21°00′–22°30′N and 88°00′–89°28′E) is a tide-dominated anthropocene megadelta belonging to the low-lying coastal zone, formed at the estuarine phase of the Hugli (Ganges) River. It is part of the world's largest delta (80,000 km^2) formed from sediments deposited by three great rivers, the Ganges, Brahmaputra and Meghna, which converge on the Bengal Basin. The whole Sundarban area is intersected by an intricate network of interconnecting waterways, of which the larger channels are often a mile or more in width and run in a north–south direction. A number of southerly flowing rivers, viz., Hugli, Baratala, Saptamukhi, Jamira, Bidyadhari, Matla and Gosaba (as shown in Fig. 1) traverse the wetland from the west to the east [23]. This is one of the most dynamic, complex and vulnerable zones in typical tropical geographical locations in the northeastern part of Bay of Bengal. Geomorphologically, mangrove swamp, tidal marsh, intertidal mudflats, sandy beaches, tidal creeks and inlets characterize the estuarine wetland. The entire mangrove forest extends over 4262 km^2 of which 2320 km^2 is forest and the rest is water [24], and is called Sundarban owing to the dominance of the tree species *Heritiera fomes*, locally known as "Sundari" because of its elegance [25].

Both plant samples and host sediments were collected from three sampling sites of diverse environmental stress located along the east–west gradient of Indian Sundarban and a brief description of each site is furnished below:

Jharkhali (S_1)—This site is characterized with the following features: (a) this is surrounded by Herobhanga Reserve forest. (b) This is the confluence of Bidya and Matla rivers (c) reduced forested area due to severe human pressure and (d) a famous tourist spot where the pollution stress is higher as thousands of people used to gather here. Moreover, this is a wide scale fishery catchment area and mechanized boats are used for fishing which helps to contribute trace metals to water mainly due to complete lack of standard norms and regulation. Rich and diversified luxuriant mangrove vegetation with high diversity of speciesis distinct mainly due to extensive afforestation program.

Gangadharpur (S_2)—It is situated on the western bank of Saptamukhi River, a major tidal inlet in the Hugli–Matla delta complex. Natural mangrove vegetation of mixed type can be seen here. Agricultural runoff, boating and domestic use of water bodies, leaching from domestic garbage dumps are the major sources of metal pollution in this area. Moreover, unawareness of the local people about the mangrove plants and their importance is leading the gradual destruction of this natural habitat. A major section of the natural habitat is already lost due to deforestation by the local people for timber, house making, boat making, etc.

Gangasagar (S_3)—It is an offshore island located open ocean at the extreme southern tip of the estuary mouth, experiencing direct wave and marine influences. The eastern bank of this triangular island faces meso-macrotidal Muriganga River and the western bank faces macrotidal Hugli estuary. In addition to the annual "Sagar

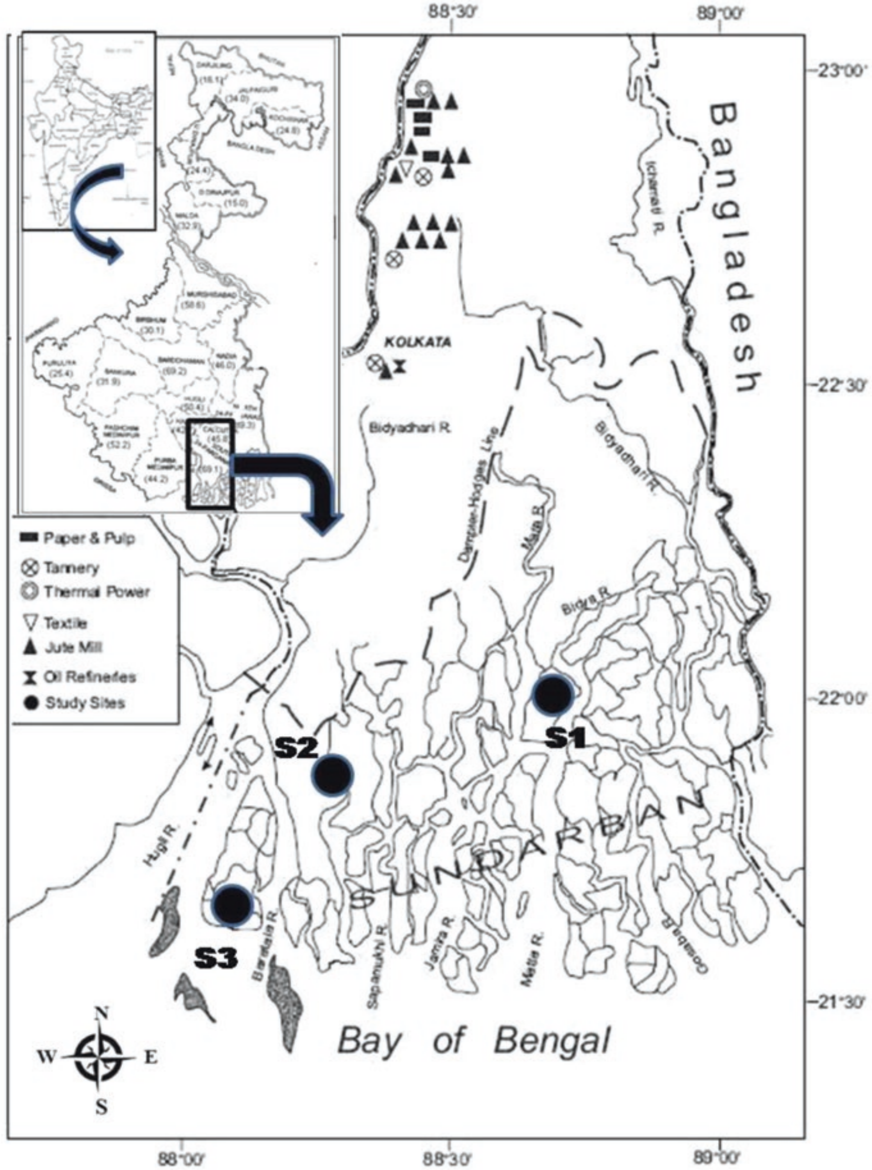

Fig. 1 Map of Indian Sundarban showing the location of the study sites (S_1–S_3). Location of multifarious industries are also shown in the upstream of Hugli river

Mela"—a pilgrim fare of over half a million of people—the area is impacted by anthropogenic stresses arising from rapid growth of settlements, aquaculture practices and tourism throughout the year [26]. Due to which the natural habitat of mangrove plants at this site is degrading though afforestation programme have been initiated by Govt. of India very recently. Two stations (S_1, S_2) are located on the main banks of the River Hugli (Ganges), while the third site (S_3) is located at the southern tip of Sagar Island, the largest delta of Indian Sundarban. The stations maintain a difference in the context of geomorphic and tidal set up that have different wave energy fluxes and distances from the sea (Bay of Bengal) and have diverse human interference with a variable degree of exposure to trace metal contamination.

2.2 Sample Collection and Processing

Surface sediment samples were collected in triplicate from top 0–5 cm at each sampling site covering an area of 1 m × 1 m using a clean, acid-washed fabricated polyvinyl chloride (PVC) scoop. Samples were stored in clean plastic zip lock pouches and transported to the laboratory. Individual sediment samples were placed in a ventilated oven at low temperature (~45 °C) [27] until completely dried, as high temperature may contribute to the alteration of volatile and even non-volatile organics of the sample [28], until they get completely dried. Samples were pulverized using an agate mortar and pestle, sieved through 63 μm mesh for homogenization since this fraction contains more sorbed metals per gram of sediment due to its larger specific surface area. Then, individually transferred into pre-cleaned, inert polypropylene bags and stored at room temperature until subsequent extraction and chemical analyses. Redox potential and pH was measured (T = 25 °C), using a glass electrode (HI 98160, HANNA Instruments, USA, Accuracy: 0.1 pH–0.01 pH, 1 mV (± 2000 mV), 0.1 °C) by inserting the probes directly into the fresh sediment sample. The electrode was calibrated using 4.01, 7.01 and 10.01 buffer solutions (HANNA instruments, USA).

Electrode were inserted for several minutes in the mud until stable values were reached, it was thoroughly washed and subsequently rubbed with fine tissue paper after each measurement in order to prevent the poisoning of electrodes by sulphide [29]. Organic carbon (C_{org}) content of the sediments was determined following a rapid titration method [30]. All the experiments were repeated three times with triplicate samples. The sand fraction was separated by wet sieving using a 63-μm mesh sieve. The silt (4–63 μm) and clay (<4 μm) fractions were determined using the pipette method [31] in which a sample suspension is prepared using sodium hexametaphosphate as the dispersing agent, and aliquots are pipetted at different time intervals from different depths, dried and weighed for mass determination. Statistical computation of textural parameters was done by using the formulae of Folk and Ward [32] following standards of Friedman and Sanders [33].

In each study site, mature mangrove trees of similar size and health condition selected for sampling. Live plant parts (young, mature and yellow leaves, bark, root/pneumatophore) were collected, from ten different mangrove plant species, namely, *Avicennia alba Blume., Avicennia officinalis L., Avicennia marina Forssk.* (Avicenniaceae), *Aegialitis rotundifolia Roxb.* (Plumbaginaceae), *Aeigeceros corniculatum L.* (Myrsinaceae), *Bruguiera gymnorrhiza L., Ceriops decandra Griff.* (Rhizophoraceae), *Exocaria agallocha L.* (Euphorbiaceae), *Sonneratia apetala*

Buch.- Ham. (Lythraceae) and *Xylocarpus mekongensis Pierre* (Meliaceae). A young leaf was selected as the leaf most proximal to the shoot apical meristem. The largest fully expanded leaf immediately distal to the shoot apical meristem was designated as mature [34]. Yellow leaves which are ready to fall from trees were also picked [35]. A sterilized knife was used to remove the bark from the tree trunks. Around the sampled trees, we excavated root system of the trees during low tide and collected pneumatophore/root as applicable. Samples were washed by deionised water in the laboratory thoroughly to remove any adhering dirt or dust particles. These were then grinded and oven-dried to constant weight under 50 °C till they became completely dry and subsequently homogenized adopting the methods performed by MacFarlane et al. [36].

2.3 Plant Description

The term mangroves collectively refers to woody halophytic angiosperm trees inhabiting in the intertidal zone of coastal estuarine regions in the tropics and subtropics, especially between 25°N and 25°S where the winter water temperature remains not less than 20 °C. Mangrove has a worldwide circumtropical distribution, the highest concentration being located in the IndoPacific region. The mangroves dominate almost 1/4th of world's tropical coastline. The total mangrove area which spans 30 countries including various island nations is about 100,000 km^2 [37]. The ten mangrove plants in consideration are thoroughly distributed in Indian Sundarban and form a mangrove bioassemblage in this sector. The most dominant plant *Avicennia* has a wide geographical distribution, with members found in intertidal estuaries along many of the world's tropical and warm temperate coasts. *Avicennia alba* and *A. officinalis*, distinctive genus in eastern tropics, are woody, possess stilt roots and are provided with pencil-like pneumatophores for aerial respiration [38].

Avicennia marina, which is a facultative halophyte that has various adaptations for hypersaline environments [39, 40], is a widely distributed species [36]. *Bruguiera gymnorhiza* was selected for investigation because it is an evergreen mangrove tree widely distributed in intertidal areas of tropical and subtropical coastlines of Asian, southern and eastern Africa, and northern Australia [41]. *Sonneratia apetala* is naturally distributed in India (the Bengal region) as a dominant species in local mangrove communities [42]. It is highly adaptable, fast growing and is used as a pioneer species in ecological succession in many degenerated mangrove forests [43]. Due to its high adaptability and seed production capacity, it has been utilized for restoration purposes in many other places besides its original locations [44, 45]. *Ceriops decandra* is an evergreen small, much branched tree which is very common in Indian Sundarban. *Aegialitis rotundifolia* is a characteristic mangrove associate but does not itself occur within closed mangrove communities, since it prefers or even requires exposed sites.

It is a low growing treelet having distinctive features like anomalous secondary thickening, abundant sclereids and incipiently viviparous seeds. *Aegiceras corniculatum* (Black mangrove), one of the most common and dominant mangrove plants, is usually 1–3 m tall. It often grows together in the intertidal habitat to form *A. corniculatum* communities in the wetland [46]. *Excoecaria agallocha* (Milky mangrove), belonging to family Euphorbiaceae [47], is found near the bank of tidal rivers in brackish water and almost all the places in the above study area of Sundarban. *Xylocarpus mecongenesis* is a woody, perennial, deciduous tree distributed throughout the mangrove habitats of Indian coasts, deltas and Andaman and Nicobar Islands [38].

2.4 Chemical Analysis

Plant and sediment samples were digested by using a microwave system (MARS Xpress, CEM Corp., USA) in automatic mode, with constant control of temperature and pressure. Sediment or dry plant material (200 mg) was quantitatively transferred to Teflon containers for mineralization, after which 8 mL of 10 M HNO_3 (Suprapur®, Merck) and 3 mL of H_2O_2 (30 %, analytical grade) were added. The containers were left to stand for 15 min to achieve preliminary acid digestion and then were placed in a microwave oven for mineralization. The digests were reconstituted with ultrapure deionized water to 20 mL for subsequent analyses of total metals. The element concentration in the solutions was determined by atomic absorption spectrometry (Thermo Scientific ICE 3500). For preparing calibration standards, certified reference AAS element standards solution (TraceCERT®, Sigma-Aldrich) was used.

2.5 Mangrove Microstructure Analysis

SEM analysis was performed to study the morphological characteristics of salt glands formed on the upper surface of the mangrove leaves. For studying the surface morphology of the leaves, scanning electron microscopy Model EVO 18 special edition (Carl Zeiss, Inc., Germany) was used. Samples of dried leaves were placed on double-sided carbon adhesive tape, which had previously been secured to aluminium-alloy stubs. These were metalized with gold coating with a sputter coater and analysed at 10 kV acceleration voltage, and the photomicrographs were taken at suitable magnifications.

2.6 Assessment of Sediment Contamination

In order to assess the level of contamination and the possible anthropogenic impact in the sediment samples, the contamination factor (CF), pollution load index (PLI) geoaccumulation index (I_{geo}) and enrichment factors (EFs) were estimated for some selected potentially hazardous trace metal evaluated in this study.

2.6.1 Contamination Factor (CF)

Metal concentration in a given environment is controlled by varied parameters like nature of substrate, physico-chemical conditions controlling the dissolution and precipitation of metals, and closeness to the pollution sites. Sediment has the capability to record the history and indicate the degree of pollution [48]. Different metals have synergetic and antagonistic effects on the prevailing environment. Concentration factor is considered to be an effective tool in monitoring the pollution over a period of time. The CF is the ratio obtained by dividing the concentration of each metal in the sediment by the baseline or background value (concentration in unpolluted sediment):

$$\mathrm{CF} = C_{\text{trace metal}} / C_{\text{background}}$$

The contamination levels may be classified based on their intensities on a scale ranging from 1 to 6 (0 = none, 1 = none to medium, 2 = moderate, 3 = moderately to strong, 4 = strongly polluted, 5 = strong to very strong, 6 = very strong) [49]. The highest number indicates that the metal concentration is 100 times greater than what would be expected in the crust [50].

2.6.2 Pollution Load Index (PLI)

For the entire sampling site, PLI has been determined as the *n*th root of the product of the *n*th CF:

$$\mathrm{PLI} = \left(\mathrm{CF}_1 \times \mathrm{CF}_2 \times \mathrm{CF}_3 \times \cdots \times \mathrm{CF}_n\right)^{1/n}$$

This empirical index provides a simple, comparative means for assessing the level of trace metal pollution. When PLI > 1, it means existence of pollution; in contrast, PLI < 1 indicates metal pollution [51].

2.6.3 Geoaccumulation Index (I_{geo})

The geoaccumulation index (I_{geo}) [52] was used to evaluate the degree of elemental pollution in the sediments from the study area. Mathematically, I_{geo} is given as:

$$I_{\text{geo}} = \log_2 [(C_n) / 1.5(B_n)]$$

where C_n is the concentration of metals examined in sediment samples, and B_n is the geochemical background concentration of the metal (n). Factor 1.5 is the background matrix correction factor introduced to account for possible differences in the background values due to lithospheric effects. The geoaccumulation index consists of seven classes [52] Class 0 (practically unpolluted): $I_{geo} \leq 0$; Class 1 (unpolluted to moderately polluted): $0 < I_{geo} < 1$; Class 2 (moderately polluted): $1 < I_{geo} < 2$; Class 3 (moderately to heavily polluted): $2 < I_{geo} < 3$; Class 4 (heavily polluted): $3 < I_{geo} < 4$; Class 5 (heavily to extremely polluted): $4 < I_{geo} < 5$; Class 6 (extremely polluted): $5 > I_{geo}$ [53].

2.6.4 Enrichment Factor

The behaviour of a given element in the sediment (i.e. the determination of its accumulation or leaching) may be established by comparing concentrations of a metal with a reference element. The result obtained has been described as enrichment factor (EF), which was calculated using the following equation:

$$\mathrm{EF} = \left(C_n / C_{\mathrm{ref}}\right) / \left(B_n / B_{\mathrm{ref}}\right)$$

In which C_n is the content of the examined element in the sediment, and C_{ref} is the content of the examined element in earth crust. B_n is the content of the reference element in the sediment, and B_{ref} is the content of the reference element in earth crust. In the present study, Fe was used as reference element because of the following reasons (a) Fe is associated with fine solid surfaces; (b) its geochemistry is similar to that of many trace metals and (c) its natural concentration tends to be uniform [54]. The world average elemental concentrations reported by Turekian and Wedepohl [55] in the Earth's crust were used as reference in this study because regional geochemical background values for these elements are not available. EF values less than 5.0 are not considered significant because such small enrichments may arise from differences in the composition of local sediment material and reference sediment used in EF calculations [56]. However, there is no accepted pollution ranking system or categorization of degree of pollution on the enrichment ratio and/or factor methodology. Five contamination categories are recognized on the basis of the enrichment factor: EF < 2 states deficiency to minimal enrichment, EF = 2–5 moderate enrichment, EF = 5–20 significant enrichment, EF = 20–40 very high enrichment and EF > 40 extremely high enrichment [57]. EF can easily be used to differentiate between elemental concentrations from anthropogenic source and those from natural origin. EF values between 0 and 1.5 indicate the metal is entirely from crustal materials or natural origin, while EF > 1.5 suggests that the sources are more likely to be anthropogenic. EFs greater than 10 are considered to be non-crusted source [58].

2.6.5 Potential Ecological Risk Index

The potential ecological risk index (PER) was also introduced to assess the contamination degree of trace metals in the studied sediments. The equations for calculating the PER were proposed by Hakanson [59] as follows:

$$E = \mathrm{TC}$$

$$C = C_a / C_b$$

$$\mathrm{PER} = £E = £\,\mathrm{TC}$$

where C is the single element pollution factor, C_a is the content of the element in the samples and C_b is the reference value of the element. The sum of C for all the metals examined represents the integrated pollution degree (C) of the environment. E is the potential ecological risk factor of an individual element and T is the biological toxic factor of an individual element, which is set at Cu = Pb = 5, Zn = 1, Cr = 2 and [59]. PER is a comprehensive potential ecological index, which equals the sum of E. It represents the sensitivity of a biological community to toxic substances and illustrates the potential ecological risk caused by contamination.

2.6.6 Sediment Quality Guidelines

Sediment quality guidelines (SQGs) are very useful to screen sediment contamination by comparing sediment contaminant concentration with the corresponding quality guideline [60]. These guidelines evaluate the degree to which the sediment-associated chemical status might adversely affect aquatic organisms and are designed to assist in the interpretation of sediment quality. Such SQGs have been used in numerous applications, including designing monitoring programmes, interpreting historical data, evaluating the need for detailed sediment quality assessments, assessing the quality of prospective dredged materials, conducting remedial investigations and ecological risk assessments and developing sediment quality remediation objectives [60]. The consensus-based sediment quality guidelines (SQGs) were used in this study to assess possible risk arises from the trace metal contamination in sediments of the study area. The SQGs were developed from the published freshwater sediment quality guidelines that have been derived from a variety of approaches [60]. These synthesized guidelines consist of a threshold effect level (TEL) below which adverse effects are not expected to occur and a probable effect level (PEL) above which adverse effects are expected to occur more often than not. Long et al. [61] also identified two guideline values: the effects range-low (ER-L) and the effects range-median (ER-M). Concentrations below the ER-L value were rarely associated with biological effects. Concentrations in the range between ER-L and ER-M were found to occasionally co-occur with biological effects. Biological effects were also often found to co-occur with concentrations above the ER-M value.

2.7 Bioaccumulation Indices for Hyperaccumulation

Three internationally recognized hyperaccumulator indices were used to evaluate the hyperaccumulator species listed as follows:

2.7.1 Translocation factor (TF)

$TF_{leaf} = C_{leaf}/C_{root}$, where C_{leaf} and C_{root} are the trace metal concentrations in the leaf and root, respectively [62, 63]. A translocation factor greater than 1 indicates preferential partitioning of metals to the shoots [64].

2.7.2 Extraction Coefficient (EF)

$$EF = C_{shoot} / C_{sediment}$$

It evaluates the ability of the plant to accumulate heavy metals in shoot biomass [64] and extraction coefficient more than 1 is one of the criteria for identifying hyperaccumulator plants [65].

2.7.3 Bioaccumulation Factor (BCF)

$$BCF_{leaf} = C_{leaf} / C_{sediment}; BCF_{bark} = C_{bark} / C_{sediment}; BCF_{root} = C_{root} / C_{sediment}$$

where C_{leaf}, C_{bark} and C_{root} are the trace metal concentrations in the leaf, bark and root, respectively, and $C_{sediment}$ is the extractable concentration of trace metal concentration in the sediment. It is used for quantitative expression of accumulation [64].

2.8 Statistical Analysis

To identify the relationship amongst trace metals in sediments, Pearson's correlation coefficient analysis and cluster analysis (CA) were performed using the commercial statistics software MINITAB version 13 for Windows. The correlation coefficient measures the strength of interrelationship between two trace metals. Data were analysed using student's test (t-test) and a one-way analysis of variance (F-test). Independent variables examined with exponential accumulation relationships were log transformed ln $(x+1)$, prior to statistical calculation. The logarithm-transformed data were applied to eliminate the influence of different units of variance and give each determined variable an equal weight [66].

Cluster analysis classifies a set of observations into two or more mutually exclusive unknown groups based on a combination of internal variables. This is often coupled with PCA to check results and to group individual parameters and variables [67]. The purpose of CA is to discover a system of organizing observations, where a number of groups/variables share observed properties. Dendrogram is the most commonly used method of summarizing hierarchical clustering. In the current study, CA was used to evaluate the sources similarities of trace metals in sediment samples.

3 Results and Discussion

3.1 Sediment Geochemistry

Physical properties of coastal sediments are important variables in order to understand geological events in coastal environments [68]. Sediment grain size distribution was generally homogenous in the rhizosediments, which ranged between 58.76–60.00 %, 15.10–41.40 % and 0.40–26.14 %, respectively, for the proportion of clay (<2 μm), silt (2–63 μm) and fine sand (63–250 μm) with slightly basic pH varying between 7.22 and 7.66 which is the characteristic of coastal sediments suffering from marine influence and limited buffer capacity. The highest percentage of organic carbon (0.95 %) was obtained in station Gangasagar (S_3) and the lowest (0.50 %) was found in Jharkhali (S_1). These low values of C_{org} are probably related to the poor absorbability of organics on negatively charged quartz grains, which predominate in the rhizosediments of this estuarine environment [23, 69]. The prevailing pH and organic carbon (C_{org}) content in the rhizosediments affect the availability and mobility of trace metals [70]. Since mangrove sediments are generally anoxic and waterlogged, trace metals are precipitated as insoluble sulphides [71]. The redox potential (E_h) values ranged between −7.6 mV and −33.5 mV. These negative potentials indicate the natural Eh oscillation [72]. The oxidation/reduction state (redox potential, Eh) of sediment is an important parameter affecting As transformation. Sediment redox levels can greatly affect toxic metals uptake by plants [73]. However, there is little information on redox chemistry of metals in rhizosediment from West Bengal (Table 1).

3.2 Metals in Sediment

The average concentrations of trace metals ($n = 3$) in mangrove sediments are summarized in Table 2 along with a comparative account in selective mangrove wetlands around the world. Concentration of majority of the trace metals (Cr, Pb and Zn) was very much similar to Yellow Sea, China [74], but the value for Cr was slightly higher than N. America [75], Korean Coast, Korea [76] and Pichavaram mangrove forest, India [77]. Metal concentration was found lower than the study carried out by Suresh et al., 2015 [78] in Kerala, India and Chakraborty et al. [79] at Cochin Estuary, India but higher (except Cu) than the study of Kathiresan [77] at

Table 1 Descriptive statistics of the studied trace metals in mangrove plant organs (values expressed in mg kg^{-1})

Organs		Cr	Cu	Fe	Mn	Pb	Zn
Young leaf	max	27.4	21.09	1130.85	2089.84	2.55	32.3
	min	1.04	4.008	106.99	25.58	0.02	4.87
	median	5.78	10.81	286.68	86.97	0.31	16.98
	mean	8.42	12.27	437.08	239.78	0.64	17.59
	SD	± 7.06	± 5.22	± 338.12	± 519.03	± 0.81	± 8.26
Mature leaf	max	22.9	16.46	1610.13	2298.77	4.98	30.19
	min	2.45	4.53	80.41	17.07	0.09	5.70
	median	8.78	9.49	328.03	114.96	0.46	12.50
	mean	9.34	9.75	484.52	323.61	0.92	16.82
	SD	± 5.55	± 3.59	± 454.57	± 585.67	± 1.20	± 8.95
Bark	max	61.3	36.51	1796.47	436.53	2.49	33.13
	min	2.6	3.35	188.42	9.58	0.13	4.43
	median	8.85	9.93	463.72	67.39	0.46	12.73
	mean	16.1	13.29	748.17	115.96	0.83	13.88
	SD	± 16.7	± 9.79	± 563.11	± 127.92	± 0.87	± 8.12
Root/pneumatophore	max	25.6	14.72	1380.64	137.20	1.46	16.55
	min	2.59	4.66	257.02	19.12	0.03	3.29
	median	8.36	8.82	628.27	42.31	0.23	6.84
	mean	9.61	9.87	750.24	62.37	0.39	9.56
	SD	± 7.27	± 3.32	± 407.57	± 44.91	± 0.43	± 5.34

Pichavaram mangrove forest, India. In the present study, values of Cu and Pb were found similar with the results of Hawaii Beach, Malaysia [80] but higher than Saudi coastline, Saudi Arabia [81].

The concentration of most of the metals is greater than the concentration of plant organs (see Fig. 2), as mangrove sediment is rich in sulphide or due to the effect of chelating substances such as humic acids [82]. They therefore favour the retention of waterborne trace metals [2], and the subsequent oxidation of sulphides between tides allows element mobilization and bioavailability [83]. The maximum concentrations of majority of trace metals were recorded at Gangadharpur (S_2) resulting deposition of metals from intensive human activities like agriculture practice, aquaculture practice, use of antifouling paints wood polishing work, etc. throughout the year. The average total contents of trace metals were in the following descending order of Fe (11,097.11 mg kg^{-1}) > Mn (709.04 mg kg^{-1}) >> Cr (76.63 mg kg^{-1}) > Ni (45.89 mg kg^{-1}) > Zn (40.42 mg kg^{-1}) > Cu (36.03 mg kg^{-1}) > Pb (14.09 mg kg^{-1}) > As (9.45 mg kg^{-1}) > Co (7.25 mg kg^{-1}). The observed high concentration of Fe might be a result of the textural and mineralogical characteristic of marine sediments [84].

In the present study, the concentration of Fe (11,097.11 mg kg^{-1}) at Gangasagar (S_3) is maximum and shows higher concentration in sediment than mangrove organ. High concentrations of Fe might be due to the precipitation of Fe as iron sulphide which is common in mangrove ecosystems. Iron is generally described as the principal metal

Table 2 The minimum, maximum and average concentrations of trace elements (mg kg^{-1}) in the rhizosediment of the present study and selective mangrove wetlands around the world

Location	Cr	Cu	Mn	Fe	Pb	Zn	References
Gulf of Guayaquil (N. America)	48.36	139.46	359.06	13,431.1	37.66	331.31	Fernandez-Cadena et al. [75]
Yellow Sea. China	–	15.1	410	1.33	12.3	47.3	Jiang et al. [74]
Hawaii Beach, Malaysia	254	32.24	–	–	18.6	18.7	Nagarajan et al. [80]
Saudi coastline, Saudi Arabia	295	7.39	–	–	9.51	36.5	Al-Trabulsy et al. [81]
Korean Coast, Korea	58.3	36.5	–	–	35	122	Ra et al. [76]
Kerala, southern part India	80.94	76.73	–	–	189.64	127.6	Suresh et al. [78]
Pichavaram mangrove forest, south eastern India	17	46	25	1770	8	25	Kathiresan et al. [77]
Kochi Estuary, south west India	131.9	43.4	–	–	39.8	422.7	Chakraborty et al. [79]
Sundarban Wetland, India	76.63	36.03	709.06	11,097	14.1	40.42	Present study

that precipitates with sulphidic compounds in anaerobic sediments [85], and these sulphides form a major sink for metals in the mangrove area. According to Badr et al. [86], rhizosediment was enriched with some trace metals such as Mn mainly due to discharge of untreated industrial and sewage wastes. The use of gasoline may be considered as a possible reason for the Pb contamination 4.98 mg kg^{-1} in mature leaf of *X. mecongenesis* at Jharkhali (S_1) [87]. Several researchers have previously measured elevated concentrations of trace metals in mangrove sediments over the world, reflecting the long-term pollution caused by human activities [2, 88]. Elements of natural origin reach coastal areas from rivers in the form of particulate material. These elements are mainly chemically bound to aluminosilicates and are therefore lowly bioavailable. On the other hand, anthropogenic elements are more loosely bound to the sediments and may be released back to the aqueous phase with the change of physical and chemical characteristics (E_h, pH, salinity and the content of organic chelators) [89].

3.3 Potential Risk Assessment

On the basis of their average geoaccumulation Index (I_{geo}) values, The trace metals can be arranged in the following sequence Fe > Zn > Pb > Cr > Cu > Mn. In the present work, I_{geo} showed very high values except lead at two stations indicating that

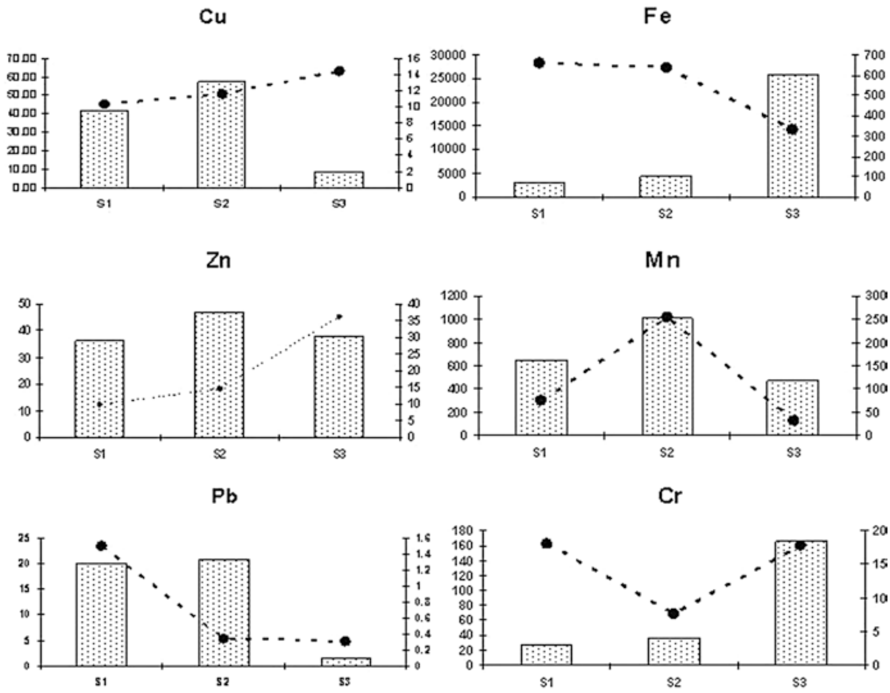

Fig. 2 Pooled mean value (expressed in mg kg^{-1}) of trace metals in rhizosediment (Y axis, column) and plant organs (X axis, discontinuous line) concentrations found in rhizosediments (mg kg^{-1}, columns and *Y* axis) and the average metal concentrations measured in plants considering pooled mean values of all studied plants collected at each site (mg kg^{-1}, discontinuous line and *Y* axis)

sediments are strongly polluted [53]. The results from EF (as shown in Fig. 3) indicate that the highest EFs values (>10) for Cu, Mn and Pb were obtained in Jharkhali (S_1) and Gangadharpur (S_2). The high EF values for these metals in sampling sites suggests the presence of contaminated sediments derived from various sources like domestic sewage, power-plant operation, major storm events, or dumping of dredged sediments dredging along the international shipping zones [90]. The highest CF values for most of the metals (Cu, Mn, Pb) studied were found at Gangadharpur (S_2), which receives a huge amount of agricultural and domestic discharge in regular basis along with aerial particulate Pb [91] from nearby road. The CF values for these trace metals were $1 < CF < 3$ and indicate moderate contamination in sediments. Effect range-low (ER-L) and threshold effect level (TEL) values were exceeded by Cr and Cu implying that adverse consequences to biota may occasionally occur (as shown in Fig. 4). Chromium comes from the untreated industrial effluents from steel and tannery industries [91]. The potential sources of Cu in this coastal region might be due to antifouling paints [92] and extensive use of fertilizers and pesticides for agricultural needs. However, exceedance of SQGs

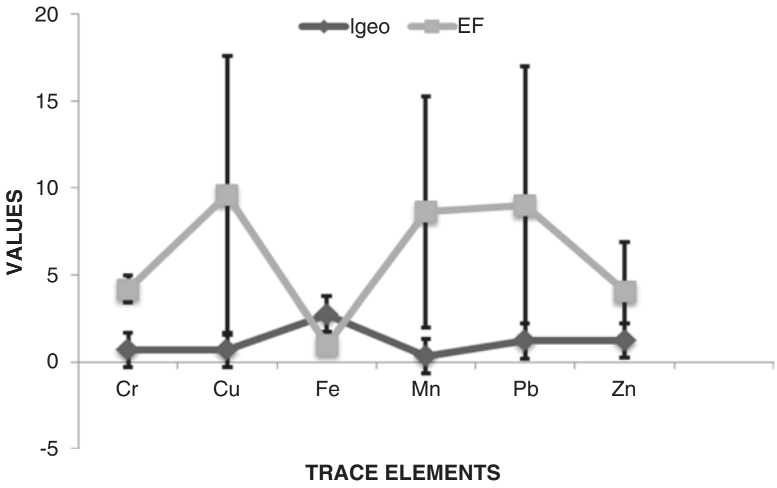

Fig. 3 Pooled mean values of Index of Geoaccumulation (I_{geo}) and Enrichment Factor (EF) considering three study sites of Sundarban (Average ± SD)

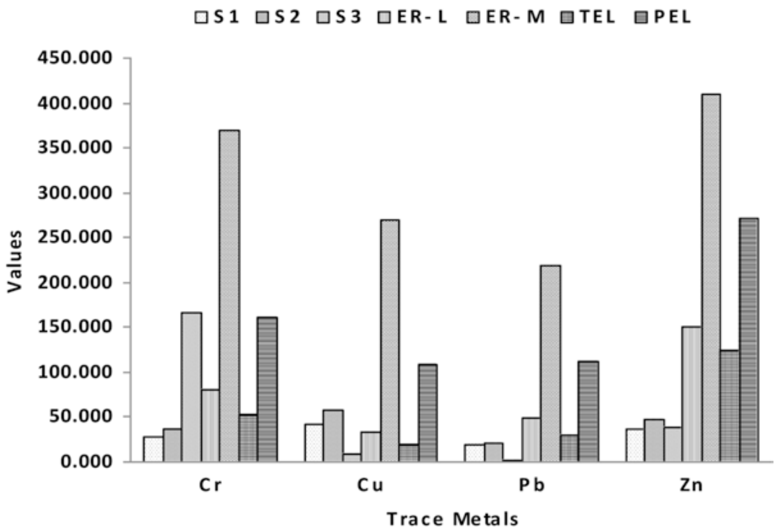

Fig. 4 Distribution of studied trace metals, ER-L, ER-M, TEL and PEL (SQGs) in rhizosediment

is not necessarily due to human stress and may be inherit from the local geological background and depositional settings [93].

Potential ecological risk was used to evaluate the potential risk of one metal or a combination of multiple metals. According to Hakanson [59], the potential ecological risk that trace metals pose in coastal sediments can be classified into the following categories: Low risk: E<40, PER<150. Moderate risk: 40≤E<80, 150≤PER<300. Considerable risk: 80≤E<160, 300≤PER<600. High risk: 160 < E<320, PER≥600. Very high risk: E≥320. It was found that the single risk factors (E) of trace metals were ranked in the order of Cu>Pb>Cr>Zn. The average ecological risk (E) for all metals in most surface sediments was less than 40, indicating a low risk to the local ecosystem [94].

3.4 Metals in Mangroves

There exists wide range of variations for trace metal uptake and distribution in three aerial tissues, and this might be due to complex physiological mechanisms involving cell wall immobilization, complexes with humic substances and presence of barrier at the root epidermis [95] (see Fig. 5). The trend of accumulation of trace metal maintained the following descending order (average for all four study sites): Fe (656.01 mg kg^{-1})>Mn (193.28 mg kg^{-1})>Zn (14.54 mg kg^{-1})>Cr (11.12 mg kg^{-1})>Cu (11.07 mg kg^{-1})>Pb (0.68 mg kg^{-1})>Co≥Ni≥As~BDL.

The maximum concentration of Fe in mangrove tissue are associated with the highest concentrations in the surrounding sediments which may be related to the precipitation of iron as iron sulphides in these mangrove sediments which might act as the potential source of this enrichment. Iron is an essential micronutrient and constituent of cytochromes and of nonheme iron proteins involved in photosynthesis, nitrogen fixation and respiration. Wide range of variations (from 53.78 mg kg^{-1} in bark of *A. alba* to 1796.47 mg kg^{-1} in bark of *E. agallocha* at S_2) of Fe was observed in the present study. Manganese, an essential element showed a wide range of variations (from 24.32 mg kg^{-1} in bark of *A. rotundifolia* at Gangasagar (S_3) to 2298.77 mg kg^{-1} in mature leaf of *S. apetala* at Gangadharpur (S_2)). Generally, Mn^{+2} is taken up by root/pneumatophore of the plant and mostly required in leaf for photosynthesis and nitrogen and carbohydrate metabolism [96]. Also, precipitation of authigenic Mn carbonate in coastal sediments acts as a potential source of Mn [97].

Trace metals can be absorbed by plants using their roots, or via stems and leaves, and stored into different plant parts. Moreover, the distribution and accumulation of trace metals in the plants depend on plant species, metal sources as well as metal concentration in sediments [98]. The maximum values of essential metals like Cu (24.17 mg kg^{-1} in *S. apetala* at Jharkhali (S_1)), Fe (1796.47 mg kg^{-1} in *E. agallocha* at Gangadharpur (S_2)) as well as non-essential metal Cr (61.26 mg kg^{-1} in *A. rotundifolia* at Gangasagar (S_3)) were recorded in trunk bark. Trunk bark is lipophilic in nature and readily adsorbs and collects metals as an excellent passive atmospheric sampler as endorsed by Fu et al., 2014. Previous reports also support the phytoextraction capacity of bark in mangrove plants in other Indian estuaries (Kathiresan et al. [77] at Cuddalore and Pichavaram estuary, southern part of India and Chowdhury et al. [3] from Indian Sundarban). Copper is required in chloroplast reactions, enzyme systems, protein synthesis, growth hormones and carbohydrate metabolism [99]. It is also required in various redox reactions in photosynthesis and

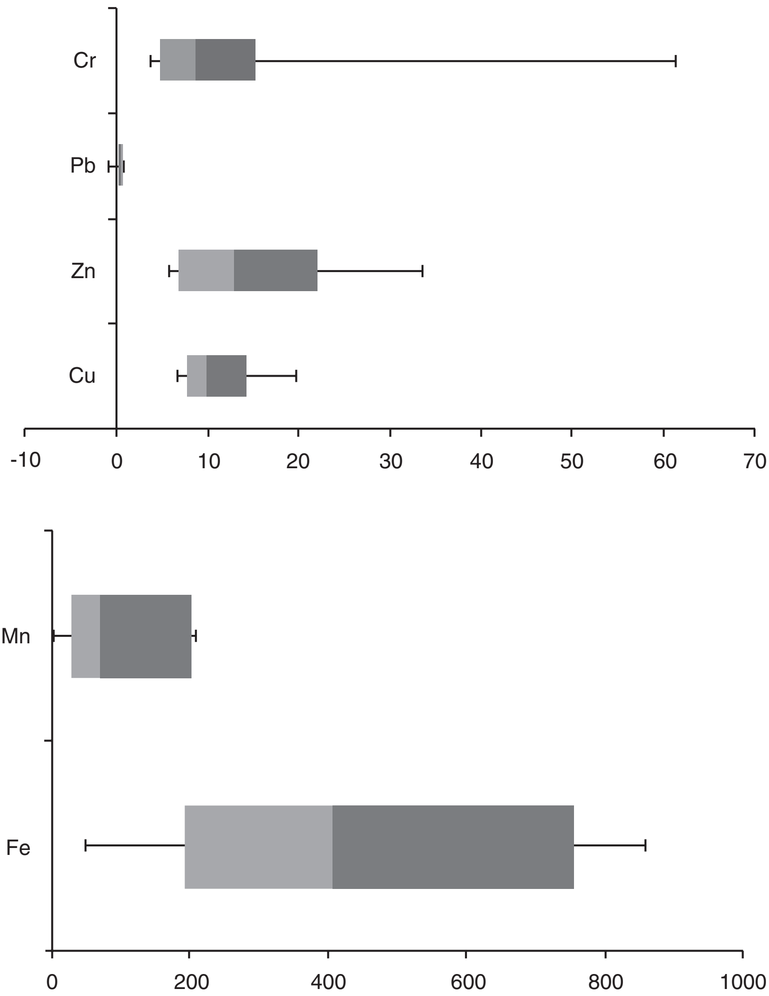

Fig. 5 Box-Whisker plots of metal concentration found in mangrove organs. All the boxes show the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest coefficients, while the line inside the boxes expresses the median

respiration [100]. Chromium is toxic to plant growth and also easily taken up and translocated [101]. The high concentration of Cr inhibits the growth of plants causing chlorosis and necrosis [102]. However, no apparent adverse effects were detected in this study, which may be due to mangrove's high tolerance to Cr stress.

Another essential metal Zn (55.80 mg kg^{-1} in *A. rotundifolia* at Gangasagar (S_3)) and Mn (2298.77 mg kg^{-1} in *S. apetala* at Gangadharpur (S_2)) along with toxic metal Pb (4.98 mg kg^{-1} in *X. mekongenesis* at Jharkhali (S_1)) showed a common tendency of accumulation in leaves, which may be attributed to acropetal movement of elements through translocation [103]. Mangrove plants are known to accumulate considerable amount of metals in leaves and other vegetative parts [77]. Nonetheless, it might indicate that the leaves of mangroves are able to take up and store certain trace metals. Moreover, the sampled leaves did not show any sign of injury in cases where concentrations were high. This suggests that leaves were tolerant to the trace metals by imparting minimal physiological effects to the leaves [104]. According to Verkleij and Schat [105], the translocation of excessive metals into mature leaves shortly before their shedding can also be considered as a tolerance mechanism, as can the increase in metal-binding capacity of the cell wall [106]. With the development of leaves from young to old, the changes in concentrations of metals in leaves indicated that Zn, Mn and Pb were apt to be accumulated in older leaves. Higher concentrations of these essential metals in leaf tissue may be because they were translocated to above ground parts and reused in plant system. It has been reported that some essential metals were transferred and reutilized in many plant species before defoliation, while toxic materials were accumulated in older leaves and then removed via defoliation [107].

In our study, *S. apetala* exhibited its capacity to absorb Cu in its bark (24.17 mg kg^{-1}) at Jharkhali (S_1) and Mn in mature leaf (2249.77 mg kg^{-1}) at Gangadharpur (S_2) S_2. According to the studies on leaf anatomy [108], different leaf morphology features were observed in *S. apetala* [109]. Epidermal trichomes were located outside *S. apetala* upper and lower epidermis while they were not observed on other mangrove species; stomatas distributed in both the upper and lower epidermis of *S. apetala* while only in the lower epidermis of other species. Such features might affect metal uptake and maintain process [22]. Chua and Hashim [110] also reported foliar absorption of certain elements especially in polluted industrial area. It was seen that only *S. apetala* absorbed higher magnitude of Fe and Mn than other mangroves in all the cases. For both the elements, the concentration varied more than ten times. For translocation factor, *S. apetala* exhibited highest TF values for Mn (31.99) and Pb (18.01) at Gangadharpur (S_2) and 9.95 for Zn at Jharkhali (S_1), respectively, where the highest value for translocation for other plant was 8.00 for Cr in case of *A. corniculatum*. Similar results were found by Sinegani and Ebrahimi [111] who observed significant metals mobilization between the plant parts above and below the surface of the sediment with translocation factor (TF) > 1. This indicates that the plant translocates elements effectively from root to the shoot and hence they could be labelled as accumulators of pollution as described earlier [112]. The prevalent trend justifies in considering the species as an effective indicator of trace metal contamination which was also endorsed by Nazli and Hashim [113] from Peninsular Malaysia.

3.5 Biological Risk Assessment

Hyperaccumulator plants can accumulate concentrations of trace metals in their aerial tissues far in excess of normal physiological requirements and above the levels found in most plant species [114]. An ideal plant for metal phytoextraction has to be tolerant to high levels of the metal and must accumulate high metal concentrations in its organs. Additional favourable traits are fast growth, easy propagation and a profuse root system [115, 116]. Translocation factor is considered as a potential tool for the determination of hyperaccumulator plants. A translocation factor greater than 1 indicates preferential partitioning of metals to the shoots [117–119]. Translocation factor values of the present work shows that *S. apetala* exhibited high values for Mn (4.48 and 31.99), Zn (9.95, 3.25) and Cu (3.42, 3.47) and Pb (1.84, 18.01) for Jharkhali (S_1) and Gangadharpur (S_2), respectively. *Aegiceros corniculatam* recorded high TF values for Cr (1.67, 8.00) and Pb (6.68, 6.25) at Jharkhali (S_1) and Gangadharpur (S_2), respectively. Members of family Avicenniaceae, *A. Alba* and *A. officinalis* showed high values for Fe (4.36, 2.78), Mn (9.53, 26.10), Pb (5.28, 5.93), and Cu (2.18, 2.23) at Gangadharpur (S_2). Bioconcentration factor, which is also considered as a tool for hyperaccumulation indicator, presented high values for *S. apetala* at Jharkhali (S_1) (5.99 for Mn and 10.7 for Fe in bark) and Gangadharpur (S_2) (2.28 for Mn in leaf). *Aegialitis rotundifolia* also showed high values for Mn (1.94 in bark), Cu (1.77 in leaf) and Zn (1.68 in bark) at Gangasagar (S_3). As stated earlier, extraction coefficient (EF) reflects the ability of plant shoot to accumulate metals and our study shows that *S. apetala* recorded the highest value for Mn (4.92) at Gangadharpur (S_2) and for Cu (1.73) and for Cr (3.01) at Jharkhali (S_1). *Aegialitis rotundifolia* recorded high value for Cu (6.51) at Gangasagar (S_3). Highest value of EF for Cr (4.22) was recorded in *X. mecongenesis* at Jharkhali (S_1). Thus, in the present study, *S. apetala* could be considered as hyperaccumulators as it fulfils most required criteria and is suitable for phytoextraction of metal-contaminated soils.

3.6 Result of Statistical Analysis

Pearson's correlation coefficient gives an idea about the possible relationships between metals: common origin, uniform distribution, similar behaviors and relationships amongst metals. The linear correlation coefficients calculated for metals in the mangrove organ samples indicated that a significant positive correlation existed amongst the metals. Significant correlation of Cu-Fe was found in case of all organs (Jharkhali (S_1): young leaf: $r=0.899, p<0.05$; mature leaf: $r=0.931, p<0.05$, Gangadharpur (S_2): mature leaf: $r=0.790, p<0.05$; root: $r=0.763, p<0.05$). Copper also showed significantly positive correlation with Manganese at Jharkhali (S_1) (young leaf: $r=0.873, p<0.05$; mature leaf: $r=0.939, p<0.05$). All mangrove plants showed significant differences between element concentrations in monitored plots (One-Way ANOVA: −df = 5; $F=20.26$; $P<0.01$).

Table 3 reflects the factor loadings, variance percentages and cumulative percentages corresponding to principal components after varimax rotation was performed to secure increased environmental significance. The analysis resulted in the explanation of 81.1 % of variances in the data. The first factor (factor 1) explains 26.5 % of total variance and is related to the variables Mn, while the parameters Pb,

Table 3 Results of factor analysis (after Varimax rotation) considering different organs of all the mangroves

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Cu	–0.048	0.717	0.516	0.167
Fe	–0.029	–0.326	0.737	–0.554
Zn	0.414	0.733	–0.027	–0.143
Mn	0.514	0.114	–0.379	–0.67
Pb	–0.758	0.2	–0.311	–0.184
Cr	–0.758	0.244	–0.021	–0.338
% variance	26.5	21.2	17.5	15.9
Cumulative var %	26.5	47.7	65.2	81.1

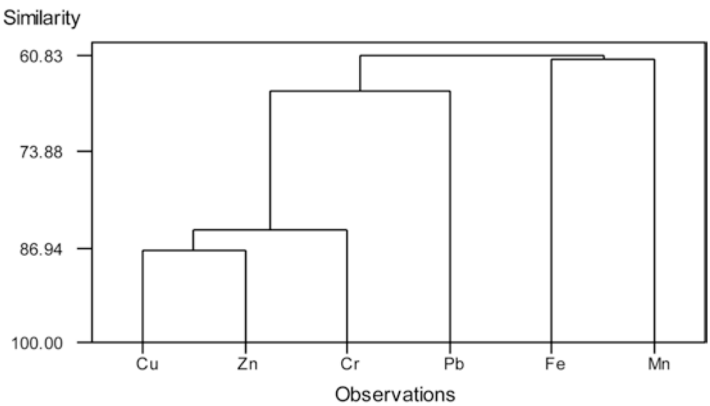

Fig. 6 Dendrogram showing the relationship between sediment samples in terms of trace metals at three study sites

Cr are negatively loaded with this factor. This may be due to very low or below detection limit of the element concentration in different plant organs of the studied mangroves. Factor 2 represents 21.2 % of the total variance and is positively loaded with Cu and Zn. Factor 3 explains 17.5 % of the total variance and is loaded with Cu and Fe. On the other hand, factor 4 represents 15.9 % of the total variance and is negatively loaded with Fe and Mn.

A hierarchical cluster analysis was carried out to identify any anomalous behaviour pattern in the mangrove plant organs. As shown in Fig. 6, which could be grouped into two clusters of Cu-Zn and Fe-Mn have been identified explaining that they are mainly generated from natural sources such as surface runoff and the presence of some metal bearing minerals in different locations of the study area. The Euclidean distance of the standardized data was chosen as dissimilarity measurement.

4 Conclusion

The present study has demonstrated the efficient role of *S. apetala* in accumulating the trace metals especially in pneumatophores and barks from a highly stressed estuarine mangrove system. This was mainly done through phytoextraction by adopting complex and cohesive processes and mechanisms. This dominant true mangrove species acts as both physical and biogeochemical barriers to trace metal mobility and hence has the potential to protect Sundarban ecosystem. Trace metal concentration in rhizospheric sediment are mainly controlled by the presence of finer particle sizes as well as organic carbon. In plants metal contamination is mainly concerned in root/ pneumatophore which is due to the formation of iron plaques on root surfaces. This tropical mangrove region is getting critically polluted due to severe anthropogenic stresses, and an extensive study is required to understand the role of rhizosphere processes in accumulation of trace metals in potential mangrove plants such as *S. apetala, A. alba and A. officinalis*.

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Fate of Phenolic Compounds in Constructed Wetlands Treating Contaminated Water

Alexandros I. Stefanakis and Martin Thullner

Abstract Phenolic compounds are used in many industrial processes and, thus, are found in various wastewaters of industrial origin. Their main source is the chemical and the petrochemical industry, but they are also present in many agro-industrial wastewaters (e.g., olive mill effluents). Phenolic compounds are considered priority pollutants with potential toxic and carcinogenic effects and should be treated and removed from water sources. Advanced oxidation processes have been applied for their removal, but their high operation and maintenance costs, complexity, and energy-intensive nature make these technologies unsustainable and often infeasible. On the other hand, Constructed Wetlands are characterized by lower operational costs, low energy consumption, and green appearance, which make them a sustainable, environmentally friendly treatment method. These systems have also been tested for the treatment of waters containing phenolic compounds. Current experience implies that Constructed Wetlands can effectively remove a series of different phenolic compounds from wastewaters, even at high concentrations. This chapter summarizes the state-of-the-art knowledge regarding the range of applications and the overall effectiveness of phenolic compounds treated in different Constructed Wetland systems.

Keywords Constructed wetlands • Phenols • Horizontal flow • Vertical flow • Removal processes • Biodegradation • Sorption • Plant uptake • Efficiency

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1 Introduction

Phenolic compounds comprise a class of chemical substances ranging from phenol to complex polyphenolic molecules, which are commonly found as pollutants in industrial wastewaters of different origin. These compounds are in use for more than 180 years in various industrial processes, such as weathering of railway ties or odor control during sewage treatment. Phenolic compounds can be produced both naturally, e.g., by isolation from coal tars, and synthetically, e.g., by toluene oxidation. Phenol is also produced from benzene during the manufacture of certain polymers such as polyamides, polycarbonates, and resins through the cumene process, which involves the production of cumene and its conversion to hydroperoxide, which is then decomposed to cumene hydroperoxide. One alternative production method is benzene oxidation with nitrous oxide (N_2O).

The main use of phenolic compounds is to produce bisphenol A (a compound used to produce polycarbonates), cyclohexanol (for the production of polyamides), and other nylon intermediates [1]. Phenol is also used as slimicide, in medicinal products (e.g., ear and nose drops, throat lozenges, mouthwashes), explosives, paints, perfumes, textiles, and drugs [2]. Phenolic compounds can be toxic for many bacteria species, which enabled their use as antiseptics and in pesticides [3]. The annual world phenol production was estimated at 8.34 million tons in 2010 and more than 8.9 million tons in 2012, while leading countries in phenol production are the USA, China, Japan, Taiwan, and South Korea (more than 60 % of the annual global production). World phenol production is anticipated to exceed 10.7 million tons by 2016 [1].

1.1 Phenol Sources, Effects, and Treatment Technologies

Industrial processes involving the production and/or the use of phenolic compounds may result in their release into the environment and in a contamination of surface and groundwater bodies. Significant phenolic compound concentrations can be found in various wastewaters from industries such as oil industry and refineries, chemical industry, pharmaceutical industries, pulp and paper mills and tanneries [4], as well as in some agro-industrial wastewater, e.g., from olive mills [5, 6] and cork processing [7]. There are several phenol-related aromatic compounds such as tannins, chlorophenol, *m*-cresol, nitrophenol, and polycyclic aromatic hydrocarbons in wastewaters, depending on its source [8]. In many wastewaters (e.g., in olive mill wastewater) phenolic compounds represent the most toxic pollutants [5]. Thus, all waters containing phenolic compounds (especially industrial wastewaters) should be properly treated to prevent the release of these toxic compounds into the environment.

Phenolic compounds can cause some adverse effects to human health, such as skin and eye irritation, irregular breathing, muscle weakness, among other symptoms, while chronic exposure at higher concentrations possess even higher risks [9]. According to USEPA, phenolic compounds are considered priority pollutants and are characterized as toxic, mutagenic, carcinogenic, and teratogenic compounds [10, 11]. Phenolic compounds are persistent pollutants and accumulate in the environment, which makes them a hazard for the ecosystems and for human health. Phenolic compounds are often the target pollutant for removal from various industrial wastewaters. Different removal technologies have been used: advanced oxidation processes [12], such as electrochemical oxidation using Ti/PbO_2 [13], Fenton process [14] and hydrogen peroxide oxidation [15], adsorption to various materials [16–18], membrane separations [19, 20] and optimized UASB reactors [21]. However, most of these technologies, although generally effective in the removal of phenolic compounds, have certain disadvantages such as high investment and operational costs, high energy input requirement, increased demand for operation and maintenance, mechanical complexity, and use of chemicals. These issues often are insurmountable obstacles towards the implementation of these technological solutions, especially in small-scale facilities. As a consequence, Constructed Wetlands are gaining more attention as an alternative technology for the treatment of waters containing phenolic compounds.

1.2 Constructed Wetlands Classification

Constructed Wetlands (CWs) are established today as an effective eco-tech treatment method. They belong to the wide category of natural treatment systems. The concept of these systems is the utilization of naturally occurring processes under controlled conditions for water treatment purposes, without the need for external energy input or use of chemicals. Overall, CWs are characterized as ecological, i.e., environmentally friendly, treatment systems with lower investment and—especially—lower operational costs [8]. Thus, they provide a series of environmental, economic, and social advantages over conventional methods such as activated sludge, or advanced methods, e.g., advanced oxidation. CWs serve the decentralized approach, i.e., introducing several smaller treatment plants for onsite wastewater treatment rather than a big end-of-the-pipe centralized plant with the accompanied extended sewer network. These are the main benefits of the wetland technology, which make them particularly appropriate for single households, small-medium settlements, remote or mountainous regions, and point pollution sources. The main disadvantage is that they have higher area demands, which means that space availability is a controlling factor of the application scale. However, continuous technological advances (e.g., promotion of vertical flow systems, artificial aeration) have managed to reduce the high area demands [22].

CWs are commonly classified according to the direction of the flow, their hydraulic characteristics, and the type of vegetation used. There are two general CW types [8, 23]: (a) Free Water Surface Constructed Wetlands (FWS CWs) and (b) Subsurface Flow Constructed Wetlands (SSFCWs). Based on the flow direction, SSFCWs can be

Table 1 Application of Constructed Wetlands for treatment of phenol containing waste water

Source of wastewater	Reference
Olive mills	[5, 6, 24–30]
Refinery effluents, contaminated groundwater, and chemical industry	[31–35]
Pulp and paper mill industry	[36–39]
Cork processing industry	[7]
Confined livestock	[31, 40]
Coffee processing	[41, 42]
Wood waste leachate	[43]
Wineries	[44–47]
Municipal with phenolics	[48–52]

either horizontal (HSSF CWs) or vertical (VFCWs). Considering the vegetation type, CWs are also classified into (a) emergent macrophyte wetlands, (b) submerged macrophyte wetlands, and (c) floating treatment wetlands. CWs with rooted emergent macrophytes are the most widely used [8]. CWs have been effectively applied for the treatment of domestic and municipal wastewater. Over the last years, focus has been shifted to the various industrial wastewaters, and waters containing phenolic compounds are among them. Table 1 presents studies on various CW applications for the treatment of phenolic compound containing waters. It is clear that management of olive mill wastewater is a big issue for many countries, especially in the Mediterranean basin, due to its high organic matter and phenolic compound concentrations. Thus, there is large number of CW studies dealing with this wastewater. Additionally, refinery effluents and wastewater from the chemical industry in general also attract the interest for CW applications. Wastewater with phenolic micro-pollutants has also been investigated for their treatment in CW systems.

2 Phenol Transformation Processes in Constructed Wetlands

Phenolic compounds have been effectively treated in various CW systems. Current knowledge on the phenol transformation/removal processes indicates that they include both biotic and abiotic mechanisms, such as biodegradation, sorption, plant uptake, and volatilization [8, 11, 47, 53, 54]. Although many studies investigated the various removal mechanisms, it is not yet completely clear which one is dominant. Biodegradation, i.e., microbial degradation of phenolic compounds, seems so far to be the main pathway for phenol breaking-down [8, 53]; however, the extent and the exact role of each process, especially in the long-term, are still to be defined.

2.1 Biodegradation

Biodegradation of phenolic compounds occurs at both aerobic and anaerobic conditions [11]. Under aerobic conditions, available oxygen is used as an electron acceptor by the microorganisms. Degradation of phenolic compounds takes place through the activity of various microorganisms such as bacteria, fungi, and actinomycetes [55], which utilize phenolic compounds as a carbon and energy source. Known bacteria species that degrade phenolic compounds are *Pseudomonas* spp., *Bacillus* spp., and *Acinetobacter* spp., among others [55]. The genera *Pseudomonas* and *Streptomyces* spp. have been found in subsurface CW systems and have been identified as phenol degraders [56, 57]. Even in olive mill wastewater that contains high concentrations of phenolic compounds, certain bacterial species have been identified as phenol degraders [58]. Anaerobic phenol degradation takes place under methanogenic, nitrate-reducing, sulfate-reducing, and iron-reducing conditions, where instead of oxygen other constituents (e.g., nitrate) serve as electron acceptors. Compared to aerobic degradation, anaerobic degradation is still less investigated.

The complexity of degradation processes increases with specific phenolic compounds; i.e., it has been found that biodegradation of bisphenol A occurs under aerobic conditions but not under anaerobic conditions [59]. Additionally, it is reported that denser bacterial biomass present in subsurface flow CW promotes a higher phenol removal rate in these systems than in surface systems [56]. Based on these findings, it is believed that aerobic biodegradation is the main removal mechanism of phenolic compounds, especially in VFCW, which are dominated by aerobic conditions [8, 47]. Moreover, recent studies also indicated that the same mechanism regulates phenol removal in HSSF CW [35, 41].

2.2 Plant Uptake

Phenolic compounds, along with other organic pollutants, can be assimilated by plant roots in CWs through phytodegradation, phytostabilization, and phytoaccumulation [50, 53, 60–63]. Generally, plant uptake occurs for compounds with a log K_{ow} in the range of 1–4 [49]. Given that phenol is a moderately lipophilic compound (log $K_{ow}=1.46$), plant uptake should take place in CW systems to some extent. Polprasert et al. [48] report on phenol uptake by *Typha* roots, and Tee et al. [50] and Rossmann et al. [41] also imply the same mechanism. The exact pathway and the extent of plant uptake are not yet clear. However, it is believed that the amount of phenolic compounds that plants can assimilate in CWs is relatively small [56], although it could be higher compared to other common wastewater constituents such as nitrogen and phosphorus [33].

It is also reported that high phenol concentrations can affect the growth and transpiration rate of certain plant species such as *Juncus effusus*, although other species such as willow trees (e.g., *Salix viminalis*) have been proved more tolerant [63]. Generally, common plant species used in wetland systems have been proved tolerant enough to phenol toxicity. However, the exact toxicological effects of phenolic compounds on plant species used in CWs are still not clearly defined. Moreover, many authors indicate that the major role of plants and their respective root system is to facilitate microbial degradation [5, 41, 50].

2.3 Adsorption and Precipitation

Adsorption is another phenol removal mechanism in CWs, mainly controlled by the characteristics of the filter media used as substrate, e.g., their physicochemical structure, as well as the pH of the solution [11, 53]. Additionally, sorption to organic matter particles and clay particles, as well as to plant roots, also plays a role in the removal of phenolic compounds [50, 64]. Phenol adsorption could also be antagonistic to any heavy metals present in the water, e.g., through competition for the adsorption sites on humic acid or clay particles [47]. Historically, activated carbon is used for phenol removal. In CWs, zeolite has been found to improve the phenol removal rate from olive mill wastewater [54]. Tee et al. [50] report that the use of rice husk (instead of gravel) enhances the phenol removal, while planted beds with LECA as substrate were also found to remove phenolic compounds from olive mill wastewater [65].

As for other compounds (mainly phosphorus), adsorption is a finite process and depends on the sorption capacity of the media used. Thus, this process is more intense and faster during the first operation lifetime of a CW system, due to the higher availability of sorption sites on the filter media grains [41]. The process is then gradually limited with time. Studies have shown that the addition of lime to the wastewater resulted in increased pH and enhanced the sedimentation of particulate organic matter and the subsequent removal of phenolic compounds [41, 66]. Soil adsorption/absorption has also been found to remove chlorophenolics from an aqueous solution [38], the magnitude of the effect depending on the characteristics of the organic compounds and the solid surface (e.g., plants, substrate, and litter; [53]). In general, phenol adsorption is recognized as a removal mechanism, but its exact extent is not yet clearly defined, while very few materials have been tested in CWs regarding their phenol sorption capacity.

2.4 Volatilization

Volatilization rates are directly related to the volatility of the compounds. Direct volatilization and phytovolatilization are expected to be moderate for hydrophilic compounds such as phenol [48, 63]. Phytovolatilization seems to be insignificant for phenolic compounds, as implied by the very low emission rate reported by [67] for 2,6-dimethylphenol. In subsurface flow systems, direct volatilization is restrained due to the limited compound diffusion rate in the unsaturated zone and the respectively limited mass transfer [53]. On the other hand, in surface beds volatilization may be more intense, given that there is direct contact of the water surface with the atmosphere.

3 Constructed Wetlands for Phenolic Compounds Removal

3.1 Wetland Types and Characteristics

Table 2 presents an overview of studies conducted in different CW systems for the treatment of wastewater containing various phenolic compounds. These studies were found online and, to the best of our knowledge, cover the whole range of CW applications regarding phenolic compounds treatment and removal. It is worth mentioning that for some wastewater types, e.g., pulp and paper industry and coffee processing industry, only one or two studies are available.

As Table 2 clearly indicates, the majority of the studies investigate HSSF CW systems; more than half of the reviewed studies addressed this wetland type. Then, VFCW systems follow as second largest group, while only two studies implemented FWS systems. However, it should be noted that the studies using VFCWs were conducted mainly over the last 5 years. This tendency coincides with the intense increase of studies on VFCWs that has been observed over the last 10 years [8], implying the increasing interest for this specific wetland type. Almost all of the investigated systems were one-stage CW beds. A four-stage VFCW system was implemented by Herouvim et al. [5] to treat highly phenol-polluted olive mill wastewater. Given that a pretreatment stage is usually included in the design for the treatment of phenol containing waters, a single CW bed appears in general as the main treatment stage. Pretreatment stages vary and may include lime addition [25, 46], electrochemical oxidation [27], trickling filter [5], aerobic [31] or anaerobic ponds [52], or sedimentation tanks [34, 38, 49, 50]. However, phenol containing wastewater has also been directly applied to CWs [26, 33, 40, 44, 48].

Table 2 also presents the range of hydraulic residence times (HRT) that have been applied in the various wetland systems. Obviously, most of the studies apply a HRT close to 6 days, although lower and higher values have been tested, too. However, for the proper HRT selection many parameters should be taken into account, such as the nature of the specific phenolic compound under investigation, the loading rate of the compound, and the characteristics of the wastewater, among others. Moreover, the common use of relatively low flow rates implies that most of the referring studies are small-scale or pilot-scale units. The data in Table 2 should be used only as an indication of the work done on phenolic compounds and CW systems. It is possible that larger systems exist in the industrial sector, but usually data from these full-scale facilities are rarely published.

Table 2 Basic information of studies on Constructed Wetland systems treating wastewaters containing one or several phenolic compounds and the observed removal efficiencies

Ref.	Type	Media	Inflow (m^3/d)	HRT (d)	Plants	Phenol substances	Inflow conc. (mg/L)	Removal (%)
[31]	HSSF/FWS	Gravel/soil	5–100,000	1–2	-	(tot.) Phenols	(a) 4, (b) 2.3, (c) 24, (d) ≤80	(a) 57, (b) 26, (c) 44, (d) ≤94
[44]	HSSF	Pea gravel	145–164	<1	*Typha dominicus, Scirpus acutus,* and *Sagittaria latifolia*	(tot.) Phenols	0.1	100
[25]	HSSF	Gravel	0.024	3	*Phragmites australis*	(tot.) Phenols	1500	83
[36]	HSSF			3–5	*Cyperus papyrus; Phragmites mauritianus; Typha domingensis*	(tot.) Phenols	0.5	73–77
[34]	VFCW	Gravel, sand	0.1		*Phragmites karka*	(tot.) phenols	6	?
[46]	HSSF	Gravel	0.002	20–40	*Phragmites australis; Schoenoplectus validus; Juncus ingens*	(tot.) Phenols	3	>95
[26]	VFCW	Gravel, sand, zeolite	0.051	3	*Typha latifolia; Cyperus alternatifolius*	(tot.) Phenols	?	?
[5]	VFCW	Gravel, sand	0.013		*Phragmites australis*	(tot.) Phenols	2800	70
[28]	FWS	Soil	3.15	5	*Phragmites australis*	(tot.) Phenols	1000	74
[41]	HSSF	Pea gravel	0.02	12	*Lolium multiflorum Lam.*	(tot.) Phenols	30	58–72

[42]	HSSF	Gravel	0.023–0.056	2–7	*Alternanthera phyloxeroides*; *Typha* sp.	(tot.) Phenols	≤65	≤80
[39]	VFCW	Gravel, sand	3.6	1	*Typha angustifolia; Erianthus arundinaceus; Phragmites australis*	(tot.) Phenols	5	70–82
[29]	FWS			60	*Phragmites australis*	(tot.) Phenols	4800	86
[48]	FWS	Soil		5–7	*Typha*	Phenol	≤380	98–100
[50]	HSSF	Gravel	0.04–0.06	7	*Typha latifolia*	Phenol	(a) 300, (b) 500	(a) 100, (b) ≤90
[68]	Mesocosms	Gravel			*Phragmites australis*	Phenol	100	100
[54]	VFCW	Gravel, sand, zeolite	0.005	3	*Cyperus alternatifolius*	Phenol	20	73–90
[35]	HSSF	Gravel	0.25–0.5	5–10	*Phragmites australis*	(a) Phenol, (b) m-cresol	(a) 15, (b) 2	>99
[40]	HSSF	Gravel	0.096	1.2	grasses	p-cresol	375 ppm	83
[33]	VFCW	Soil	240	5	*Phragmites* sp.	(a) Dinitrophenol, (b) Trinitrophenol	(a) 2, (b) 30	>99
[43]	FWS	Soil		7	*Typha latifolia*	Tannin and lignin	1000	31–44
[45]	HSSF	Pea gravel		5–10	*Typha dominicus and Scirpus acutus*	Tannin	47	46–78
[51]	HSSF	Gravel	0.028	3.5	*Phragmites australis*	Bisphenol A	0.0015	99
[52]	HSSF	Gravel	3.5	1.8	*Phragmites; Heliconia psitocortum*	(a) Bisphenol A, (b) Nonylphenol	(a) 0.009, (b) 2	52–73
[63]	PFBR	Gravel	0.002	5	*Juncus effusus*	Dimethylphenols	40	98
[38]	HSSF	Gravel, sand	0.067	5.9	*Canna indica*	Chlorophenolics	0.04	90

3.2 Phenolic Compounds Applied in Constructed Wetlands

Depending on the origin of the wastewater and on the concept of the specific studies, a large variety of phenolic compounds at various concentration ranges have been applied to CW systems (Table 2). Most studies monitored the fate of total phenols (i.e., the sum of all phenolic compounds) in the used wetland system with concentrations of total phenols ranging from below 1 mg/L [36, 44] to several 1000 mg/L [5, 29]. Studies focusing on specific groups of phenolic compounds addressed the fate of phenol [48, 50, 54, 56, 57], *m*-cresol [35], *p*-cresol [40], tannin [43, 45], lignin [43], dimethylphenols [63], nonylphenol [52], and di- and trinitrophenol [33]. These compounds were typically applied at concentrations in the order of 10–100 mg/L with highest reported values reaching 1000 mg/L [43]. In contrast, the fate of chlorophenolic compounds [38] and Bisphenol A [51, 52] in wetlands was investigated at concentrations in the order of 10 μg/L. An overview on the fate of these compounds in the tested wetland systems is given in Table 2.

In addition, pentachlorophenol [49], o-diphenol [24], 4-nitrophenol [69], pentachlorophenol [70], and vanillin and gallic acid [47] have also been applied to wetlands or to experimental setups mimicking conditions in wetlands. The large differences between the concentrations for a given phenolic compound (group) are partly caused by differences between the used wastewater. Furthermore, some studies spiked the applied wastewater with specific phenolic compounds (e.g., [50, 52]), while in turn other studies pretreated (as mentioned above) or diluted the applied wastewater before its application to a wetland system (e.g., [25, 28]).

3.3 System Efficiency

Available literature studies show that phenolic compounds can be well degraded in CWs (Table 2). Significant concentration reductions were reported for total phenols found in wastewaters of different origin, as well as for tested specific phenolic compounds even if applied at concentrations of 1000 mg/L and above. The only apparent exception is tannin (and lignin) which showed only moderate concentration reduction in two studies [43, 45], both using CWs planted with *Typha*. For these compounds, further studies would be needed to determine if this is a general trend. Most literature studies focus on the removal of total phenols or phenol from wastewater showing that such removal can be achieved with HSSF [25, 50], VFCW [5], or FWS [28, 29] wetland types. For the later type volatilization and/or photo-oxidation contributed to the observed removal due to the open water interface to the atmosphere. Removal rates associated with the observed concentration reductions are highly variable reaching values of up to 400 mg/L/d [25]. The highest areal phenol removal rate is reported for a 4-stage VFCW system (almost 750 g/d/m^2; [5]).

It should be noted that most studies report on a removal of typically 70–90 % of the total phenols, but rarely on a (nearly) full removal of these compounds. While for high concentration this might be explained with insufficient HRT of the treated water in the wetland, the incomplete removal observed also for low concentrations might suggest that some more recalcitrant fractions of the total phenols (e.g., tannin or lignin) exist in the applied wastewaters. The abundance of such less degradable total phenol fractions in the applied wastewater must be assumed to vary between studies (even when using the same wastewater type) and might be an important factor for the overall removal of total phenols in wetland systems. When phenol as a compound was applied with the wastewater, removal of up to 100 % has been reported [48, 50, 54] even for applied concentrations of 100–500 mg/L. The associated removal rates were up to 70 mg/L/d and, as for total phenols, removal could be achieved with different wetland types.

The above studies do not suggest specific key factors controlling the removal of (total) phenols from wetlands. The presence of plants is certainly promoting the removal but not required to achieve it (e.g., [35, 39, 50]) since studies showed high removal also for unplanted control systems. The most commonly used plant species is *Phragmites australis*, which resulted in slightly better performance when directly compared to *Typha angustifolia* and *Erianthus arundinaceus* [39], but other plant species also promoted high removal in other studies (e.g., *Typha latifolia*; [50] or *Cyperus alternatifolius*; [54]). In turn, in some cases the application of high concentrations was reported to have negative effects on plant health and growth [46, 48], which might be caused by the phenolic compounds or by other compounds present in the applied wastewater. Other factors found to promote phenol removal were the use of special substrate materials such as zeolites [54] or rice husk [50]. For the removal of other specific phenolic compounds the limited number of studies (1–2 for each compound only) does not allow to identify further specific factors promoting their removal.

4 Conclusions

Constructed Wetlands present an efficient near-natural option for the removal of phenolic compounds from various kinds of wastewater. Different wetland designs and operation modes were found to promote high removal rates of total phenols as well as of many specific phenolic compounds. While such high removal rates have been shown to effectively reduce even high concentrations, less attention has been given so far to their ability to reach legal concentration limits. More research would be needed to determine if such limits can be met using Constructed Wetland systems or if the effluent of the wetlands would need further treatment using other technologies.

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Removal of Pathogenic Bacteria in Constructed Wetlands: Mechanisms and Efficiency

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Abstract Sanitation efficiency is an important parameter in wastewater treatment. Removal of pathogenic microorganisms is crucial to prevent water resources contamination and to limit any risks for human health. Constructed Wetlands are today a well-established technology for wastewater treatment. Although very effective in the removal of organic matter and nutrients, pathogen removal is seldom the main target in these systems. Current experience shows that Constructed Wetlands can be very effective in the removal of pathogens from wastewater with removal rates up to 99 %. This review chapter provides information about sanitation practices using different technologies, focusing on the sanitation efficiency of Constructed Wetlands, the removal processes and the design and operational parameters that affect the removal of pathogens in Constructed Wetland systems.

Keywords Constructed Wetlands • Sanitation • Pathogens • Processes • Microorganisms • Bacteria • Fecal indicators • Coliforms

1 Introduction

Continuous pressure to available fresh water resources, overexploitation and extended contamination of water sources due to rapid human population increase, resulted in respective increasing demand for fresh, clean water, while at the same time available water per capita decreases [1]. Adding to these the gradual increase of wastewater volumes generated, the issue of providing clean water becomes

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more and more difficult to address. Therefore, proper and effective treatment of wastewater is required, especially considering that treated wastewater could represent an additional water source in the water cycle. Conventional centralized treatment methods (mostly activated sludge systems) have been widely applied. However, the global treatment capacity still remains below the produced wastewater volume. Especially in developing countries, where economic issues are more intense, wastewater reuse often takes place with little or even without prior treatment [2]. Under this frame, new technologies are needed that will fulfill certain criteria, namely appropriate performance, cost-effectiveness, sustainable character, and easiness to build.

Wastewater of human origin contains various pollutants such as organic matter (BOD and COD), nutrients (nitrogen and phosphorus), and pathogenic microorganisms. However, focus is usually given on organic matter and nutrients and the majority of effluent quality limits from wastewater treatment plants (WWTPs) refer to these pollutants. Pathogens are usually not the main target pollutant, and sanitation control is not the main interest, despite the fact that pathogenic microorganisms pose a risk to public health when discharged untreated to surface waters or even when they are not treated effectively and residual concentrations remain in the effluent water after the treatment process. These facts represent a major hygienic concern, which needs to be handled. The cell density of these pathogens should be reduced below a certain level during the treatment process, before the final discharge to the aquatic receiver or the reuse of the treated wastewater, e.g., for irrigation.

It is clear that the need for sustainable and appropriate technologies to eliminate the risks related to pathogenic microorganisms is still high and growing. All these gradually shift the interest to more sustainable and ecological treatment systems, such as Constructed Wetlands (CWs) and to their performance for pathogens removal. Therefore, the technology of Constructed Wetlands appears more and more as an attractive solution.

1.1 Pathogen Sources and Fecal Indicators

Ideally, before treatment process design takes place, a full characterization of the microbial community should be carried out. Of course, this is practically infeasible since the related costs for the identification of all microorganism species present in a wastewater sample would be too high. From a technical point of view, this would also be very difficult. The microbial environment, especially in Constructed Wetland systems, is complex and continuously changing; it is estimated that up to 1000 distinct bacterial species can be found in a single gram of soil [3] and/or 50 bacterial species in a single millimeter of water [4]. Thus, it is common practice to quantify specific indicator bacteria group. Basic prerequisite for this is that the indicator bacteria chosen should be easy to measure and identify through a reliable method that will provide acceptable correlation with the total number of pathogen population. Of course, no indicator microorganisms or bacteria group can be characterized as a perfect indicator. However, some bacteria groups can be used for this.

When investigating pathogens, we should not forget that pathogenic microorganisms are biological agents that can cause an illness to their host. Wastewater contains a wide and diverse range of different microorganisms. There are five main categories of pathogens, which are related to waterborne diseases: enteric bacteria, protozoa, helminthes, fungi, and viruses [2]. Fecal pathogens in wastewater can cause waterborne diseases such as diarrhea, typhoid fever, dysentery, cholera, and ineffective hepatitis [5, 6]. Diarrhea is the third largest morbidity cause and the sixth largest mortality cause worldwide [7]. It should also be noted that almost 80 % of global diarrhea cases are attributable to unsafe/untreated water, improper sanitation, or insufficient hygiene [8].

Generally, measuring of pathogenic microorganisms in wastewater of human origin can be expensive and with high technical complexity. Therefore, common indicator microorganisms are used in wastewater quality measurements, which can be easily measured and give a good correlation with the total number of pathogenic microorganisms in the specific wastewater and provide a good indication for fecal contamination. Coliform bacteria group represents the most common indicator microorganisms and are usually measured and expressed as total (TC) and fecal coliforms (FC). *Escherichia coli* (*E. coli*) is the most common indicator bacterium used and is a member of the fecal coliform group. *E. coli* is an intestinal pathogen of both human and warm-blooded animal origin. These bacteria and their different strains are considered a public health concern and many outbreaks have been recorded worldwide in both developed and developing regions [9]. Typical concentration of *E. coli* in domestic wastewater varies between 10^6 and 10^9 CFU/100 mL [2].

TC group includes a variety of other bacteria of the *Enterobacteriaceae* family that indicates both human and animal contamination. TC measurement represents a general indication of fecal contamination, but it does not provide a specific indication for human pollution. TC group includes many different fecal coliforms with the most common genera being *Escherichia coli*, *Enterobacter*, *Citrobacter*, and *Klebsiella*, but again they do not provide an indication only for human contamination, although the FC group is apparently smaller than TC group [10]. The fecal coliform group includes bacteria such as *E. coli* and *Klebsiella pneumonae*. A typical figure for *E. coli* counts is about 20–30 % of the TC group in raw domestic wastewater [11]. Some TC group member, e.g., *Klebsiella*, can also grow in industrial and agricultural waste under specific environmental conditions. Coliforms can also reproduce in the environment, and they cannot be used as indicators of viruses and protozoan cysts in aquatic environments [12].

Moreover, along with *E. coli* is the most common bacteria pathogen. This enteric pathogenic bacterium also originates from food industries, e.g., meat industry, livestock farms [13]. It can cause food-borne salmonellosis and other food-borne diseases such as typhoid fever [14]. This bacterium has a long survival period once introduced into water, which is of great interest regarding public health. Typical numbers in raw wastewater vary between 10^3 and 10^4 CFU/L [15]. Another commonly used indicator group for fecal contamination is fecal streptococci (FS). FS provide another indication of human or animal contamination, but they are more stress-resistant (e.g., to temperature variations) bacteria and do not reproduce in the environment (or their multiplication rate is low) compared to FC. FS are used as a second indicator group of fecal contamination and provide an indication of long-living virus presence. FS survival rate in water is usually shorter than bacteria of the *Enterobacteriaceae* family, thus, FS are often considered as indicator of relatively fresh pollution. FS can be found at relatively high density in the feces of both humans and warm-blooded animals [10].

The ratio of FC to FS can also provide indication regarding the origin of fecal contamination and an index to distinguish between human and animal pollution, given that FS concentrations in animal feces are usually higher than in human ones. Therefore, an FC to FS ratio greater than 7.0 is indicative of human contamination while a ratio of up to 1.0 indicates animal contamination [15] although a ratio of 4.0 is also reported as indicative of human contamination [16]. FS group includes *Streptococcus faecalis*, *S. bovis*, *S. equinus*, and *S. avium*. Enterococci (*E. faecalis* and *E. faecium*, *E. durans*, *E. gallinarum* and *E. avium*) are also a subgroup of fecal streptococci and is often used as an indicator of virus presence in marine environment and biosolid material. Other anaerobic bacteria are also used as indicators of fecal contamination. The most common is *Clostridium perfringens* (*C. perfringens*). Other clostridia such as tetanus (*C. tetani*), botulism (*C. botulinum*), and acute colitis (*C. difficile*) are related with human diseases too [17]. *C. perfringens* is found in the colon and represents the 0.5 % of the fecal microflora of both humans and animals. The spores of this bacterium are considered too resistant in aquatic environment, therefore is often used as an indicator of past pollution.

1.2 Analytical Methods

Two are the most commonly applied methods to enumerate pathogenic bacteria: the most probable number method and the membrane filtration method. These are classical culture-based methods based on the assumption that bacteria can grow and multiply on supplied biochemical substrates under specific physicochemical conditions. Generally, these methods are relatively cheap and do not demand high level of expertise, which makes them the most commonly used methods for fecal contamination monitoring [18]. Improved measurements of pathogenic microorganisms can be carried out through molecular techniques, though this is not always feasible due to technical or other constraints [19]. The main limitation of culture-based methods is that measured cultivability is not always guaranteed, given that only a small fraction (between 1 and 15 %) of the total microbial density can produce cultures in environmental samples [20].

Membrane filtration is probably the most widely used method to estimate bacterial populations in water samples. It is an easy and fast method, which is very important when the set of samples to be evaluated is large. The concept is to filter a sample volume through a membrane filter with a small pore size (0.45 μm) to retain the bacteria present in the sample. Then, the filter is placed on an appropriate substrate (culture medium) for coliform growth [21]. The most probable number (MPN) method is a simple, useful method that estimates the concentration of pathogenic microorganisms in a specific sample by means of replicate liquid broth growth in tenfold dilutions. MPN method can be particularly useful with samples containing high particulate material that interferes with plate count enumeration methods. The MPN method relies on the assumption that the bacteria follow Poisson statistics. This method is a good alternative when the classical plate count method is not applicable [22]. Total Coliforms are able to ferment lactose with simultaneous gas production within 48 h at 35 °C, forming anaerobic rod-shaped bacteria. Fecal Coliforms are measured through a similar procedure using a lactose substrate within 24 h at 44 °C. Both methods are described in detail in Standard Methods for the Examination of Water and Wastewater [23].

1.3 Conventional Pathogen Removal Methods

Conventional treatment methods such as activated sludge systems are usually applied worldwide for wastewater treatment, but they cannot completely remove pathogenic microorganisms from wastewater [21, 24–26]. Typical removal rates are close to 99–99.99 %. A common practice in conventional WWTPs is to upgrade the facility with a final tertiary or polishing/disinfection treatment step, in order to eliminate as many microorganisms from wastewater as possible. This modification becomes increasingly a necessity, given that more stringent water quality limits are introduced concerning pathogen concentration in the outflow of WWTPs.

Chlorination is the most widely used method for pathogen elimination. It is a simple, effective, and relatively cheap method, which can also provide a residual chlorine concentration in the distribution system or in the outflow for additional protection from pathogen growth [26]. However, the reaction of chlorine with natural organic matter results in the formation of toxic disinfection by-products, i.e., trihalomethanes and haloacetic acids [27], which are also environmental pollutants and are considered carcinogenic [28]. These have pointed out the need for the development of other, more safe methods and technologies for pathogen elimination. Other options for disinfection include advanced oxidation processes, such as ozonation or UV radiation, but they are more expensive compared to chlorination mainly due to high energy consumption and maintenance needs [26]. These methods also have technical issues, e.g., when the wastewater still contains turbidity at some level.

1.4 Constructed Wetlands Technology

Today, Constructed Wetlands are considered an established "green" technology for the treatment of wastewaters of various kinds and origins and are recognized as an effective technological solution in the field of ecological engineering. It was the last 10–15 years that this technology met a tremendous increase in the worldwide interest and the respective number of full-scale applications, while research focus on these systems was also significantly intensified. Their noticeable expansion is mainly attributed to their multiple economic and environmental benefits, especially compared to other conventional treatment technologies [25, 26]. To name a few, CWs possess lower operational costs due to the minimum (or even no) external energy input they require, there is no large and complex mechanical equipment, use of chemical substances in the treatment process is avoided, while specialized staff is also not necessary to run the facility [25].

Constructed Wetlands are classified into three main categories, based on their hydraulic characteristics [25]: (a) free water surface (FWS), (b) horizontal subsurface flow (HSF), and (c) vertical flow (VF). Different CW types can be combined to achieve higher treatment efficiency (hybrid systems). Their very good performance enabled the investigation of their usage in a continuously growing range of contaminated waters and wastewaters. They have been applied for the treatment of various wastewaters, such as domestic, municipal, agro-industrial, industrial, urban/agricultural runoff, as well as for sludge dewatering [25, 26]. The CW concept serves the decentralized approach, which makes them particularly appropriate for single households, small/medium settlements, remote, rural, or mountainous areas. As a relatively cheap technology that can be built using local materials, Constructed Wetlands can be an ideal solution for developing countries, where almost half of the global population lives [29]. Especially, South East Asia and Africa are the regions where more than 50 % of the population are not served by proper sanitation practices [30]. Particularly, subsurface flow wetlands that limit the direct contact of humans with the wastewater are a suitable solution for wastewater treatment in areas where it is difficult to control public access. Additionally, conventional centralized facilities can be economically infeasible, especially for small-scale applications and remote-rural regions, particularly in developing countries, which makes even more attractive the wetland technology.

1.5 Constructed Wetlands and Sanitation

Constructed Wetlands are currently under investigation concerning their capacity to remove various pollutants, including microbiological pollution from domestic/municipal wastewater. Historically, CWs have been mainly designed for the removal of common target pollutants such as organic matter (BOD and COD), nitrogen (mainly ammonia), and suspended solids. Removal of pathogenic germs is usually not the main design target although pathogen removal is often required when domestic and/or municipal wastewater is treated. Few CW systems have been specifically designed for the removal of pathogenic microorganisms from wastewater, since microbiological pollutants are seldom the main target pollutant. Generally, only few studies investigated the diversity of bacterial community in CWs.

It is only the last decade, more or less, that the capacity of CWs systems to remove pathogenic bacteria from wastewater is being investigated in a more systematic way. However, respective knowledge regarding the fate of pathogenic microorganisms in Constructed Wetlands is generally limited. Until recently, most of the available studies would only refer to common microbial indicators and their removal rate in wetland systems. It is a common practice for wastewater quality estimation that common fecal indicators would be used due to the relative easiness of their analytical measurement, as already mentioned above [31]. Based on these, similar approach is also utilized in this chapter to assess the fate of main fecal indicator microorganisms in Constructed Wetlands systems.

2 Removal Mechanisms of Pathogens in Constructed Wetlands

The removal of pathogenic microorganisms in Constructed Wetlands is accomplished through a complex of chemical (e.g., oxidation, UV radiation by sunlight, exposure to plant biocides, adsorption to organic matter and biofilm), physical (e.g., filtration, sedimentation), and biological (e.g., predation, biolytic processes, antibiosis, natural die-off) factors, which often act in combination for the removal of pathogenic bacteria [25, 32–34]. Although all these mechanisms have been identified, the extent and the exact role of each one of them is not yet clear. Many studies report a high efficiency of Constructed Wetland systems in the removal of pathogens from wastewater, yet the number of studies investigating the removal processes is limited. A summary of the removal mechanisms and the controlling factors is presented in Table 1. A further classification of the removal mechanisms can be made based on the parameters that regulate these mechanisms and whether living organisms are involved. Thus, the above-mentioned mechanisms can also be classified into abiotic and biotic mechanisms [25].

2.1 Sedimentation

Sedimentation has been proved as a removal mechanism, controlled by the sediments and media grains used in the CW substrate [35–37]. The particle size and density controls this process; higher sizes result in higher sedimentation rates [38]. Bacteria are accumulated on media grains and sediments, which means that

Table 1 Pathogens removal mechanisms in constructed wetlands

Removal mechanism	Process	Parameter
Physical	Sedimentation	System setup, substrate media
	Filtration	System setup, substrate media
Chemical	Oxidation	System setup, plant presence
	UV radiation by sunlight	System setup
	Exposure to plant biocides	Plant species
	Adsorption to organic matter	Wastewater characteristics
Biological	Predation activity	Microbial ecology
	Exposure to root exudates	Plant species
	Biolytic processes	Microbial ecology
	Retention in biofilm	Microbial ecology
	Natural die-off	Hydraulic retention time

bottom layers in CWs could act as pathogen sink. Bacteria such as fecal coliforms, fecal streptococci, and helminths have a higher settling velocity compared to other bacteria (e.g., protozoa cysts) and viruses, thus, they are more efficiently removed [39].

2.2 Mechanical Filtration

A pretreatment (primary) step (i.e., sedimentation tank) is also contributing to the removal of pathogenic microorganisms at some extent. In this step, usually large organic particles are retained, thus, in the following CW bed pathogens will be associated with smaller organic particles [38]. Pathogen association with smaller particles, e.g., colloidal ones, which remain in suspension are filtered in the wetland bed [33, 34]. Filtration of common indicators such as *E. coli*, total coliforms, fecal streptococci and enterococci has been characterized as the main removal mechanism in VFCWs [40, 41]. Generally, subsurface flow systems tend to remove more bacteria through the filtration process [36].

2.3 Adsorption

Adsorption of microorganisms is closely related to filtration and is caused by various interactions between the plant roots, the filter media grains, and the associated biofilm [25, 41, 42]. Adsorption of coliforms and viruses is affected by the characteristics of the media particles, e.g., grain size, type of media, ionic strength, and electrochemical charge [33].

2.4 Oxidation

Redox conditions could also affect bacteria removal in wetland systems. Plants species used in wetlands are capable of transferring oxygen from the atmosphere to their roots and exude it to the rhizosphere, creating this way of aerobic microsites [25]. Coliforms and enteric bacteria are generally facultative and/or obligate anaerobes. Oxygen availability plays an important role in the survival and growth of these bacteria, which are capable of growing under anaerobic conditions [43]. Hence, provision of dissolved oxygen by plant roots negatively affects the removal of enteric bacteria, as already shown in some studies [44].

2.5 UV Solar Radiation

Studies have shown that solar radiation can have an effect on coliform bacteria removal, especially if it is combined with high dissolved oxygen concentrations [45, 46]. Wavelengths around mid-UV (290–320 nm) and near-UV (320–400) have been shown to have lethal effects on coliform bacteria [47]. Few studies investigated the role of this process in Constructed Wetlands. In general, solar radiation is considered to be a removal mechanism in CWs, which can cause mortality to coliform bacteria in CW systems, especially at low temperatures [47–49].

2.6 Exposure to Plant Biocides

Certain plants species used in wetland systems (e.g., *Phragmites australis*, *Scirpus lacustris*) have been reported to excrete some substances through their root system that can be fatal to fecal coliforms and generally to pathogenic bacteria [50]. Soto et al. [51] reported that bactericidal excretions in the biofilm by plant roots could be considered as a removal mechanism for total and fecal coliforms in planted gravel CW beds.

2.7 Predation Activities and Biolytic Processes

This biological mechanism refers to the elimination of pathogenic bacteria by other microorganisms such as protozoa, bacteriophages, and *bdellovibrio*-like organisms (BLOs) [34, 52]. Predation depends on the characteristics of the prey-bacteria, e.g., population density and species present, and the predator, e.g., morphology, physiology [26, 53]. Predation activities are reported as the main removal bacteria mechanism in CWs [34]. Protozoa predation has been recorded in various CW systems [34, 54–56]. Bacteriophage and BLOs activities in CWs are less investigated [26] although some studies indicated the relation between these predator groups and pathogenic bacteria [57].

2.8 Biofilm Retention

The biofilm layer along plant roots and on the filter media grains may facilitate pathogen removal through bacteria attachment and protozoa grazing. Pathogens that are associated to smaller particles and suspended solids can also be retained in the biofilm layer [26]. The gradual creation of the biofilm in the sand layer has been found to enhance the removal rate of pathogenic bacteria [58].

2.9 Natural Die-Off

Natural die-off is considered as an important elimination mechanism of pathogens in CW systems [26, 34, 44, 54], which can be related to parameters such as the HRT applied, the predation activities, and starvation of microorganisms. Especially in free water surface CWs, natural die-off has been found to be the most important mechanism for the removal of coliform bacteria [38]. It is also reported that die-off rates for bacteria are higher in the water column than in the sediment [36].

3 Pathogen Removal in Constructed Wetlands

Table 2 presents an overview of selected representative publications concerning pathogen removal in various CWs systems. From the results reported in this table, it is obvious that CWs can achieve high pathogen removal efficiencies (up to 99 %). Nevertheless, pathogen effluent concentrations do not always comply with legislation limits, since CWs usually remove pathogens from 2-log [59, 60] to 4-log [41, 61], resulting in effluent concentrations above 10^3 counts/100 mL [62, 63].

3.1 Effect of Constructed Wetland Type

The majority of CWs applications include hybrid CWs systems, which consist of a VF stage and an HSF stage (Table 2) and are commonly used for domestic wastewater treatment. Hybrid CW systems remove pathogens up to 4-log [64], while they can receive higher hydraulic loading rates than single beds. Concerning the more efficient CW type, VFCWs have been reported to be more efficient that HSF beds [37, 65, 66], while FWSCW systems appears to be the less effective CW type for pathogen removal. The high removal rates of hybrid CWs could be attributed to the following:

- The combination of different CWs types take advantage of all potential pathogen removal mechanisms.

Table 2 Constructed wetland studies reporting pathogen removal

Reference	CW type	Wastewater type	Vegetation	HRT (d)	TC		FC		Salmonella	
					Inflow (CFU/100 mL)	Rem (%)	Inflow (CFU/100 mL)	Rem (%)	Inflow (CFU/100 mL)	Rem (%)
[74]	VF-HSF	Domestic	*B. reptans, T. portulacastrum*	4–28			1630	84		
[59]	VF	Domestic	*Phragmites* spp.	3 h			3×10^6–10^7	91–99		
[82]	VF	Grey water	*J. alpigenus*, *C. haspen*	12 h	10^7	99				
[79]	VF	Domestic	*P. australis*	1	10^5	99				
[77]	VF-HSF	Surface water	*Iris*		3–5	99	2–4	99		
[71]	VF-HSF	Domestic	*P. australis*			99		99		
[84]	HSF	Domestic	*V. zizanioides*, *M. giganteus, A. donax*, *P. australis*		10^6	99				
[62]	HSF	Domestic	*P. karka*	4	2×10^6	93	10^6	98		
[83]	HSF	Domestic	*C. papyrus*, *Pontederia* spp. *Canna* spp., *P. australis*	2–3			1.7×10^6–1.3×10^9	99		
[66]	VF-HSF	Domestic	*P. australis*	2	7×10^6	96				
[85]	HSF + maturation ponds	Domestic	*P. mauritianus*				7400	48	424 MPN/4 g	45

(continued)

Table 2 (continued)

Reference	CW type	Wastewater type	Vegetation	HRT (d)	TC Inflow (CFU/100 mL)	TC Rem (%)	FC Inflow (CFU/100 mL)	FC Rem (%)	Salmonella Inflow (CFU/100 mL)	Salmonella Rem (%)
[37]	VF-HSF		*P. australis*		10^8	99				
[73]	VF-HSF	Domestic	*P. australis*, *Juncus* spp., *Scirpus* spp., *T. angustifolia, T. latifolia*, *I. pseudacorus, A. calamus*, *G. maxima, Carex* spp.			99				
[76]	VF-HSF	Domestic	*P. australis*	1.5	7.6×10^6	99				
[64]	VF-HSF	Domestic	–	–	6×10^4	99				
[78]	VF-HSF	Domestic	*P. australis*	2	10^3–10^4	99	10^3–10^4	99		
[86]	FWS	Domestic		7	10^2–10^4	20				
[75]	HSF	Domestic	*Typha* spp., *Scirpus* spp., *Juncus* spp.	6–8			10^8	99	10^5	99
[87]	VF-HSF	Domestic	*Phragmites australis*				–	97	–	100
[60]	HSF	Domestic	*Phragmites* spp., *Phalaris* spp., *Glyceria* spp.	3–5	10^{14}	96				
[41]	VF	Domestic	*M. gigantea*	-	10^7	99				
[80]	HSF	Domestic	*P. australis*		4×10^7	99				
[63]	VF	Domestic	*Z. mays*	–			3×10^6	99		
[70]	VF-HSF	Domestic			10^6–10^7	99				
[88]	FWS	Livestock manure	*L. minor*	6–9	10^3–10^5	99				

[65]	VF-HSF	Domestic	*Canna* spp., *P. australis*	7–11	2×10^5–7×10^7	99	10^3–6×10^6	99		
[89]	VF	Domestic	*Canna* spp., *P. australis, C. papyrus*	7.7	2.8×10^7	94–99	2.3×10^6	94–99		
[61]	VF-HSF	Domestic	*Phragmites* spp.		10^7	90	8×10^5	90		
[63]	VF	Domestic	*Z. mays*				3×10^6	99		
[34]	VF	Domestic	*J. effusus, P. australis*		9×10^4	99			3×10^4	99
[72]	VF	Domestic	*P. australis*	0.5			10^5	99		
[90]	VF	Grey water		2-3	10^5	99				
[40]	VF	Domestic	*P. australis*		$4–84\times10^6$	99	$3–56\times10^6$	99		
[91]	VF-HSF	Domestic	*Z. aethiopica, S. reginae, A. andreanum, C. hybrids, H. dumortieri*	4	$5–8\times10^6$	99				
[92]	VF-HSF	Domestic	*Phragmites* spp.					95		
[93]	VF-HSF	Domestic	*Phragmites* spp., *Typha* spp.					99		
[94]	HSF	Domestic	*P. australis, T. latifolia, I. pseudacorus*	8	7×10^5	99				
[95]	FWS, HSF	Domestic		2–16			8×10^4	82–99		
[67]	VF, HSF	Domestic	*Z. aethiopica, S. reginae, A. andreanum, A. africanus*		5×10^6	92–97				

- In VFCW stages higher dissolved oxygen concentrations occur, which favor pathogen removal [67].

The substrate type is also important. Finer substrates (e.g., sand) achieve higher pathogen removal rates than coarser substrates (e.g., gravel; [66]). Apparently, filtration and sedimentation, along with adsorption, are the main mechanisms in this case, which are affected by the characteristics of the filter media [68, 69]. Pathogen areal load also affects the CW efficiency, since extremely high loads result in lower pathogen removal rates [70]. Another factor, which should also be examined, is the CW bed depth, since shallow VF and HSF CWs usually receive lower pathogen areal loads [66, 71]. It is reported that a VFCW bed with a depth of 65 cm achieved significantly higher pathogen removal rates compared to a bed with a depth of only 25 cm [72]. On the other hand, in horizontal subsurface systems, a higher depth seems to negatively affect the removal of *E. coli* [66].

3.2 Effect of Vegetation

Vegetation effect is always a subject of argument in CWs operation; its exact role in pollutant removal processes is still under investigation. While numerous plant species have been used in CWs application for pathogen removal (Table 1), different plant species don't seem to have any significant effect on pathogen removal [67]. There are several studies reporting that vegetation does not have a significant effect on pathogen removal [37, 41, 66, 72–74], while only one reported a significant contribution of plants to enhanced pathogen removal in CWs [75]. Regarding pathogen removal, as for organic matter removal, vegetation mainly provides higher area for microorganism growth and higher DO concentrations. Nevertheless, the positive effect of vegetation is not so intense, in order to be statistically significant, thus, the majority of the related published studies conclude that vegetation effect is minor.

3.3 Effect of Hydraulic Residence Time

Hydraulic residence time (HRT) has been reported to significantly affect pathogen removal [72, 74, 76–78], since prolonged HRTs provide longer contact time between pathogens and biofilm [74]. The main reason of HRT effect on pathogen removal is that prolonged HRTs minimize wastewater flow, enabling wastewater to flow in a capillary film around substrate particles and not in the macro-pores [76]. The need of prolonged HRTs in pathogen removal is one of the reasons, which lead to the use of hybrid CWs systems, since VF units ensure high DO concentrations, while HSF ensure higher HRTs.

3.4 Effect of Temperature

Temperature and seasonal effects play a significant role in pathogen removal by CWs [26, 35, 40, 70, 79, 80]. Generally, there is an ongoing discussion concerning the belief that higher temperatures enhance pathogen removal. It is true that pathogens are most active at temperatures around 37 °C (similar to that found in the internal human body), which could mean that lower temperatures promote pathogen inactivation. Although all the above-mentioned studies in Table 2 report the positive significant effect of temperature on pathogen removal, these studies only focused on seasonal effects and not temperature. Specifically, Gikas and Tsihrintzis [79] stated that pathogen removal is enhanced, when temperatures are above 15 °C, while Morato et al. [80] and Hagendorf et al. [70] stated that pathogen removal rates increased during summer and decreased during winter, due to the respective effects on the biological processes responsible for pathogen removal. However, biological processes in CWs are affected by temperature only in a certain thermal range, since above and below a certain temperature value these processes reach their maximum or minimum performance, respectively. Additionally, vegetation is also affected by season and temperature [81].

3.5 Effect of Post-Treatment

Although CWs achieve high pathogen removal rates, effluent concentrations are often above legislation limits, making imperative a post-treatment stage in order to further remove pathogen load. Usually, a disinfection step (e.g., chlorination or UV radiation) is used after CWs systems [82, 83]. The advantages of the CW treatment stage before UV radiation concentrate on: (a) the reduction of the contact time (down to 1.5 min; [82]), and (b) the increase of UV performance, due to the lower suspended solids concentration in CWs effluent. Thus, the combination of CWs and UV radiation achieve extremely low effluent concentrations, while minimizing operational cost.

4 Conclusions

CWs appear to be extremely sufficient in pathogen removal, as their removal rates are up to 99 %. Although all CWs types have been reported as able to achieve high pathogen removal rates, hybrid CW systems are preferred, since they combine the advantages of both VF and HSF CWs. Specifically, high DO concentrations in VFCWs and the prolonged HRT in HSF CWs seems to enhance pathogen removal. While several vegetation species have been used in CWs, neither vegetation nor the use of different plant species seems to significantly affect pathogen removal. On the other hand, pathogen removal shows seasonal variations as temperature increase is positively affecting pathogen removal. However, pathogen effluent concentrations in CWs are usually above legal limits for reuse, which means that a final post-treatment stage is often necessary in order to safely discharge the effluent or reuse it.

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Part IV
Phytoremediation for Reclamation and Restoration

Low-Tech Alternatives for the Rehabilitation of Aquatic and Riparian Environments

Gabriel Basílico, Laura de Cabo, Ana Faggi, and Sebastián Miguel

Abstract The rehabilitation of degraded riparian environments seeks to recreate natural ecosystems by the reintroduction of native plant species, among other actions. This reintroduction could be conducted from seed, saplings, and planted rolls and blankets. The advantages of the planted rolls and blankets are the ease of field installation and a rapid establishment of vegetation due to a better protection of the roots. In addition, rolls and mats reduce erosion, stabilize slopes, and retain sediments. The creation of wetlands in the coastal zone and the use of artificial vegetated floating islands can contribute to improving water quality. The establishment of buffer areas adjacent to the shore zone acts as a biological filter retaining diffuse pollution associated with surface runoff. In this chapter are described some low-tech alternatives and strategies that can be used in the rehabilitation of riparian zones, including a case study in the Matanza-Riachuelo River (Argentina), one of the most polluted in the world.

Keywords Riparian buffers • River rehabilitation • Restoration • Matanza-Riachuelo River

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1 Introduction

Riparian areas are complex hydrological systems. It is widely accepted that hydrological flow paths increase the spatial variability of the system by creating topographic heterogeneity through processes of sediment deposition and erosion [1]. In fact, the life cycles of many riparian species are timed to overlie with periods of high flow, or flood pulses, to optimize their dispersal to suitable habitats [2, 3]. Also, aquatic and riparian environments are local sources of biodiversity that act as biological corridors and provide ecosystem services at different scales, such as the provision of water for human consumption, agriculture, industry, commerce and recreation, transport, dilution and treatment of nutrients and pollutants, food supply and flow regulation, among others [4]. In this context, an open mosaic pattern of vegetation may develop in riparian areas that correspond to differences in topography at a small spatial scale [5]. In addition, flooding can supply seeds from upstream reaches and theoretically disperse species from the whole catchment species pool, thereby promoting high species input in hydrologically restored areas [6, 7]. Benefits of this kind are among the main reasons that highlight the importance of managing these environments properly.

Globally, the prevailing model of production and consumption of goods and services does not contemplate renewal rates of natural resources and the integral management of solid and liquid waste, conditions that threaten the integrity of natural environments, including aquatic and riparian environments. As a result, these systems are very frequently impacted through the contamination of water, sediment and soil, erosion, geomorphological modifications, increase of impervious surfaces, loss of habitat for flora and fauna, anthropic occupation of floodplains, and biological invasions [8, 9].

Ecological restoration, in a broad sense, is a branch of ecology that studies strategies and techniques for the recovery of degraded ecosystems and the services they provide. It draws on the physical, chemical, and biological characteristics of undisturbed landscape, taking as reference slightly degraded environments from the same region. The selection of technologies to be used is performed in order to simultaneously improve different aspects of the ecosystem, using those with less environmental and economic cost. Usually, there is substantial uncertainty about the success of the proposed restoration efforts. The main reason of this uncertainty is that the restoration of ecological systems will involve multiple objectives with conflicting interests. Then, the learning about the project impacts on biophysical systems and their potential economic and social outcomes are critical for an adequate management plan. Making choices will involve finding ways to work collaboratively with a diversity of people and organizations who care both about the outcome of restoration decisions and the process by which such decisions are made [10]. Depending on the particular impact and the characteristics of the environment, ecological restoration of aquatic and riparian ecosystems often holds as its main objectives the recovery of the water body morphology, the elimination of domestic and industrial effluents and/or the management of biotic components such as riparian vegetation

[11] and the reestablishment of biological, chemical, and physical linkage between aquatic and riparian ecosystems [12]. In many cases, due to its expensive nature, restoration is unrealistic. Rehabilitation, establishing a community similar to the original, is a proper alternative when it is impossible to restore a site to its original condition. Rehabilitation actions improve the environment from a degraded state.

Remediation of contaminated water, soils, and sediments is usually part of a comprehensive strategy for ecological restoration and rehabilitation. There is a large and diverse list of practices, techniques, and strategies for the remediation of contaminated sites although the current trend is to select those that maximize the benefits to the environment, within the approach known as "green remediation." From this point of view, it seeks to reduce the impact of remediation actions on the environment by protecting natural resources and optimizing their use, applying environmentally friendly products, reducing, reusing and recycling materials, minimizing energy use and improving efficient energy, and reducing emissions of polluting gases [13].

Remediation phytotechnologies are the techniques that use plant species for the extraction, degradation, containment, or immobilization of contaminants in different environmental matrices. Contaminants that may be treated by means of these techniques include organics such as volatile organic compounds (VOC), polycyclic aromatic hydrocarbons (PAH), petroleum hydrocarbons, munitions constituents, metals and radionuclides [14], and nutrient excess. Management of plant biomass produced during remediation is a critical issue when pollutants such as metals accumulate in tissues. Phytotechnological applications for the remediation of contaminants use and enhance mechanisms such as phytoextraction, phytosequestration, rizodegradation, phytohydraulics, and phytovolatilization. In each case, one or more mechanisms may be involved depending on the pollutant, the plant species and environmental conditions of the soil, sediment, or water. For a description of each mechanism is suggested, besides this book, consult the work of the ITRC [15].

The aim of this chapter is to review the impact of some low-tech alternatives commonly used in the restoration and rehabilitation of wetlands and riparian environments.

2 Applications of Rehabilitation Phytotechnologies

Various criteria influence the selection of plant species for revegetation of riparian and aquatic environments besides the biological considerations that define native species per ecoregion:

- Native species that can produce erosion control, slope stabilization, habitat creation, stabilization of contaminants in soils and sediments, and protection of water bodies against diffuse pollution sources by creating buffer zones (riparian buffers).
- Tolerant native species found in highly degraded wetlands and riparian environments.

- Species with short life cycle, of predominant vegetative propagation form, high productivity, able to interact with other species of flora and fauna.
- Plant species that are easy to grow and handle in the natural environment.

Phytotechnologies for restoration and rehabilitation of riparian environments can include direct seeding or planting, the installation of fiber rolls and mats preplanted with selected species and vegetated floating islands for aquatic environments.

2.1 Direct Seeding and Planting

The most common way to revegetate degraded sections of banks and wetlands edges is the direct seeding or planting of native vegetation in the riparian zone. Prior land preparation may include the management of invasive and adventitious vegetation using mechanical or biological control [16], avoiding the use of herbicides, due to its toxicity in aquatic organisms [17].

The culture of the selected species can be done under greenhouse conditions, but it is frequently performed outdoors. For the cultivation of marsh species, a water pond with saturated substrate or a nutrient solution (hydroponics) are required. Waterproof materials as clay or high- and low-density polyethylene (HDPE, LDPE) geomembranes [18] should be used for the construction of ponds. In the case of larger projects, the supply of plants from a specialized nursery could be required. When the species required are not commercially available, it will be necessary to collect individual samples or other vegetable materials from natural areas in the region, with the permission of local authorities.

Vegetative propagation is one of the most interesting alternatives in many plant species. This propagation can be done by cuttings, bulbs, rhizomes, and division of bushes [19]. The advantage of this system consists in the short time required to achieve an optimum size for transplantation and the simplicity of techniques. Its main disadvantage is the low genetic diversity obtained. Direct seeding in the banks is only justified if the species selected have a high germination rate, are fast growing, and seeds are readily available. The increased incidence of pests and diseases of the natural environment itself may be critical for the survival of seeds and seedlings, while some contaminants in the soil could inhibit germination.

In general, the best time for seeding or planting is early spring, although in many cases it is also possible to perform it in early autumn in order to ensure that the plants will adapt to the new environment before winter. It should be noted that many phytoremediation mechanisms depend on the growth of plant biomass and therefore are more significant during spring and summer. The planting of herbaceous and woody species in heavily contaminated riparian soils usually require the addition of substrates such as humus or compost in the immediate environment of the roots, in order to facilitate the adaptation stage.

Once the plantation is established irrigation may be required, depending on the selected species, growth stage, seasonal and meteorological factors. One advantage that the use of native species adapted to the local climate conditions brings is that they reduce the need for irrigation. Pruning and disease control may be needed, but be reserved only for severe cases where nonintervention means plants death. The application of biopesticides is preferred, which have the advantage of being less risky than conventional pesticides, attack only the target species and are often effective even at low doses [20].

2.2 Prevegetated Rolls and Mats

Rehabilitation and restoration of river banks generally tend to choose geotextiles or pre-vegetated seeding rolls. The advantages of these techniques are the ease of field installation and a rapid establishment of vegetation due to a better protection of the roots. In addition, rolls and mats reduce erosion, stabilize slopes, and retain sediments. Moreover, they can be manufactured with local labor and plant materials [21].

The vegetated rolls, also known as coir fiber rolls, are cylindrical structures built with biodegradable materials that support the growth of reeds, cattails, reeds, lilies, and other plants. They consist of a mesh of plant fiber with slow degrading rate as jute, coconut, sisal, and cereal straw but also burlap or polypropylene may be used. The function of the mesh is to contain filler that serves as support to the plants and allow root growth rapidly through the fabric (Fig. 1). Coconut fiber is the most often used filler material though local availability of other fibers and plant materials must be assessed in order to reuse waste from other activities (industry, urban pruning, etc.) and reduce the economic costs of the restoration project. The selection of materials for the mesh and the filler should consider the absence of industrial pollutants, pesticides, and propagules of alien plant species. The most common dimensions are 0.1–0.3 m in diameter and 3 m long to the most and can be purchased pre-vegetated with selected species or to be vegetated by means of seedlings, cuttings, or stakes. In some countries, the commercial development of this technology often use standard measures 0.2–0.3 m in diameter and 3 m long, with a filling of coconut fiber densely packed. Still, rolls can be built with other dimensions and materials (Fig. 2).

The fiber mats, or coir mats, also known as pallets or coir blankets, are geotextiles made with natural or other biodegradable materials which allow the growth of vegetation through the fibers. They may have different dimensions, with a variable width usually less than 3 m and a thickness of 9–15 mm. As commercial products, mats can be purchased without vegetation or pre-planted or sown, in cloth or rolls up to 30 m long. It is also possible to build them locally, resulting in social benefits such as job creation [22]. The application of coir mats allows for slope stabilization and erosion prevention [23]. Moreover, they can be used to moderate soil temperature; reduce surface runoff; increase water infiltration and soil moisture content. Durability of natural geotextiles is strongly inferior to that of synthetic ones [24] but their biodegradation adds organic matter to the soil contributing to further plant growth [25].

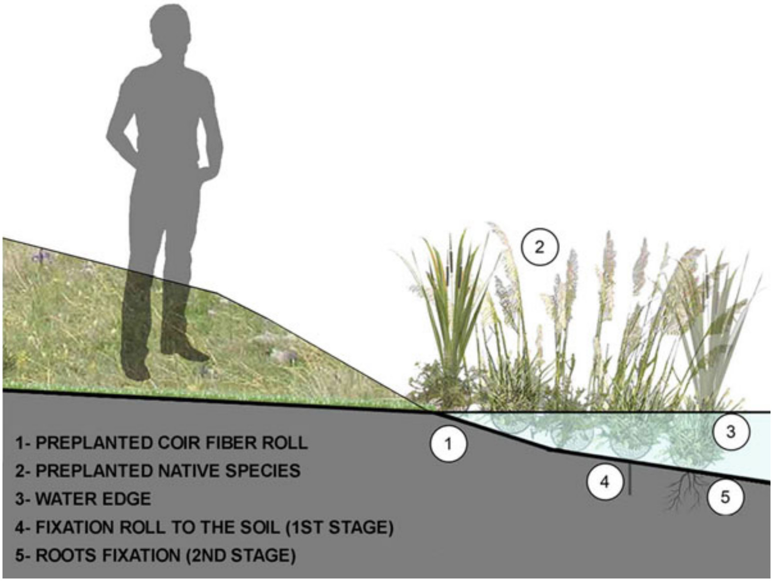

Fig. 1 Vegetated roll plantation cycle

Fig. 2 Vegetated roll assembly sequence. Roll design: Sebastián Miguel

Rolls and geotextiles fixation to riparian soil is generally performed by stacks of wood or metal, the latter should be removed after the rooting of plants. Rolls use is often limited to the water edge or riverbank foot, by reference to the average height of the water. By their shape and flexibility they can easily adapt to the irregular contours of banks. Vegetated mats are often set on steep slopes, eroded and/or unvegetated soil.

2.3 Floating Islands

Floating islands exist naturally in different ecosystems and consist of accumulations of decaying plant biomass colonized by macrophytes and small shrubs [26]. Several species of floating macrophytes like *Eichhornia crassipes*, *Pistia stratiotes*, *Spirodela intermedia*, and *Salvinia molesta* may themselves cover large areas of water bodies to form real floating islands with a very rapid proliferation associated with increased water temperature and nutrients levels. In this case, the presence of these species is often associated with negative effects on water resource management, conservation and biodiversity, among others [27]. However, many species of macrophytes including the above mentioned have been successfully studied or used in phytoremediation water and effluents [28, 29].

The presence of macrophytes in a water body promotes phytoextraction of dissolved nutrients and solids settling rhizofiltration. Simultaneously, the periphyton established over the roots and the submerged organs contributes to the removal of dissolved and suspended nutrients and pollutants [30]. These natural processes can be harnessed to the ecological improvement of eutrophic or contaminated aquatic environments through the construction of floating vegetated structures with purely aquatic and/or marsh species that act as true biological filters and improve water quality [31].

Installation of floating islands in a water body has the additional advantages of habitats creation for aquatic life and an economic cost that is generally lower than conventional water treatment. In an experience of ecosystem restoration that used floating islands among other technologies, Da and Guo [32] found that unit costs were four and six times lower than traditional technologies for the removal of nitrogen and phosphorus, respectively. The islands can be built based on a modular system that can be installed according to the shape and size of the water body or design objectives.

Structurally, the floating islands consist of three parts: a floating system, a holder for planting macrophytes, and an anchoring or fixation system. The flotation system can be built with plastic tubing and even through the reuse of PET bottles, while the support matrix can be performed with fiber mats. The roots grow through the fiber matrix to reach the water, promoting the phytoremediation through several mechanisms, such as phytoextraction. The submerged plant tissues and structures allow the growth of algae, bacteria, and fungi that contribute to increased water quality. The anchoring can be achieved using ropes or chains attached to a stake or an anchor depending on the size of the island.

Figure 3 shows a floating island prototype built using plastic fabric, PET bottles as floats and wood shavings as supporting matrix. This prototype allows the reuse of about 11 PET bottles of 1.5 L capacity per square meter. It has now tested the adaptation and growth of different hydrophytes and helophytes species in mesocosm conditions: *Echinodorus grandiflorus*, *Hydrocotyle ranunculoides*, *Sagittaria montevidensis* and *Pontederia rotundifolia*. In all cases, it was possible for the roots to penetrate the matrix and reach a length of more than 0.5 m; however, the growth of helophytes species (*E. grandiflorus* and *S. montevidensis*) was very low, almost zero in the *S. montevidensis* case. This finding highlights that levels of dissolved nutrients were very low and the culture pond received few hours a day of direct sunlight. The design and installation of floating islands in lotic environments and their fixation must consider the maximum flow during floods because the water can kill plants and promote the accumulation of sediment on the structures and then collapse [33] or directly drag or destroy the entire structure.

Fig. 3 Floating island prototype built with plastic fabric, PET bottles, and wood shavings as filler, vegetated with *E. grandiflorus*, *H. ranunculoides*, *S. montevidensis*, and *P. rotundifolia*. Prototype design: Gabriel Basílico

2.4 Constructed Riparian Wetlands and Buffers

The vegetated filter strips or riparian buffer strips (RBS) are banks or adjacent zones in which the vegetation is preserved, either due to the fact that the land cannot be tilled, or by the constant presence of water, or by legal regulations on environmental issues. These areas play key roles such as sequestration of nutrients excess and pollutants, sediment retention from runoff water and secondarily promote biodiversity, water regulation, shading, hydrological connectivity, biomass production, and provide cultural services [22, 34]. Nitrogen is one of the pollutants that can be efficiently removed by the RBS, with a decrease of the concentration of nitrates in the water body itself [35] and in shallow groundwater [36] associated with the establishment of these vegetated areas.

Another ecotechnology used for nitrogen removal is the creation of wetlands within the floodplain of rivers and streams. As in the case of RBS, the anaerobic environment of waterlogged soils promotes denitrification and adsorption of ammonia to the soil particles, which generally have negative charges [35].

Wetlands creation in degraded flood plains improves structural aspects of aquatic ecosystems, such as other technologies. However, this technology also contributes to the recycling of nutrients and organic matter and the taxonomic and functional richness in constructed wetlands could be higher than in natural degraded wetlands in the same basin [37]. On the other hand, the design of riparian wetlands should consider the complex dynamics of these systems, where water quality responds to the coverage of macrophytes, water metabolism, accumulation of sediments, changes in redox potential and occurrence of flood pulses, among other factors [38].

3 A Case Study: Rehabilitation of River Banks in the Matanza-Riachuelo River

For more than a century, the Matanza-Riachuelo River has been heavily managed in its lower reaches for the purpose of flood defense and is contaminated specially by metals. The river located in the metropolis of Buenos Aires is approximately 70 km long and discharges in the La Plata River Estuary (Argentina). The lower part of the Matanza-Riachuelo watershed (MRW), Riachuelo, was characterized in 2013 by Green Cross Switzerland as one of the ten most polluted sites in the world [39], but the degradation of the basin is old. In 1801, the first salting factory settled on the banks of the river began throwing debris leather, meat, bones, and fat of animals to the Riachuelo waters. Ten years later, the river was already polluted by the activity of the tanneries and slaughterhouses. Then, economic and social growth gave way to the industrial boom, and industrial effluents reached the river polluting its waters, riverbed, and riparian environments [40]. Nowadays, the river basin is subjected to different types of contaminants from agricultural and urban runoff, industrial effluents, sewage treatment plants and leaching from domestic garbage dumps [41].

In 2006, 17 residents of the lower part of the basin filed a lawsuit against the national state, the province of Buenos Aires, the city of Buenos Aires, 14 municipalities, and several companies in order to be compensated for damages caused by pollution and to stop the contamination. In an unprecedented verdict, the Argentina Supreme Court ruled that the national, provincial, and municipal authorities should improve the quality of life of MRW residents, restore the environment, and prevent any further damage. The Authority of the MRW was created and is responsible for the compliance of this ruling [42]. A comprehensive rehabilitation plan has been enforced since 2009 in order to: (1) improve the quality of life of the inhabitants of the basin (more than eight million inhabitants); (2) the environmental recovery in all its components (water, air and soil); and (3) to prevent future damage. It includes the conversion of industries, expansion of the water supply and sewage system, monitoring of water and sediment quality, relocalization of slums, cleaning up the riverbanks and beds, and environmental education [40]. Considering these objectives, the *Gerencia Operativa de Riachuelo y Borde Costero*, belonging to the *Agencia de Protección Ambiental* (*APRA*) of the *Gobierno de la Ciudad Autónoma de Buenos Aires*, convened specialists of the *Universidad de Flores* for an ecological rehabilitation project in the riparian area.

A plan for the revegetation of a pilot area in the lower reach of the river was prepared and developed into a detailed design incorporating objectives with the site constraints. The aims of the scheme were to rehabilitate the floodplain wetlands, which had been largely impacted, to improve the water and riparian soils quality, and to recover a place where people could experience a more natural landscape.

3.1 Site Characterization

Due to the scarcity of existing information a diagnosis of contaminants in soil and existing vegetation in the river banks was carried out in February 2015. Some metals accumulating native plants (*S. montevidensis*, *Schoenoplectus californicus*, *H. ranunculoides*, *H. bonariensis*, *Tradescantia fluminensis* and *P. rotundifolia*) were identified. They provide ecological services such as stabilization of contaminants in their roots [43, 44]. The main pollutants identified in the soil of the riverbanks were metals chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn). When these levels were compared to a reference soil according to Mendoza et al. [44], a moderate to extremely high degree of contamination was established (Table 1).

However, soil characteristics evaluated (soil rich in sulfides, carbonates, and organic matter) allowed inferring that the metals were adsorbed to the soil matrix, reducing its availability to plants. Furthermore, the predominance of silt and clay over the sand and gravel increased the soil ability to bind metals and decreased the risk of their mobility.

Metal levels were also determined in belowground and aerial structures of two native plants: *S. montevidensis* and *T. fluminensis*. Both species accumulated Cr, Cu, Pb, and Zn in the roots and metals were undetectable in leaves except for zinc. The highest val-

Table 1 Concentrations of metals in soils of the pilot area of Matanza-Riachuelo River ($Cm_{M\text{-}R}$) and in a reference soil (Cm_{ref})

Metal	$Cm_{M\text{-}R}$	Cm_{ref}	$Cf = Cm_{M\text{-}R}/Cm_{ref}$	Classification
Cr	122.3	19.3	6.3	Extremely high
Cu	81.6	23.0	3.5	High
Pb	51.4	31.0	1.7	Moderate
Zn	318.1	74.1	4.3	High
$Dc = \Sigma Cf_i$			**15.83**	**Extremely high**

Concentration factor (Cf) for each metal, where Cf < 1: low contamination; Cf 1–3: moderate contamination; Cf 3–6: high contamination and Cf > 6: extremely high contamination. Degree of contamination (Dc), where Dc < 6: low degree of contamination; Dc 6–12: moderate degree of contamination and Dc 12–24: extremely high degree of contamination [44]. Metal concentration unit is mg/kg. Cf is a dimensionless factor

Table 2 Concentrations of metals in leaves and root of *S. montevidensis* and *T. fluminensis* and bioconcentration (BCF) and translocation factors (TF) in the pilot area of the Matanza-Riachuelo River

	S. montevidensis				*T. fluminensis*			
Metal	Leaves	Roots	BCF	TF	Leaves	Roots	BCF	TF
Cr	2.5	16.1	0.472	0.155	<0.5	192	6.465	–
Cu	2.5	18	0.272	0.134	<0.5	73.5	1.397	–
Ni	<0.5	<0.5	–	–	<0.5	8	0.976	–
Pb	<0.5	10.1	0.075	–	<0.5	41.3	1.125	–
Zn	7.1	90	0.216	0.079	11.5	280	1.029	0.041

Metal concentration unit in leaves and roots is mg/kg. BCF and TF are a dimensional factors

ues of bioconcentration factor (BCF) for all the metals show that *T. fluminensis* is more effective in stabilizing these pollutants in soils, moreover, the low translocation factors (TF) imply that there is a low risk of exposure for wildlife (Table 2). These results allowed us to define the most appropriate species for planting.

3.2 *Proposals for the Revitalization of the Pilot Area*

The landscape proposal for the pilot area was designed to be materialized in several stages. This small didactic park promotes the care of the environment, the contact with the riparian flora and allows visitors to learn about remediation techniques. This type of project uses the natural elements of the site and incorporates the minimum artificial components to preserve the environment [45]. The intervention proposes the connection of the different parts of the upper bank and the water edge through a natural didactic sidewalk made with recycled materials. The recent restored upper sidewalk and bicycle path give the visitors the general view of the revitalization area (Fig. 4).

Fig. 4 Project section

The selection and distribution of the native species allow obtaining different scales and perceptions of the park: sunny and shadowed areas, green density, and variety of textures.

Two plantation zones were defined and waste was removed before plantation in May 2015 (Figs. 4 and 5):

- Preplanted burlap rolls (*S. californicus*, *S. montevidensis*, *H. ranunculoides*, *P. punctatum*, *T. fluminensis*) were installed at the water edge, to give stability to riverbed, preventing water erosion of the margins and providing habitat for wildlife.
- On the upper part of the bank, fast-growing native trees and shrubs were planted. The riparian soil was not seeded to allow natural revegetation. On the site exposed to the sun *Salix humboldtiana*, *Syagrus romanzoffiana*, and *Erythrina crista-galli* were used. Under the bridge, species accustomed to the shade were preferred (*Sambucus australis*, *Solanum granuloso-leprosum*, *Myrceugenia glaucescens*, *Allophylus edulis*, *Cestrum parqui*). Between the trees, herbs with highest rates of removal of contaminants were planted (*T. fluminensis*, *H. ranunculoides*).

3.3 Preliminary Evaluation of Actions

During the first months after revegetation actions, the area was monitored every 15 days. The area showed signs of trash accumulation on the rolls and of rising water by the action of rain and tides. Therefore, manual scavenging was reinforced. A layer of silt was accumulated on vegetated rolls by floods infilling the spaces between rolls and stabilizing them. This caused the death of susceptible species like

Fig. 5 General view of plantation zones in the pilot area

H. ranunculoides and *P. punctatum*. In consequence, some repositioning was made in the plant selection for the second revegetation action. Those species that survived the flood action are only: *S. californicus*, *T. fluminensis*, and *S. montevidensis*. Also, levels of metals uptake and detoxification mechanisms involved were analyzed. The results indicate that environmental conditions are inducing the antioxidant system in the three species.

3.4 Concluding Remarks

The rehabilitation of degraded riparian zones can use several technics to reintroduce native vegetation, such as direct planting and preplanted rolls and mats. Various native plant species also contributes to water and soil remediation. The use of floating islands and constructed riparian wetlands and buffers can ameliorate water contamination by nutrients and metals from point and diffuse sources.

The implementation of several of these techniques in Matanza-Riachuelo River has not shown negative outcomes so far, native plants are useful but adaptive management is needed, showing that the restoration processes are dynamic and must adapt to local conditions. The applied techniques have caused a local change in attitude promoted by the *Gerencia Operativa de Riachuelo y Borde Costero* of the

APRA which has launched similar projects in other sites of the river for the coming future. The project aroused the interest of the community with many articles published in newspapers and radio and TV interviews. The project team has committed to continuing the survey work to enable critical assessment of the results.

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Proposed Rehabilitation Method of Uncontrolled Landfills in Insular Communities Through Multi-Criteria Analysis Decision Tool

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Abstract Landfills (controlled and uncontrolled) receive today a combination of municipal, commercial, and mixed industrial waste, typically producing a wide range of pollutant compounds influencing nature and human health in many ways. Despite the promotion of waste management being held throughout the world, the disposal of untreated waste into final landfill sites is unfortunately the most wide solution these days. Uncontrolled management of waste in landfills leads to negative environmental issues, and there is a need to undertake an environmental analysis of existing facilities and services in order to analyze the problems they present and take the necessary measures for reducing adverse effects in order to propose the most valuable restoration solution especially in insular communities. This chapter indicates a rehabilitation method of uncontrolled landfill sites in insular communities, using Cyprus as a case study, using different management plans through multi-criteria analysis and Analytic Hierarchy Process (AHP).

Keywords Uncontrolled landfills • Restoration techniques • Rehabilitation • Multi-criteria analysis • Phytoremediation • Analytic Hierarchy Process

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1 Introduction

In developing countries, natural urban population growth and immigration from rural areas are contributing to a substantial increase in urbanization levels [1]. In the last 30 years, the generation of municipal solid waste was increased from 0.2–0.5 to 0.5–1.2 kg/inhabitant/day and this is the result of urbanization and globalization. While the percentage of organic matter in municipal waste has fallen, the percentage of voluminous and nonbiodegradable components such as plastic, metals, glass, etc. has increased. According to Eurostat statistics, the total waste generation in the EU-27 was 2.62 billion t with an increasing trend (continually). Ninety-eight million t or 3.7 % were categorized as hazardous waste, which means that during the year 2008 (as base) each European citizen produced approximately 5.2 t of waste (196 kg were hazardous) [2, 3]. Shifting the focus to how the municipal waste generated in the period 2001–2010 was managed, there is clearer evidence of a modification up the waste hierarchy. According to Fig. 1, municipal solid waste landfilling reduced by almost 40 million t, whereas incineration increased by 15 million t and recycling grew by 29 million t. Observing at the EU-27 only, landfilling decreased by 41 million t, incineration by 15 million t, and composting with recycling increased at the same time by 28 million t. Moreover, Fig. 1 indicates that the total amount of municipal solid waste (MSW) recycled has declined slightly since 2008 [4].

Word bang report [5] focuses on waste generation (projection for 2025) indicated that in all regions we will have a continual waste amounts (Table 1). The per capital waste production varies from 0.77 kg/day for SAR (South Asia Region) to 2.1 kg/day for OECD (Organisation for Economic Co-operation and Development, Region). At the same time, the SAR population on 2025 is estimated to be 734 million (426 million on 2012) with the urban waste generation on 2025 to be 0.77 kg/day than 0.45 kg/day on 2012. On the other hand, OECD region produced 2.2 kg/day in 2012 with total population to 729 million while in 2025 the population is

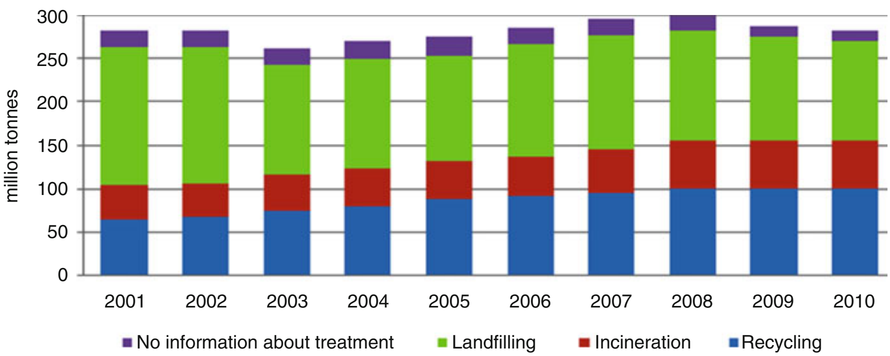

Fig. 1 Development of municipal waste management in 32 European countries, 2001–2010

Table 1 Waste generation projections for 2025 by region

Region	Available data			Projections for 2025			
		Urban waste generation		Projected population		Projected urban waste	
	Total urban population (millions)	Per capital (kg/capital/day)	Total (t/day)	Total population (million)	Urban population (million)	Per capital (kg/capital/day)	Total (t/day)
AFR	260	0.65	169,119	1152	518	0.85	441,840
EAP	777	0.95	738,958	2124	1229	1.5	1,865,379
ECA	227	1.1	254,389	339	239	1.5	354,810
LCR	399	1.1	437,545	681	466	1.6	728,392
MENA	162	1.1	173,545	379	257	1.43	369,320
OECD	729	2.2	1,566,286	1031	842	2.1	1,742,417
SAR	426	0.45	192,410	1938	734	0.77	567,545
TOTAL	2980	1.2	3,532,252	7644	4285	1.4	6,069,703

AFR Africa Region, *EAP* East Asia and Pacific, *ECA* Europe and Central Asia Region, *LCR* Latin America and the Caribbean region, *MENA* Middle East and North Africa region, *OECD* Organisation for Economic Co-operation and Development, *SAR* South Asia region estimated to be up to 842 million. According to the OECD [6], the generation of municipal solid waste increased about 54 % in the Denmark, Switzerland, Netherlands, Portugal, and Greece between 1980 and 2000. In the OECD countries, an increase of 18 % has been reported between 1995 and 2007 [6].

MSW landfills represent the leading choice for waste disposal in all around the world. The comparatively high costs of treatment and disposal options are considered being the major reason for the reliance on MSW landfills, mainly in developing economies [7–9]. However, even some highly industrialized countries like USA, UK, Finland, and Australia mostly depend on landfilling. For example, in the USA, 54 % of the 250 Tg (1 Tg = 106 metric tons) of MSW generated was landfilled during 2008, with composting and recycling being around 33 % of MSW management [10]. 70 % of MSW in Australia has been forwarded to landfills without pretreatment stage in 2002 [11]. The direct disposal of MSW in Japan was less than 30 % of MSW generation in 2000 with high incineration rates during the last decades due to the historic scarcity of land [12]. Greece, UK, and Finland are some of the most European Countries which landfilling is the main solution. According to Eurostat [13] MSW landfilled in 2008 was 77 % in Greece, 55 % in the UK, and 51 % in Finland while in Germany, Netherlands, Denmark, Austria, and Sweden was <5 %.

MSW impacts on landfills have received social and environmental attention the last years. Landscape appearance, environmental degradation, heavy traffic load, dusts, noise odor emissions, infect these facilities environmental stressor with significant impact on quality of life as well as on the nearby communities [14]. Several studies indicated that waste facilities are incommensurate located in the areas where more deprived or minority groups live [15–17], with consequent pollutant exposure. Several studies show relations between adverse health effects and the residence distance from the landfill site [18, 19]. However, the level of epidemiological evidence is insufficient, with a general lack of consistency in the results for cancer incidence and mortality [20, 21]. Although there is lack of evidence on the health effects and consequence, citizens are concerned with potential toxic compounds and unpleasant odors produced by the production of landfill gas (LFG), which include gases generated by the biodegradation of waste and those arising from chemical reactions or volatilization from waste [22]. It is well known that LFG mainly consists of CH_4, CO_2, water vapor as well as [23] volatile organic compounds (VOCs), hazardous air pollutants, and odorous compounds [24, 25].

Globally the per capita amount of MSW generated on a daily basis varies significantly. Economic standing is one primary determinant of how much solid waste a city produces. An important dimension of wastes growing is the today economic crisis which starts from 2008 and continues until today, and secondly is the waste distancing. In today's world, there is a growing distance, geographically as well as mentally, between consumers and their waste [26]. First is the fact that when decision makers have little knowledge of the ecological and social impact of the wastes related with goods they produce or purchase, they have little incentive or ability (as producers or consumers) to change their habits based on waste consideration. Secondly is the fact that such distancing consumers waste (sink capacity) both the social capacity to deal with waste in manner that minimizes harm and that is far;

and the ecological capacity to adapt waste that toxic contamination and intergenerational effects. Thirdly, there is economic pressure from the citizens in local authorities to reduce waste taxes and especially in insular communities (which there is limited space for waste landfills and the gate fees from central units is extremely high) and as a result local authorities develop uncontrolled landfills sites to dump their wastes [8, 27]. According to Cyprus National Law N(I) 111/1985 (Clauses 84Z) [28], any local authority is authorized to charge waste fees according to the following principle: (1) for houses (regardless of square meters) maximum 171 €/y; (2) for stores, shops, coffee shops, and similar activities up to 855 €/y; (3) for bars, restaurants, and tourist facilities (apartments) up to 6848 €/y; (4) for hotels up to 17,100 €/y; and (5) for private hospitals, industrial activities, or other not included above the fees is up to 13,680 €/y. The only existing Municipal Waste Treatment plant (MWTP) in Cyprus charges (until the end of 2012) 54.8 €/t for the mix waste (mostly household), 46.8 €/t for green, and 80.80 €/t for the recyclable waste. For the residual waste, there is a charge up to 100.80 €/t f (like furniture, equipment, etc.) until 2012 [8]. Now, according to the New Policy of the Ministry of Interior Affairs the per ton fees are highly decreased and is less than 450 €/t regarding the mix waste.

While the use of landfills as a solution is decreasing in many parts of the world, there are nonetheless thousands of closed landfills and thousands more that are operating but will close over the next 10–30 years [29]. In United States, there are more than 1800 MSW landfills which during 2008 were operated than 6300 from 1990 [10]. In Germany, during 2009, the MSW landfills have decreased from 560 (in 1993) to 182 [29]. More than 2000 MSW landfills were operating in April 2004 in UK, but by the end of 2009 only 465 remained in operation with a Landfill Directive (EC, 1999) [30] compliant permit [22]. Usually, aftercare and monitoring of closed landfills comprises monitoring of emissions (e.g., leachate and gas) and receiving systems (e.g., surface water, groundwater, soil, and air) and maintenance of the cover and leachate and gas collection systems. Regarding the European Landfill Directive (EC, 1999) [30] specifies a period of at least 30 years of aftercare and maintenance as a basis for the buildup of financial provisions. This for many EU countries typically means that requires at least 30 years aftercare.

Uncontrolled waste disposal on landfill sites causes pollution that can be hazardous on the environment and human health and introduces several problems on socioeconomic welfare of the planet. Waste disposal pollution is caused by either normal operations, such as the emissions of landfill gas into the atmosphere and the leakage of leachate in subsoil (and then to the aquifer), or the accidence from subsidence and fires; either way they constitute serious threats to public health and the environment. The environmental impacts from an illegal/uncontrolled landfill are the same as those from landfills that are not subject to the engineering and management controls detailed in the Landfill Directive. The main impacts include (1) groundwater and surface water contamination, (2) emission including CH_4 and VOCs depending on the waste deposited, and (3) infliction creation which affects the surrounding environment through the production of litter, odor, pests and degradation of the visual urbanity [31–35].

Council Directive 99/31/EC of 26 April 1999 (EC, 1999) [30] on the landfill of waste entered into force on 16 of July 1999. The deadline for implementation of the legislation in the Member States was 16 of July 2001. The main objective of the 99/31 Directive is to reduce or to prevent negative effects on the environment from the landfilling of waste, by introducing stringent technical requirements for waste and landfills. The specific directive gives emphasis on the protection of surface water, groundwater, soil, air, and human health from landfilling. The directive expresses the different categories of waste (municipal waste, hazardous waste, nonhazardous waste, and inert waste) and applies to all landfills, as well as waste disposal sites for the deposit of waste onto or into land. There are three main landfill categories which are for hazardous waste, nonhazardous waste, and for inert waste. The directive does not cover the spreading on the soil of sludges, the use in landfills of inert waste for redevelopment or restoration, rehabilitation work, the deposit of unpolluted soil or of nonhazardous inert waste resulting from prospecting and extraction, treatment and storage of mineral resources as well as from the operation of quarries and for the deposit of nonhazardous dredging sludge alongside small waterways from which they have been dredged and of nonhazardous sludge in surface water, including the bed and its subsoil. According to the directive, wastes must be treated before being landfilled. Liquids, flammable waste, explosive or oxidizing waste, clinical waste, used tires, and any other type of waste which does not meet the acceptance criteria laid down in Annex II are not accepted to landfills. Member States must ensure that all the existing landfill sites may not continue to operate unless they comply with the provisions of the directive the soonest possible.

2 Area Description and the Problem as Presented in Cyprus

The annual per capita production of waste in Cyprus according to previous studies [9, 13, 36] is estimated at 468 kg for residential areas and 670 kg for tourist areas. The total waste was estimated at the end of 2012 up to 630,000 t (Fig. 2). Figure 3 presents the waste production per economical sector in Cyprus. The existing MSW is disposed of, in 117 landfills (Fig. 4), of which five are known to be operating currently in Nicosia, Limassol, Larnaka, Paphos, and Paralimni (closed on 2011) [37]. Only 75 % of the population of Cyprus is served by those landfills. The other 25 % (mainly in residential rural areas) dispose of their waste uncontrolled.

The current implemented solid waste management plan (SWMP) (Fig. 5) in Cyprus includes the door by door collection from any houses or organization. Recyclable materials (such as papers, PMD, plastics, glass, including WEEE, and batteries) are collected by Green Dot Cyprus. More than 30 Green Points (GP) has been designed and promotes from the Ministry of Interior Affairs and will be established before 2020 around Cyprus. In the GP any citizen could transfer and leave their specific waste stream like WEEE (refrigerators, washing machines, etc.), furniture, etc. Several composted units exist in Island, but the organic wastes that are forwarded for composting are considered to be limited (less than 5 % of the total organic waste).

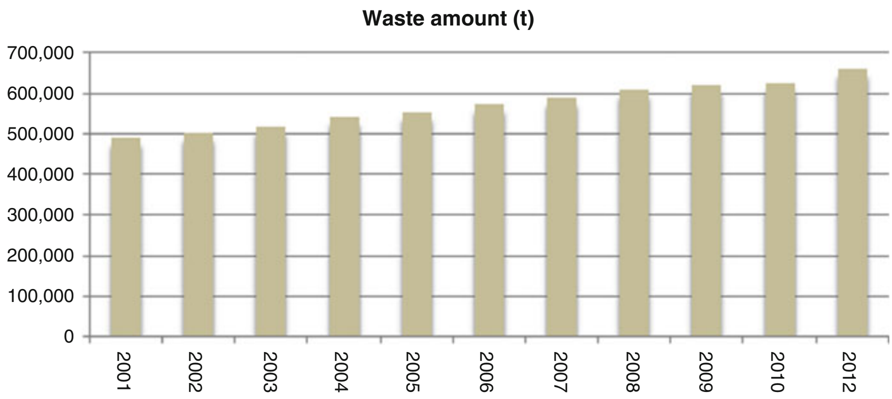

Fig. 2 Waste generation in Cyprus from 2001–2012

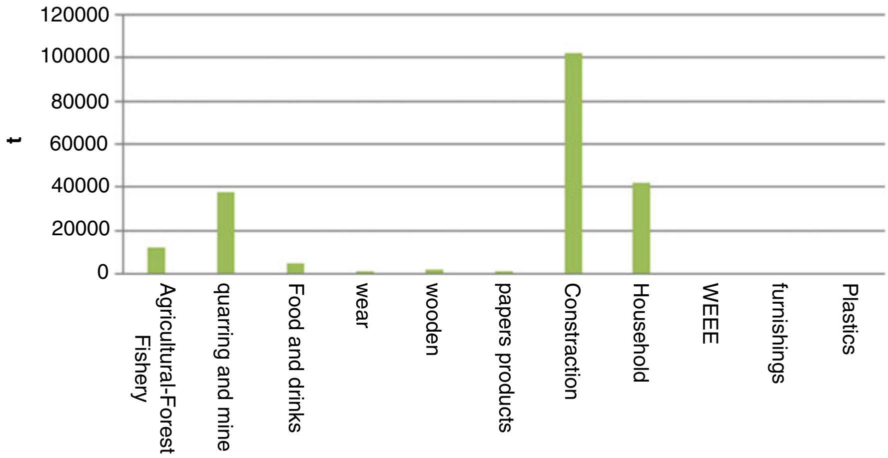

Fig. 3 Waste production per economical sector 2010 [13]

Until the end of 2013 there exist 117 illegal landfilled site areas in which 113 have been closed (the other two must be closed until the end of 2015). One waste management plant (WMP) exists which has the ability to treat the wastes that are produced from two districts (Famagusta and Larnakas) which are equal with 150,000 t/year. One more WMP will be established by 2016–2017 for the District of Limassol and Pafos (in south west), which will have the ability to treat almost 200,000 t/year. The SWMP includes apart implementation of pay as you throw principles and zero waste management approach in smaller municipalities [8, 9]. A typical waste compositional analysis of the uncontrolled landfills is presented in Table 2.

Fig. 4 Uncontrolled landfills on Southern Cyprus (there is no any available quantitative or qualitative data for the North part of Island since 1974)

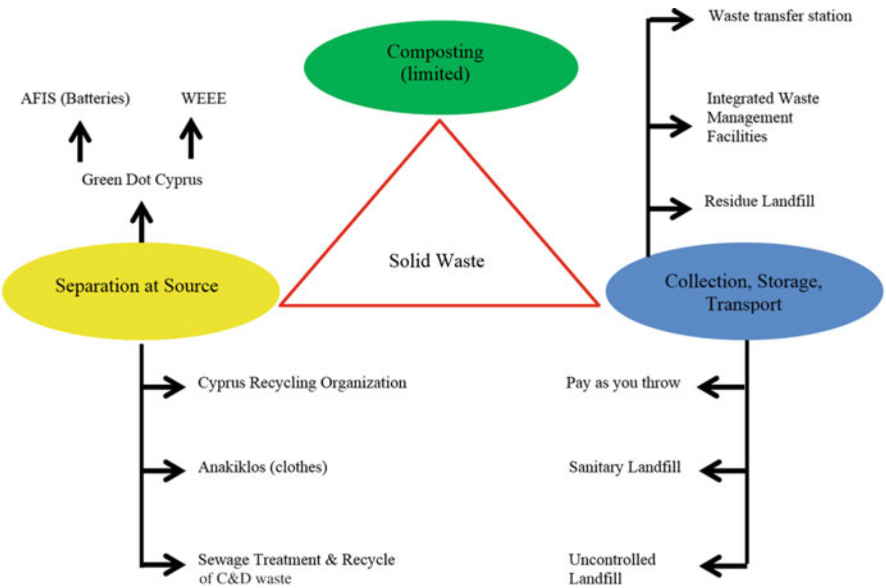

Fig. 5 Current state of solid waste management in Cyprus

Table 2 Qualitative and quantitative composition analysis of waste [36]

Paper	22.8 %
Plastic	17.0 %
Metal	2.6 %
Glass	2.5 %
Organic	42.0 %
Aggregates	1.5 %
Other	11.6 %

3 Multi-criteria Analysis

The multi-criteria analysis models of Analytic Hierarchy Process (AHP) were applied, for the pairwise comparison of the candidate proposed rehabilitation solutions. The objectives served by the application of AHP model include the determination of the most viable methods in terms of low operating costs combined with the high rate of degradation, thereby making it attractive for implementation in industrial scale and simultaneously satisfying regulatory requirements for the restoration of uncontrolled landfills. Additionally, it serves the identification of technology with lower environmental footprint, ensuring the protection of the environment and to achieve social acceptance. The application of the methodology of AHP is performed by using the software Make It Rational Professional [38].

The AHP analysis is based on three fundamental principles: (a) the breakdown of the problem into subproblems, (b) pairwise comparison of criteria and the various alternative scenarios, and (c) the composition of preferences. The analysis is completed through four steps as follows: (1) the degradation of the problem into subproblems and the formation of an hierarchical structure, (2) the pairwise comparison of decision elements used to derive normalized absolute scales of numbers whose elements are then used as priorities, (3) the calculation of the priorities for the data of the problem, and (4) the composition of preferences for alternative scenarios to solve the problem. The key element, of the method, is the pairwise comparison of the components at each level of the hierarchical structure, namely the criteria and sub-criteria of the alternative scenarios, which affect the problem. For this purpose, comparison matrices are structured for the comparison of the elements of a level of the hierarchy with the elements of the next higher level and so. The data input in comparison matrices, which represent the expression of preferences of the decision makers, resulting from the fundamental scale of Saaty, which is a qualitative scale that includes values from 1 to 9. These values are used by the decision makers for the purpose of benchmarking as equal (1), moderately strong (3), strong (5), very strong (7), and very (9) important. Based on the scale preferences Saaty, all possible gradations of preference is $P = \{1, 2, 3, 4, 5, 6, 7, 8, 9, 1/2.1/3.1/4.1/5.1/6.1/7.1/8.1/9\}$ [38–42].

To ensure consistency in the pairwise comparisons, during AHP analysis, the calculation of the consistency ratio (CR) was necessary to take place in order to assess any discrepancies in matrices of pairwise comparisons that should lead the decision makers to revise their initial estimates. According to Zorpas and Saranti

[38], any pairwise comparison matrix is considered to be consistent and hence acceptable when CR is less than 10 %. In addition to that, a sensitivity analysis on the AHP weights is unfolded to show the impact of varying weights to the final outcome. The much-criteria analysis aims to prioritize in order of preference of alternative scenarios (the best choice) by resolving conflicting parameters. The analytical hierarchy process developed by Saaty T. in 1980 [39] (established as one of the most commonly applied analytical techniques [43, 44] was applied to evaluate the rehabilitation methodology.

The evaluation criteria that were set out divided in the following categories as indicated below:

1. Economics: (K1) cost of recyclable management (this criterion includes the cost of the material recovery from the soil, the transportation cost, and the recycling cost), (K2) recovery cost (this criterion evaluates the total costs that are needed for recovery), (K3) aftercare costs (This cost includes the cost of maintenance of the rehabilitated area, checking for landslides, slope failures and damage to infrastructure maintenance, water analysis, maintenance of bio-filters), (K4) Land Usage (This criterion reflects the choices of land use after the restoration of the waste disposal site), (K5) Income from Generated Energy (Financial benefits may arise from the management of biogas and the photovoltaic systems covering the area).
2. Environmental Criteria: (K6) Emission of Greenhouse Gases, (K7) Avoiding pollution (This quality criterion refers to the efficiency of each method in rehabilitation response to pollution caused by uncontrolled landfills).
3. Technical Criteria: (K8) Simplicity of Technique
4. Social criteria: (K9) Social acceptance (This criterion is the acceptance of this methodology by society, which can exert considerable pressure by reactions that occur due to disturbance of the applicable technique).

The selection criteria, according to Turcksin et al. [45], were the four criteria of multi-criteria analysis (social, environmental, operational, and financial). The evaluation of criteria to determine the optimal methodology was determined through the Analytic Hierarchy Process, which shows the relationships between the target criteria, the sub-criteria, and the alternative scenarios. Five alternatives scenarios were tested (Table 3), represented different solutions to the same problem.

4 Proposed Scenarios

Based on the estimated score criteria, economic and environmental criteria have equal weight (41.86 %) while social (4.06 %) and technical (12.22 %) criteria have lower weights. Figures 6 and 7 present the ranking of the proposed scenarios.

The simple extraction and transportation of all the material to a controlled landfill (S4) obtains the highest rate of satisfaction of criteria and sub-criteria evaluation. Second ranks the scenario of phytoremediation (S1) and third the mining and recovery of materials (S5). The scenario for covering the area with photovoltaic

Table 3 Proposed scenario for rehabilitation in Cyprus

S1	Phytoremediation of uncontrolled landfills	The technique of phytoremediation requires molding the relief to settle and compress the waste in order to avoid the exposure and smoothing the waste relief by creating uniform gradients and fencing. The wastes are covered by a layer of fine soil material and humus
S2	Simple covering of uncontrolled landfills	Covering uncontrolled landfills includes molding the relief to settle and compact the waste in order to avoid exposure, smoothing the waste relief by creating uniform gradients in and covering them with several layers (for sealing, biogas collection), and finally overlying them with humus soil material, required for the region of planting
S3	Coverage and energy utilization of uncontrolled landfills using photovoltaic	Covering uncontrolled landfills includes the molding of the relief with the settlement and compaction of the waste in order to avoid the exposure, smoothing the waste relief by creating uniform gradients and covering them with several layers (for sealing, biogas collection), and on top, a final layer of soil material where photovoltaic panel is installed
S4	Extraction and transportation of wastes to a controlled landfill	In this scenario, the wastes are extracted and transferred to other controlled landfills
S5	Extraction and recovery of recyclable materials and transfer of the remaining waste to a controlled landfill	In this scenario, from the extracted waste, the recyclable materials are recovered. Recoverable materials are considered those that contain iron and aluminum; plastics and glass do not have the necessary quality to be recycled [76]

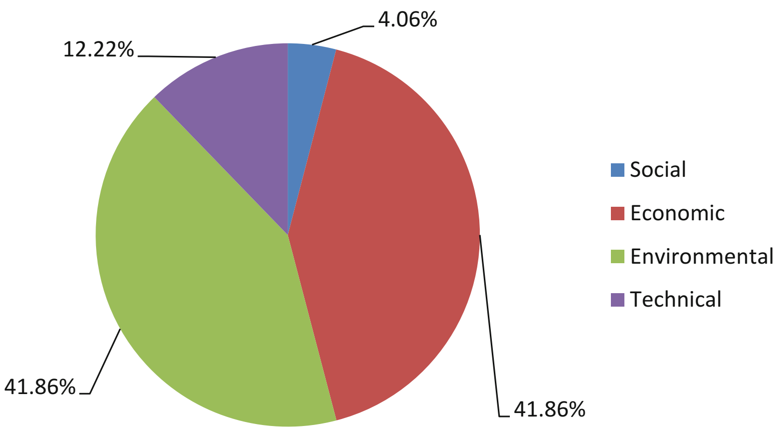

Fig. 6 Basic criteria for the evaluation of the alternative scenarios

(S3) ranks forth while the simple coverage scenario (S2) ranks fifth. The satisfaction rankings for each sub-criterion are indicated in Table 4. Although the S1 scenario (phytoremediation technique—Fig. 8) are in the third place, is consider to be among the most effective and applicable for the restoration of uncontrolled landfills as the Ministry of Interior Affairs in Cyprus after taking into consideration

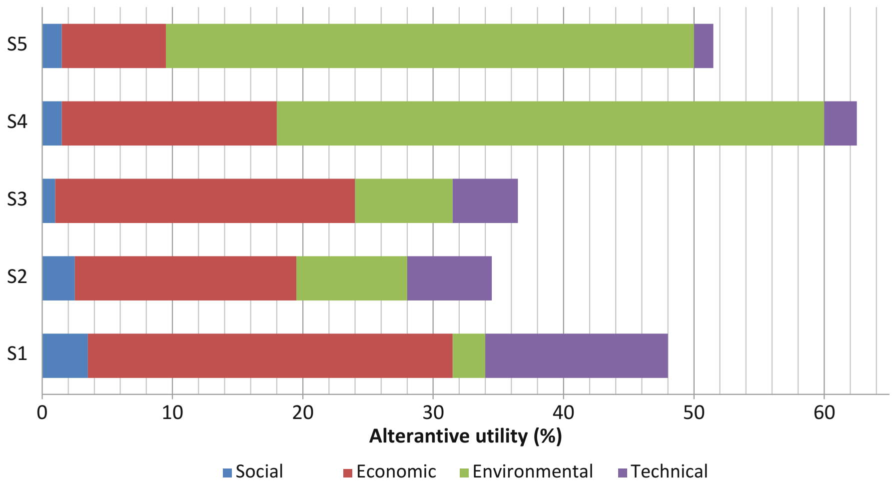

Fig. 7 Ranking of alternative scenarios

Table 4 Satisfaction rankings for each sub-criterion

Proposed scenarios	Economic (%)	Social (%)	Technical (%)	Environmental (%)
S4	16.84	2.33	1.1	41.86
S1	8.19	0.9	0.88	40.63
S5	28.91	12.22	2.98	4.06
S3	23.49	5.02	0.58	7.69
S2	17.98	6.3	2.4	7.57

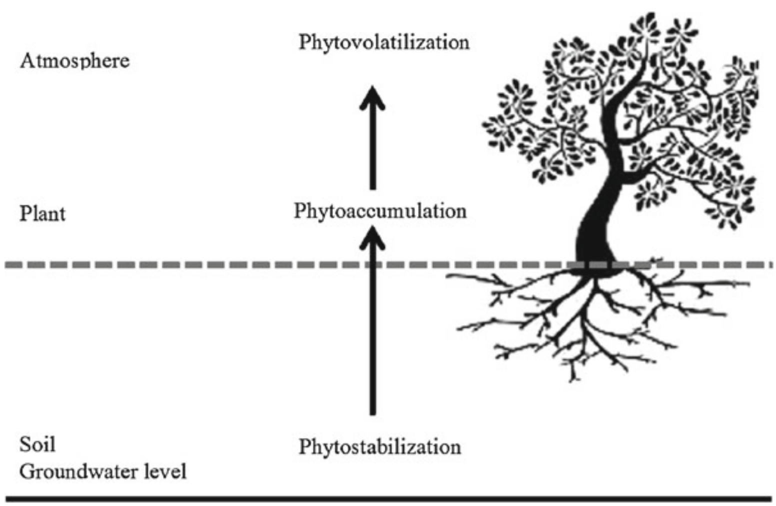

Fig. 8 Mechanisms of the phytoremediation process for metal uptake

the police of green public procurement implement this solution. Phytoremediation basically refers to the use of plants and associated soil microbes to reduce all kinds of concentrations including toxic substances [46].

The methods are suitable for heavy metal removal as well as for organic pollutants (like polynuclear aromatic hydrocarbons pesticides and polychlorinated biphenyls). The methods according to several studies [47–59] present ungues characteristics, which among those are efficient, cost-effective, easy to apply as in situ, environmental, and eco-friendly. Plants generally handle the contaminants without affecting topsoil, thus conserving its utility and fertility. The phytoremediation process produces at the end less secondary waste in contrast to other methods of decontamination and is applied on site (in situ technique) with the landscape without requiring extra works (like excavation, pumping or buildings). It is also a cost-effective method of treatment as it can remove significant quantities of pollutants from large volumes of water or soil without allowing any further spread of pollutants and protect the soil from erosion and precipitation. The type and quantity of plants depend on the climate, the type of metal, the area, and the physicochemical properties of the surface.

The areas where the vegetation cover occurs require fencing, especially if it is covered by small plants and not trees which can be used as animal/bird food, to prevent the transfer of toxic substances in the food chain. The cost of the procedure may increase due to lack of pre-existing applications lesion plant (anaerobic digestion, thermal methods), but part of it returns through the high biomass produced. The application is unfortunately effective at shallow depths, approximately 1 m in soils and 3 m in aquifers [60], although pollution occurs in most cases of uncontrolled disposal of waste. The application of phytoremediation has experimentally proved that can be used for treatment of organic (e.g., insecticides) and inorganic (e.g., heavy metals) contaminants [61]. The *hyper-accumulators* have the ability to collect the biomass of up to 5 % of their dry weight in heavy metals [62]. The heavy metals amount in the environment are reacting with the soil components and imparting toxic nature to plants, animals, microorganisms, and humans. Metals and metalloids are toxic and can cause undesirable effects and severe problems even at very low concentrations and that's why deserve special attention [63–67].

A good example is the *Thlaspi caerulescens*, which can accumulate in its mass up to 26,000 ppm zinc (Zn) and large amounts of cadmium (Cd), without lesions [68]. The *Helianthus annuus* (sunflower) is known to adsorb strontium, cesium, and uranium, and has been used in the past in soils with high levels of radioactive elements e.g., Chernobyl [68]. Some studies indicated that *nicotiana glauca* has the ability to absorb several elements: Pb, Cd, Zn, and Hg, while *Alyssum Baldacci* absorbs Ni and Pb. The *trifolium* (alfalfa) can be used to absorb lead, *Amaranthus* to absorb Cobalt, and many other plants can be used for phytoremediation [68]. Rapid growth trees, resistant to metals, with deeper root systems, suitable for planting in poor soils, can be used as an alternative hyper-accumulator plants [69]. The *Populus nigra* and *populus tremula* (White) are fast-growing plants that thrive in wet soils (e.g., Riverbed) and are known to absorb arsenic (As) and Cd [70], same as the willows. The *Paulownia tomentosa* is of great interest in phytoremediation techniques because of its rapid growth, high biomass production, and the great resistance encountered in soils with high concentrations of metals [71, 72]. It is a consolidation of contaminated soils and water bodies (surface and groundwater) through procedures exist plants (EPA). The procedure is based on the natural ability of certain (hyper-accumulate) plants to break the steady state of the contaminants, to absorb through their roots high concentrations of metals, and to accumulate them in their mass, thus removing them from the soil and drainage network [68, 73].

The modelling of an environmental policy and methodology for the restoration of uncontrolled landfills is a complex process [74, 75] as there are many alternative techniques for remediation disposal, each method/recovery system has advantages and disadvantages, technical, economic, environmental, etc. Therefore it requires benchmarking to be as reliable and scientifically correct as possible. The appropriateness of each recovery method depends on the local circumstances and the characteristics of each region (climate, geology, hazards, hydrology, etc.), which pose physical and technical constraints. To obtain an evaluation for the various scenarios, it is not sufficient to compare a parameter, but requires analyzing and scoring a series of conflicting parameters. All scenarios were analyzed based on the same criteria, which incorporate a weighting factor. To extract the best solution—the script must be run a sufficient number of representative criteria both qualitative and quantitative, and also relates to the three pillars of sustainability (economic, environmental, social). For the implementation of multi-criteria analysis and the determination of the best methodology for the rehabilitation of uncontrolled landfills, the following were taking into consideration: (1) evaluate the characteristics of Cyprus landfills, (2) determinate the potential management of recovery plans (scenarios), specifically: mulch, phytoremediation, photovoltaic, recovery, extraction (with or without recovery of materials), (3) identify benchmarks per recovery scenario: Specific restoration and aftercare costs, greenhouse gas emissions, energy recovery, materials recovery, land use perspective, further pollution, simplicity of implementation, social acceptance, and (4) estimate the incidence of management methodologies within the hierarchy sensitivity analysis.

The test cases of this research are investigating phytoremediation, surfacing and extraction of areas of uncontrolled landfills. The case of surfacing includes planting the sites and covering the areas with photovoltaic panels while the case of extraction includes (a) extracting and transporting all materials to a controlled landfill and (b) extracting and recovering recyclable materials while transferring the rest to a controlled landfill. Phytoremediation is a simple method to reduce pollution and to upgrade the landscape. The planting sites are a common practice, applied to prevent further pollution, to control of leachate and biogas (where appropriate), and to upgrade the landscape. Covering of the site with photovoltaic cells can prevent pollution and enhance the landscape with the energy utilization at the same time. The extraction of the uncontrolled landfill and the transfer of all solid materials in landfills were also evaluated while the recovery of recyclables scenario along with the transfer of the remaining fraction in a controlled landfill proved unprofitable, as reported by the USEPA in 1997 [76]. Thermal processing scenarios were not taken into consideration due to the current lack of thermal treatment plants (incineration, gasification) in waste site in Cyprus and as there is no any specific strategy on this section until now. If landfill sites are problematic in mainland scenarios, in large islands the situation is even worse. The limited surface of islands, the waste volume that is steadily increased (due to fishing, tourism, or other sources) [77, 78], and the waste washed up from open seas magnify the problem and make them a priority [79].

5 Conclusions

The uncontrolled disposal of waste is daily poisoning the planet, threatening the viability of human nature. The use of multi-criteria analysis was a powerful tool for organizing the rehabilitation work on uncontrolled landfills; it indicated as optimal recovery method the mining of the disposal site and transferring the material to a controlled landfill. In cases where the extracted waste is at the appropriate quality, instead of deposited in landfills can be transferred to existing plants to be use for the production of energy (RDF, SRF). This methodology is followed by the extraction method with material recovery, which has essentially the same results (with a significant difference in cost for recovery), for cleaning and management of materials recovered from the soil. The phytoremediation is a very promising technique, since it presents several advantages over the other techniques. Plants can stabilize and bind pollutants in their bodies and their management can result in energy savings due to the high-energy characteristics of the biomass. In Cyprus, due to the absence of public facilities for heat treatment, the methodology can be applied to small uncontrolled landfills by planting appropriate trees rather than small plants. Trees can remain for years to form a small forest without the need for continuous seeding, harvesting, and heat treatment. This methodology can be applied in parallel with the creation of certain infrastructure space, available as permeable walls or through applications that satisfy the environmental criteria. Through the techniques of single coverage and coverage of areas with photovoltaic, further spread of contamination can be prevented. The collection and treatment of leachate and biogas can bring economic and environmental benefits as the use of recycled water can help water conservation and the energy use of biogas can bring positive effect as well.

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Suitability of Different Mediterranean Plants for Phytoremediation of Mine Soils Affected with Cadmium

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Abstract Mine residues dumped on the environment as overburden or tailings ponds show environmental and human health hazards by the transfer of heavy metals through erosion or leaching. The objective of this study was to assess the potential use of different Mediterranean plant species for phytostabilization or phytoextraction of cadmium in acidic mine residues. For this purpose, a reclamation strategy was carried out in a mine tailing based on the use of phytoremediation aided with three different amendments (pig slurry, pig manure, and marble waste). –Six Mediterranean species were introduced: *Lygeum spartum*, *Atriplex halimus*, *Helichrysum stoechas*, *Dittrichia viscosa*, *Piptatherum miliaceum*, and *Limonium cossonianum*. Soil and plant samples were collected 24 months after remediation works. Results showed that the characteristics of the mine residue improved with the reclamation developed, with increased pH, organic matter and fertility, and decreased salinity. The extractable and exchangeable fractions of Cd decreased 85 % and 96 %, respectively. The tested species (except for *A. halimus* and *L. cossonianum*) may be potential candidates for the objectives of Cd phytostabilization since they showed low translocation and bioaccumulation factors. *P. miliaceum* was the best candidate owing to its lower translocation and bioaccumulation factors, higher biomass, and higher colonization of the area. *A. halimus* seems a potential candidate for phytoextraction rather than for phytostabilization of soil Cd, with high translocation and bioaccumulation factors, high biomass, and fast growth.

Keywords Cadmium • Tailing pond • Amendments • Phytoextraction • Phytostabilization

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1 Introduction

Soil pollution by heavy metals is a major environmental problem, since it can decrease the ecosystems productivity and enhance the risks for the environment and humans [1]. In mining areas, many tailings ponds, which may extend in some cases over hundreds of hectares, are now abandoned without any particular safety measures and with a high environmental impact on the surrounding ecosystems and populations [2]. In these tailings, mine residues usually contain acid materials rich in Fe-oxyhydroxides, sulfides, sulfates, and heavy metals such as Cd. As a consequence, these materials show no colonization by vegetation and have low fertility [3]. Hence, there is an urgent need to carry out measures to remediate these sites, since environmental hazards, especially water and wind erosion, stand out with propensity to adversely affect both human health and the functioning of ecosystems [4].

Conventional remedial approaches to soils affected by heavy metals usually involve removal and replacement of soil with clean materials [5], although it is not considered the most economically or environmentally sound solution available [6]. Phytoremediation takes its place as a feasible reclamation technique in the in situ stabilization/immobilization or extraction of heavy metals by the use of vegetation. Soil amendments are also required to improve the soil growing limiting factors. Recent research has shown that the reclamation of abandoned mine sites relies on achieving optimal conditions for plant growth by improving the soil's physical, chemical, and biological characteristics by using different amendments [7, 8]. As a general rule, for the creation of a vegetation cover through the phytoremediation, native species that are adapted to the specific conditions of the area are preferred, as their use prevents the introduction of nonnative and potentially invasive species that may result in decreasing local plant diversity and endangering of the harmony of the ecosystem [9, 10]. Plant screening is a prerequisite for successful use of phytoremediation, since the selected plants have to be able to grow in a growing limiting substrate, develop a proper root system and aerial biomass and immobilize metals in the roots and rhizosphere if phytostabilization is selected, or have a high translocation of toxic elements to the aerial tissues if phytoextraction is selected.

Soil organic matter is universally recognized to be among the most important factors responsible for soil fertility and land protection from contamination, degradation, erosion, and desertification [11]. Organic residues such as sewage sludge, urban solid waste, crop residues, or animal manure can be used as a source of organic matter and nutrients which stimulate the formation of soil aggregates [12] and thus the creation of a soil from mine residues. Incorporation of organic residues as amendments into mine residues has been proposed as a feasible, inexpensive, and environmentally sound disposal practice as such residues improve the soil's physical and chemical properties and activate soil microbial communities [13, 14]. Organic residues can also reduce the availability of toxic metals through complexation, or increase their availability by formation of mobile chelates, depending on the quality of the organic compounds supplied [15]. Alkaline materials, such as marble wastes, are commonly used as an amendment for ameliorating the acidic conditions of many acid-generating mine residues. Correction of acidity not only enables a wide range of plants to be established but also mitigate metal toxicity and increases plant nutrient availability such as potassium, calcium, magnesium, molybdenum, and phosphorus, which are more mobile at neutral pH [16]. The use of organic amendments and materials rich in carbonates has been successfully used to reclaim mine soils and restore the ecological function of contaminated soils [6, 17–19]. In the current study, different plant species were tested in a field experiment as potential metal-tolerant plants for phytostabilization or phytoextraction in acidic mine residues, aided with the use of pig slurry and manure as organic amendments and marble waste as alkaline amendment. The objective of the study was to assess the effectiveness of the use of the selected species and amendments for the immobilization or extraction of Cd in the mine soil.

2 Materials and Methods

2.1 Study Site

The experiment was established in a tailing pond at Cartagena-La Unión Mining District (SE Spain; 37°35′38″N, 0° 3′11″'W), where great mining activities had been carried out for more than 2500 years, although the activity ceased in 1991. The climate of the area is semiarid Mediterranean with mean annual temperature of 18 °C and mean annual precipitation is 275 mm, with rainfall events occurring mostly in autumn and spring. The tailing pond has an area of 5800 m^2, and the soil remained bare, with sandy loam texture, pH 5.8, 0.73 % soil organic matter, and high levels of heavy metals. The total concentration of Cd was 20.82 mg kg^{-1}.

2.2 Experimental Design

The tailing pond was ploughed and leveled to create uniform surface soil conditions prior to conducting the experiment. A reclamation strategy was carried out in ~2/3 of the total surface based on the use of three different amendments (pig slurry (PS), pig manure (M), and marble waste (MW)), in order to facilitate vegetation growth. The characteristics of the amendments are given in Table 1.

The marble waste (8.6 kg m^{-2}) was formed by particles of 5–10 μm diameters and was applied in September 2012. This rate was calculated to establish the quantity of calcium carbonate required to neutralize all the potential acid according to the percentage of sulfides present in the mine soil [20]. We applied the organic amendments in two different episodes to favor a suitable stabilization of organic matter in soil before introduction of vegetation. We applied 2.5 L m^{-2} PS in September 2012 and 4.5 kg m^{-2} M in October 2012. The PS dose was based on the agronomic rate established by Spanish legislation RD 261/1996 (framed within the

Table 1 Main characteristics of the amendments used for reclamation purposes

Parameters	Pig slurry	Pig manure	Marble waste
pH	7.8	9.1	8.0
Electrical conductivity (dS m^{-1})	39.1	10.2	2.2
Calcium carbonate (%)	–	–	99
Moisture (%)	96	10	1
Total organic carbon (g L^{-1}/g kg^{-1})	17.8	171	–
Total nitrogen (g L^{-1}/g kg^{-1})	5.1	13.6	–
C/N	3.5	12.5	–
Available phosphorus (mg L^{-1}/mg kg^{-1})	623	9.64	<d.l.
Calcium (mg L^{-1}/mg kg^{-1})	249	855	2190
Magnesium (mg L^{-1}/mg kg^{-1})	14.4	802	347
Sodium (mg L^{-1}/mg kg^{-1})	459	4280	69
Potassium (mg L^{-1}/mg kg^{-1})	1059	15,700	59

<d.l.: below detection limit

European Directive 91/676/CEE), to avoid contamination of groundwater by nitrates. The M dose was calculated on the basis of its organic carbon content to increase soil organic carbon >5 g kg^{-1}. This solid manure was obtained after separation of the solid phase of the PS from the liquid phase using a physical phase separator. The solid fraction was air-dried outdoor under environmental conditions for 4 weeks. After the mechanical application of the amendments, all materials were mixed to a depth of 0–50 cm to incorporate the amendments into the soil.

Six Mediterranean native species were introduced in December 2012 for phytoremediation. Seedlings (15–20 cm in height) of *Lygeum spartum* (L.) Kunth, *Atriplex halimus* L., and *Helichrysum stoechas* (L.) Moench were manually and randomly planted at a density of 1 plant per m^2 to create a mosaic landscape. The species *Dittrichia viscosa* (L.) Greuter, *Piptatherum miliaceum* Beauv., and *Limonium cossonianum* Kuntze were homogenously sown covering the surface of the amended area. One irrigation event was carried out after planting; after that, no water was added and plants were exposed to the semiarid climatic conditions of the study area. Approximately, a third of the tailing pond surface was kept unamended and unplanted, acting as control. In each of the two different study areas (unamended tailing pond and amended tailing pond), we established four plots (8 m × 8 m) to monitor soil and vegetation evolution. Plots in the unamended area of the tailing pond had no vegetation; the plots located in the amended surface of the tailing pond had the six introduced plant species.

2.3 Soil and Vegetation Sampling

Soil samplings were carried out in September 2012 (before the remediation works) and in October 2014 (24 months after remediation works) in the plots located in the amended surface. Three random soil samples per plot were taken at two different depths (0–15 cm (surface) and 15–30 cm (subsurface)) to obtain a composite

Table 2 Aerial dry weight and density of the studied plant species growing in the amended tailing pond

Plant species	Aerial dry weight g plant^{-1}	Density plants ha^{-1}	Aerial biomass kg ha^{-1}
A. halimus	124	2390	296.4
H. stoechas	18	94	1.7
D. viscosa	124	80	9.9
L. cossonianum	13	142	1.8
P. miliaceum	139	377	52.4
L. spartum	12	157	1.9

Values are average of four 8 × 8 m^2 plots

sample per plot and depth. Thus, four composite samples per sampling date were used for analyses. Samples were air-dried for 5 days, sieved <2 mm, and stored at room temperature until analyses. Biochemical properties were also measured in air-dried samples since these properties from Mediterranean semiarid soils are medium-term stable in stored air-dried samples [21–23]. A plant of each species was completely taken from each plot. The species *A. halimus*, *H. stoechas*, *D. viscosa*, and *L. cossonianum* were divided in roots, stems, and leaves for analyses. The Poaceae species *P. miliaceum* and *L. spartum* were divided in roots and shoots. The characteristics of the vegetation in the plots from the amended tailing pond are given in Table 2. Plant samples were carefully washed with deionized water and then dried at 55 °C for 72 h. The dried material was ground using a mill (A11 Basic, IKA). For each sample, 0.7 g was incinerated prior to a metal redilution using 0.6 N HNO_3. Plant extracts were stored at 4 °C until analysis of Cd concentration.

2.4 Analytical Methods

Soil texture was determined by the Bouyoucos method [24]; pH and electrical conductivity (EC) were measured in deionized water (1:2.5 and 1:5 w/v, respectively); total organic carbon (TOC) and total nitrogen (Nt) were determined by an elemental analyzer CNHS-O (EA-1108, Carlo Erba); cation exchange capacity (CEC) by the method of Chapman [25]; available phosphorus (P) was determined by the Burriel–Hernando method [26]; urease activity was measured according to the method of Nannipieri et al. [27]; the activity of β-glucosidase was determined according to Tabatabai [28]; arylsulfatase activity was measured by the method of Tabatabai and Bremner [29]; total Cd concentration was determined using HNO_3/$HClO_4$ digestion at 210 °C for 1.5 h [30]; for soil extractable Cd, DTPA was used in the ratio of 1:2 soil extractant [31]; the exchangeable Cd fraction was determined using 0.01 M $CaCl_2$ (1:10 soil-extractant ratio) [32].

The Cd concentrations in soil and plant samples were measured using ICP-MS (Agilent 7500CE). The standard concentrations of the calibration curve were 0, 2, 5, 10, 25, 50, 100, 200, 500, and 1000 μg L^{-1}. When the sample concentration was higher than the highest standard, a corresponding suitable dilution was made. The detection limit of the equipment was 0.02 μg L^{-1} for Cd. The methodology for total Cd concentration was referenced using the Certified Reference Material BAM-U110 (Federal Institute for Materials Research and Testing, Germany). The reference sample was analyzed in triplicate. The overall recovery ratios were 96–126 %. Certified internal standard solutions of Ga, Rh, and Tl (1000 mg L^{-1} High Purity Standards, Charleston, USA) were used as quality control for each sample measured in the ICP-MS, with overall recovery ratios of 97–108 % for Ga, 94–115 % for Rh, and 99–117 % for Tl. In addition, every ten samples run in the equipment, the calibration standard of 50 μg L^{-1} was measured to assess that values were within the range of ±10 %. Should some value be outside this range, the calibration was remade. In order to evaluate whether the plant species could selectively accumulate Cd from soil in their tissues, or block the translocation to the aerial tissues, the bioaccumulation factor ($BF = [Cd]_{\text{shoot or leaf}}/[\text{extractable } Cd]_{\text{soil}}$) and the translocation factor ($TF = [Cd]_{\text{shoot or leaf}}/[Cd]_{\text{root}}$) were calculated. The Cd shoot concentrations were used for *P. miliaceum* and *L. spartum*, while the Cd leaf concentrations were used for the rest of species.

2.5 Statistical Analysis

The fitting of the data to a normal distribution for all properties measured was checked with the Kolmogorov–Smirnov test. Since data followed no normal distribution despite log or root transformations, nonparametric statistic tests were used. The soil data were submitted to the Kruskal–Wallis test to assess the differences found before and after application of amendments and between depths. The Cd concentrations in the plant samples were submitted to the Kruskal–Wallis test to assess the differences found among plant tissues and plant species. The separation of means was made according to the Mann–Whitney U test at $P < 0.05$. Relationships between properties were studied using Spearman correlations. These analyses were performed with the software IBM SPSS Statistics 20.

3 Results and Discussion

3.1 Soil Properties and Cd Mobility

Soil properties and Cd mobility before and 24 months after the remediation works are shown in Table 3. Soil pH was increased after the application of amendments. In surface, pH significantly increased from 5.80 before remediation to 7.71 after 24 months of the first addition of amendments, while in subsurface it increased from 5.85 to 7.69. These increments in pH are due to the presence of carbonates in soils,

Table 3 Soil physicochemical and biochemical properties and available fractions of Cd in the amended soil before the remediation strategy and 24 months after the initiation of remediation

Soil property[a]	Before reclamation		24 months after reclamation		
	Surface	Subsurface	Surface	Subsurface	χ^{2b}
pH	5.80 (5.04–6.33) a	5.85 (5.18–6.24) b	7.71 (7.60–7.85) c	7.69 (7.53–8.06) d	26**
EC (mS cm^{-1})	2.72 (2.49–2.95) a	2.74 (2.52–2.99) a	2.60 (2.48–2.78) b	2.74 (2.54–2.90) a	5**
TOC (g kg^{-1})	7.54 (2–19.14) a	7.05 (1.58–14.70) b	8.97 (6.34–11.98) c	8.54 (5.01–11.20) c	2**
Nt (g kg^{-1})	0.32 (0.17–0.63) a	0.29 (0.19–0.41) b	1.28 (1.05–1.69) c	1.13 (1.03–1.21) d	27**
CEC ($cmol_+ kg^{-1}$)	5.13 (3.25–8.64) a	5.46 (2.8–10.40) a	13.50 (8.32–18.05) b	13.63 (8.59–15.98) b	24**
P (mg kg^{-1})	0.012 (0.002–0.032) a	0.033 (0.002–0.091) b	7.830 (5.468–9.507) c	5.059 (3.060–6.636) d	29**
β-glucosidase (μg PNP g^{-1} h^{-1})	10.45 (3.18–24.61)	7.04 (3.39–10.89)	15.38 (3.99–15.38)	11.22 (3.37–23.73)	6 ns
Urease (μg NH_4^+ g^{-1} h^{-1})	0.25 (0–1.69) a	0.57 (0–2.47) b	5.14 (3.61–6.89) c	3.29 (0.01–6.55) d	24**
Arylsulfatase (μg PNP g^{-1} h^{-1})	3.25 (0.16–7.33)	2.42 (0.22–6.30)	2.49 (0–6.47)	1.09 (0–2.99)	6 ns
DTPA–extractable Cd (mg kg^{-1})	16.55 (11.12–27.23) a	18.24 (7.76–30.86) b	2.90 (0.91–4.93) c	2.15 (1.40–3.07) d	26**
Exchangeable Cd (mg kg^{-1})	2.55 (0.08–7.90) a	2.78 (0.05–10.37) b	0.08 (0.02–0.18) c	0.11 (0.02–0.28) d	14*

Values are mean and range ($n=4$)

[a]*EC* electrical conductivity, *TOC* total organic carbon, *Nt* total nitrogen, *CEC* cation exchange capacity, *P* available phosphorus, *PNP* *p*-nitrophenol

[b]Significant at $*P<0.01$ and $**P<0.001$; ns: not significant ($P>0.05$). Different letters indicate significant differences ($P<0.05$) among means

which reacted with acidity turning soil close to neutrality. The EC decreased in surface samples with the application of amendments (Table 3), due to the application of the marble waste.

This was likely due to the reaction of Ca^{2+} with sulfates forming mineral precipitates or by precipitation of ions by increments in soil pH [33, 34]. TOC increased in surface from 7.54 g kg^{-1} before remediation works to 8.97 g kg^{-1} after 24 months, while in subsurface it increased from 7.05 to 8.54 g kg^{-1}. This fact is due to the application of the organic amendments and to the development of vegetation. However, the content of TOC after remediation works was lower than expected taking into account the organic amendment rate applied. Thus, most of the organic C applied with the amendments was mineralized. This has been previously reported, since the normal trend under semiarid conditions is soil organic matter mineralization [14, 35]. However, the increased content of TOC after 24 months of reclamation works with regard to the initial state may suggest that the development of a vegetation cover is maintaining TOC levels by root exudates and litter accumulation [36].

In surface, Nt significantly increased from 0.32 to 1.28 g kg^{-1} after 24 months of remediation works. In subsurface, Nt increased from 0.29 to 1.13 g kg^{-1}. These results are very positive indicating a significant increment in a macronutrient like N, essential for the development of microbial communities and vegetation. Thus, soil fertility was increased which was promoting the growth of the introduced species. CEC showed no significant differences between surface and subsurface samples, with significant increments after 24 months (Table 3). CEC increased from 5.3 to 13.6 $cmol_+ kg^{-1}$. The application of the amendments positively contributed to the improvement in this indicator of soil fertility, owing to increments in organic compounds with higher exchangeable positions. Available P increased in surface from 0.012 mg kg^{-1} before remediation works to 7.830 mg kg^{-1} after 24 months of remediation, while in subsurface it increased from 0.033 to 5.059 mg kg^{-1}. Thus, the application of the organic amendments positively contributed to the improvement of soil fertility, since pig slurry and manure are sources of essential nutrients for vegetation like N and P [13, 14, 35].

No significant changes were observed in β-glucosidase and arylsulfatase activities with the remediation works. However, urease activity significantly increased after 24 months, from 0.25 to 5.14 μg PNP g^{-1} h^{-1} in surface and from 0.57 to 3.29 μg PNP g^{-1} h^{-1} in subsurface. Thus, the activation of urease activity may be indicating a need of microorganisms and vegetation for inorganic N. Plants and microorganisms may be releasing this enzyme to mineralize organic N and make it available for their nutrition. The absence of increments in enzyme activities after addition of amendments to mine soils is not common, since most authors have reported increased activities after addition of different organic and inorganic amendments [6, 13, 17, 18, 37]. The lack of differences in β-glucosidase may indicate exhaustion of labile organic compounds in soil, since this enzyme is implied in the last steps of degradation of polysaccharides. Arylsulfatase releases sulfate by mineralization of organic S. The absence of shifts in this activity with the remediation carried out may indicate that the sulfate contents in soil are high, and plants and biota have it quite available. The mine residues contain high quantities of metallic sulfides (pyrite, blenda, sphalerite, etc), which rapidly oxidize in oxidizing conditions to release sulfates.

All enzyme activities were negatively correlated with the exchangeable and DTPA-extracted fractions of Cd ($P<0.01$). This may suggest a direct inhibition of the enzymes by the available fractions of Cd (and other possible metals present). Enzyme activities are inhibited by metals which may form a complex with the substrate, compete with metallic enzyme cofactors, combine with the active site of enzymes, or react with the enzyme–substrate complex [38]. Thus, the presence of Cd may be also negatively affecting enzymes in the amended soil. The bioavailability of Cd decreased 24 months after the initiation of the reclamation strategy (Table 3). The DTPA-extractable Cd decreased ~85 %, while the exchangeable Cd decreased ~96 %. The decrease in the Cd bioavailable fraction is mainly due to a direct effect of pH, which favors the processes of adsorption, precipitation, and co-precipitation with oxyhydroxides; the formation of chelates; the formation of metallic carbonates (provided by the marble waste); and the formation of metallic phosphates (provided by the pig slurry and manure) [6, 14, 34, 39]. Zornoza et al. [14] reported that organic complexation and precipitation as phosphate was very effective for Cd immobilization. Additionally, vegetation can also contribute to Cd immobilization by the creation of an appropriate rhizospheric environment. In the rhizosphere, processes such as precipitation and stabilization of heavy metals are very intense [10]. Kabas et al. [4] observed that the decrease in the available fraction of Cd, Cu, and Pb in a tailing pond was higher when vegetation was present in comparison with addition of amendments with absence of vegetation. Thus, those results highlight the need to create a vegetation cover to ensure soil stabilization of heavy metals (phytostabilization).

3.2 Cd Content and Distribution in Plants

Concentrations of Cd in plant tissues after 24 months of experiment are shown in Table 4. The concentration of Cd was relatively low (<3 mg kg^{-1}) and below toxicity limits for all the studied species except for *A. halimus*. Variability of data was high, but *A. halimus* accumulated, as an average, 18.8 mg kg^{-1} of Cd in the stems and 32.64 mg kg^{-1} of Cd in the leaves, without symptoms of toxicity or disease. No correlation between the exchangeable and extractable fractions of Cd in soil and the Cd concentrations in the different plant tissues was found. In addition, no significant correlation was observed between the content of Cd in the different plant tissues and any physicochemical or biochemical soil property. The BF was <1 in all species except for *A. halimus* that showed a BF = 11.6 (Fig. 1). The TF was ≤1 for all species except for *A. halimus* (TF = 17.1), *L. cossonianum* (TF = 5.8), and *L. spartum* (TF = 1.6) (Fig. 1). Thus, all species except for *A. halimus* tended to exclude accumulation of Cd from soil to the aerial tissues. In addition, *P. miliaceum*, *H. stoechas*, and *D. viscosa* showed Cd exclusion from roots to aerial parts, while *A. halimus*, *L. cossonianum*, and *L. spartum* in a lesser extend accumulated Cd in the shoots.

P. miliaceum, *H. stoechas*, *D. viscosa*, and even *L. spartum* must have strategies for protecting themselves against absorption of toxic elements and for restricting their transport within the plant. In fact, this is supported by the absence of correlations between plant Cd and soil Cd concentrations, something previously reported in literature (e.g., [19, 40]). These strategies include the subcellular compartmentalization of the metal (normally in vacuoles) and the sequestration of the metal by organic

Table 4 Cadmium concentration in roots and aerial parts of the studied species after 24 months after the initiation of remediation

Species	Plant part	Cd (mg kg^{-1})
A. halimus	Root	6.14 (1.99–12.73) b
	Stem	18.88 (1.93–48.19) bc
	Leaf	32.64 (4.43–82.39) c
H. stoechas	Root	1.85 (1.72–2.05) ab
	Stem	1.95 (1.79–2.04) ab
	Leaf	2.09 (1.07–2.86) ab
D. viscosa	Root	2.53 (0.69–4.37) ab
	Stem	1.58 (0.17–2.99) a
	Leaf	1.29 (0.40–2.19) a
L. cossonianum	Root	0.59 (0.44–0.73) a
	Stem	0.35 (0.19–0.51) a
	Leaf	3.00 (2.28–3.72) b
P. miliaceum	Root	2.68 (1.26–3.64) b
	Shoot	0.47 (0.25–0.64) a
L. spartum	Root	1.37 (0.81–2.00) ab
	Shoot	1.99 (1.89–2.12) ab
χ^2		25 ($P<0.001$)
Plant leaf toxicity limits[a]		5–30
Domestic animal toxicity limits[a]		10

Values are mean and range ($n=4$)
Different letters indicate significant differences ($P<0.05$) among means
[a]Based on data provided by Mendez and Maier [10]

compounds, like phytochelatins, concentrating metal in roots [41]. Thus, the tested species (except for *A. halimus* and *L. cossonianum*) may be potential candidates for the objectives of Cd phytostabilization, which are drought and pollution resistant, can be grown in deficient soils, and restrict the transport of Cd within the plant. In addition, Cd concentration in aerial tissues in all these plants are below toxicity limits for domestic animals, and so the use of these species would not significantly increase their transfer from the soil to animals via the food chain. *P. miliaceum* and *D. viscosa* were the best candidates for phytostabilization, since they accumulated higher quantities of Cd in roots than in shoots (TF=0.18 and 0.54 for *P. miliaceum* and *D. viscosa*, respectively) and showed an efficient restriction of Cd from soil (BF=0.11 and 0.44 for *P. miliaceum* and *D. viscosa*, respectively). In addition, these species are the ones with the highest biomass and are able to colonize the surface of the tailings ponds (Table 2). In this sense, *P. miliaceum* showed a density of 377 plants ha^{-1}, the highest value after *A. halimus*, which enhances its potential for soil phytostabilization since it can cover a vast extension of polluted soils to stabilize Cd. These results confirm previous findings by Zornoza et al. [19], who concluded that *D. viscosa* and especially *P. miliaceum* had potential for the phytostabilization of heavy metals (Cd, Cu, Pb, and Zn) in a stream affected by mine residues.

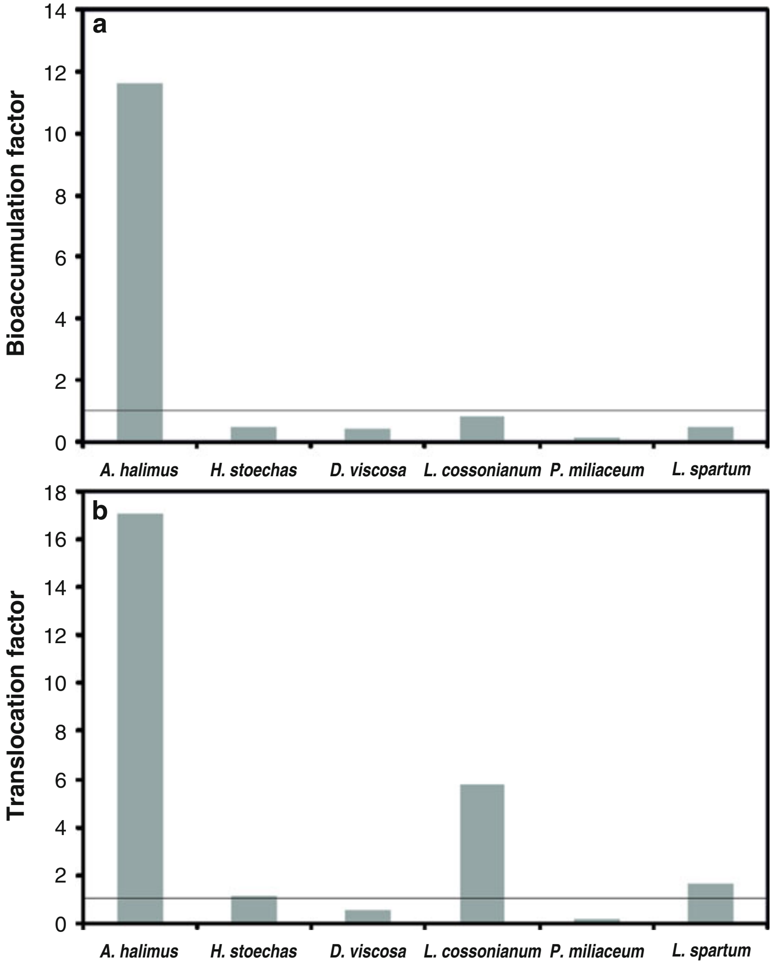

Fig. 1 Bioaccumulation factor (BF = $[Cd]_{shoot\ or\ leaf}$/$[extractable\ Cd]_{soil}$) (**a**) and translocation factor (TF = $[Cd]_{shoot\ or\ leaf}$/$[Cd]_{root}$) and (**b**) in the different plant species studied. *Horizontal line* indicates BF/TF = 1

Contrarily, *A. halimus* showed important translocation and bioaccumulation of Cd in the aerial tissues. Cd is not considered an essential element, being toxic to plants [1]; however, Cd is thought to enter root cells by means of the same uptake processes that move essential micronutrient metal ions [42]. Walker et al. [43] reviewed the biology and uses of *A. halimus* and reported high Cd tolerance by means of precipitation with oxalate; synthesis of the endogenous antioxidants glutathione, ascorbic acid, and α-tocopherol; and stimulation of glutathione reductase. Although variability of data is high, one plant specimen was able to accumulate 82.39 mg kg^{-1} of Cd in the leaves. This level is so high that is almost reaching the threshold proposed for hyperaccumulation of Cd (100 mg kg^{-1}) [44]. In addition, this species was the one with the highest biomass and the highest density in the study area (Table 2). So, although *A. halimus* should not be considered for phytostabilization of Cd, it has the potential to be used for phytoextraction, since it has fast growth, high biomass, easy harvesting, and can naturally accumulate heavy metals in the aboveground tissues without developing toxicity symptoms, with high BF [44–46]. Previous studies also observed that *A. halimus* accumulated higher quantities of heavy metals in shoots than in roots [4, 47], but the Cd concentrations in the plant tissues were as average lower than those reported in this study. Pérez-Esteban et al. [48] observed in a pot experiment that plants of *A. halimus* managed to reduce heavy metal concentration in soil, but concluded that its phytoextraction capacity was insufficient to remediate contaminated soils in the short-to-medium term. Contradictory results could be due to different soil characteristics such as nutrient levels, organic matter, Cd speciation and pH, and different genotypes. Plant genotype is considered as the most important factor affecting heavy metal uptake by plants [49]. Several studies have shown the presence of genotypic differences in the heavy metal uptake and distribution within species [50–52]. Thus, the efficiency for phytoextraction could be increased by selection of suitable agronomic practices and finding and breeding the most accumulator genotypes.

4 Conclusions

The remediation strategy developed on a tailing pond, based on phytoremediation aided with pig slurry, pig manure, and marble waste was effective to reduce the bioavailability of Cd and increase soil fertility to promote the development of vegetation. However, soil organic matter and biochemical activity are still low. Among the plant species studied, *P. miliaceum*, *H. stoechas,* and *D. viscosa* showed Cd exclusion from roots to aerial parts, while *A. halimus*, *L. cossonianum*, and *L. spartum* in a lesser extend accumulated Cd in the shoots. Thus, the tested species (except for *A. halimus* and *L. cossonianum*) may be potential candidates for the objectives of Cd phytostabilization, *P. miliaceum* being the best candidate owing to the higher concentration of Cd in roots, higher biomass, and rapid colonization of the soil. Oppositely, *A. halimus* should not be considered for phytostabilization of Cd, but has the potential to be used for phytoextraction, since it has fast growth, high biomass, easy harvesting, and have high translocation and bioaccumulation factors without developing toxicity symptoms.

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