The Handbook of Environmental Chemistry 44
Series Editors: Damià Barceló · Andrey G. Kostianoy

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Wastewater Reuse and Current Challenges

Springer

The Handbook of Environmental Chemistry

Founded by Otto Hutzinger

Volume 44

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Wastewater Reuse and Current Challenges

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ISSN 1867-979X ISSN 1616-864X (electronic)
The Handbook of Environmental Chemistry
ISBN 978-3-319-23891-3 ISBN 978-3-319-23892-0 (eBook)
DOI 10.1007/978-3-319-23892-0

Library of Congress Control Number: 2016930825

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

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Aims and Scope

Since 1980, *The Handbook of Environmental Chemistry* has provided sound and solid knowledge about environmental topics from a chemical perspective. Presenting a wide spectrum of viewpoints and approaches, the series now covers topics such as local and global changes of natural environment and climate; anthropogenic impact on the environment; water, air and soil pollution; remediation and waste characterization; environmental contaminants; biogeochemistry; geoecology; chemical reactions and processes; chemical and biological transformations as well as physical transport of chemicals in the environment; or environmental modeling. A particular focus of the series lies on methodological advances in environmental analytical chemistry.

Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last three decades, as reflected in the more than 70 volumes of *The Handbook of Environmental Chemistry,* there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry,* the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of

"pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Editors-in-Chief

Volume Preface

Water is basic for all life and also for several physicochemical processes that directly and indirectly support life and shape our planet. With an increasing population as well as with climate change, there will be an increasing shortage of water in different qualities and for different purposes such as for drinking water and for irrigation. Access to clean and safe drinking water is a human right. However, it is not yet accessible to all people. Growing humankind needs more and more resources. This holds for water that is indispensable as drinking water as well as for food production – with or without irrigation. Water shortage by volume is the case particularly in arid regions; however, such a shortage is also more and more observable in the so-called water-rich regions. Additionally, there is a shortage of clean water, i.e., water of sufficiently high quality – not just for drinking water purposes but also for high-tech industrial production – and sufficient quality is also indispensable for safe food.

Therefore, increasing reuse of reclaimed water in different qualities is necessary. There are, however, several challenges to implement this on a large scale. Depending on its further use, water needs to comply with different quality levels needed to be met for its usage, respectively. Reclaimed water for irrigation and agriculture needs to meet certain standards as water contaminants can be taken up by plants/crops and/or accumulate in non-target organisms. Current challenges include the removal of microbial contaminants such as bacteria (including antibiotic resistant bacteria), viruses, protozoa, and other microorganisms, mobile-resistant elements, and also organic contaminants of emerging concern and other organic and inorganic constituents. As for the chemical compounds, it is anticipated that their usage and introduction into the aquatic environment via various routes will increase in the future, as will do their production and application in various products and processes. This holds true for the amount but also for the number of compounds. As for (micro)organisms, the effect of climate change and increase of human population on them is expected to be significant.

One of the most important challenges for water reuse is therefore enabling wastewater reuse in sufficient quality and quantity in the most sustainable manner.

This book address the most important related current challenges including analytical chemical methodologies for the identification and quantification of contaminants of emerging concern and also of their transformation products, the various bioassays applied for the assessment of the biological potency of treated wastewater, and the bioavailability and uptake of organic contaminants during crop irrigation. It also addresses emerging issues like antibiotic resistance, both in wastewater and in soil in downstream environments. It presents the current situation in various countries that suffer from water scarcity and various other important issues like water recovery systems. The potential for other reuse practices like in the paper industry and in landfill management is also presented.

The editors would like to acknowledge all the scientists involved in the development of the book and for creating the opportunity for fruitful discussions and exchange of ideas and knowledge and their patience with the editors. They would also like to thank warmly their co-workers of their research groups for their support in the daily working routine for giving them time to edit a book in such a vital field for the sustainable development of the urban environments and societies. Special thanks go to Dr. Lida Ioannou and Mr. Toumazis Toumazi (Nireas-International Water Research Center, University of Cyprus), Dr. Oliver Olsson (Institute of Sustainable and Environmental Chemistry, Leuphana University Lüneburg), and Ms. Xiaodi Duan (University of Cincinnati) for their significant contribution and administrative work and support during the development of the book.

The editors would like to express their gratitude to Dr. Andrea Schlitzberger and their team at Springer Publisher who supported in such a wonderful and constructive way the idea to realize this book.

Nicosia, Cyprus — Despo Fatta-Kassinos
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November 2015

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Scope of the Book *Wastewater Reuse and Current Challenges*

Klaus Kümmerer, Dionysios D. Dionysiou, and Despo Fatta-Kassinos

Abstract This volume offers an overview of current challenges related to the wastewater reuse practice, including analytical methodologies, bioassays, uptake of organic contaminants during crop irrigation, and antibiotic resistance-related issues. It also offers information on various wastewater reuse cases under various scenarios.

Keywords Antibiotic resistance, Bioassays, Chemical analysis, Uptake, Wastewater reuse practices

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 1–6, DOI 10.1007/698_2015_448,

Water and wastewater reuse is a long-established practice used for irrigation especially in arid countries. With the advent of modern industrial societies and modern agriculture, the advent of modern chemical and microbiological analytical methods and instruments, and increased knowledge related to health and well-being brought the quality of water to be reused within recent decades more and more into focus. The need for reuse of water is increasingly fuelled by the climate change and increasing population. This in turn has increased the interest on safeguarding the quality of water for reuse – not only in arid countries. The presence of "new" contaminants in treated wastewater has led to an increased concern about the potential direct and indirect effects to the environment and possible implications to human health.

In response to the increasing problem of water shortage, treated urban wastewater is currently widely reused and considered to be a reliable alternative water source. Regions inhabited by more than 40% of the world's population already are in a situation where water demand exceeds supply. The shortage of water and the increasing need for food due to the expanding world population and for irrigation water, both in respect to sufficient quality and quantity (see also Sustainable Development Goal No. 6 of the United Nations), render reuse an indispensable practice. Nowadays, closing the urban water cycles is of high priority on the policy agendas of many countries around the world.

Although reuse is accompanied by a number of benefits, and major advances have been made with respect to producing safe treated effluents for reuse (e.g., successful removal of nutrients and metals, strongly reducing chemical oxygen demand), several important questions are still unanswered, and others were recognized with the advent of modern chemical and microbiological knowledge, methods, and apparatuses. It has been learned that still barriers exist regarding the safe and sustainable reuse practices. Available and applied technologies fail to completely remove many of the contaminants of emerging concern, while no consolidated information exists concerning the efficacy of the treatment technologies to remove bacteria resistant against antibiotics and the related genetic material. The contamination of the environment, the food chain, drinking water, etc., with antibiotic-resistant bacteria and resistance genes is presently considered as a serious public health problem. For this reason, the World Health Organization (WHO) identified the development of antibiotic resistance as one of the major global threats to humankind and recommends intensive monitoring for the identification and surveillance of critical hot spots such as wastewater treatment plants, aiming at reducing its propagation.

Other current challenges include the analytical methods to identify and quantify such contaminants in complex matrices like wastewater; the development of bioassays that can be applied to assess the effects of such contaminants and of treated wastewater as complimentary or alternative methods to the chemical methodologies, in order to evaluate the potential of the treated flows to cause harm to the human and environmental health; and the potential crop uptake during wastewater reuse for irrigation of such chemical and biological microcontaminants.

The various chapters of this book address these important issues along with other related issues and present specific examples [1].

The second chapter of the book aims at giving an overview of the analytical methodologies and techniques currently applied while providing a discussion on their requirements, potential, and limitations [2]. The presence of organic microcontaminants in wastewater represents a significant challenge to wastewater reclamation. Problems associated to the repeated release of treated wastewater in the environment for reuse applications, such as infiltration into the underground including pollution of groundwater or accumulation in soil and plants, are still scarcely investigated. Consequently, comprehensive and high-throughput analytical methods have to be developed and validated to provide a comprehensive evaluation of these microcontaminants in water, soils, and crops.

The development and application of bioassays able to identify and quantify the biological potency of treated wastewater are an ongoing research effort, especially when taking into consideration that a plethora of biological contaminants exist and interact in the complex wastewater matrix and also with other environmental parameters when in nature. The third chapter of the book summarizes the available literature regarding the sensitivity of currently applied bioassays for assessing biological effects of treated wastewater and their correlation with chemical analysis [3].

Organic microcontaminants occurring in reclaimed water can be introduced into soil, where they can interact with inorganic constituents, organic matter such as humic compounds or anthropogenic organic matter depending on their physicochemical properties. In the soil water, a fraction of them can be more or less completely biodegraded or mineralized, while another fraction including products of incomplete mineralization can be taken up by plants and translocated further. Once incorporated in the plant, a fraction can be metabolized to again new compounds. These processes are tackled by the fourth chapter of the book [4].

Wastewater reuse for irrigation, apart from the introduction of some biological and chemical hazardous agents in the environment, is a process that can potentially cause the disturbance of the indigenous soil microbial communities. The consequences of these disturbances, e.g., for soil fertility or human health, are still poorly understood. These alterations, which involve a high complexity, may have impacts on soil quality and productivity. In addition, possible health risks may arise, in particular, through the direct or indirect contamination of the food chain with micropollutants, pathogens, or antibiotic resistance determinants. The fifth chapter summarizes the physicochemical and microbiological alterations in soil that can result due to the irrigation with treated wastewater [5].

The sixth chapter summarizes the current understanding of antibiotic resistance in wastewater treatment plants and downstream environments, presents knowledge gaps that need to be bridged in order to better understand the potential ramifications of this phenomenon [6], overviews the effect of disinfection treatments on antibiotic resistance elements, and finally discusses policy guidelines that should be implemented in the future to reduce the risks of antibiotic resistance from wastewater treatment plants.

The incomplete elimination of contaminants of emerging concern during conventional wastewater treatment constitutes a major issue and possible limitation for water reuse, because these compounds can undergo transformation in the environment or during disinfection and other treatment if reclaimed water is used for drinking water production. Different emerging contaminants, e.g., perfluorinated compounds, pharmaceuticals, antibacterials, plasticizers, preservatives, flame retardants, dyes, and the products of their transformation and incomplete mineralization (transformation products) which are in some cases more toxic than original compounds, have been occasionally found in finished drinking waters. The seventh chapter reviews the contaminants detected in drinking water and the disinfection by-products generated by many of them present in the aquatic environment [7]. Moreover, the potential toxicologic effects that these pollutants and their transformation products pose for human health are also reviewed.

The growing need for better water management leads concurrently to the need for development of various process integration tools for resource conservation. In the past three decades, process integration techniques such as *pinch analysis* and *mathematical optimization* have been developed to address various resource conservation issues, ranging from energy, materials, and more specifically water recovery. The eighth chapter presents one of the major process integration tools [8], known as *water pinch analysis*, for the design of water recovery system. A water recovery case study of a steel plant is used for illustration.

Based on research findings during the last decades, the presence of micropollutants in reclaimed water has gained interest not only in developed countries but also elsewhere. In North African and other arid countries, in view of the prevailing quality of reclaimed water and its current usage for growing crops, the occurrence of such contaminants has recently raised concern with an increasing number of research works and publications. However, it remains challenging to identify, quantify, and prioritize the most relevant to be regulated. The ninth chapter aims at shedding light on the usage of reclaimed water for irrigation in Algeria, Egypt, Libya, Morocco, and Tunisia while pinpointing the potential sources of contaminants of emerging concern in wastewaters [9].

Various industrial sectors are water intensive. Pulp and paper industry is one of them. Sustainable water management has been achieved by following the principle of water fit for use, which has mainly been developed through the optimization of water circuits, the cascade use of water, etc. In fact, this sector is nowadays regarded as a reference for water reuse. Chapter ten discusses the various opportunities that exist in closing the water cycle in this type of industry [10].

Another important example of wastewater reuse is presented in the eleventh chapter of the book [11]. It refers to the possibility of reusing leachate substances for agronomical purposes, which might be of interest, especially in arid areas when used in addition to the leachate water content. The study presents a simple procedure for the revegetation of the walls of closed landfills, reusing the leachate as a fertigant.

An outlook that provides the reader with information on the potential strategies that could be applied in order to tackle the problems related with the presence of

contaminants of emerging concern and wastewater can be found at the end of the companion volume *Advanced Treatment Technologies for Urban Wastewater Reuse* [12].

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New Challenges for the Analytical Evaluation of Reclaimed Water and Reuse Applications

Ana Agüera and Dimitra Lambropoulou

Abstract Presence of unregulated and not assessed organic microcontaminants in wastewater effluents represents a significant challenge to wastewater reclamation, especially if intended for human consumption or irrigation practices. Problems associated to the repeated release of treated wastewater in the environment for reuse applications, such as infiltration into the underground including pollution of ground water or accumulation in soil and plants, are still scarcely investigated. Consequently, comprehensive and high-throughput analytical methods have to be developed and validated to provide a comprehensive evaluation of these micro-contaminants in water, soils and crops. This chapter aims to give an overview of the analytical strategies currently used in this field, its requirements and limitations.

Keywords Mass spectrometry, Organic microcontaminants, Screening analysis, Transformation products, Wastewater

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 7–48, DOI 10.1007/698_2015_364,

1 Introduction

Reuse of wastewater is nowadays accepted as a strategy that can contribute significantly to an efficient and sustainable water usage [1]. However, the inefficient removal of a large number of organic contaminants of emerging concern (CECs) during wastewater treatments can represent a limitation. Pharmaceuticals, perfluorinated compounds, artificial sweeteners, hormones, disinfection by-products, UV filters, brominated flame retardants, benzotriazoles, naphthenic acids, siloxanes, musk fragrances, etc. are among the long list of CECs reported, to which we must add the transformation products (TPs) generated during water treatment processes or by natural processes [2]. It is nowadays accepted that the removal of these compounds prior to discharge of treated water is essential to avoid damage of water resources. However, increasing reuse practices involve new exposure routes, such as soils and crops. Consequently, the accumulation of contaminants in soils after irrigation practices and the evaluation of the uptake by crop plants are new insights that demand research.

In the last decades, scientific community has made a great effort to provide analytical methods able to accurately determine CECs and their TPs in different environmental matrices, overcoming limitations associated to analytes (high polarity, instability, etc.) and matrix nature (matrix effects). This effort has been possible thanks to significant technological advances in analytical instrumentation. Chromatography–mass spectrometry coupled systems have emerged as the undisputed leaders in this field, due to their large separation and identification capacity for, in principle, an unlimited number of compounds. Multi-stage mass analysers like triple quadrupole (QqQ-MS) or quadrupole linear ion trap (QqLIT-MS) have dramatically enhanced the sensitivity, specificity and quantitative performance of target analyses. Likewise, the increasing availability and application of high-resolution mass spectrometry (HRMS) has contributed in the expansion of the scope of analyses, by implementing wide-scope screening methods for non-target analytes.

HRMS has also provided an excellent platform for identification studies of unknown TPs, although in many cases results are insufficient for a reliable identification and structure allocation.

This chapter intends to provide an overview of the main analytical methodologies currently applied for the evaluation of CECs and their TPs in wastewater, reclaimed water, soils and plants. Analytical methods are discussed considering new developments in sample preparation and determination. Finally, the current contamination status by different groups of organic contaminants in soils and crops is overviewed.

2 Determination of Organic Microcontaminants in Wastewater and Reclaimed Water

Trace analysis of organic contaminants in raw and treated wastewater is usually performed to assess the contaminants present in the effluents and identify the formation of possible TPs. Nonselective extraction/preconcentration steps are usually combined with extensive target analyses, non-target screening methods and/or with analytical strategies for the identification and structure elucidation of unknown compounds, by using different chromatography–mass spectrometry approaches.

2.1 Sample Extraction

Low concentrations of contaminants in wastewater make the sample enrichment a crucial step. However, the comprehensive evaluation of the samples entails great difficulty due to the large number of compounds involved and the differences in their properties. Some examples are included in Table 1.

Traditional liquid–liquid extraction (LLE) has been successfully applied for the extraction of less polar contaminants. However, new developments tend to focus on reducing solvent usage and improving extraction of more polar or ionic compounds.

Solid phase extraction (SPE) is the most comprehensive and widely used technique. To extend its applicability to more hydrophilic compounds, traditionally used sorbents have been replaced by new polymeric materials. Oasis HLB (hydrophilic–lipophilic balance) sorbent, containing lipophilic (divinylbenzene) and hydrophilic (*N*-vinyl-pyrrolidone) groups in its structure, and Strata-X material, based on a polydivinylbenzene resin containing piperidone, have been extensively used [3–6]. Both provide large capacity and high retention of a broad type of compounds. However, the lack of selectivity causes undesirable matrix effects when working with MS.

To improve selectivity and retention for ionic compounds, mixed-mode polymeric sorbents exhibiting both hydrophobic and ion-exchange properties have been developed. These materials base their structure on a polymeric skeleton chemically modified in its surface with strong and weak cationic or anionic functional groups. Commercially available sorbents, such Oasis MAX, Oasis MCX, Strata X-C, Strata

Table 1 Analysis of organic microcontaminants in wastewater

Analytes	Water matrix	Extraction	Separation	Analysis	Reference
Multi-class emerging organic pollutants (53)	Tap, surface and wastewater	SPE Oasis HLB	SymmetryShield RP18 (2.1 × 150 mm, 3.5 µm) in positive mode	LC-(ESI+/−) TQ-MS/MS	[3]
			Luna Phenyl-Hexyl (150 × 2 mm, 3 µm) in negative mode		
Pharmaceuticals (38), metabolites (10), pesticides (6), disinfectants (2)	Wastewater	SPE Oasis HLB	Zorbax SB C18 (250 × 3.0 mm, 5 µm)	LC-(ESI) QqQLIT-MS/MS	[4]
Pharmaceuticals (50)	River water and wastewater	SPE Oasis HLB	Acquity UPLC HSS T3 (100 × 2.1 mm, 1.8 µm)	UPLC-(ESI) TQ-MS/MS	[5]
Pharmaceuticals (18)	Wastewater	SPE Strata X	Gemini C18 (150 × 2 mm, 3 µm) ESI +	LC-(ESI+/−) TQ-MS/MS	[6]
			Synergi MAX-RP (100 × 2 mm, 2.5 µm) ESI−		
Multi-class emerging organic pollutants (pharmaceuticals, personal care products and illicit drugs) (50)	River water and wastewater	SPE Oasis MCX	Acquity UPLC	UPLC-(ESI +/−)TQ-MS/MS	[7]
			BEH C18 column (100 × 1 mm, 1.7 µm)		
Pharmaceuticals (48) and metabolites (6)	Surface water and wastewater	SPE Oasis MCX	BEH C18 column (100 × 1.0 mm, 1.7 µm)	UPLC-(ESI +/−)TQ-MS/MS	[8]
Basic, neutral and acidic pharmaceuticals (15)	Wastewater	SPE in series Oasis MCX and Oasis MAX	Waters Acquity HSS T3 (100 mm × 2.1 mm, dp 1.8 µm)	LC-QTOF-MS	[9]

Amphetamine drugs	Wastewater	MISPE SupelMIP-Amphetamine	Halo C18 (100 × 2.1 mm, 2.7 μm)	LC-(ESI)TQ-MS/MS	[10]
Selected psychoactive pharmaceuticals (7)	River water and wastewater	MISPE SupelMIP-antidepressant	Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm)	UPLC-(ESI+)TQ-MS/MS	[11]
Non-steroidal anti-inflammatory drugs (15)	Wastewater	MISPE Affinilute MIP-NSAIDs	Fused-Core™ Ascentis Express C18 (100 × 4.6 mm, 2.7 μm)	LC-UV LC-(ESI)TQ-MS/MS	[12]
β-blockers (8)	Wastewater	MISPE MIP4SPE™ β-blockers	Purospher Star RP-18 endcapped column (125 × 2.0 mm, 5 μm)	LC-QqLIT MS	[13]
Pharmaceuticals (56), metabolites (10) and corrosion inhibitors (2)	Hospital wastewater	On-line mixed-bed multilayer SPE Isolute ENV+ and Oasis HLB	Atlantis T3	LC-TQ-MS/MS	[14]
Polar organic micropollutants (88)	Ground, surface and wastewater	On-line mixed-bed multilayer SPE Oasis HLB, Strata XAW, Strata XCW and Isolute ENV+	Atlantis T3 (150 × 3.0 mm, 3 μm)	LC-(ESI)TQ-MS/MS	[15]
Cytostatics (13) and metabolites (4)	Groundwater, surface water, and raw and treated wastewater	On-line SPE PLRP-s (crosslinked styrene-divinylbenzene polymer)	Purospher STAR RP-18e (125 × 2 mm, 5 μm)	LC-(ESI) QqQLIT-MS/MS	[16]
Estrogens (estrone, estradiol, estriol and ethinylestradiol) (4), perfluoroalkyl carboxylates (C4–C11) (8), and perfluoroalkyl sulphonates (5)	Wastewater	LVI (900 μL)	ZORBAX Eclipse Plus-C18 (75 × 3.5 mm, 3.5 μm)	LC-(ESI)TQ-MS/MS	[17]
Illicit and legal drugs and urinary indicators (cotinine, caffeine and creatinine)	Wastewater	LVI (1,800 μL)	Atlantis T3 C18 (150 × 4.6 mm, 5 μm	LC-(ESI)TQ-MS/MS	[18]

(continued)

Table 1 (continued)

Analytes	Water matrix	Extraction	Separation	Analysis	Reference
Endogenous androgens (2) and synthetic androgens (9)	Wastewater	LVI (1,800 µL)	ZORBAX Eclipse Plus C18 (150 × 4.6 mm, 3.5 µm)	LC-(ESI)TQ-MS/MS	[19]
Drugs of abuse, along with some of their respective metabolites (22)	Wastewater and surface water	Direct injection (10 µL)	ZORBAX Eclipse XDB C8 (150 × 4.6 mm, 5 µm)	LC-(ESI) QqQLIT-MS/MS	[20]
Micropollutants (76)	Surface water, sea water and wastewater	HS-SPME (PDMS/DVB)	TRB-5MS (60 m × 0.32 mm, 1 µm)	GC-Q-MS	[21]
VOCs (20)	Surface water and wastewater	HS-SPME (CAR/PDMS)	BP624 (30 m × 0.25 mm, 1.4 µm)	GC-TQ-MS	[22]
Anti-inflammatory drugs (6)	Surface water and wastewater	HS-SPME (PDMS)	HP-5MS (30 m × 0.25 mm, 0.25 µm)	GC-Q-MS	[23]
Musk fragrances (9)	Wastewater	HS-SPME (PDMS/DVB)	ZB-50 (30 m × 0.25 mm; 0.25 µm)	GC-IT-MS	[24]
Endocrine-disrupting compounds (14)	Wastewater, solids and sludge	SBSE (PDMS)	DB-5MS (30 m × 0.25 mm; 0.5 µm)	GC-Q-MS	[27]
Insect repellents and synergists (8)	Surface water and wastewater	SBSE (PDMS)	HP-5MS (30 m × 250 µm, 0.25 µm)	GC-Q-MS	[38]
Chlorinated chemicals	River water and wastewater	SBSE (PDMS)	HP-5MS (30 m × 250 µm, 0.25 µm)	GC-TQ-MS	[39]
Pharmaceuticals and personal care products	Wastewater	SBSE (EG Silicone)	Kromasil 100 C18 (150 × 4.6 mm, 5 µm)	LC-(ESI)TQ-MS/MS	[28]

Pharmaceuticals and personal care products (16)	Wastewater	SBSE (poly-PEGMA-co-PETRA)	Kromasil 100 C18 (150 × 4.6 mm, 5 μm)	LC-(ESI)TQ-MS/MS	[29]
Nitro musks	Surface water and wastewater	DLLME	HP-5MS (30 m × 0.25 mm, 0.25 μm)	GC-Q-MS	[30]
Volatile siloxanes (8)	Wastewater	USA-DLLME	DB-624 (60 m × 0.25 mm, 1.40 μm)	GC-Q-MS	[31]

MISPE molecularly imprinted SPE, *SBSE* stir-bar sorptive extraction, *SPME* solid-phase microextraction, *MWCNTs* multi-walled carbon nanotubes, *LVI* large volume injection, *HS-SPME* headspace solid-phase microextraction, *CAR/PDMS* carboxen/polydimethylsiloxane, *PEGMA-co-PETRA* poly(ethylene glycol) methacrylate-co-pentaerythritol triacrylate, *DLLME* dispersive liquid–liquid microextraction, *USA* ultrasound-assisted

X-WA or Bond Elut Plexa PCX, have yielded good extraction for both charged and neutral compounds in wastewater [7, 8].

Although extraction in one single step is the most common approach, serial SPE separation using different sorbents also represent an interesting alternative. Relying on ion-exchange and reversed-phase mechanisms, different groups of compounds can be isolated in separate fractions by the application of different elution conditions. Thus, increasing recoveries for specific compounds and cleaner extracts can be simultaneously obtained. Lavén et al. [9] report simultaneous extraction of 15 basic, neutral and acidic pharmaceuticals in wastewater using mixed-mode cation- and anion-exchange SPE in series.

Another type of selective sorbents is based on molecularly imprinted polymers (MIPs). MIPs are synthetic polymeric materials with specific molecular-recognition properties that can specifically rebind a target molecule. The inherent specificity prevents their application to multiresidue extraction, but the high potential for single group analysis has contributed to their widespread use [10, 11]. As an example, a commercial MIP specific for non-steroidal anti-inflammatory drugs was successfully compared with three common sorbent (Oasis HLB, Oasis MAX and Oasis WAX), proving to be very effective in the reduction of matrix interferences and the selective extraction of 15 acidic pharmaceuticals from effluent wastewater samples [12]. Reduced matrix effects and higher sensitivity was also reported by molecularly imprinted solid phase extraction (MISPE) of 8 beta-blocker drugs, comparing with Oasis HLB [13].

An advantageous alternative to classical SPE, in terms of labour and time-consuming are on-line SPE methods. They usually involve a two-step procedure including automated sample loading in an extraction cartridge and subsequent elution directly onto the analytical column. This procedure provides similar or better detection limits than off-line methods using smaller sample and organic solvents volumes, in a shorter analysis time, with minimal interferences and good performance, largely due to easier handling and higher automation [14]. A recent application of online SPE-LC–MS/MS has been reported by Huntscha et al. [15] for the simultaneous enrichment and analysis of 88 neutral, cationic and anionic microcontaminants in wastewater. In this study a single mixed-bed multilayer cartridge was used, containing four different extraction materials: Oasis HLB, Strata XAW, Strata XCW and Isolute ENV+ in order to cover the different physical–chemical properties of the analytes. The majority of compounds was quantified with high precision and relative recoveries between 80% and 120%, using a sample volume of only 20 mL. The effort for manual sample handling was limited to filtration, reducing the whole analysis time to only 36 min. Other on-line SPE configurations use robotic systems working in parallel mode [32]. This means that one sample is loaded in one cartridge while another one is eluted into the HPLC system [16]. These sample preparation units use single-use cartridges, avoiding problems associated to the reusability of the pre-columns, such as changes in selectivity and capacity, or cross-contamination.

In contrast to SPE, large volume injection methods combined with liquid chromatography–mass spectrometry (LVI-LC-MS) are rapidly gaining acceptance,

because of their simplicity and good performance [33]. The method basically consists of injecting up to a few millilitres of a filtrated or centrifuged sample directly into a chromatographic column. This method presents clear advantages over SPE: (1) it reduces material and solvent consumption; (2) it increases sample throughput; and (3) it eliminates analytes losses associated with the extraction procedures. Despite its apparent simplicity, LVI also requires adequate optimization of the operating conditions to avoid effects related with overloading of the analytical column (poor peak shapes), lack of retention of more polar analytes or matrix effects associated to the absence of pre-treatment. Although the application of LVI-based methods to complex matrices such as wastewater is still limited, recent studies have demonstrated to produce analytical signals of similar quality to SPE-based methods [17–19].

To overcome limitations of LVI and to take advantage of the increasing mass spectrometers' sensitivity, direct injections of smaller volumes have been assayed. An example has been reported by Martinez Bueno et al. [20] for the simultaneous identification/quantification of 22 drugs of abuse and their major metabolites, in sewage and river water. The absence of pre-concentration and the use of 10 μL injection volumes resulted in a reduction of matrix effects, with LODs ranging from 1 to 700 ng/L in wastewater.

Another group of extraction techniques includes sorptive extraction methods, which are based on a partitioning equilibrium of analytes between the aqueous sample and a solid sorbent supported in different devices. They mainly include solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE) [34]. Both are based on the same principles and their merits yield on their simplicity of operation, solvent-free nature of the process, possibility of full automation and easy coupling with gas chromatography (GC).

In SPME a fine fused silica fibre coated with a polymeric stationary phase is used to extract and concentrate analytes directly from a sample. The choice of commercial fibre coatings is limited to poly(dimethylsiloxane) (PDMS), divinylbenzene (DVB), polyacrylate (PA), Carboxen (CAR) and poly(ethylene glycol) (PEG), while combinations of polar/nonpolar sorbents such as PDMS/DVB, PDMS/CAR, or CW/DVB have been designed for extracting more polar compounds [21, 22, 35]. However, increasing the polarity of the sorbent also increases the affinity for the matrix, leading eventually to the leaching of the analytes. Another choice to expand the applicability of SPME-GC is to decrease the polarity of analytes previously or simultaneously to the microextraction process by in situ [23] or on-fibre derivatization [36].

Although SPME is a widespread technique, most of the studies are devoted to natural water samples and only a little percentage of them deals with complex wastewater samples. Applications are frequently focused to determination of specific groups of compounds [37]. Headspace mode coupled to GC is the preferred configuration to minimize matrix interferences. Musk fragrances [24], benzotriazole UV stabilizers [25], nonylphenol ethoxylates [26], anti-inflammatory drugs [23] and parabens [36] are among the groups of compounds analysed.

SBSE is expected to provide higher sample capacity and extraction efficiency than SPME derived from the larger volumes of extraction phase contained in the SBSE device, consisting of a magnetic stir bar covered with a polymeric coating. SBSE can be used in combination with thermal desorption and GC analysis or, in a more simple and versatile way, by liquid desorption using a small volume of an organic solvent, eliminating the need for specific devices and permitting GC or LC analysis [27, 38, 39]. Until recently, the only commercially available coating was based on PDMS, thus limiting the application of the technique to the extraction of apolar or moderately polar analytes (generally with log $K_{o/w} > 3$). Very recently, novel polar coatings like poly(ethyleneglycol) (PEG)-modified silicone (EG Silicone Twister) and polyacrylate (PA) with a proportion of PEG (Acrylate Twister) have been marketed and applied to the analysis of pharmaceuticals and personal-care products (PPCPs) in wastewaters [28]. However, commercial coatings are still limited in terms of the more polar analytes. Novel approaches applied on the development of in-house coatings such as sol–gel technology, the synthesis of monolithic materials and polyurethane foams (PUFs) have yield promising results in the analysis of CECs in wastewater [29, 40, 41], but new polar monomers and novel formats need to be explored to improve extraction of polar compounds from complex matrices [42].

Finally, another group of miniaturized methodologies, included under the term liquid-phase microextraction (LPME) has emerged. They are based on the use of negligible volumes of a water-immiscible solvent (μL or sub-μL) and an aqueous phase containing the analytes of interest [43]. Simplicity of operation, speed, low cost and high enrichment factors are the main strengths of these techniques. Developments have led to different approaches of LPME, namely, single-drop microextraction (SDME), hollow-fibre LPME (HF-LPME), dispersive liquid–liquid microextraction (DLLME) and solidified floating organic drop microextraction (SFODME). Some applications of these techniques have been reported in the analysis of CECs in wastewater, being HF-LPME and DLLME the approaches most widely used [30, 31].

2.2 Chromatographic Separation and Determination

Liquid and gas chromatography coupled to mass spectrometry are by far the analytical techniques most often used for the analysis of wastewater and reclaimed water. In most cases the choice between GC and LC is based on the physicochemical properties of the selected analytes. LC is the preferred choice for polar and less volatile compounds (e.g. pharmaceuticals, transformation products), while GC allows the determination of less polar and volatile analytes (e.g. fragrances, UV filters, fire retardants and antioxidants). The definition of the objective of the analysis is crucial for the choice of the most appropriate instrumentation and/or analysis strategy. Three approaches can be considered: (1) analysis of target

compounds, (2) comprehensive analysis of target and non-target analytes by screening methods and (3) identification of unknown TPs.

2.2.1 Analysis of Target Compounds

The analysis of CECs in wastewater often focuses on quantitative assessment of a selected group of compounds [44, 45]. In the last few years, there is a trend to expand the number of compounds included in the methods, with the aim to provide a more comprehensive assessment.

GC approaches typically use quadrupole, ion trap or triple quadrupole analysers working in selective ion monitoring (SIM) or tandem-mass-spectrometry (MS/MS) modes to enhance sensitivity and selectivity. These methods rely on only a few ions and are not designed to find compounds unless they are on the target list. Full-scan analyses improve confirmation and allow analysis of non-target compounds but the methods are less sensitive and prone to matrix interferences.

Recent progress in instrumentation has increased the use of time-of-flight (TOF) mass analysers coupled to GC [142]. The main advantage of TOF-MS relies on the full spectrum acquisition, with better sensitivity than conventional instruments. The high acquisition speed (100–500 spectra/s) provided by some instruments, make them suitable for coupling to ultra-fast GC or comprehensive two-dimensional gas chromatography (GC × GC). GC × GC-TOF-MS has emerged as a good alternative to analyse complex samples because it offers increased peak capacity, improved resolution and enhanced mass sensitivity. Sample preparation procedures can be minimized or eliminated due to the superior separating power, although at the expense of a more frequent maintenance and cleaning. In addition, the ability of GC × GC to produce structured two-dimensional (2-D) chromatograms or "fingerprint" of a sample opens up the opportunity for sample comparison protocols. These advantages make GC × GC-TOF-MS a very interesting tool in the evaluation of wastewater treatments. However, its application to this type of studies is still limited. Examples recently published include the application of a sensitive multiresidue method to assess the removal of a group of 55 contaminants (PCPs, PAHs and pesticides) in wastewater using ozonation, UV and visible light irradiation and TiO_2 photocatalysis [143]. Due to the enhanced separation capacity, GC × GC-EI-TOF-MS has been also successfully applied to the identification of enantiomeric species (R) and (S) of HHCB-lactone and other relevant TPs of the synthetic musk HHCB during its degradation by various oxidative and irradiation processes [144].

The application of LC-MS to the quantitative evaluation of degradation processes usually is based on the use of hybrid triple quadrupole (QqQ) or quadrupole linear ion trap (QqLIT) analysers, which exhibit excellent performance working in the multiple reaction monitoring (MRM) mode. New generations of instruments allow ultrafast MRM acquisition speeds and ion polarity switching, which ensures compatibility with UHPLC analyses and get maximum response simultaneously for higher number of analytes.

In QqLIT analysers the third quadrupole (Q3) can be operated in the linear ion trap mode, leading to a unique tandem mass spectrometer capable of functioning as either a triple quadrupole for quantitative workflows or as a highly sensitive linear ion trap for qualitative workflows. Both capabilities can be combined in one analysis by operating under the Information Dependent Acquisition (IDA) mode. In this case, the MRM mode is used to screen for target compounds and whenever the MRM signal is above a specified threshold automatically enhanced product ion (EPI) spectra are acquired. These spectra can then be searched against a mass spectral library thus improving qualitative capabilities [4, 145]. In this way accurate quantitative and reliable qualitative information can be simultaneously acquired. LC-QqLIT-MS/MS based methods have been applied to monitor degradation of selected CECs after different wastewater treatments [44, 45].

As a consequence of the increasing interest of using accurate mass high resolution mass spectrometers (HRMS), e.g. Orbitrap and time-of-flight (TOF) instruments, in environmental analysis, recent studies have explored the quantitative potential of these instruments. Compared with first-generation instruments, the latest TOF instruments provide increased sensitivity and resolving power, and a wider linear dynamic range, which provides adequate quantitative skills [46, 47]. In addition, HRMS overcomes limitations of using MRM methods, such us the limited number of transitions that can be registered without damage in accuracy or sensitivity, the non-specificity of the MRM transitions or the absence of a second MRM confirmatory. Virtually all compounds present in a sample can be determined simultaneously operating in full-scan mode, making no pre-selection of compounds and associated MRM transitions necessary. Hybrid instruments, like quadrupole/time-of-flight (QTOF) or linear ion trap (LTQ) Orbitrap, have improved the capacities as screening tools for target compounds with respect to single ones, due to the combination of mass accuracy, for both precursor and product ions, and improved sensitivity. Furthermore, their high mass resolving power enhances the identification of isobaric compounds since they can distinguish between compounds of identical nominal masses. These instruments also offer the possibility of information dependent MS/MS acquisition, i.e. an MS/MS analysis is triggered if a target compound is detected in the full scan. As an example, Fig. 1 shows the identification of nicotine from a river water sample [46] in a QTOF system, based on (1) the measured mass of nicotine at m/z 163.1229, which matches the calculated mass 163.1222 with an error of −4.5 ppm, and (2) mass spectral library searching of the MS/MS spectrum (purity score = 68.5).

But, despite the reported improvements of modern instruments applied to target analysis, the matrix effects remain the main pitfall in target quantitative analysis of complex samples [48]. The suppression or, less frequently, the enhancement of the analytes signal is frequently observed. Standard addition is the most suitable method for compensating matrix effects in quantitative analysis, but it is time-consuming and laborious. Matrix-matched calibration has been widely used [146], but the absence of blanks and the variability of the matrix throughout the set of samples analysed, represents a drawback.

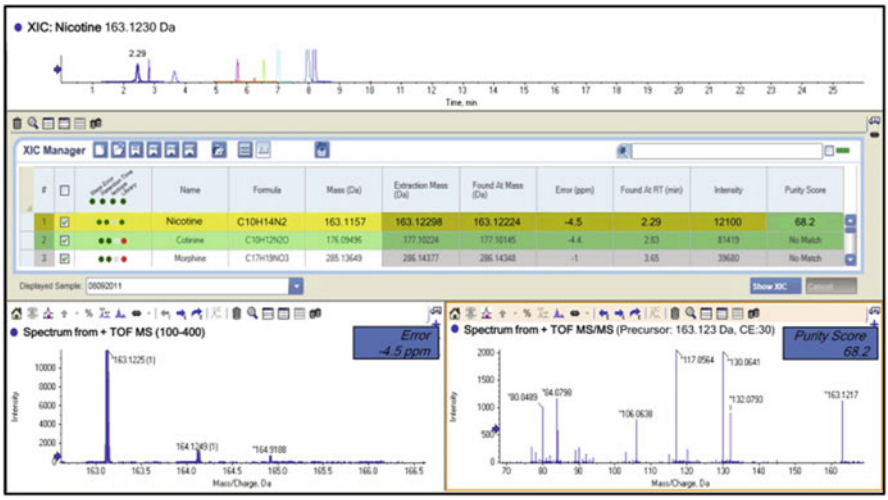

Fig. 1 Example of identification of the targeted compound nicotine in a river water sample based on accurate mass MS and MS/MS information (from Panditi et al. [46])

The use of internal standards (IS) also reduces matrix effects since the analyte-to-internal standard response ratio compensates for any ion suppression/enhancement that may be present. Use of isotopically labelled internal standards (ILIS) is the most recognized technique. Panditi et al. [49] report signal suppression/enhancement values lower than 20% in most cases in the LC-MS/MS analysis of 31 antibiotics in reclaimed water. Ibañez et al. [50] also report the use of ILIS to evaluate the efficiency of ozone treatment in the removal of a set of pharmaceuticals and drugs of abuse. A detailed study of matrix effects in wastewater samples [5] also highlight the use of ILIS, demonstrating that the selection of an analogue eluting at close retention time did not always ensure adequate correction.

2.2.2 Screening Methods

The target approach involves the purchase and measurement of hundreds of compounds, coming along with increase in time, effort and money. In addition, wastewater effluents contain a multitude of organic contaminants and TPs, which escape the target analysis alone. Thus, a good choice is combining extensive target analysis for the most relevant analytes and screening analysis, to identifying other potentially relevant compounds. In this sense, capabilities of HRMS are gaining in relevance together with novel data processing approaches to complement an extensive target analysis.

Krauss et al. [51] differentiate between "suspect screening", looking for compounds that are expected to be in the samples, and "non-target screening" when no prior information about the identity of the compounds is available. Suspect

compounds can be screened using databases containing the exact mass of expected ions, calculated from the molecular formula. However, limitations rely on the limited availability of databases for LC-MS/MS and the lack of reproducibility between spectra obtained with different instruments. Some authors propose the creation of home-made suspect lists to occur in water samples [52]. A general weakness of the approach is the peak detection, which provides an extensive list of suspected peaks, which in many cases derived from matrix background. Thus, an extensive compound filtering has to be applied to discard false positive detections based on retention time prediction, the evaluation of isotope patterns, ionization behaviour, and HRMS/MS spectra.

"Non-target screening" involves masses that are detected in the samples, but where no a priori information on the underlying compound is available. Identification of masses of interest is possible when the MS is operated in a data-dependent acquisition (DDA) mode in which both MS and MS^n spectra are acquired without the need to specify parent masses. In this mode, the instrument is initially set to operate in full-scan ("survey") and the acquisition software looks for the MS spectra in real-time on a scan-by-scan basis to select the most intense parent ions for MS^n analysis. This technique is capable of finding true unknowns, as long as they are ionized and behave accordingly in the chromatographic process, since the method does not require any pre-selection of masses. From the measured exact mass, the elemental compositions of non-target ions are calculated with a high degree of certainty (maximum deviation of 5 ppm is generally admitted). This elemental composition can be used to search electronic databases (NIST Library, Chemfinder or Chemspider) in order to provide a reliable structure assignation if the compounds are present there. Finally, the structures found in the libraries are evaluated based on the fragmentation patterns observed in the simultaneously acquired product-ion spectra [53].

2.2.3 Identification of Unknown Transformation Products

Currently it is becoming evident that the absence of parent contaminants in the analysis of wastewater does not guarantee the quality of treated or reclaimed water and the absence of an impact in the environment. During wastewater treatment, many organic microcontaminants undergo transformation reactions resulting in the appearance TPs [54]. Despite efforts in the identification, only a small portion of possible TPs that can be generated during treatments have been investigated, mainly because of labour-intensive and time consuming experimental and analytical steps and the frequent absence of analytical standards for an unequivocal confirmation.

HRMS represents an interesting choice for this kind of analysis because of the ability of providing accurate mass and elemental composition of both molecular and MS/MS product ions [55], although structural isomers cannot be distinguished. Figure 2 shows an example of identification of the TP thiazole-4-carboxamide generated by Fenton oxidation treatment of the pesticide thiabendazole in water

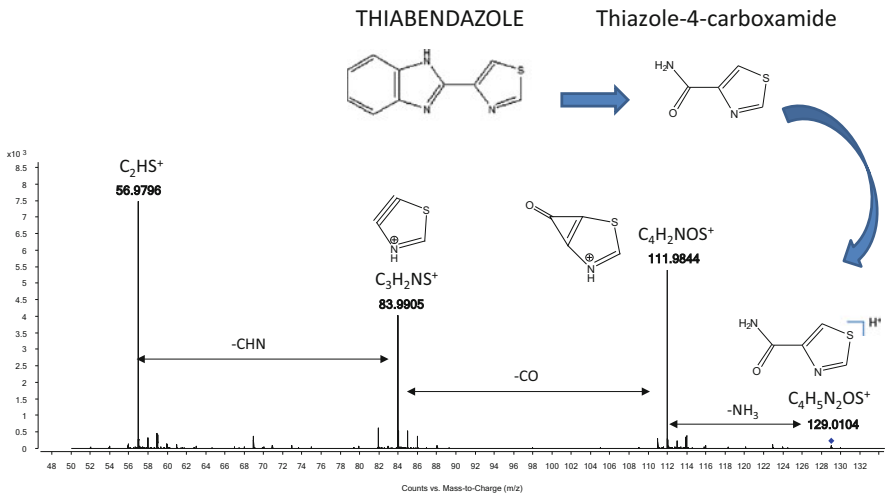

Fig. 2 Identification of the TP thiazole-4-carboxamide generated by Fenton treatment of the pesticide thiabendazole in water solution based on the accurate mass spectrum obtained by LC-QTOF-MS/MS

solution. Structural elucidation was based on the accurate mass spectrum obtained by LC-QTOF-MS/MS and then confirmed by analysis of the analytic standard.

Most published studies dealing with the identification of TPs are performed with individual compounds and under laboratory conditions (distilled water and high initial concentration of the contaminant studied). However, the "ideal" conditions applied hardly are comparable to those that occur in real processes. Methods are needed allowing high-throughput elucidation of TP structures in real waters. With this objective in mind, a systematic approach has been proposed [154]. This approach is based on the use of characteristic fragmentation undergone by organic contaminants during MS/MS fragmentation events, and its relationship with the transformations experimented by these chemicals in the environment or during water treatment processes [56]. Thus, a database containing accurate-mass information of 147 compounds and their main fragments generated by CID MS/MS fragmentation experiments was created using an LC-QTOF-MS/MS system. This database was applied to the identification of tentative TPs and related unexpected compounds in wastewater effluent samples. The approach comprises the automatic extraction of compounds using the "Molecular Feature Extraction (MFE)" algorithm to search and create a list of all the peaks that represent real molecules. This list is compared to the database to identify possible matches. Once the potential TPs have been tentatively identified, confirmation of their identity is obtained by MS/MS fragmentation.

Another strategy has been proposed by Helbling et al. [147]. In this case, candidate TPs were preliminarily identified with an innovative post-acquisition data processing method based on target and non-target screenings of the full-scan MS data obtained by an LTQ Orbitrap system. For the target analysis, single ion

chromatograms were extracted at the exact masses of plausible TPs predicted by the University of Minnesota Pathway Prediction System (UM-PPS) [57]. In addition, non-target screening was based on full-scan MS data obtained from two samples obtained at $t = 0$ and $t > 0$ to identify compound masses that formed during the biotransformation experiment. A series of mass filters (mass and retention time domain constraint, a background subtraction algorithm, a constrained molecular formula fit, presence of ^{13}C monoisotopic masses) was applied to reduce the number of extracted masses. The list of candidate TPs must be further analysed through manual inspection of the XICs, MS spectra and MS/MS spectra. This procedure yielded the identification of 26 TPs but the extent of TP formation remains unknown. Additional TPs may have formed but remained undetected because of different causes, such as low concentration levels, limited ionization efficiency, and poor separation in the LC system.

3 Determination of Organic Microcontaminants in Soils Associated with Reclaimed Wastewater Reuse

3.1 Analytical Methods/Sample Preparation in Soil Samples

Solid–liquid extraction (SLE) is the oldest sample preparation technique for extracting organic microcontaminants from soil and other solid matrices. It is still used mainly because of its easy use and procedural simplicity. However, two main disadvantages, the lengthy time and low extraction efficiency, have been pointed out for this technique. To this purpose, ultrasound treatment is often used to accelerate and favour the extraction process. Ultrasound-assisted solvent extraction (USE) is considered a good option for organic-compound extraction from soil matrices, as it provides more efficient contact between sample and solvent due to an increase of pressure (which favours penetration and transport) and temperature (which improves solubility and diffusivity). Thus, USE is one of the most widely used techniques due to its distinct advantages, such as low cost, easiness of use, wide-ranging applicability and availability. The extraction solvents employed are usually mixtures of buffer solutions and organic solvents such as acetonitrile, methanol, acetone and ethyl acetate. Ethylenediaminetetraacetic acid (EDTA) and McIlvaine buffer solutions (mixture of citric acid and Na_2HPO_4, (e.g. 0.1 M Na_2EDTA–pH 7 McIlvaine buffer, 50:50, v/v) a) are used as chelating agents in order to improve the isolation of some antibiotic compounds from solid samples, like tetracyclines (TCs) (e.g. tetracycline, chlortetracycline and oxytetracycline) which tend to form chelate complexes with metal ions and are strongly sorbed to soil [63–65]. In most cases, extraction conditions such as pH must be controlled in order to enhance analyte extraction. Thus, for pharmaceutical compounds with acidic (e.g. inflammatory drugs) or zwitterionic characteristics (fluoroquinolones—FQs), the extraction is usually carried out at acidic pH. For

example, Chen et al. [66] reported the acidification of ethyl acetate with formic acid for the extraction of 19 pharmaceuticals from soils. Golet et al. [67] demonstrated that the adjustment of pH at 2 is necessary for simultaneous determination and high extraction yields of FQ analytes from soils. At low pHs, FQs present a higher water solubility as they are then mainly present as cations that enhances the extraction efficiency. Moreover, both FQs and soil surface are protonated and, therefore, electrostatically repulsed favouring the extraction [63]. On the other hand, for basic or neutral compounds higher pHs are required to improve the extraction efficiency [68]. Overall, pH should be chosen according to pK_a value, since for some antibiotics like β-lactams, hydrolysis may occur below or above neutral pH.

In addition to conventional SLE, instrumental methods such as pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), and microwave-assisted extraction (MAE) have attracted growing interest in CEC analysis of soil samples (Table 2). They have the advantages of easier automation and higher extraction throughput, whereas they require smaller volumes of solvent and provide better extraction efficiencies (in terms of extraction yield and/or recovery) when compared to conventional SLE. PLE has been successfully employed for the determination of different groups of ECs [79]. A very interesting feature of this technique is the possibility of full automation and many samples can be extracted sequentially. The amount of time spent for method development can therefore be significantly reduced compared to other techniques. In general, the extraction is carried out with methanol [86, 99], mixtures of methanol (MeOH) and hexane/acetone [85], dichloromethane (DCM) and acetone [92], water [69], or mixtures of water with organic solvents, such as acetonitrile [100], isopropanol [101], acetone/hexane [95] or MeOH [102, 103]. When water is used as extraction solvent, pH is also controlled in the case of analytes with acid–base properties, as in the case of macrolide, sulphonamide and β-lactam antibiotics (MeOH–citric acid (0.2 M, pH 4.7) [102].

The extraction efficiency of PLE is dramatically influenced by extraction pressure and temperature, and therefore, both parameters must be carefully optimized. Extraction pressure is usually kept in the range of 500–1,500 psi. The extraction is commonly carried out at temperatures ranging from 60 to 100°C because at higher temperatures thermal degradation of analytes can occur and more matrix components can be co-extracted affecting the extraction efficiency and leading to interfering signals in MS chromatographic systems. Other particular variables of PLE that are usually studied are the number of cycles and/or extraction time. Usually, one to five cycles are carried out, although two cycles are mostly used [94]. Extraction time of 5 min is commonly used [103], whereas longer extraction is employed in dynamic mode as in the case of static extraction process.

Another interesting and environmental friendly instrumental approach which nowadays attracts considerable attention for the determination of CECs in solid matrices is MAE. MAE simply involves placing the sample with the solvent in specialized containers and heating the solvent using microwave energy. Hence, extraction solvents available for MAE are limited to those solvents that absorb microwaves (solvents with a permanent dipole). The use of solvent mixtures with

Table 2 Analysis of ECs in soil and crops samples

Analytes	Matrix	Sample preparation	Instrumentation	Rec.	LOD/LOQ (ng/g)	Reference
Pharmaceuticals						
Pharmaceuticals (19)	Soil	USE: 10 mL EtAc–formic acid) (50:1, v/v) (×3)	LC-ESI-MS/MS	43–245	0.02–4.20	[66]
		Clean-up: Silica gel				
Pharmaceuticals (6) and metabolites (2)	Soil	PLE: acetone–hexane–HAc (50:50:2, v/v/v), 100°C	GC-MS (MTBSTFA)	62–118	0.5–2	[148]
		Clean-up: Oasis HLB				
Pharmaceuticals (32)	Soil	PLE: 0.1 M ammonium–MeOH (1:1 v/v), 80°C	LC-ESI-MS/MS	66–114	0.1–1.5	[149]
		Clean-up: MAX-HLB in tandem				
Pharmaceuticals (19)	Soil	PLE: ACN–water (7:3,v/v), 130°C	LC-ESI-MS	63–113	0.76–5.46	[100]
Pharmaceuticals (20)	Crops (pepper collard, lettuce, radish, tomato	USE: two-step extraction; 20 mL MTBE; 20 mL ACN	LC-ESI-MS/MS	56.3–129.6	0.04–3.0	[96, 140]
		Clean-up: OASIS HLB				
Pharmaceuticals (17)	Soil	PLE: water, 90°C	LC-ESI-MS/MS	34–105	0.1–6.8	[69]
		Clean-up: SAX + HLB in tandem				
Pharmaceuticals (18)	Soil	MAE: 10 mL MeOH–water (3:2, v/v)	GC-MS (BSTFA + 1% TMCS)	91–101	0.8–4.7 ng/kg	[75]
		Clean-up: SPE (Oasis HLB)				
Anti-inflammatory drugs (4), Clofibric acid	Soil	USE: 9 mL acetone + 9 mL EtAc	GC-MS (MTBSTFA)	52–11	70.2–0.4	[77, 105]
		Clean-up: C18				
Anti-inflammatory drugs (3), Diphenhydramine hydrochloride	Soil	MAE: 10 mL DCM–MeOH (2:1, v/v) (×3), 115°C	GC-MS (pyridine–BSTFA (2:1))	<40		[72]
		Clean-up: Silica microcolumns				

PPCPs (118)	Biosolids	USE: buffered ACN + ACN	LC/ESI-MS/MS		0.03–5,080	[150]
		Clean-up: SPE				
PPCPs (118)	Crops (tomatoes, carrots, potatoes and sweet corn)	USE: buffered ACN + ACN	LC/ESI-MS/MS		0.24–54.15	[150]
		Clean-up: SPE				
Ibuprofen, Ciprofloxacin	Soil	Shaking: ACN	LC-UV	28–97	0.27–25.56	[131]
		USE: 50 mL Na_2-EDTA phosphate buffer pH 3–ACN (1:1, v/v)	GC-MS (MSTFA)			
		Clean-up: Oasis HLB				
Diclofenac sodium, Naproxen	Crops (lettuce (Lactuca sativa) and collards (Brassica oleracea	USE: two-step extraction; 20 mL MTBE; 20 mL ACN	HPLC-UV	74–85.8		[76]
		Clean-up: OASIS HLB				
Carbamazepine	Crops (cucumber)	USE: 12 mL Methanol	LC/ESI-MS/MS		70 and 40 ng/L	[151]
Carbamazepine	Soil	USE: 15 mL isopropanol–water (8:2, v/v) (×2)	GC-MS	67–96	110	[107]
		Clean-up: Oasis HLB – Florisil in tandem				
Antibiotics						
Antibiotics (15)	Soil	USE: 10 mL citric acid buffer (0.2 M, pH 4.4) + 10 mL	LC-ESI-MS/MS	64–245	0.08–4.20	[66]
		ACN (×3)				
		Clean-up: Oasis HLB				
Antibiotics (7)	Soil	USE: 90 mL MeOH + 45 mL acetone + 45 mL EtAc	LC-ESI-MS/MS	38–121	3–20[a]	[78]
		Clean-up: LiChrolut C18				

(continued)

Table 2 (continued)

Analytes	Matrix	Sample preparation	Instrumentation	Rec.	LOD/LOQ (ng/g)	Reference
Antibiotics (13)	Soil	SAs + TCs	SAs + TCs	61–94	0.8–23	[64]
		USE: 10 mL EDTA-McIlvaine buffer–MeOH (1:1, v/v)	LC-UV			
			QNs			
		Clean-up: C18 + SAX in tandem	LC-FL			
		QNs				
		USE: 5 mL 50% $MgNO_3$ aqueous solution containing 4% aqueous ammonia				
		Clean-up: C18 + SAX in tandem				
Antibiotics (11)	Soil	TCs + SAs	TCs + SAs	61–105	100[a]	[63]
		USE: 30 mL MeOH–EDTA-MacIlvaine buffer pH 6 (9:1, v/v) (×3)	LC-ESI-MS/MS			
		Clean-up: C18	FQs			
		FQs	LC-ESI-MS			
		USE: 30 mL ACN acidified with formic acid 2% + 0.5 g organic substratum (×3)				
		Clean-up: LLE (n-hexane)				
Antibiotics (14)	Soil	USE: 10 mL citric buffer (0.2 M, pH 4)–ACN (1:1, v/v) (×3)	LC-ESI-MS/MS	48–160	0.08–4.2	[152]
		Clean-up: Oasis HLB				

Antibiotics (6)	Soil	PLE: MeOH–citric acid (0.2 M, pH 4.7) (1:1, v/v), RT Clean-up: SAX + HLB in tandem	LC-MS/MS	50–100	0.6–5.6	[102]
Quinolones (10)	Soil	USE: 8 mL $MgNO_3$ aqueous solution (50%, w/v) + 4% ammonia	LC-UV	82–104	40–80	[74]
Fluoroquinolones (5)	Soil	USE: 8 mL $MgNO_3$ aqueous solution (50%, w/v) + 4% ammonia Clean-up: MISPE Ciproflaxin	LC-UV	75–85	40–70	[153]
Tetracyclines (3), Tylosin	Soil	Shaking: 1.2 mL citrate buffer (1 M, pH 4.7) + 6 mL EtAc	LC-ESI-MS/MS	33–127	1–2	[70]
Enrofloxacin, Ciprofloxacin	Soil	Shaking and USE: 15 mL phosphate buffer (pH 3)–ACN (1:1, v/v) Clean-up: SAX + Oasis HLB in tandem	LC-FL	61–100		[71]
Oxytetracycline Norfloxacin Chlortetracycline	Soil	USE: 25 mL MeOH–EDTA-MacIlvaine buffer pH 6 (9:1, v/v) (×3)	HPLC-DAD	65–78	0.08–0.50 mg/Kg	[65]
Oxytetracycline Norfloxacin Chlortetracycline	Crops	USE: 25 mL MeOH–EDTA-MacIlvaine buffer pH 6 (9:1, v/v) (×3) Clean-up: (Strata-X)	HPLC-DAD	65–78	0.08–0.50 mg/Kg	[65]
Sulphonamide antibiotics and their metabolites	Soil	PLE: MeOH–water (90:10, v/v), (×3), 100°C; Clean-up: OASIS HLB	LC-MS/MS	60–130	0.01–4.19 ng/g	[103]
Estrone 17-Estradiol, 17-ethynylestradiol estriol	Soil	PLE: DCM–acetone (3:1, v/v), 60°C; Clean-up: Strata X	GC-MS (BSTFA)	71–118	0.02–0.19 ng/g	[92]

(continued)

Table 2 (continued)

Analytes	Matrix	Sample preparation	Instrumentation	Rec.	LOD/LOQ (ng/g)	Reference
Bisphenol A (BPA)	Soil	PLE: DCM–acetone (3:1, v/v), 60°C; Clean-up: Strata X	GC-MS (BSTFA)	90–128	0.37	[92]
Ionophores						
Salinomycin A	Soil	PLE: 30 g sample with MeOH (1% NH_4OH)	APCI(+)	76 ± 32	5.3[a]	[79]
		Clean-up: UCT Diol SPE 2 g	Triple quad (QqQ)			
		Elution with 0.1 M	SRM mode			
		NH_4Ac–MeCN (2:3)				
Monensin A	Soil	SLE: 1 g sample LLE with EtOAc (NH4-citrate, NH_4OH, pH 5.8)	ESI(+)	75	2,000	[82]
			Triple quad (QqQ)			
			SRM mode			
Lasalocid, Monensin, Salinomycin and Narasin	Soil	PLE: MeOH–water (1:1, v/v), 50°C; Clean-up: OASIS HLB	LC-MS/MS	71–123	0.64–0.98 µg/kg	[94]
EDCs						
Bisphenol A	Biosolids	USE: buffered ACN + ACN	LC/ESI-MS/MS		5,080	[150]
		Clean-up: SPE				
Bisphenol A	Crops (tomatoes, carrots, potatoes and sweet corn)	USE: buffered ACN + ACN	LC/ESI-MS/MS		396	[150]
		Clean-up: SPE				
Bisphenol A	Crops (lettuce (Lactuca sativa) and collards (Brassica oleracea	USE: two-step extraction; 20 mL MTBE; 20 mL ACN	HPLC-UV	81.5		[76]
		Clean-up: OASIS HLB				

UV filters and parabens						
BPs (5)	Soil	USE-clean-up: 16 mL EtAc–MeOH (90:10, v/v), C18	GC-MS-EI-SIM (BSTFA)	89–105	0.07–0.28	[87]
BPs (7)	Soil	Shaking: 20 mL MeOH + 20 mL EtAc	GC-MS (MSTFA)	60–125	0.1	[88]
Parabens (6)	Soil	SAESC: 4 mL ACN (×3)	LC-MS/MS	83–110	0.04–0.14	[89]
Parabens (7)	Soil	SAESC: 4 mL ACN (×3)	HPLC-UV	80–90	1	[90]
		Clean-up: MISPE	LC-MS/MS			
Triclosan, Triclocarban	Soil	PLE: 70% MeOH, 100°C	LC-ESI-MS/MS	80–142	0.1–5.1	[99]
		Clean-up: pH 4 cartridge ABN				
Parabens (6)	Biosolids	USE: buffered ACN + ACN	LC/ESI-MS/MS		3.5–14.3	[150]
Triclosan, Triclocarban		Clean-up: SPE				
Parabens (6)	Crops (tomatoes, carrots, potatoes and sweet corn)	USE: buffered ACN + ACN	LC/ESI-MS/MS		3–175	[150]
Triclosan, Triclocarban		Clean-up: SPE				
Triclosan	Crops (Bean)	SLE: 10 mL ACN–water, 1:1(v/v)	HPLC-UV	76.5	0.12 mg/g dw	[131]
		Clean-up: SPE C_{18}				
Triclosan	Soil	SLE: 50 mL ACN	HPLC-UV	93	1.04 ng/g dw	[131]
Triclosan	Soil	PLE: water–isopropanol (80:20, v/v), 100°C	LC-ESI-MS/MS	87	2[a]	[101]
		Clean-up: Oasis HLB cartridge				
Triclosan	Soil	MAE: 10 mL MeOH–water (3:2, v/v)	GC-MS (BSTFA + 1% TMCS)	92	3 ng/kg	[75]
		Clean-up: SPE (Oasis HLB)				
Musk fragrances						
Nitro musk	Soil	MAE: 30 mL DCM–MeOH (2:1, v/v), 160°C	GC-MS (BSTFA)	90		[72]
		Clean-up: Silica gel				

(continued)

Table 2 (continued)

Analytes	Matrix	Sample preparation	Instrumentation	Rec.	LOD/LOQ (ng/g)	Reference
Polycyclic musk	Soil	PLE-clean-up: DCM, silica gel + hydromatrix, 60°C	GC-MS	>80	1	[91]
Estrogenic compounds						
Estrone	Soil	USE:10 mL EtAc–acetone (1:1, v/v)	GC-MS (MTBSTFA)	63–110	1.2	[105]
		Clean-up: C18				
Estrone	Soil	MAE: 10 mL MeOH–water (3:2, v/v)	GC-MS (BSTFA + 1% TMCS)	92–96	4.7–5.1 ng/kg	[75]
17β-Estradiol		Clean-up: SPE (Oasis HLB)				
17α-Ethinylestradiol						
Hormones (17)	Biosolids	USE: buffered ACN + ACN	LC/ESI-MS/MS		–	[150]
		Clean-up: SPE				
Hormones (17)	Crops (tomatoes, carrots, potatoes and sweet corn)	USE: buffered ACN + ACN	LC/ESI-MS/MS		19.0–44.8	[150]
		Clean-up: SPE				
17a-ethynylestradiol	Crops (Bean)	SLE: 10 mL ACN–water, 1:1(v/v)	HPLC-UV	71.9	0.10 mg/g dw	[131]
		Clean-up: SPE C_{18}				
17a-ethynylestradiol	Soil	SLE: 50 mL ACN	HPLC-UV	99.8	0.96 ng/g dw	[131]
Alkylphenols						
AEOs, ANEOs	Soil	PLE: (A) MeOH and (B) hexane–acetone (1:1, v/v), HAc (75 mmol/L) and TEA (100 mmol/L), 150°C	LC-APCI-MS	27–109	7–43	[85]
		Clean-up: Porapak RDX				
APs (3), APEOs (7)	Soil	PLE: MeOH, 70°C	GC-MS	97–104	3–38	[86]
		Clean-up: Isolute ENV+ cartridges	(BSTFA–TMCS, 1:1,v/v)	96–104	6–60	
			LC-FL			

APs, APEOs, AEOs,	Soil	PLE: acetone–hexane (1:1, v/v), 60°C Clean-up: C18	LC-APCI-MS	89–102	0.3–30	[85]
4-Nonylphenol	Crops (lettuce (Lactuca sativa) and collards (Brassica oleracea	SLE: 50 mL Hexane	HPLC-UV	66.8	–	[76]

MTBE methyl *tert*-butyl ether)
[a]LOQ

and without dipoles opens up a variety of potential solvent mixtures. As in the case of PLE, to develop a successful MAE, several parameters (i.e. solvent volume and composition, extraction time and temperature, pressure, water content, matrix characteristics, etc.) that influence the extraction yield of MAE has to be studied and optimized.

In the case of solvent mixtures, the most popular is the MeOH–H_2O mixture, which has been applied to the extraction of pharmaceuticals, triclosan and estrogenic compounds [75]. DCM–MeOH has also been applied for extracting nitromusks and anti-inflammatory drugs [72] from soils. According to the literature, the extraction times used in MAE for CECs in soil and plant samples are within 6 and 15 min. Concerning extracting volumes, they range from 10 to 60 mL, whereas extraction temperature and pressure ranged between 110 and 130°C and <10 mPa, respectively. In the case of microwave treatment, values in the 150–1,600 W were applied for closed systems, while 500 W is most common used.

The MAE technique is more environmental friendly than the others as it uses substantially smaller amounts of solvents, reduces sample consumption, waste production and shortens extraction times, thereby reducing overall energy input and costs [98, 104]. However, similarly to PLE, additional clean-up is usually needed prior to chromatographic analysis. Moreover, special care with temperature and irradiation time is required to avoid degradation of analytes. For instance, accelerated decomposition of pharmaceuticals such as clofibric acid, metoprolol and propranolol has been observed at high microwave powers, in combination with long extraction times [75].

3.1.1 Clean-Up Methodologies for Soil Samples

One of the problems with most methods used for extracting organic pollutants most prominent in solid samples is that large amounts of co-extracted compounds will add to the complexity of the chromatograms and interfere with detection of analytes. Thus, after the target compounds are extracted from the sample into the liquid phase, a further sample clean-up step is necessary to enable a robust analysis. Solid-phase extraction (SPE) is currently the most widely used choice to prepare extracts from solid samples for instrumental analysis. Before SPE, the organic-solvent content of the extract has to be reduced to less than 5% to prevent early breakthrough of analytes from the cartridges. The majority of studies performed SPE by using predominantly Oasis-HLB sorbent [66, 69, 76, 93, 96] that due to its hydrophilic–lipophilic balance allows the separation of compounds with a wide range of polarity. Other sorbents such as C18 [63, 78, 105], silica [66], Strata X [69], SAX and alumina [106] have also been used. Methanol is the main solvent used in the elution of these cartridges. Although the clean-up is, in general, carried out using one cartridge, some authors have performed two successive clean-up steps using SPE cartridges with different functionalities [102, 107]. For example, PLE extracts were further cleaned by a two-step SPE clean-up using SAX and HLB sorbents for the analysis of antibiotics [102].

3.2 *Occurrence of Microcontaminants in Soils Associated with Reclaimed Wastewater Reuse*

The use of reclaimed water may often provide a technically and economically feasible solution [73]. Nevertheless, its use in irrigation and/or aquifer recharge can introduce a range of CECs into the terrestrial environment, if these are not effectively removed during WWTPs. In addition to irrigation with reclaimed water, the application of sludge or manure to amend land and to fertilize agricultural soils can be another major pathway into the terrestrial and subsequently again in to the aquatic environment for these chemicals [61, 62, 64, 65].

The fate of CECs in soils is mainly dependant on their physico-chemical properties, which will influence their mobility, persistence and bioavailability in the soil matrix. The physico-chemical properties of CECs can vary widely; however, many of them contain a non-polar core with a polar functional moiety which complicated their fate patterns. Prevailing climatic conditions, soil types and a variety of other environmental factors are also critical for their fate and transport processes (e.g. volatilization, transformation and plant uptake).

In general, the CEC concentrations of reclaimed water are quite low (ng/L or μg/L) and their fate and transport in the receiving soils would be difficult to track and quantify [81]. Moreover, the water quality of reclaimed water fluctuates and thus the stability and reliability of reclaimed water quality are difficult to be ensured in the long run. Consequently, very little is known about the behaviour and occurrence of such contaminants in soils associated with reclaimed wastewater reuse. Only a few specialized reports are available on exposure of receiving soils to CECs by reclaimed wastewater irrigation. While only a few studies have explored the occurrence of CECs in the soil environment, available data indicate that a broad range of pharmaceuticals and personal care product (PPCPs) classes, including non-steroidal anti-inflammatory drugs, antidepressants, anticonvulsants, musk compounds, estrogens, UV filters and antibacterial agents does occur in soils in concentrations up to the low mg/kg level [81]. For example, Xu et al. [105], demonstrated the occurrence of six different PPCPs, endocrine-disrupting compounds (EDCs) and estrogenic compounds (clofibric acid, ibuprofen, naproxen, triclosan, bisphenol A and estrone) in soil samples collected from a golf course irrigated with reclaimed wastewater in southern California at concentration levels ranging from 0.55 to 9.08 ng/g dry weight soil. The findings of this study indicate that trace organic contaminants in the reclaimed wastewater may accumulate in the top soils during irrigation with reclaimed wastewater, consequently exposing the groundwater to a potential contamination. Another interesting study by the same research group [84] found that significant amounts of reclaimed water borne PPCP and EDC compounds, such as Ibuprofen, naproxen, triclosan, bisphenol A, clofibric acid and estrone, accumulated at the top (30 cm) of an irrigated turf grass field. However, no compound was detected in the leachate draining through the 89-cm profile of a loamy sand soil and a sandy loam soil turf grass field during 4 months of irrigation. Chen et al. [77] detected six PPCPs and ECDs, namely, bisphenol-A,

4-nonylphenol, triclosan, triclocarban, salicylic acid and clofibric acid in soil samples from four irrigated plots in Guangzhou. Finally, in a recent study, Fang et al. [108] reported that gemfibrozil in reclaimed water applied on land might reach the groundwater aquifer underneath.

Occurrence of synthetic musk fragrances (SMFs) (six polycyclic musk compounds (galaxolide, tonalide, celestolide, phantolide, traseolide, cashmeran) and two nitro musk compounds (musk xylene and musk ketone) was determined in soil cores from a land application site, groundwater below as well as in plants irrigated with treated effluent [109]. For most of the target SMFs, only traceable amounts were detected in soil samples (ND to <1 ng/g, (method detection limit, soil = 0.3 ng/g)), except for galaxolide and tonalide, the concentration of which ranged from trace levels to 5.69 and 6.24 ng/g in the top six inches (15,24 cm) of soil, respectively. The findings demonstrated that there was no difference in SMF occurrence whether samples were from inside or outside the pivot irrigation system. For cashmeran, celestolide, phantolide and musk ketone the concentrations ranged from ND (method detection limit, soil = 0.3 ng/g), to 1.57 ng/g, while traseolide and musk xylene were not detected in soil samples. The results of this study are in contrast to those for a similar land application site that had similar soil type, square metres and years applied [83]. However, it should be emphasized that the volume of water applied in a study of Ternes et al. [83], was much lower than in the first study. With concentrations in discharge already being lower, the absence of SMFs in groundwater it could have been expected. In addition, other environmental factors such as climate (e.g. arid versus humid) may play a role in the differences observed between the two sites with similar land application characteristics.

Finally, in a recent review study by Li et al. [81], it was demonstrated that the antibiotics (trimethoprim, sulfadiazine and triclosan), analgesics (ibuprofen and diclofenac) and antiepileptic (carbamazepine) were among the most common PPCPs found in soils with concentration levels up to 60.1 μg/kg. Considering the data of five studies [66, 110–113], it can be concluded that among the target compounds carbamazepine is the most frequently detected compound in soil. It is worth mentioning, however, that much of the environmental occurrence of this compound is likely associated with their selection as target compound in most comprehensive monitoring studies It is resistant to degradation and can be discharged to the soil in various ways. However, according to the studies by Gibson et al. [111] and Chen et al. [66], the irrigation of reclaimed water is considered the major pathway introducing PPCPs into soils.

In addition to the aforementioned works, a number of field and laboratory-scale studies provided a snapshot of the PPCP mobility, persistence and bioavailability in soil matrix [97, 114–117]. For example, Drewes et al. [118] examined the fate of selected PPCPs during the ground water recharge at two reclaimed water reuse sites. They found that diclofenac, ibuprofen, ketoprofen and naproxen were not detectable, whereas carbamazepine and primidone were found in the recharged aquifer throughout 8 years of operation. Yao et al. [119] tested the ability of different types of biochar to sorb aqueous sulfamethoxazole (SMX) and determined the leaching and retention of SMX in simulated reclaimed water through soils

amended with selected biochar. The authors found that mobility and bioavailability of SMX in biochar-amended soils were lower than that of non-amended soils. Biochar soil amelioration, therefore, should be promoted in areas where reclaimed water or wastewater is used for irrigation.

Overall, various detailed field and screening studies have been performed during recent years, and preliminary data are available for a variety of CECs in soils including antibiotics, sulphonamides, fluoroquinolines, musks, etc. However, the fate and transport of CECs in the terrestrial pathway have not been well understood, and most emphasis should be laid on this issue.

4 Determination of Organic Microcontaminants in Crops

4.1 Extraction Methods/Sample Preparation in Crops

Similarly to analysis of PPCPs in soil, determination of trace levels of PPCPs in plants presents great challenges due to high contents of pigments, and fatty or waxy materials, which may induce severe matrix interferences. Therefore, sample preparation methods that eliminate potential interferences while permitting the improvement of isolation and extraction of these compounds are usually performed. Most of them are focused on commonly used techniques, including PLE [112, 120–122], SLE by using buffers or solvent mixtures [65, 123, 124] and QuEChERS (quick, easy, cheap, effective, rugged and safe) [125]. After extraction, purification is usually performed by using preferably SPE.

4.2 Occurrence of Microcontaminants in Crops Associated with Reclaimed Wastewater Reuse

Since residual concentrations of CECs from both human and agricultural uses can be found in soils, many of these compounds have the potential to be taken up from the soil via plant roots. Once the CEC has entered the plant, a posterior translocation, driven by the transpiration process, can take place. The extent of distribution within the plant will depend on the compound's physico-chemical properties [126]. Octanol-water partition coefficient (K_{ow}) and dissociation constant are among the most useful chemical descriptors of for organic contaminants plant uptake and distribution. If a compound is too hydrophilic, it will be unable to enter and to cross hydrophobic lipid membranes. For compounds of high lipophilicity, adsorption or "solution" in the lipid material is usually happening which reduces its ability to cross the endodermis. Hence, in general, uptake is greatest for compounds with a log K_{ow} in the range of 1–4 [127] for non-ionizable compounds. If a compound dissociates in the physiologically relevant pH range,

this will influence both uptake velocity and level [128] and log D has to be considered instead Log K_{ow}.

In the last decades, most plant uptake studies were focused on pesticides or on legacy chemicals that are often less hydrophilic organic contaminants such as PCB, dioxins and PAHs. Little attention has been paid to the plant uptake of CECs and especially to ionized compounds and zwitterionic species. However, the presence of PPCPs and other CECs in the environment and the possible transfer to the animal and human food chain, calls for a better general understanding of uptake and translocation processes in plants. Thus, the number of studies dedicated to plant uptake of CECs is steadily increasing in recent years, proving that many of the CEC groups such as musks and pharmaceuticals (fluoroquinolones, sulphonamides, tetracyclines, anti-inflammatory and other drugs) are taken up by plants [123, 129–131]. For example, Eggen et al. [124] demonstrated the uptake of metformin, ciprofloxacin and narasin in carrot (*Daucus carota ssp. sativus cvs*. Napoli) and barley (*Hordeum vulgare*), with the root concentration factors (RCF) being higher than the corresponding leaf concentration factors (LCF) for all the target pharmaceuticals. The uptake of metformin was higher compared with the other two tested pharmaceuticals for all the target plant compartments, showing a generally higher bioaccumulation pattern in roots (RCF 2–10) and leaves (LCF 0.1–1.5). Negative effects on plant growth such as reduced biomass were observed for all three studied compounds, with narasin showing the most pronounced effect. Uptake of 17-alpha-ethynylestradiol (EE2) and triclosan in bean plants (*Phaseolus vulgaris*) grown in sand and soil was demonstrated by Karnjanapiboonwong et al. [131]. According to the authors, roots were the primary plant part in which EE2 and triclosan accumulated, and the accumulation of both test compounds was higher in plants grown in low organic carbon substrate. Antibiotics such as oxytetracycline, enrofloxacin, chlortetracycline and sulfamethazine were found to be taken up by alfalfa, corn, lettuce, potato, onion, cabbage and cucumber from manure-amended soil, agar medium or nutrient solutions [132–135]. Furthermore, bioaccumulation and phytotoxicity in algae, rice, cucumber and wetland plants have been reported by other authors [80, 136–138].

The majority of the aforementioned studies, however, is focused on the bioavailability and uptake of CECs by plants grown in soil-based mediums with artificial added contaminants [129, 139] or contaminated bio-solids used to fertilize agricultural soils [137]. Up to date only a handful of studies have considered plant uptake of CECs after application of reclaimed water for crop irrigation. For instance, the uptake of eleven, frequently detected PPCPs (diclofenac, carbamazepine, clofibric acid, caffeine, ibuprofen, naproxen, triclosan, methyl dihydrojasmonate (MDHJ), galaxolide, tonalide and hydrocinnamic acid) in apple (*Malus domestica*) and alfalfa (*Medicago sativa*) was evaluated by Calderón-Preciado et al. [121] under actual field conditions. Five of the 11 target contaminants were identified and quantified, namely, ibuprofen, naproxen, MDHJ, caffeine and tonalide. Caffeine and MDHJ were found in both crops in concentration levels between <0.011 and 0.016 and 0.041 and 0.532 mg/kg (fresh weight), respectively, whereas galaxolide, ibuprofen and naproxen were detected only in alfalfa with

levels from <0.011 to 0.061 mg/kg (fresh weight). Comparing the studied crops, it seems that the occurrence of the PPCPs in alfalfa is higher than those in apple. Besides the aforementioned field study, in vitro uptake of triclosan, hydrocinnamic acid, tonalide, ibuprofen, naproxen and clofibric acid by lettuce (*Lactuca sativa L*) and spath (*Spathiphyllum spp*.) was investigated by the same research group [139] in order to evaluate the reuse of treated wastewater for irrigation of agricultural crops. The authors conclude that compounds with a carboxylic group in their structure such as hydrocinnamic acid, naproxen and clofibric acid exhibited higher uptake rates. In relation to previous study, Wu et al. [140] examined a larger suite of PPCPs (20 frequently occurring compounds in irrigation) that had different K_{ow} or pK_a values and they compared their accumulation into four staple vegetables (lettuce, spinach, cucumber and pepper) grown in nutrient solutions containing PPCPs at 0.5 or 5 μg/L. Results showed significant disparities between the studied compounds regarding their potential for root uptake and subsequent translocation. Out of the 20 PPCPs considered in this study, triclocarban, fluoxetine, triclosan and diazepam accumulated in roots at levels relatively higher than the other PPCPs, while translocation to leaves/stems was more extensive for meprobamate, primidone, carbamazepine, dilantin and diuron. The authors suggested a positive correlation between root uptake and pH-adjusted log K_{ow} (i.e. log D_{ow}) for non-ionic compounds and a negative correlation for translocation from roots and log D_{ow}, indicating that compounds with strong hydrophobicity (i.e. high D_{ow}) tended to remain in the roots with limited in-plant redistribution. Consequently, and according to the study for the later compounds higher residues may be found in tuber vegetables (i.e. carrot and radish), while for PPCPs with high translocation potential, higher levels are expected in leafy vegetables such as lettuce, spinach and cabbage.

Finally, in the field study of Jones-Lepp et al. [141], greenhouse experiments were performed in which selected food crops were irrigated with three different water types (wastewater effluent known to contain CECs, CEC-free well water and Colorado River water containing trace-level CECs) spiked with three antibiotics. The results showed the potential for uptake of one or more of the antibiotics evaluated at very low levels only. The industrial flavouring agent, *N*,*N*′-dimethylphenethylamine (DMPEA), was consistently found in food crops irrigated with wastewater effluent, whereas none of the evaluated contaminants were found in crops irrigated with Colorado River water.

In summary, biosolids seem to be a more significant reservoir or sink for plant uptake of particular compounds than reclaimed water and therefore, much of the occurrence of some CECs is likely associated with biosolids. Meanwhile, although relatively few studies have specifically examined the role of reclaimed water usage in crop irrigation, detections of trace concentrations of selected CECs in different plant species have been documented. These plant uptake studies have provided a snapshot of the CECs in plant species, but many of them have been done at unrealistic exposure concentrations (in most cases higher than those detected in real samples), and therefore, more systematic investigation under real environmental conditions is required. The data generated must be supported by an appropriate

QA/QC system, which has not always been done and experiments should integrate phytoxicity/ecotoxicity tests. In addition, further research is required to clarify the transport processes and bioavailability of CECs to plants and whether species-specific uptake patterns can occur from contaminated soil. Such information is also important for the identification and prediction of CECs with potentially high transfer to human and livestock food webs that could provide a scientific framework for establishing environmental regulations.

5 Conclusions

Over the past few years a vast amount of research has been conducted in sample preparation and instrumental analysis and a number of methods have been proposed for analysis of organic microcontaminants in reclaimed water as well as in soils and crops associated with wastewater reuse. Thus, in recent years more data and broader knowledge have become available on CECs detection and identification in these matrices. Despite, however, this effort, innovative methods combining efficient extraction and selective mass spectrometric detection have to be designed and applied to improve non-target screening and identification of unknown transformation products. Furthermore, there is an urgent need for laboratory trials and field-scale studies in order to explore the fate, distribution and uptake of a range of organic microcontaminants in soil–plant systems to provide essential data for modelling their environmental behaviour.

Acknowledgements This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program "Excellence II (Aristeia II)", Research Grant, No 4199, which is gratefully appreciated. The authors would also like to thank the Cost Action "ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS)" for support.

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Bioassays Currently Available for Evaluating the Biological Potency of Pharmaceuticals in Treated Wastewater

Marlen I. Vasquez, Irene Michael, Klaus Kümmerer, and Despo Fatta-Kassinos

Abstract Water deprivation with regard to quantity and quality is one of the most important environmental problems of the century. The increasing demand of water resources puts pressure on the utilization of alternative sources such as treated wastewater. In the context of "reduce, reuse, and recycle," the inclusion of treated wastewater in the water cycle seems a promising practice for water management. The lack of general acceptance of stakeholders and public, however, still hinders the widespread application of wastewater reuse. A reason for this is, among others, the presence of contaminants of emerging concern in treated wastewater. This has led to an increased concern about direct and indirect effects to the environment and possible implications to human health. The development and application of bioassays able to identify and quantify the biological potency of treated wastewater is an ongoing research effort, especially when taking into consideration that a plethora of contaminants exist and interact in this complex matrix. This chapter summarizes available literature regarding the sensitivity of currently applied bioassays for assessing biological effects of treated wastewater and their correlation with chemical analysis. The focus is on pharmaceuticals since they represent one of the major

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 49–80, DOI 10.1007/698_2015_417,

groups of contaminants of emerging concern with many unanswered questions currently in place.

Keywords Effect-directed bioassay, Pharmaceutical, Toxicity, Wastewater reuse

Contents

Abbreviations

CEC	Contaminants of emerging concern
COD	Chemical Oxygen Demand
COX	Cyclooxygenase
DTA	Direct toxicity assessment
EC	Effect Concentration
EROD	Ethoxyresorufin-*O*-deethylase
ISO	International Organization for Standardization
LC	Lethal Concentration
LOEC	Lowest Observed Effect Concentration
MIC	Minimum Inhibitory Concentration
NADPH	Nicotinamide adenine dinucleotide phosphate
NOEC	No Observed Effect Concentration
OECD	Organization for Economic Cooperation and Development
PGE_2	Prostaglandin E_2
PSII	Photosystem II
TU	Toxic unit
USEPA	US Environmental Protection Agency
WET	Whole effluent toxicity

1 Introduction

The history of environmental toxicology is a quite short one since it was not until the mid-1900s that environmental effects of chemicals became a concern [1, 2], mainly regarding the effects of industrial wastes. Standardization and international

acceptance of protocols for ecotoxicological testing has improved the quality of the data produced. Organizations, such as the International Organization for Standardization (ISO), the US Environmental Protection Agency (USEPA), and the Organization for Economic Cooperation and Development (OECD), have contributed to this direction making ecotoxicological testing nowadays a very important part of environmental and chemical legislation such as the Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation (REACH).

Ecotoxicological testing seems attractive because toxicity to living organisms is more comprehensible than the concentration of a chemical or an effluent for non-specialists and often is less expensive than chemical analysis. It can be used in a "weight of evidence" approach and as a complementary tool of analytical measurements. Nowadays the "environmental safe levels" are derived by taking into account the ecotoxicity of the substance, its persistence, and its ability to bioaccumulate. They should also include a broad scope of effects such as mutagenicity, carcinogenicity, and reproductive impairment [3].

In principle, ecotoxicological testing can be carried out at any biological level of organization. The endpoints to be studied in each organization level can be selected based on the objectives of the study. As a rule though, the majority of pollutants act initially at the molecular level following accumulation in the exposed organism, with effects then becoming apparent as physiological changes and effects on key individual parameters, such as growth, reproduction, and survival [4]. These may exert effects on population level and then potentially on ecosystem level. As a general rule, the higher the organizational level, the more complicated, the longer lasting, and the more expensive are the experiments required for assessing effects. Within an ecosystem, the flow of energy and cycle of materials lead to the development of trophic structures, biotic diversity, and nutrient cycles, as shown in Fig. 1 [5].

A key corollary in ecotoxicological testing regarding the hierarchical levels of ecological organization is that detrimental effects at a given level of organization can propagate to higher organization levels. However, in reality, effects at any organizational level may or may not propagate at higher levels. Similarly, neither an effect at a lower organizational level may be easier to detect, nor an effect at a higher organizational level may be easier to interpret. Organisms vary in sensitivity and the single-species approach has limitations in population and ecosystem extrapolation. For this, the need to evaluate effects at higher organizational levels has been acknowledged. These experiments and observations, however, require more effort and higher cost [6].

The legislation pursues protection and preservation of the whole environmental entity and not that of single species. In practice, ecotoxicological testing still focuses on the organismic level, relying on the data generated from single-species toxicity tests. Ecotoxicological testing may differ according to its (1) duration, short to long term; (2) method of exposing the organisms to test chemicals, static, recirculation, or flow through; (3) type of the test, in vitro or in vivo; and (4) purpose of the study, screening, research, surveillance, etc. [7]. It should be noted that the point times evaluated at each bioassay are intrinsically connected to the

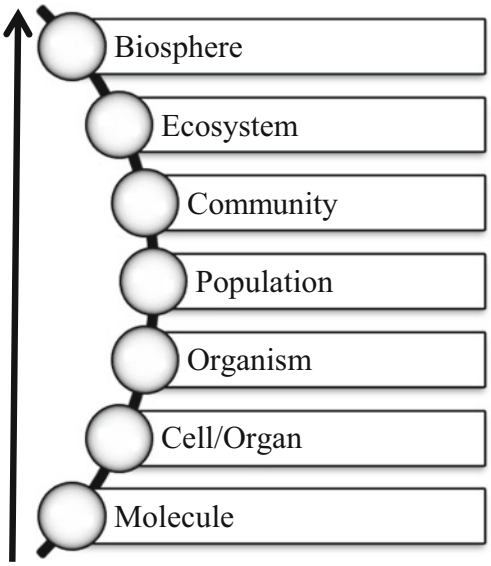

Fig. 1 Hierarchical view of levels of biological organization

organizational level under study [8, 9]. For instance, it can be performed before/after a treatment process and/or at different point times in order (1) to estimate the toxicity of a flux as a whole for research or compliance purposes and (2) to investigate the effectiveness of mitigation measures.

To this end, this chapter aims at:

- Providing an up-to-date compilation of the most widely applied bioassays and their endpoints for the assessment of effects of wastewater in general and pharmaceuticals present in wastewater
- Bridging the gap between chemical and biological assessment by extracting knowledge from relevant studies
- Identifying the usefulness and limitations of current practices of assessing effects of treated wastewater when the target contaminants of emerging concern (CEC) are pharmaceuticals

2 Toxicity Testing Strategy for the Assessment of Wastewater and Contaminants

Scrutinizing possible adverse effects of treated wastewater and its contaminants is one of the prerequisites to increase trust, credibility, and confidence in favor of wastewater reuse, the overall objective being the protection of human and ecological health. The incorporation of treated wastewater in the water cycle includes all types of its direct or indirect use. The amount of treated wastewater can be substantial, and its utilization may moderate the ongoing water demand of most

developed and developing countries. Common issues that need to be tackled in order to increase the amount of wastewater reuse have been successfully identified by Bixio et al. [10]. They consist of measures toward (1) reorientation of the water governance, (2) strengthening cooperation among stakeholders, (3) establishing guidelines and criteria for wastewater reclamation and reuse, (4) providing economic benefits from wastewater reuse, and (5) building trust and confidence.

In order to benefit from any wastewater reuse scheme, it is necessary to develop effect-directed bioassays able to pinpoint even subtle changes at any organizational level. Taking into consideration the fate of CEC during wastewater treatment, a battery assay with endpoints for chronic toxicity sensible even at ng/L should be developed.

The assessment of adverse effects of complex matrices such as effluents of sewage treatment plants is a significant application of the ecotoxicological testing. To this end, the whole effluent toxicity testing strategy was developed since the early 1990s by the USEPA with the main objective that "discharge of toxic pollutants in toxic amounts is prohibited" [11]. The term "whole effluent toxicity" (WET) refers to assessing effects on whole organisms toward broad endpoints such as mortality, growth inhibition, and reproduction impairment. The WET approach entails various bioassays for acute and chronic toxicity determination and was formalized by the USEPA since 1985 [12]. The basic step is to test the effluents in their initial conditions without any treatment and dilution. Other terms used worldwide are "whole effluent assessment" (Europe), "direct toxicity assessment" (DTA-Australia, New Zealand, the UK), "effluent toxicity testing" (Canada), "whole effluent environmental risk" (Denmark), and "integrating controlling of effluents" (Germany) [13]. Even though the WET approach was developed for the assessment of effects of wastewater, it can be applied to practically all aquatic and terrestrial samples (groundwater, wastewater, drinking water, sediments, soils, etc.). A review regarding the legislative requirements for WET in various countries has already been published [13]. The WET approach has been included in the legislation of various countries as a tool for assessment of effects of real matrices and environmental protection. For instance, in 1988, Environment Canada undertook a 5-year study to quantify and regulate toxicity of industrial effluents discharged into the St. Lawrence River [14]. Yi et al. [15] pointed out that the Korean Ministry of Environment announced that a new standard protocol and legislation using *D. magna* acute toxicity tests would be gradually implemented from 2011 onward to regulate wastewater effluent. For discharging effluents from sewage treatment plants, the new legislation states that the toxic unit (TU) of 24 h should be less than 1. However, at European scale, there are no standard toxicity tests yet or defined limits for the monitoring of effluents with the exception of Italy (DLgs152/2006, use of *D. magna*) [16] and Cyprus (Law 106 (I)/2002 use of *D. magna*, *P. subcapitata*, *V. fischeri*) [17].

Municipal sewage treatment plants usually receive high loads of effluents of temporal variable qualitative characteristics from different origins such as industrial, hospital, touristic, and commercial human activities. An increasing number of contaminants exist in the urban flows that are suspected or already proved to be able

Table 1 Bridging chemical and biological assessment of wastewater

Sampling information	Phase I	Phase II (target)	Phase III	Toxicity reduction evaluation (TRE)	Toxicity test applied	Identified contaminants	Reference
Grab influent and effluent of industrial wastewater (piggeries)	pH adjustment	Graded methanol elution	Mass balance	γ-ray treatment	*D. magna* 48-h immobilization test	Cr(VI)	[22]
	pH adjustment/ aeration	IC (anions)	Spiking approaches	γ-ray treatment + O_3		*o*-Toluidine (influent only)	
	pH adjustment/ filtration	GC/MS (nonpolar organics)		Coagulation + γ-ray treatment (influent only)		Anionic organic and inorganic chemicals (effluent only)	
	pH adjustment/SPE	ICP/AES (metals)					
	Graduated pH						
	EDTA chelation						
	Oxidant reduction manipulations						
	Ion exchange manipulations						
Grab effluent of industrial wastewater (tank truck)	Activated carbon	Quantification of nitrates, nitrites, orthophosphates, sulfates			*P. subcapitata* 72-h algal growth inhibition test	Acetochlor	[23]
	EDTA chelation	ICP/MS			*D. magna* 48-h immobilization test		
		ELISA kit (acetochlor and acetanilide)			*V. fischeri* 30-min bioluminescence inhibition test		
					30-min bacterial nitrification inhibition test		

Grab effluent of municipal and industrial wastewater (dyeing and textile, pulp and paper mills, electronic and electroplate factories, chemical factories)	pH, conductivity, DO, ammonium anions	ICP/MS (metals)			*C. dubia* 48-h immobilization test	4-nonylphenol, 4-nonylphenol-ethoxylate, phthalates in the textile and dyeing industry. 4-Nonylphenol, bisphenol A, phthalates, and sterol derivatives in the paper and pulp industry. Metals in the electronic and electroplate factories	[24]
	Aeration	GC-NCI-MS (endocrine disruptors)			*D. magna* 48-h immobilization test		
	EDTA addition	GC/MS (PAH, PCDD/F, PCB, PBDE)			*L. minor* 7-day growth inhibition test		
	Sodium thiosulfate addition				*D. rerio* 96-h lethality test		
	Filtration and EDTA addition				*P. subcapitata* 72-h growth inhibition test		
	Filtration and sodium thiosulfate addition				Recombinant *E. coli* luminescence inhibition test (5 and 15 min)		
	SPE and EDTA addition						
	SPE and sodium thiosulfate						
Grab influent	Fractionation SPE				*O. mykiss* hepatocytes for EROD activity (biomarker for the aryl-hydrocarbon (Ah) - receptor-mediated toxicity, 48 h), vitellogenin production (estrogenicity), and cytotoxicity (48 +48 h)	17b-estradiol, estriol, alkylphenols, benzophenone and methylparaben as estrogen receptor agonists. Polycyclic aromatic hydrocarbons (PAHs), alkyl-substituted PAHs, nitro-polycyclic aromatic compounds (nitro-PACs), carbazoles and alkyl-substituted carbazoles for EROD activity	[25]
	Reverse-phase HPLC						
	GC/MS						

(continued)

Table 1 (continued)

Sampling information	Phase I	Phase II (target)	Phase III	Toxicity reduction evaluation (TRE)	Toxicity test applied	Identified contaminants	Reference
24-h composite influent and effluent samples	Fractionation SPE				PLHC-1 cell cytotoxicity (72 h), EROD activity, and Pgp transport activity (60 min)	Polar compounds	[26]
	GC/MS				*D. subspicatus* growth inhibition test (72 h)		
	LC/Q-TOF				*S. typhimurium* genotoxicity test (48 h)		
					Yeast estrogen assay (72 h)		
24-h composite effluent of industrial and municipal wastewater (pulp and paper mill, pharmaceutical, enzyme production, oil refinery, polyester production plant, and a steel factory)	EDTA addition				Reverse electron-transport test	Metals	[27]
	Sodium thiosulfate addition				*V. fischeri* 30-min bioluminescence inhibition test	Organic substances	
	pH gradient				*P. subcapitata* 72-h growth inhibition test	Ammonia	
	pH adjustment				*D. magna* 48-h immobilization test		
	pH adjustment/aeration				*Allium cepa* root-elongation test (6 days)		
	pH adjustment/-filtration/SPE				Genetically modified *S. typhimurium* TA 104 recN2-4 strain		

to exhibit various adverse effects once released in the environment. It should be noted that some of these contaminants not only pass through the treatment processes without being removed completely, but also many of their products of incomplete degradation, i.e., transformation during biological and chemical treatment (e.g., nonylphenol, nonylphenol carboxylates [18], and treatment processes [19, 20]), may as well exhibit adverse effects.

The importance of using both chemical analyses and toxicity tests for the characterization and control of effluents of sewage treatment plants in the framework of water quality programs is widely accepted nowadays [21]. Compared to chemical analysis alone, the WET programs have advantages in that they assess the potential biological effects of the chemicals present in wastewater, as shown in Table 1. The WET approach has led to the identification of detrimental effects in the environment of CEC such as insecticides, surfactants, and treatment polymers [28, 29].

The "toxicity identification evaluation" seeks to identify contaminants (i.e., substances with unknown effects) that can be also considered pollutants (i.e., substances with known effects). In various cases, USEPA has documented that the toxicity of effluents toward freshwater, estuarine, and marine species correlates well with ecotoxicological measurements in the receiving water when effluent dilution is taken into account [21]. It should be noted though, that there are still a lot of unanswered questions when trying to correlate the biological effects of complex matrices with the chemical analysis. For example, identifying the exact compound present in a complex matrix like wastewater that causes an effect is not an easy task [30].

3 Bioassays Applied for the Assessment of Effects of Wastewater and Pharmaceuticals

The OECD and other legal entities have adopted guidelines for the testing of chemicals. Tests include the assessment of the effects to aquatic ecosystems (algae, water flea, and fish), terrestrial ecosystems (terrestrial plants, earthworms, avian), and technical systems such as treatment processes (activated sludge, respiration inhibition tests). A summary of the most common species used for the assessment of effects of treated wastewater and in studies assessing the effects of pharmaceuticals is provided in Table 2.

Pharmaceuticals represent a group of contaminants with significant chemical heterogeneity. At the same time, this group consists of compounds intentionally designed to have biological potency. Pharmaceuticals are known to be present at ng–μg/L in secondary and tertiary treated wastewater [31–36]. It is notable that several publications have been devoted to the toxicity assessment of pharmaceuticals in various model matrices (e.g., simulated wastewater, surface water, etc.) with the main focus, however, on ultrapure water. Since wastewater reuse is a strategy that is gaining wider acceptance and rapidly expanding, it is imperative to perform integrated toxicity assays in real effluents which contain all contaminants and their transformation products.

Table 2 Bioassays used for toxicity evaluation of wastewater and pharmaceutical compounds

Phylum (class)	Species	Common name	Exposure time	Endpoint
Annelida	*Eisenia fetida/andrei*	Red worm	14 days	Reproduction
Arthropoda (Branchiopoda)	*Artemia salina*	Brine shrimp	24 or 48 h	Immobilization
	Ceriodaphnia dubia	Water flea	48 h, 6 days	Immobilization
				Reproduction
	Daphnia magna	Water flea	24 or 48 h	Immobilization
			10 or 21 days	Immobilization/ reproduction
	Daphnia pulex	Water flea	24 h	Immobilization
	Moina macrocopa	–	7 days	Reproduction
	Streptocephalus proboscideus	–	24 h	Immobilization
	Thamnocephalus platyurus	Beavertail fairy shrimp	24 h	Mortality
Arthropoda (Arachnida)	*Hypoaspis aculeifer*	Mite	14 days	Reproduction
Arthropoda (Collembola)	*Folsomia candida*	Springtail	14 days	Reproduction
Arthropoda (Malacostraca)	*Gammarus pulex*	Freshwater shrimp	1.5 h	Activity
	Hyalella azteca	Lawn shrimp	14 days	Reproduction
				Biomarkers of oxidative stress
				Mortality
	Hydra vulgaris	Common brown hydra	96 h	Morphology and feeding behavior
			7 days	
Bacillariophyta	*Cyclotella meneghiniana*	Diatom	96 h	Growth
Bacteria	*Bacillus stearothermophilus*	–	3 h	Spore germination
	Blastomonas natatoria	–	24 h	Growth
	Legionella pneumophila	–	16 h	Growth
	Micrococcus luteus	–	24 h	Growth
	Pseudomonas aeruginosa	–	16 h	Growth
	Pseudomonas putida	–	16 h	Growth
	Staphylococcus aureus	–	24 h	Growth
	Vibrio fischeri	Luminescent bacteria	5, 15 or 30 min	Growth
			24 h	

(continued)

Table 2 (continued)

Phylum (class)	Species	Common name	Exposure time	Endpoint
Basidiomycota	*Ganoderma lucidum*	Bracket fungus	7 days	Biodegradation
	Irpex lacteus	Milk-white toothed polypore	7 days	Biodegradation
	Phanerochaete chrysosporium	–	7 days	Biodegradation
	Trametes versicolor	–	7 days	Biodegradation
Chlorophyta	*Chlorella vulgaris*	Green alga	48 h	Growth
	Desmodesmus subspicatus	Pond scum, green weed	96 h	Growth
			24 h	Photosynthesis rate
	Dunaliella tertiolecta	Green alga	72 h	Growth
	Pseudokirchneriella subcapitata	–	72 or 96 h	Growth
Chordata (Actinopterygii)	*Danio rerio*	Zebrafish	48, 72 or 96 h	Egg and embryo mortality
				Hatching success
				Morphology
				Behavior
				Development
	Oncorhynchus mykiss	Rainbow trout	28 days	Structural changes
	Oreochromis niloticus	Tilapia	48 h	Genotoxicity
			10 day	
	Oryzias latipes	Japanese medaka	14 days	Growth
			28 days	Reproduction
	Pimephales notatus	Bluntnose minnow	48 h	Biomarker
	Pimephales promelas	Fathead minnow	4 days	Hatching
				Survival
	Salmo salar	Atlantic salmon	5 days	Gene expression
	Salmo trutta	Brown trout	21 days	Histopathological alterations
Chordata (Amphibia)	*Xenopus laevis*	African clawed frog	96 h	Morphology
Cyanophyta	*Synechococcus leopoliensis*	–	96 h	Growth
	Synechocystis sp.	–	72 h	Growth
				Biomarkers of photosynthesis

(continued)

Table 2 (continued)

Phylum (class)	Species	Common name	Exposure time	Endpoint
Mollusca	*Dreissena polymorpha*	Zebra mussel	96 h	Oxidative biomarkers
			7 days	Cytotoxicity
				Bioconcentration
	Mytilus edulis	Baltic blue mussel	21 days	Bioconcentration
				Growth
				Byssus strength
				Mortality
	Mytilus galloprovincialis	Mediterranean mussel	7 days	Cell signaling
	Planorbis carinatus	–	72 h	Mortality
			21 days	Growth
				Mortality
				Hatching success
	Potamopyrgus antipodarum	New Zealand mud snail	42 days	Growth
				Reproduction
Rotifera	*Brachionus calyciflorus*	Marine rotifer	24 h	Immobilization
			48 h	
			7 days	
Streptophyta	*Lactuca sativa*	Lettuce	14 days	Emergence
				Biomass
	Lemna gibba	Swollen duckweed	7 days	Weight
				Frond number
				Chlorophyll a
	Lemna minor	Common duckweed	7 days	Reproduction of fronds
	Lepidium sativum	Garden cress	72 h	Emergence
				Growth
Tracheophyta	*Brassica napus*	Rape	14 days	Emergence
				Biomass
	Triticum aestivum	Spelt wheat	14 days	Emergence
				Biomass
	Vicia sativa	Vetch	14 days	Emergence
				Biomass

A mini-review of the biological effects toward pharmaceuticals is presented below. A selection of eight compounds belonging to the β-blockers (atenolol, metoprolol, and propranolol), nonsteroidal anti-inflammatory drugs (diclofenac and ibuprofen), and antibiotics (erythromycin, ofloxacin, and sulfamethoxazole) was made. Their widespread use, their existence, and in some cases their persistence in the environment along with substantial literature were among the criteria for their selection as examples. The bioassays, endpoints, and effective

concentrations of each trophic level, producers, consumers, and decomposers are discussed. It should be noted that the vast majority of studies investigated the species organizational level. When applicable, other organizational levels such as molecular, cellular, etc. are also presented. Information is provided on the parent compounds and, if reported, on their transformation products as well.

3.1 β-Blockers: The Example of Atenolol, Metoprolol, and Propranolol

According to the published ecotoxicological studies available so far, propranolol exhibits higher acute toxicity than other β-blockers. This could be partly due to the relatively higher value of the pH-dependent octanol-water distribution coefficient (log D_{OW}) and the fact that propranolol is a strong membrane stabilizer [37].

Atenolol was not found to be toxic to microbial respiration at concentrations up to 100 mg/L and genotoxic using the umuC test. No mutagenicity was observed when the Ames test was applied to metoprolol [38]. Although metoprolol is considered as non-readily biodegradable [39], the biotransformation of its transformation products was accelerated under light conditions, implying that photo-induced intermediates could be more easily biodegraded in river water [40]. Growth was inhibited after exposing *P. putida*, *P. aeruginosa*, *M. luteus*, and *B. natatoria* at the highest concentration of propranolol tested (130 μg/L). In most cases the death rate increased; associated changes were observed in the metabolic fingerprints [41].

When tested to the algae *D. subspicatus*, atenolol almost failed to register a toxic effect with an EC_{50} value of 620 mg/L. Metoprolol and propranolol were found to be more toxic with EC_{50} values of 7.9 and 7.7 mg/L, respectively. In the same study, *L. minor* was not affected to concentrations of metoprolol up to 320 mg/L [42]. Propranolol was shown to have an EC_{50} value of 668 μg/L toward the blue-green alga *S. leopoliensis* after a 96-h exposure time, whereas the most sensitive organism within the phytoplankton was the diatom *C. meneghiniana* with an EC_{50} value of 244 μg/L [43]. Propranolol caused a specific photosynthesis inhibition after a 24-h exposure time of *D. subspicatus* with an EC_{50} value of 0.7 mg/L [44].

No effects were found to terrestrial organisms at concentrations of atenolol up to 1000 mg/kg. The tests included the evaluation of the emergence and the production of biomass of the plants *T. aestivum*, *B. napus*, and *V. sativa* after a 14-day exposure time, the reproduction of the springtail *F. candida*, the compost worm *E. fetida/andrei*, and the predatory mite *H. aculeifer*. In the same study, it was shown that atenolol was not toxic to the amphipod *H. azteca* and the snail *P. antipodarum* to the highest concentrations tested (~10 mg/L) after 14- and 42-day exposure times, respectively [45].

At the same range with the terrestrial organisms, an effect was observed only with the *Daphnia* reproduction test, in which the mortality of the offspring of the second generation (F_2) increased [45]. The cnidarian *H. vulgaris* showed similar effects at the same concentrations after a 7-day exposure time [46].

The acute toxicity of metoprolol after an exposure time of 48 h was found to be 8.8 mg/L for *C. dubia* and higher than 100 mg/L for both *H. azteca* and *O. latipes* [47]. The chronic effects after an exposure time of 28 days indicated that at concentration of 1 μg/L, ultrastructural changes occurred to the liver and kidney of the rainbow trout *O. mykiss* and even to the gills, if exposed at 20 μg/L metoprolol [48].

The EC_{50} value of propranolol toward the rotifer *B. calyciflorus* was 2.59 mg/L after an acute 24-h exposure time and 1.9 mg/L toward the crustacean *S. proboscideus* [49]. For the same exposure time, propranolol was found to have an EC_{50} value of 3.8 mg/L toward *D. pulex*. Following a 48-h exposure time to propranolol, LC_{50} values of 29.8 and 0.8 mg/L were obtained, whereas reproduction decreased with NOEC values of 1 and 125 μg/L for *H. azteca* and *C. dubia*, respectively [47].

Atenolol at the highest concentration tested (100 mg/L) was not found to cause any effects when cytotoxicity on hemocytes, gill, and digestive gland primary cell cultures of the zebra mussel *D. polymorpha* was investigated [50]. The most sensible organism for atenolol was found to be *P. promelas* with a 4-day NOEC for hatching and survival of 10 mg/L and a 28-day NOEC for growth of 3.2 mg/L [51]. Furthermore, it was found to produce differences in the expression of 480 candidate genes of the Atlantic salmon *S. salar* when exposed for 5 days at 11.1 ± 8 μg/L. The effects and bioconcentration of metoprolol on the mussel *D. polymorpha* after an exposure time of 7 days were investigated at concentrations in the range of 0.5–534 μg/L. Gene expression in gills and the digestive gland at higher concentrations was altered and a 20-fold bioconcentration at low concentrations was observed, even though metoprolol is water soluble.

From a 2-week study, it was observed that exposure to 500 μg/L of propranolol was able to reduce growth rates of the Japanese medaka *O. latipes* [47]. Propranolol was found to bioconcentrate in the Baltic Sea blue mussels *M. edulis* even at 1 μg/L when exposed for 3 weeks. Furthermore, a significantly lower scope for growth was observed when exposed to 1–10 mg/L, which indicated that the organisms had a smaller part of their energy available for normal metabolism, and secondly, they had lower byssus strength and lower abundance of byssus threads, resulting in reduced ability to attach to the underlying substrate. Higher mortality was observed at these concentrations, whereas lower concentrations (1–100 μg/L) tended to differ from the controls [52].

A subchronic test of 7 days with propranolol to the Mediterranean mussel *M. galloprovincialis* demonstrated that propranolol at concentrations of 0.3 ng/L was able to affect cell signaling and interacted with specific and evolutionally conserved biochemical pathways. It also induced a stress response and affected its physiology by interacting with the same molecular targets as in humans [53]. According to Solé et al. [54] an exposure time of 10 days to the same species provoked a decrease in the feeding rate with an NOEC value of 11 μg/L and an LOEC of 147 μg/L. These concentrations caused a decrease of acetylcholinesterase activity and an increase of the carboxylesterase and glutathione-*S*-transferase activity in gills. An increase in the lipid peroxidation levels in gills and a decrease

of the glutathione-*S*-transferase activity in the digestive gland were also observed. The LC_{50} of survival after a 24-h acute exposure time was 10.3 mg/L for *T. platyurus*.

Measurement of ethoxyresorufin-*O*-deethylase (EROD) activity as a biomarker for CYP1A activity was used to investigate propranolol effects on the rainbow trout *O. mykiss*. It was found to provoke an increase in EROD activity in the liver and gill at 200 μg/L, in both in vivo (albeit nonsignificantly in the liver) and in vitro, thus supporting the use of the latter as a surrogate of the former [55]. The in vitro EROD induction was previously reported by Laville et al. at concentrations of 8 mg/L [56].

A 96-h exposure time of *O. latipes* toward propranolol had an LC_{50} of 11.4 mg/L [57] and a 48-h exposure time an LC_{50} of 24.3 mg/L [47]. When the fish juvenile growth test was applied to *O. mykiss*, the LOEC of propranolol was 10 mg/L and the NOEC 1 mg/L [58]. Furthermore, as *O. mykiss* is considered to have many additional β-receptor subtypes for different physiological functions, propranolol has the potential to cross over into non-cardiovascular systems such as homeostasis, immunocompetence [59], and O_2 chemoreceptor activity [60].

According to Huggett et al. [47], a 2-week exposure time to 500 μg/L propranolol reduced growth rates and a 4-week exposure time to 0.5 μg/L decreased fecundity of *O. latipes* with a decrease in the total number of eggs produced and the number of viable hatching eggs. Regarding *P. promelas*, a 3-day exposure time to 3.4 mg/L propranolol caused 100% mortality or severe toxic effects that required euthanasia. The most sensitive endpoints in the study though were the hatchability and the female gonadal somatic index with an LOEC of 0.1 mg/L. Furthermore, plasma concentrations of propranolol in male fish exposed to concentrations of 0.1 and 1 mg/L were 0.3 and 15 mg/L, respectively, which constitutes 436 and 1,546% of measured water concentrations [61].

Atenolol was found to create toxic by-products to the dicotyledonous *L. sativa* when chlorinated in an aqueous solution [62]. The study tried to simulate common wastewater disinfection procedures. However, the tested concentrations used were higher than those usually present in effluents of sewage treatment plants. Possible bioaccumulation caused by continual irrigations was not assessed and the dangers this may enclose should not be neglected.

A peculiarity has been reported regarding the toxicity of propranolol enantiomers on *D. magna* [63]. The immobilization percentages at 24 h of both enantiomers were similar with S-enantiomer being slightly more toxic than the R-enantiomer with EC_{50} values of 1.4 and 1.6 mg/L, respectively. When the enantiomers were examined for their chronic effects (21 days), the R-enantiomer was found to be more toxic than the S-enantiomer regarding immobilization. Furthermore, with regard to the reproduction rate, an increase of the total number of neonates was observed for both enantiomers and a decrease on the number of neonates when exposed to 869 μg/L of R-enantiomer.

Regarding the effects for humans, atenolol has been found not to cause DNA damage (DNA strand brakes) at concentrations of 7,990 μg/L, whereas it was found to cause long-term carcinogenic effects to both male and female rats when they were exposed at 500 mg/kg/day [64]. In the same review metoprolol is reported not

to cause effects on DNA strand breaks. In the long-term carcinogenesis assay, no effects were observed when performed on male mice, CD-1 mice, and rats at concentrations up to 750 mg/kg/day, whereas when evaluated on female mice at the same concentrations, lung adenomas were detected.

Metoprolol did not show significant genotoxic effects using the micronucleus test [38]. In a recent study, atenolol has been found to cause chromosome loss detected as micronuclei in the peripheral lymphocytes of treated patients with chromosomes 7, 11, 17, and X being preferentially present in the micronuclei [65].

Propranolol was found to cause DNA strand breaks to rat primary hepatocytes at concentrations of 7,880 μg/L [64]. Its photo-transformation products did not exhibit any acute toxicity in mice or significant binding to β-adrenergic receptors using rat cerebellum cortex membranes and their binding to β-adrenergic receptors [66].

To sum up, the following main findings are listed:

- The lowest concentration able to demonstrate an effect was at the very low ng/L level of propranolol.
- Cell signaling and conserved biochemical pathways of *M. galloprovincialis* were found to be very sensitive endpoints to assess the effect of propranolol.
- A substantial exposure period of greater than 21 days is needed to identify effects toward mussels and fish at low μg/L concentrations for β-blockers.
- Disinfection techniques such as chlorination applied on wastewater treatment may create more toxic transformation products for these compounds.

3.2 Nonsteroidal Anti-inflammatory Drugs: The Example of Diclofenac and Ibuprofen

Diclofenac was found to inhibit the growth of the marine phytoplankton *D. tertiolecta* at concentrations of 25 mg/L and above [67]. It was shown to have an acute toxicity of 224.3 mg/L toward *C. dubia* when exposed for 48 h. Furthermore, it demonstrated sublethal effects at 25 mg/L toward *B. calyciflorus* exposed for 48 h, 2 mg/L toward *C. dubia* exposed for 7 days, and 8 mg/L toward *D. rerio* exposed for 10 days [68, 69].

Biomarkers of oxidative stress in *H. azteca* such as lipid peroxidation, protein carbonyl content to evaluate oxidized protein content, and the activity of superoxide dismutase, catalase, and glutathione peroxidase were significantly altered by the exposure of diclofenac to a concentration of 46.7 μg/kg. The LC_{50} value was much higher (0.5 mg/kg) [70]. In chronic toxicity tests of the reproduction of *D. magna*, the LOEC of diclofenac was found to be 0.2 mg/L [71]. Diclofenac inhibited the growth of the marine phytoplankton *D. tertiolecta* at concentrations of 25 mg/L and above [67].

No effects were observed in a study using eight biomarkers of the freshwater bivalve *D. polymorpha* when exposed for 96 h to concentrations up to 592 ng/L diclofenac [72]. Another biomarker, lipid peroxidation, was found to be affected at

concentration of 1 μg/L in *D. polymorpha* exposed for 96 h [73]. The previous biomarker was sensitive for blue mussels *Mytilus* spp. when exposed for 96 h at the same concentrations 1 μg/L [74]. Ibuprofen was found to inhibit *D. subspicatus* algal growth with an EC_{50} value of 342 mg/L after an exposure time of 96 h [75], whereas it inhibited the duckweed *L. minor* growth causing an EC_{50} value of 22 mg/L after an exposure time of 7 days [76]. Ibuprofen in a different study was found to have an EC_{50} value of 4 mg/L toward *L. minor* [77].

When *D. magna* was exposed to 40 and 80 mg/L of ibuprofen for 10 days, the number of offspring reduced significantly. Interestingly, when a recovery period of 10 days followed the exposure period, ibuprofen-stressed daphnids produced offspring faster and by the end of the experiment the average growth was comparable with control populations. This suggested that daphnids were susceptible during egg maturation [78].

Detrimental effects were observed for exposure time of 21 days at 1 μg/L diclofenac toward the rainbow trout *O. mykiss* to which induced tubular necrosis in the kidney, hyperplasia, and fusion of the villi in the intestine were observed. Furthermore, the expression levels of cyclooxygenase (COX) in the liver, gills, and kidney were significantly reduced, and it was found that diclofenac was able to bioaccumulate in the bile by a factor of 509–657 [79]. A 28-day exposure time of diclofenac resulted in renal lesions and alterations of the gills at concentrations of 5 μg/L and bioconcentration of 12–2,732 in the liver, 5–971 in the kidney, 3–763 in the gills, and 0.3–69 in the muscle, respectively [71]. Furthermore, cytopathology effects in the liver, kidney, and gills were observed at concentrations of 1 μg/L diclofenac [48].

Effects on the gene expression profile of *O. mykiss* were found at concentrations of 1.6 μg/L and the bioconcentration factor was found to be 4.02 ± 0.75 for the blood plasma and 2.54 ± 0.36 for the liver for diclofenac [80]. Laville et al. [56] demonstrated that diclofenac was cytotoxic to the PLHC-1 cell line with an EC_{50} value of 5.6 mg/L and estrogenic to a primary rainbow trout hepatocytes cell line with an EC_{50} value of 18.6 mg/L.

The effects of diclofenac to the brown trout *S. trutta* were observed at concentrations of 0.5 μg/L. At 7 and 14 days, the hematocrit levels were affected, whereas after 21 days histopathological alterations were observed in the liver, gills, and kidney. Moreover, diclofenac was able to hinder the stimulation of prostaglandin E_2 synthesis in head kidney macrophages in vitro [81]. Biomarkers of cellular toxicity (cytochrome P450 1A gene), p53-related genotoxicity (p53 gene), and estrogenicity (vitellogenin gene) were overexpressed in *O. latipes* after a 4-day exposure time to 1 μg/L diclofenac [82]. Nanoinjection of diclofenac resulted in a decrease of the survival of injected embryos of *O. latipes* at hatching with an EC_{50} value of 6 ng/egg [83]. At 1 mg/L acute effects on the feeding behavior (time to eat midge larvae) of *O. latipes* were monitored [84].

A decrease of reproduction was observed at 25 mg/L diclofenac for *D. magna* after a 21-day exposure time and at 50 mg/L for *M. macrocopa* after a 7-day exposure time. Furthermore, a 3-month exposure time of fish to 0.001–10 mg/L of diclofenac caused a lower hatching success and a delay in hatch [85]. Diclofenac

did not cause any effects to early-life stages of *D. rerio*. The parameters investigated were egg and embryo mortality, gastrulation, somite formation, movement and tail detachment, pigmentation, heartbeat, and hatching success after 48–96-h exposure times to up to 2,000 μg/L [86]. In another study of *D. rerio*, specific effects were observed for hatching, yolk sac, and tail deformation at concentrations above 1.5 mg/L when exposed for 72 h [87].

Ibuprofen was reported to have an LC_{50} of 19.6 mg/L toward *T. platyurus* after an acute 24-h exposure time and an LC_{50} of >100 mg/L toward *O. latipes* after a 96-h exposure time [57]. Ibuprofen had no effect on the oxidation rate of nicotinamide adenine dinucleotide phosphate (NADPH) and lipid peroxidation when *O. mykiss* hepatocytes were exposed for 60 min at concentration of 100 μM [88]. *O. latipes* demonstrated an alteration of the spawning behavior when exposed to 0.1 μg/L ibuprofen for 42 days, indicating that a different reproduction pattern was developed [89]. A delay in egg hatching was also observed when *O. latipes* was exposed for 120 days to concentrations of 0.1 μg/L [90]. *O. mykiss* fry were exposed to ibuprofen solutions for 4 days. Even at 1 μg/L the heat shock protein70 was induced in the trout liver [91].

At 1 mg/L, ibuprofen was shown to disturb the seawater-induced elevation in plasma osmolality and concentrations of Cl^- and K^+. This was accompanied by enhanced gill glycolytic capacity and reduced liver glycogen content suggesting enhanced metabolic demand to fuel ion pumps induced elevation in gill Na^+/K^+-ATPase activity [92]. After a 48-h exposure time of *P. notatus* to 50 μg/L and 100 μg/L, a significant reduction (30% and 80%, respectively) of the prostaglandin E_2 (PGE_2) concentration of gill tissue was observed [93]. The results from daily observations of *D. rerio* for a total period of 7 days indicated that developing embryos tolerated lower (1 and 5 μg/L) doses of the ibuprofen readily, but exposure to higher doses (>10 μg/L) caused retarded development, decreased hatching rate and growth, cardiac anomalies, spinal curvature, pectoral fin malformation, and behavioral alterations resulting in higher mortality of experimental embryos [94].

Ibuprofen was found to have an EC_{50} value of 22.4 mg/L when exposed for 96 h to the cnidarian *H. vulgaris*, whereas its morphology and feeding behavior was affected when exposed at 1.65 and 3.9 mg/L, respectively [95]. The most sensitive of the cnidarians was found to be *H. vulgaris* with an effect on feeding behavior when exposed for 7 days and an LOEC for ibuprofen of 10 μg/L [46]. Ibuprofen had an EC_{50} value of 72.6 mg/L when the immobilization of the cladoceran *M. macrocopa* was monitored for 48 h and an NOEC of 25 mg/L when the reproduction was assessed after a 7-day exposure time [90].

Ibuprofen was found to increase the frequency of micronuclei to the *O. niloticus* fish (tilapia) at 300 ng/L in both acute (48-h) and subchronic (10-day) exposure times, hence inducing genotoxicity potential [96]. *X. laevis* was investigated and an EC_{10} of 30.7 mg/L was calculated for a 96-h exposure time when deformity was investigated as an endpoint [97].

The behavior of the amphipod *G. pulex* was found to be affected by ibuprofen by quantifying its movements using a multispecies freshwater biomonitor in a test chamber. In particular, exposure to low concentrations (10–100 ng/L) resulted in a

significant decrease in activity, whereas the activity of *G. pulex* at higher concentrations (1 μg/L to 1 mg/L) was similar to the control [98]. Ibuprofen had an LC_{50} of 17.1 mg/L toward the mollusk *P. carinatus* when its survival was monitored for 72 h and an NOEC of 1, 2.4, and 5.4 mg/L when exposed for 21 days when the wet weight, the hatching success, and the survival were monitored, respectively [99]. Exposure of *M. edulis* to 10 mg/L resulted in lower byssus strengths and byssus thread abundance compared to the control treatment when exposed for 3 and 2 weeks, respectively. The scope of growth of this organism was influenced at lower concentrations when exposed to 1 mg/L for 2 weeks [52]. The effects of ibuprofen at 0.2–8 μg/L were addressed to the bivalve *D. polymorpha* exposed for 96 h, which demonstrated a slight cytogenotoxic effect on the mussel hemocytes at the lowest concentration tested, whereas higher concentrations tested were able to significantly increase both genetic and cellular damage [100]. In addition, ibuprofen was suggested to have a considerable effect on the activities of antioxidant and detoxifying enzymes due to the notable oxidative status imbalances of the exposed specimens.

The nature of the bacterial community on a river biofilm was influenced by diclofenac at 10 and 100 μg/L [101]. Lotic biofilms (bacteria and algae) were found to be negatively affected when exposed to diclofenac at 100 μg/L for a 5-day exposure time [102]. The effects of 10 μg/L ibuprofen to a riverine microbial community were monitored for 8 weeks indicating a toxic effect. *Cyanobacteria* were suppressed and bacterial biomass was reduced. The live-dead ratio was affected by the exposure [103]. Ibuprofen was not able to inhibit a number of endpoints monitored in *L. gibba* after a 7-day exposure time [104]. An older study indicated that ibuprofen had antibacterial activity suppressing the growth of *S. aureus* when exposed to 150 mg/L [105]. Ibuprofen at 10 mg/L was able to be biodegraded by the rot-white fungi *T. versicolor*, *I. lacteus*, *G. lucidum*, and *P. chrysosporium* after an exposure time of 7 days [106].

Recently diclofenac has been qualitatively detected in the hair of Eurasian otters *Lutra lutra* indicating that wildlife is being exposed to this compound [107]. The most severe adverse effects of diclofenac though were found in three species of vultures the Indian white-rumped one (*Gyps bengalensis*), the Indian one (*Gyps indicus*), and the slender-billed one (*Gyps tenuirostris*) in India and Pakistan causing a population decline [108, 109]. Renal failure and visceral gout were observed due to their scavenging behavior feeding on carcasses of domestic cattle treated with diclofenac [110]. The LOEC causing renal failure was 0.007 mg/kg. Adverse effects on the same concentrations were found for the African vultures *Gyps coprotheres* [111].

The effects of diclofenac on four avian species: broiler chicks (*Gallus gallus*, 15 days old), pigeons (*Columba livia*, 3 months old), Japanese quail (*Coturnix japonica*, 4 weeks old), and myna (*Acridotheres tristis*, independent young) when exposed to concentrations 0.3, 2.5, 10, and 20 mg/kg body weight for 7 days were depression, somnolence, decreased body weight, and mortality. Serum creatinine levels were elevated and kidneys and livers were enlarged. Histologically, the kidneys showed acute renal necrosis and the livers had fatty change and necrosis of hepatocytes. The kidneys and livers of broiler chicks and pigeons given 10 and 20 mg/kg diclofenac exhibited uric acid crystal aggregates (tophi) and associated

lesions in the parenchyma [112]. When turkey vultures *Cathartes aura* were exposed to diclofenac, no signs of toxicity, visceral gout, renal necrosis, or elevate plasma uric acid were observed at concentrations greater than 100 times the estimated median lethal dose reported for *Gyps* vultures, showing a different sensitivity among avian species [113].

Diclofenac was not found to cause bacterial mutation, cytogenotoxicity in vitro and in vivo, gene mutation in the mouse lymphoma cells, and carcinogenicity on mouse at concentrations of 0.02–0.04× the high animal dose (mg/m^2/maximum recommended human dose) and on rats at concentrations up to 0.09×. Furthermore, no cell transformation and no effect on the dominant lethal assay were observed [114]. Ibuprofen was reported to be non-mutagenic using the Ames mutagenicity assay (in strains TA97a, TA100, and TA102) and weak genotoxic when using the in vivo genotoxicity test of sister-chromatid exchange in bone marrow cells of mice [115].

As a conclusion, the following remarks should be made:

- Detrimental effects have been reported at low μg/L concentrations for both diclofenac and ibuprofen.
- Acute and chronic adverse effects were observed at low μg/L concentrations.
- The lowest concentration reported of ibuprofen able to cause an effect is 10 ng/L.
- The most sensitive endpoint was the quantification of movements of amphipods.

3.3 Antibiotics: The Examples of Erythromycin, Ofloxacin, and Sulfamethoxazole

Erythromycin has been found to inhibit the growth of the *Cyanobacteria Synechocystis* sp. by 70% when exposed for 5 days to 1 mg/L; *L. minor* was found to be inhibited by 20% when exposed for 7 days at the same concentrations [77]. *L. gibba* was not inhibited using a 7-day static renewal test at concentrations up to 1 mg/L [104]. Ofloxacin was found to be phytotoxic to *L. gibba* at μg/L when exposed for 7 days with an EC_{50} value of 532–1,374 μg/L, depending on the endpoint assessed [104]. *L. minor* when exposed to ofloxacin had an inhibition on the reproduction of fronds after a 7-day exposure time with an EC_{50} value of 126 μg/L. Sulfamethoxazole could inhibit *L. gibba* after a 7-day exposure time to seven endpoints evaluated (e.g. wet weight, frond number, chlorophyll a, chlorophyll b, carotenoids) with EC_{50} values ranging from 0.8 to 81 μg/L [104]. The concentration of *para*-aminobenzoic acid was found to increase when *L. gibba* was exposed to sulfamethoxazole suggesting a specific mode of action at concentrations of 100–1000 μg/L during the 7-day exposure time [116].

The effects of erythromycin to *P. subcapitata* have been recently studied using a biomarker battery that included photosynthetic rate, chlorophyll fluorescence, Hill reaction activity, photophosphorylation activity, and ribulose-1.5-bisphosphate carboxylase activity, and it was found to cause acute effects (96 h) at concentrations of 0.6 mg/L [117]. Levofloxacin was found to inhibit the O_2 evolution and the

photosystem II (PSII) activity of the *Synechocystis* sp. at concentrations of 0.1–10 mg/L after 12-h exposure time [118]. The spore germination of *B. stearothermophilus* was inhibited by a 3-h exposure time with an LOEC of 23 μg/L [119]. The growth of *C. meneghiniana* and *S. leopoliensis* was found to be inhibited when exposed for 96 h to sulfamethoxazole with an EC_{50} value of 2.4 and 26 ng/L, respectively [43]. Sulfamethoxazole was toxic to *C. vulgaris* when exposed for 48 h with an EC_{50} value of 6.2×10^{-3} mM [120].

Erythromycin was found to immobilize *B. calyciflorus* and *T. platyurus* exposed for 24 h and *C. dubia* exposed for 48 h with EC_{50} values of 27.53, 17.68, and 10.2 mg/L, respectively [121]. *D. rerio* was not killed when exposed for 96 h to concentrations up to 1000 mg/L [121]. No effects on the immobilization and morphology of adults and neonates, adult length, resting egg production, brood size (fecundity), and proportion of male broods produced (sex ratio) when *D. magna* was exposed to 6 and 30 days at concentrations of 1–100 μg/L [122]. The growth of *B. calyciflorus* at 48 h and the number of female rotifers of *C. dubia* at 7 days were affected with an EC_{50} value of 0.9 and 0.2 mg/L [121]. It has been found that erythromycin may affect the microbiological population in aquaculture by changing the bacterial composition, rather than the numbers of total viable aerobic bacteria or erythromycin-resistant bacteria at 25 mg/L [123]. Immobilization of *B. calyciflorus* and *T. platyurus* after 24-h and *C. dubia* after 48-h exposure time to sulfamethoxazole was observed with an EC_{50} value of 26.3, 35.4, and 15.5 mg/L, respectively. *D. rerio* was not affected when mortality was monitored after an exposure time of 96 h to 1000 mg/L. Chronic exposure times of 48 h of *B. calyciflorus* and 7 days of *C. dubia* had an EC_{50} value of 9.6 and 0.2 mg/L [121]. The morphology feeding response, hydranth number, and attachment of *H. vulgaris* were not found to be affected when exposed at 96 h at concentrations up to 100 mg/L of sulfamethoxazole [95].

Erythromycin was reported to cause membrane lysis of Gram-negative bacteria *L. pneumophila* with a minimum inhibitory concentration (MIC) of 0.5 mg/L when exposed for 16 h [124]. At subinhibitory doses of 1.5 mg/L, repression of lectin production in *P. aeruginosa* [125] and modification of the cell surface structure and hydrophobicity were observed [126]. Erythromycin was found to inhibit ammonification, nitritation, and nitratation at concentrations higher than 20 mg/L. It also affected heterotrophs, particularly filamentous bacteria by causing floc disintegration and breakage of filaments. Cell lysis was observed [127]. Adverse effects such as inhibition of the specific evolution rate of COD and $N–NH_4^+$ and destruction of flocs were observed in activated sludge when exposed at 10 mg/L erythromycin for 24 h [128].

Erythromycin was found to cause mortality to *A. salina* when exposed to 10 mg/L for 120 h [129]. The respiration inhibition test OECD 209 was applied to erythromycin for an exposure time of 20 h and the inhibition concentration 50% (IC_{50}) was greater than 100 mg/L [130]. Only part of the bacterial population of activated sludge was found to be affected by erythromycin with an EC_{50} value ranging between 39 and 43 mg/L [131]. Ofloxacin was found to immobilize *B. calyciflorus* and *T. platyurus* after an exposure time of 24 h and *C. dubia* after an exposure time of 48 h with an EC_{50} value of 29.9, 33.9, and 17.4 mg/L, respectively [121]. Lethality of

D. rerio at the maximum concentration tested (1,000 mg/L) did not increase markedly after an exposure time of 96 h [121]. Chronic exposure time of 48 h for *B. calyciflorus* in which growth was evaluated and 7 days for *C. dubia* in which the number of females was counted had an EC_{50} value of 0.5 and 3.1 mg/L, respectively. Ofloxacin was found to have genotoxic properties at concentrations of 1–2 μg/L present in hospital effluents [132].

No mutagenic effect was observed during the AMES test, to both the TA98 evaluating frameshift mutations and the TA100 monitoring base pair substitutions for erythromycin [121]. Erythromycin at 1 and 100 mg/L did not affect the methanogens of an anaerobic batch reactor and the biogas production, indicating that a substantial percentage of the population was resistant to erythromycin. The conversion of butyric acid though was inhibited when erythromycin was present, indicating that specific substrate degradation pathways can be affected [133]. *B. stearothermophilus* was inhibited to sulfamethoxazole when exposed for 3 h, and an LOEC of 132.5 μg/L to its spore germination was reported [119]. Sulfamethoxazole was found to be mutagenic using the AMES test at high concentrations 6.25 and 50 mg/L with the TA98 and TA100, respectively [121]. It was found to be unstable in anaerobic mesophilic digesters [134, 135]. Furthermore, it could inhibit the soil bacteria as means of leucine incorporation and endpoint for estimating pollution-induced community tolerance when exposed to 20 and 500 mg/kg for 30 days. An increase in the fungal and a decrease in the bacterial phospholipid fatty acids were observed [136].

Erythromycin was not able to produce an increase in the frequency of biomarkers as sister-chromatid exchanges or chromosomal aberrations in either the presence or absence of metabolic activation to Chinese hamster ovaries [137]. Ofloxacin was reported to display high activity not only against bacterial topoisomerases [138], but also against eukaryotic topoisomerases [139]. According to Li et al. [140] it could also induce oxidative stress, lipid peroxidation, and DNA oxidative damage to chondrocytes. Although ofloxacin is toxic to mammalian cells in culture, its mechanism of action is still not completely understood. A reason may be that quinolones bind cooperatively to DNA, perhaps as a consequence of π–π stacking of planar quinolone rings [141]. It should be mentioned that since the dosing period of ofloxacin is usually short, carcinogenicity studies are not always compulsory for its governmental approval. Sulfamethoxazole was found to be hepatotoxic and cause systemic hypersensitivity reactions [142]. However, the frequency of chromosomal aberrations in peripheral lymphocytes [143] and in the bone marrow [144] did not increase. An increase of the number of micronuclei was observed in the bone marrow [144]. More recently sulfamethoxazole was found to be genotoxic in lymphocytes at 500 mg/L [145].

To summarize, antibiotics were found:

- To cause chronic effects at the low μg/L toward plants, daphnids, and bacteria
- In some cases, e.g., fluoroquinolones, genotoxic at the low μg/L concentration levels

4 Future Challenges: Correlating Chemical and Biological Parameters

The difficulty of fully correlating chemical parameters of wastewater and biological effects is translated in the few publications published so far in the scientific literature. The studies included in Table 1 are successful examples, in which a TIE scheme was applied to characterize wastewater. As described by the USEPA, each TIE consists of (1) phase I, toxicity characterization procedures; (2) phase II, toxicant identification; and (3) toxicant confirmation [146].

As presented in Table 1, an accurate correlation of biological effects with chemical parameters can be drawn when toxicity is mainly caused by a limited number of compounds, for instance, in case that the main cause of toxicity is ammonia. As Ankley et al. [147] discuss this has to do with the easy characterization, manipulation, and assessment of effects of ammonia. This fact stresses the limitations of the currently applied methodologies toward fully characterizing the chemical origin of toxicity.

The TIE approach, however, has not been able to fully characterize toxicity if many contaminants are taken into account. This has to do mainly with the bioassays currently being applied in this approach, in which focus is given on acute toxicity and whole organisms' endpoints of survival, growth, etc.

The great number of components interacting in the wastewater matrix may cause a mixture effect increasing its complexity. Pharmaceuticals, as a group, have also been studied for mixture effects. Recent studies reviewed by Vasquez et al. [148] indicate that mixture effects are possible at environmental concentrations. Publications correlating mixture effects of different groups of contaminants are still lacking. The difficulty to accurately identify, quantify, and assess the effects of contaminants, their metabolites, and their transformation products is diverting research from a chemical-oriented approach to an effect-based approach. In this context, complex mixtures are not seen only as many different compounds but as a dynamic mixture. To this end, the effort is not being given in reconstituting complex mixtures but in better understanding how complex mixtures behave, as a whole. Complex mixtures have been primarily assessed as a black box and only recently tools for simultaneously assessing multiple contaminants are being developed [149].

First and foremost, a selection of relevant effect-based bioassays that constitute a battery assay should be made. Some of the criteria to be taken into account in order to select the assays are (1) sensitivity, (2) reproducibility, (3) ecological relevance, and (4) cost-effectiveness. Regarding pharmaceuticals, this chapter has identified that adverse effects have been reported at environmental concentration levels of ng–μg/L. These effects were observed mainly after chronic exposure periods. Molecular and cellular endpoints were found to be sensible enough to capture effects at these minuscule concentrations. In some cases, even acute effects were observed at the low μg/L concentrations. In general, the endpoints and bioassays

able to capture adverse effects of pharmaceuticals at environmental concentrations are not widely applied and are missing from most current monitoring programs.

As Burgess et al. [150] have recently concluded, the research effort should be driven in two directions. One direction would be trying to have a contaminant or chemical approach of complex matrices. This would necessitate development of addition of steps in the TIE approach focusing on specific chemical groups. The target however is to simplify a complex matrix and not reconstitute it by testing chemicals alone. The other direction would be trying to maintain the relevant environmental bioavailability of any of the samples resulting from the TIE approach. This may be quite challenging since bioavailability is species and even organ specific.

Whichever path a researcher decides to follow, the ultimate goal should be the protection of the environment. The precautionary principle should be considered in the case of pharmaceuticals, as the ongoing research efforts to complete the complex matrix puzzle are continued. In this context, unified and harmonized legislative tools for wastewater reuse practices are in urgent need.

5 Conclusions

Pharmaceuticals and their products of incomplete mineralization ("transformation products, TPs") are considered as CEC due to their inherent ability to affect biological systems and their occurrence and pseudo-persistence in the environment. As presented herein, a wide variety of bioassays and endpoints have been used to investigate adverse effects of pharmaceuticals. These bioassays include organisms from all trophic levels (e.g., producers, consumers, and decomposers) and various biological organizational levels (e.g., molecular, cellular, etc.). However, most of them, due to their relatively limited environmental relevance or time duration, cannot be used to fully understand how pharmaceuticals may behave in complex matrices under real conditions.

The experimental designs mostly applied have only been substance oriented rather than effect based. Moving into an effect-based direction can lead to the identification of adverse outcome pathways that may link exposure to pharmaceuticals with a molecular-initiating event leading to an adverse outcome.

Since the ultimate objective is the protection of the environment where pharmaceuticals are present in complex mixtures, this exact complexity cannot and should not be resolved by component-based approaches only. Experimental designs should embrace this complexity by simultaneously addressing multiple stressors in order to accurately assess the potential adverse effects of pharmaceuticals in the environment.

Acknowledgments This work was prepared in the framework of the PENEK/0609/24 research project "Development of novel methods for the toxicity assessment of the multi-component chemical mixtures to humans and the ecosystem" (TOMIXX), implemented within the framework

of the program for research, technological development, and innovation "DESMH 2009–2010" and stimulated by NIREAS activities, the International Water Research Center of the University of Cyprus (ΝΕΑ ΥΠΟΔΟΜΗ/ΣΤΡΑΤΗ/0308/09). These projects are funded by the Cyprus Research Promotion Foundation, which is co-financed by the Republic of Cyprus and the European Regional Development Fund. The authors would also like to acknowledge the financial support provided by COST - European Cooperation in Science and Technology, to the COST Action ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS).

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Bioavailability and Uptake of Organic Micropollutants During Crop Irrigation with Reclaimed Wastewater: Introduction to Current Issues and Research Needs

N. Cañameras, J. Comas, and J.M. Bayona

Abstract Organic contaminants occurring in reclaimed water can be incorporated in soil, where they can interact with humic compounds or anthropogenic organic matter depending on their physicochemical properties. In the soil water, a fraction of these contaminants can be biodegraded, particularly in the rhizosphere, where the process is enhanced by root exudates. Another fraction can be uptaken by plants and translocated by xylem. Once incorporated in the plant, a fraction of the incorporated contaminant is metabolized, while the rest remains unaltered. Three stages can be distinguished in the metabolization process: (1) oxidation, (2) conjugation, and (3) accumulation in the vacuole or cell wall.

Keywords Bioavailability, Metabolization, Organic contaminants, Plant uptake, Translocation

Contents

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 81–104, DOI 10.1007/698_2015_412,

Abbreviations

BCFsv	Bioconcentration factor soil-vegetal
BPA	Bisphenol A
CYP450	Cytochrome P450
DBP	Disinfection by-product
DDT	Dichlorodiphenyltrichloroethane
DEET	*N*,*N*-Diethyl-meta-toluamide
DMPEA	*N*,*N*′-dimethylphenethylamine
DMPEA	*N*,*N*′-dimethylphenethylamine
DOC	Dissolved organic carbon
D_{OW}	*pH*-adjusted octanol-water partition coefficient: $D_{\mathrm{OW}} = \frac{K_{\mathrm{OW}}}{1 + 10^{\mathrm{pH} - \mathrm{p}K_a}}$
DW	Dry weight
fw	Fresh weight
GSH	Glutathione
GST	Glutathione *S*-transferase
GT	Glycosyltransferase
HC	Hydrocarbon
HS	Humic substance
$K_{\mathrm{d,solid}}$	Soil sorption coefficient
K_{OC}	Sorption coefficient
K_{OW}	Octanol-water partition coefficient
MTBE	Methyl *tert*-butyl ether
NADP+	Nicotinamide adenine dinucleotide phosphate
NSAID	Nonsteroidal anti-inflammatory drug
OP	Organic pollutant
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCCD	Poly(1,4-cyclohexylidene cyclohexane-1,4-dicarboxylate)
PCDD	Polychlorinated dibenzodioxin
PCDF	Polychlorinated dibenzofuran
PPCPs	Pharmaceuticals and personal care products
TSCF	Transpiration stream concentration factor
UV	Ultraviolet
Xenobiotic	Exogenous organic compound

1 Introduction

Reclaimed wastewaters may contain different mineral nutrients, organic microcontaminants, and trace elements depending upon their original source (i.e., industrial, urban, or domestic) and the treatment process (i.e., secondary, tertiary, or quaternary). When reclaimed water is used in agriculture, it is subjected to additional treatment processes to meet water quality standards, including disinfection (i.e., chlorination, UV, photocatalysis, nanofiltration, etc.), to remove or attenuate microbial pathogens and salinity [1]. During these disinfection processes, by-products (DBPs) can be formed due to the reaction of the organic matter or recalcitrant contaminants with the oxidants depending on the oxidant dose and contact time between the oxidant and disinfected water [2, 3]. Some of these DBPs are of health and/or environmental concern, exhibiting genotoxicity or carcinogenicity [4]. Moreover, recalcitrant contaminants and DBPs contained in reclaimed irrigation water may be incorporated into crops and, thus, eventually into the food chain [5–7].

This chapter reviews processes affecting contaminants' availability to and their fate in plants. Factors that influence the uptake of organic microcontaminants, such as physicochemical properties, soil sorption properties, and interaction with humic substances (HSs), are evaluated. Although the primary focus is emerging contaminants of environmental and health concern such as pharmaceuticals and personal care products (PPCPs) that are frequently detected in reclaimed water, we also include published information about pesticides whenever no information regarding PPCPs is available. Moreover, although the foliar route of incorporation is also feasible [6], we pay particular attention to the radicular route, since drip irrigation is the most commonly used technique with reclaimed water and organic contaminants are mostly uptaken through the rhizosphere. This review does not cover the incorporation of organic contaminants associated with biosolids, as that is a broad topic in itself and deserves a specific attention.

2 Factors Controlling the Bioavailability of Organic Micropollutants in Soil

A variety of organic pollutants (OPs), e.g., priority organic contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dichlorodiphenyltrichloroethane (DDTs) or pharmaceutical and personal care products (PPCPs), can enter the soil through irrigation with contaminated water, dry and wet atmospheric deposition, or biosolid and manure disposal. Assessing the behavior of OPs in soil and their bioavailability by crop and soil biota is a complex task as OP dynamics are governed by their physicochemical properties, soil–root–microbiota interactions, and cropping practices (e.g., plowing

and irrigation). These interactions mostly occur in the rhizosphere and on the interface between microminerals and HSs and organic carbon from anthropogenic sources, e.g., soot and black carbon particles. The following section looks at factors affecting the bioaccessibility and bioavailability of OPs.

2.1 Soil–Water–Contaminant Interactions

Various pools of organic matter can coexist in soil. Firstly, there is labile organic matter that can be easily partitioned within soil-pore water exhibiting a very high dissolved organic carbon (DOC) concentration. Secondly, there is a high-molecular-weight organic matter known as kerogen, which cannot be extracted using conventional solvent methods. While this fraction may contain organic contaminants, these are not available to plants and thus will not be considered further. Finally, soil can also contain black carbon originating during combustion processes, which significantly impact the adsorption processes of hydrophobic contaminants and are not usually considered [8].

Organic and inorganic contaminants in soil are strongly associated with humic substances (HSs). These substances act as both temporary storage and transport agents for hydrophobic organic contaminants (HOCs) such PAHs, PCDDs, PCDFs, hormones, and fragrances, carrying them to the aquifer or surface water as colloids [9, 10]. Cations have a major influence on the surfactant character of HSs. HS aqueous solutions flocculate when the ionic strength is raised, e.g., humic polymers proceed in continuous fashion – from micelle-like assemblies to macroscopic precipitates – as the salt concentration is increased [11–13].

Lowering the pH of aqueous HS solutions has an effect similar to adding metal salts, albeit less pronounced [14]. Moreover, lowering pH causes the protonation of the HA carboxylic groups, which eventually leads to precipitation. This usually begins at pH ~ 3–2 and reaches completion at pH ~ 2–1 [15].

Due to the polarity of the aqueous soil solution in which organic contaminants are contained and the electrical charge of clays, these organic molecules tend to self-assemble into micelle-like structures, when suspended at high concentrations, or bilayer-like structures, on microminerals [16]. In both situations, hydrophilic exterior regions shield hydrophobic interiors from contact with water molecules [12]. This is evidenced by the fact that when hydrophilic mineral particles are exposed to HSs, they develop hydrophobic surfaces, rendering them more capable of absorbing HOCs [17, 18].

That sorption process shows a rather discrete zonal sequence. In the contact zone, strong organo-mineral associations are favored either by polar organic functional groups of amphiphiles that interact via ligand exchange with singly coordinated mineral hydroxyls or by protein-like substances that show a strong tendency to bind to surfaces and to resist desorption [19], thereby adding hydrophobic interactions to the electrostatic binding [20]. In fact, positively charged *N*-containing moieties show a preferential adsorption over neutral or negatively

charged organic compounds to mineral surfaces [21, 22]. This behavior is basically attributable to the electrostatic attraction of the positively charged N-containing molecules at the soil pH with negatively charged mineral surfaces such as clays and the carboxylic groups of HSs from the soil organic matter. In this regard, mineral-associated HSs exhibit C/N ratios of 7–14, whereas water-extracted HSs have C/N ratios of 26–55 [23]. Most *N*-containing products were derived from heterotrophic bacteria, rather than plant tissues [24].

The hydrophobic character of HSs is due to the presence of elongated aliphatic and aromatic functional groups, probably derived from plant waxes and cutins. Carbohydrates and their derivatives, which include functional groups such as alcohols or ethers that do not ionize under typical soil and water pH conditions, are mildly polar. The HSs' hydrophilic character is mainly due to the dissociation of carboxylic acid and phenolic groups and proton acceptance of amines, which can be positively charged under typical soil and water pH conditions. HSs have a high aromatic content, estimated to range from 20 to 60% of the carbon present, and are responsible for dispersive and dipole-induced interactions [25].

2.2 Sorption to Natural and Anthropogenic Organic Matter

As mentioned in the previous section, HSs make up the main pool of organic matter and organic contaminants in soil. They can behave as a temporary storage of organic contaminants that are released whenever the HS biodegrades or, with acidic compounds, when the pH decreases.

In addition, black carbon and kerogen exhibit a nonlinear sorption behavior and may dominate the overall sorption by soils [26]. Elemental carbon is generated by combustion of fossil fuels such as coal, diesel, or gasoline and can be advectively transported by the wind through the atmosphere and accumulate in the soil by wet or dry deposition.

Sorption and desorption of HOCs occurring in soil-pore water on to the soil organic matter is one of the most important mechanisms controlling the mobility of nonpolar and low-polarity organic contaminants in surface waters, subsurface waters, and plant uptake. Recent studies show that soils exhibit an array of hydrophobic sorption phenomena that are inconsistent with an early partitioning model. Experimental data from sorption–desorption studies reveal a nonlinear isotherm, varied sorption–desorption hysteresis, solute–solute competition, and low rates of sorption–desorption [27]. In the case of veterinary pharmaceuticals, there is a broad range of soil sorption coefficients ($K_{\mathrm{d,solid}} = 0.2$–$6{,}000$ L kg^{-1}) and those for a single compound can span several orders of magnitude depending on the soil's physicochemical properties [28] and the contaminant speciation (neutral, cationic, anionic, or zwitterionic) at the soil pH. Accordingly, for ionizable molecules, the D_{OW}, a soil pH-corrected K_{OW}, is used to evaluate their fate in soil. D_{OW} can be calculated from the following equation:

$$D_{OW} = \frac{K_{OW}}{1 + 10^{pH - pK_a}}$$

where pK_a is the ionization constant of acidic molecules. In case of neutral molecules, K_{OW} and D_{OW} are equivalent.

A number of hydrophobicity-independent mechanisms, such as cation exchange, cation bridging at clay surfaces, surface complexation, and hydrogen bonding, appear to be involved. Accordingly, different models have been proposed to deal with the high heterogeneity of rigid HCs and the limited sorption sites available of black carbon and kerogen, namely, the Langmuir, Freundlich, and composite models based on distributed reactivity [27]. However, linear partitioning coefficients corrected by the organic matter, namely, K_{OC}, are still the most widely used, particularly for nonionic organic contaminants leading to biased results especially at high pore water concentrations of organic contaminants since competitive displacement occurs when the number of contaminant molecules exceed the number of soil's active sites.

3 Uptake of Contaminants by Plants

Different experimental setups have been used to evaluate the uptake of a large variety of organic contaminants by different plant species. In the following discussion, the bioavailability of organic contaminants is classified in accordance with these experimental setups (Table 1).

3.1 Uptake from a Hydroponic Medium

Predicting the uptake of contaminants by roots and xylem translocation is of great importance in risk assessment studies and to anticipate the effectiveness of phytoremediation [38]. The root uptake of OPs can be passive or active, and a commonly used descriptor is the transpiration stream concentration factor (TSCF). It is defined as the ratio of the contaminant concentration in the transpiration stream to that in the root zone pore water or hydroponic solution. Chemicals with a TSCF > 1.0 are actively transported, while chemicals that move in plants at the same rate as water have a TSCF near 1.0. Due to the interaction of OPs with the lipid bilayer in root membranes, the TSCF is usually lower than 1. Because of the difficulty of measuring the TSCF experimentally, estimated data based on empirical relationships based on the log K_{OW} are usually used. These relationships suggest that highly lipophilic OPs ($\log K_{OW} > 4$) and highly hydrophilic OPs ($\log K_{OW} < 1$) should not be considered available to plants [39]. However, recent studies suggest that highly polar nonionic OPs with $\log K_{OW} < 1$ (e.g., MTBE) can be uptaken by

Table 1 Organic pollutants uptaken by plant from irrigation evaluated according to experimental setups

Plant specie	Target compound	References
In vitro tissue culture		
Armoracia rusticana	Ibuprofen	[29]
Linum usitatissimum	Diclofenac sodium	
Glycine max *Triticum aestivum*	2,4-Dichlorophenoxyacetic acid	[30]
Hydroponic conditions		
Brassica rapa	Carbamazepine	[31]
	Salbutamol	
	Sulfamethoxazole	
	Trimethoprim	
Lactuca sativa	20 compounds	[32]
Spinacia oleracea		
Cucumis sativus		
Capsicum annuum		
Ipomoea aquatic	Bisphenol A	[33]
Hordeum vulgare	Ibuprofen	[29]
Lupinus luteolus		
Phragmites australis	Diclofenac sodium	
4 vegetables		
Brassica oleracea	Bisphenol A	[34]
Lactuca sativa	Naproxen	
	4-Nonylphenol	
Phragmites australis	Ciprofloxacin	[35]
	Oxytetracycline	
	Sulfamethazine	
Soil test-pots in greenhouses or in field trials		
Brassica campestris	Carbamazepine	[36]
	Sulfamethoxazole	
	Salbutamol	
Daucus carota sativus	Galaxolide	[37]
	Tonalide	
	Triclosan	

plants [40, 41] probably by channel protein route (e.g., aquaporins). In fact, enhanced transport occurs for small neutral solutes along this pathway [42] but still not well understood in the case of highly hydrophilic OPs. Moreover, the integrity of the root cell membranes is also a key factor controlling root uptake of OPs. When damaged, roots are easily exposed to toxicants, and the TSCF can increase significantly [38]. The following empirical relationship has been proposed to estimate the TSCF for 25 chemicals ranging from log K_{OW} −0.8 to 5:

$$\mathrm{TSCF} = \frac{11}{11 + 2.6^{\log(K_{\mathrm{OW}})}}$$

The main limitation of this model is that all the TSCF measurements are performed under hydroponic conditions. Thus, soil interaction is not considered. Moreover, it is limited to neutral OPs, whereas a large number of pharmaceuticals are ionic or ionizable compounds. For ionizable compounds, electrostatic attraction or repulsion and ion trap may affect the accumulation of contaminants in roots [43, 44].

In the rhizosphere – the soil area that has been physically, chemically, or biologically altered by the presence of plant roots [45, 46] – roots absorb nutrients but also exude many organic compounds and oxygen. Indeed, it has been estimated that roots can release about 10–40% of their total photosynthetically fixed carbon [47]. Organic acids, amino acids, proteins, sugars, phenols, and other secondary metabolites are significant exudates extensively used by soil microorganisms and mycorrhizal fungi [48]. These components help plants to access nutrients by light-induced acidification assisted by photosynthetic activity (daytime) or alkalinization (night), changing the redox conditions (oxygen transport) within the rhizosphere or directly chelating nutrients [49].

Hydroponic experimental conditions do not simulate field conditions, but the rhizosphere remains functional. Analgesics (i.e., acetaminophen), stimulants (i.e., caffeine), anxiolytics (i.e., meprobamate), nonsteroidal anti-inflammatory drugs (NSAIDs) (i.e., diclofenac, naproxen, ketoprofen), anticonvulsants (i.e., primidone, carbamazepine, dilantin), lipid regulators (i.e., gemfibrozil, atorvastatin), polymers and surfactant-related products (i.e., bisphenol A, nonylphenol), β-agonists (i.e., salbutamol), insect repellents (i.e., DEET), triclocarban, antibiotics (sulfonamides such as sulfamethoxazole and sulfamethazine, tetracyclines such as oxytetracycline, and fluoroquinolones such as ciprofloxacin), and dihydrofolate reductase inhibitors (trimethoprim) have been widely evaluated by several authors (Table 1). The compartmentation of contaminants in the plant system depends on the physical properties of the contaminants. The highest concentrations are generally found in the root system, but some compounds can be translocated to seedpods, stem, or leaves [31]. Nevertheless, in contrast to aerial plant parts, roots accumulate too lipophilic organic compounds [50, 51]. However, taking into account the large variety of experimental setups, crops, water quality, and soil characteristics at present, it is almost impossible to draw any conclusion regarding the contaminant uptake by plants. Nevertheless, the soil organic matter and clay content lead to a decrease in the OP bioavailability. On the other hand, the DOC content in the irrigation water decreases also the uptake of the OP probably because they are associated with the colloidal organic matter becoming more mobile through soil and then less bioaccessible.

In a recent study, 20 PPCPs were evaluated in different common plant species (i.e., lettuce, spinach, cucumber, and pepper) [32]. Out of the 20 PPCPs considered, triclocarban, fluoxetine, triclosan, and diazepam accumulated in roots at higher

levels than the other PPCPs, while translocation to leaves/stems was more extensive for meprobamate, primidone, carbamazepine, dilantin, and diuron. Interestingly, all of these compounds are moderate or weak bases and can be actively transported by the ion trap effect from neutral pH (pH 7–7.5) in the cytoplasm to acidic vacuoles (pH 5.5) [44] where they tend to store. For nonionic compounds, root uptake was positively correlated to $\log K_{OW}$ which suggests that hydrophobic interactions are relevant to root uptake, but they limit the contaminant translocation (negative correlation with $\log K_{OW}$) through limited mobility either by phloem (weak acids) or xylem (weak bases).

3.2 *Uptake from Soil-Pore Water*

The uptake of organic contaminants by plants is chiefly controlled by their bioavailability in the soil-root system. Bioavailability is defined as a measure of chemicals' accessibility to plant roots or of their absorbability by living organisms [52, 53]. Usually, plant uptake is measured by dimensionless bioconcentration factor soil-vegetal (BCF_{SV}), which is defined as follows:

$$BCF_{SV} = \frac{\text{Concentration in plant shoot } \left(\text{mg kg}^{-1}\,\text{DW}\right)}{\text{Concentration in soil } \left(\text{mg kg}^{-1}\,\text{DW}\right)}.$$

However, several authors used aqueous concentration of contaminant in irrigation water instead of soil concentration, and in this case, the BCF_{SV} is expressed as L kg^{-1}. Although the latter method for BCF_{SV} is suitable for hydroponic culture when the contaminant solution is supplied at constant concentration, if it is used in soils, a significant underestimation occurs because some of the OPs exhibit a fast degradation in soil.

The availability of nutrients and other organic compounds for uptake by roots is a process that largely consists of microorganism-mediated activity, and the enhancement of the biodegradation of contaminants decreases in accordance with the distance from the roots. This is known as the rhizosphere effect in phytotechnology. Nevertheless, recent studies have demonstrated an abiotic mechanism in the case of hydrophobic contaminants. Artificial (e.g., citric and oxalic acids) or natural root exudates promote the desorption of hydrophobic contaminants such as phenanthrene and pyrene sorbed to soil by decreasing the surface tension of the pore water, and the fraction of the contaminant desorbed depends on the soil organic matter and contaminant aging [54, 55].

Several experiments have been carried out to evaluate plants' uptake of OPs from irrigation water in greenhouses, allowing for the control of experimental variables (e.g., temperature, humidity, watering, etc.) (Table 1). Wu et al. [56] compared the uptake of OPs by soybeans from spiked irrigation water and biosolids containing emerging contaminants. Carbamazepine, triclosan, and triclocarban

accumulated in root tissues and were translocated into aboveground parts, including the beans. The uptake of selected compounds differed depending on the treatment. The application of biosolids resulted in higher plant concentrations of the target contaminants, likely due to higher loading. However, organic contaminants delivered by irrigation were more easily taken up and translocated.

In another greenhouse study with unspiked irrigation waters of different quality (i.e., well water, secondary effluent, chlorinated water, photocatalytic oxidation) [5], crops grown in secondary effluent were most frequently detected in the highest concentrations, while the lowest were found in green pods. Tributyl phosphate and butylated hydroxylanisole had the highest concentration among the 21 compounds monitored in irrigation waters (up to 570 ng g^{-1} fresh weight (fw)). Concentrations for the other microcontaminants screened were found to range from 0.7 to 83 ng g^{-1} (fw) for pharmaceuticals, from 0.4 to 573 ng g^{-1} (fw) for pesticides, and from 4 to 336 ng g^{-1} (fw) for fragrances. From pharmaceuticals, carbamazepine exhibited the highest concentration in carrot (52 ng g^{-1} fw) followed by flunixin in lettuce grown in reclaimed waters (secondary treatment). Both compounds are secondary amines with basic properties. From fragrances, ambrettolide exhibited the highest concentration in carrot followed by lettuce (75–134 ng g^{-1} fw) grown with reclaimed water. All the fragrances analyzed share a $\log K_{OW} > 4$, and the uptake is closely related to their concentration in irrigation waters being tuber vegetables, the ones with the highest concentrations. Phenoxy acids and triazinic acid herbicides exhibited the highest concentrations in crops, which is consistent with their systemic behavior.

In a greenhouse and field experiment, river and wastewater effluents were used for irrigation [57]. The results showed the potential for uptake of one or more of the antibiotics evaluated (azithromycin, roxithromycin, clarithromycin) and illicit drugs (methamphetamine, pseudoephedrine), albeit at very low levels. In those food crops watered with wastewater effluent, only an industrial flavoring agent, *N*, *N*′-dimethylphenethylamine (DMPEA), was consistently found. None of the evaluated contaminants were found in crops irrigated with water from the river. However, the reported recoveries in vegetables for all the target analytes were matrix dependent and consistently low (2–50%) which could led to an underestimation of actual concentrations.

To date, very few field studies have been carried out to evaluate the incorporation of waterborne contaminants into crops. One such study was conducted in an irrigation pipe network in which reclaimed water (secondary effluent) was mixed with riverine water depending on the hydric demand and its availability [58]. Alfalfa and apple were analyzed, and 5 anthropogenic compounds, namely, hydrocinnamic acid, caffeine, ibuprofen, naproxen, and galaxolide were identified and quantitated, with concentrations ranging from 0.014 to 16.9 ng g^{-1} (fw). Due to the temporal variability of contaminants in the irrigation waters, incorporation pathways (e.g., foliar or radicular), and the different half-life in soil, no significant correlations between irrigation water and crop concentrations were found.

4 Metabolization of Organic Contaminants

4.1 Plant Detoxification

Plants develop defense mechanisms for survival under unfavorable abiotic and biotic conditions [59]. One of the plant strategies for reducing OPs toxicity is through biotransformation reactions promoted by the activation of the plant's enzymatic system. In contrast to heterotrophic organisms, plants do not completely oxidize uptaken OPs because plants do not possess the enzymatic machinery to complete the degradation of many OPs. However, plants have varying capacities to detoxify pollutants using specific enzymatic pathways, depending on the plant species and environmental conditions, as well as the structure of the organic compound [60]. After being taken up by the root, OPs can have different fates. They may be translocated [61] or transformed into less toxic compounds and confined in plant tissues as non-available forms in vacuoles or cell walls [62]. Plant cells can metabolize different kinds of OPs, but they have a limited capacity to prevent their accumulation in plant tissues. It depends on the OP's structure, its concentration in the soil, and the uptake mechanisms [63]. A plant's susceptibility to an OP can also vary according to the species and cultivars.

The complete degradation of an OP by a plant can only be accomplished in the case of low concentrations; with high concentrations, only partial mineralization is possible [64, 65]. Moreover, biodegradation depends on the chemical structure of the OP and its lipophilicity. The metabolization of OPs often produces alterations in plant morphology and physiology. Many researchers have referenced these alterations, especially in relation to pesticides and herbicides [64–66]. However, in the case of emerging OPs occurring in irrigation water, the information is scarce.

4.2 Metabolization Phases

Most OPs are transformed during a sequential metabolization into more hydrophilic and less toxic compounds. Plants usually detoxify OPs in three consecutive phases (Fig. 1):

- Phase I: Activation, transformation, or functionalization of lipophilic organic exogenous compounds, as

$$R \rightarrow R - OH$$

  where R is the organic xenobiotic and R–OH is the activated xenobiotic
- Phase II: Conjugation of metabolites formed in phase I (activated xenobiotics), then

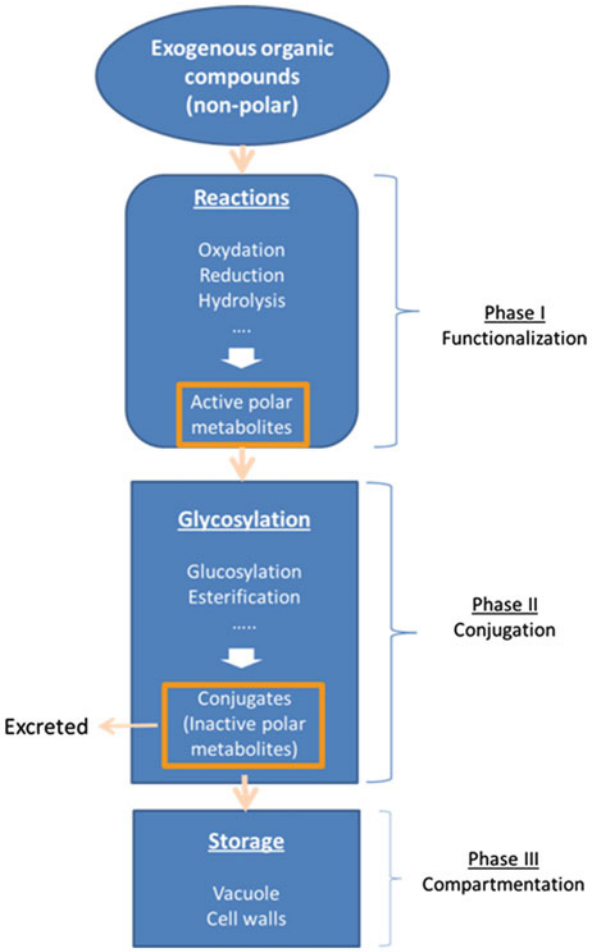

Fig. 1 Phases of metabolization of organic pollutants [67]

$$R - OH \rightarrow R - OR'$$

where R–OR′ is the conjugated xenobiotic. For example, in a glucuronidation reaction,

$$R - OH \rightarrow R - O - G$$
$$R - NH_2 \rightarrow R - NH - G$$
$$R - SH \rightarrow R - S - G$$

and in a methylation reaction

$$R - OH \rightarrow R - O - CH_3$$

- Phase III: Compartmentation of modified compounds in vacuoles or cell walls [67, 68]

Many different enzymes catalyze these metabolic reactions, as cytochrome P450 monooxygenases, peroxygenases, and nitroreductase in phase I or glutathione *S*-transferase, glucosyltransferase, and *N*-malonyl in phase II. They are synthesized in the cytosol of plant cells [69]. Sandermann [67] proposed the "green liver concept" to explain and compare the metabolization of OPs conducted by plants with that conducted by animals. Although plants involve more potential enzymes than animals [70], these enzymes have numerous similarities to both normal secondary plant metabolism enzymes and enzymes that participate in the metabolism of xenobiotics in the mammalian liver [71], so the mechanisms for the detoxification of xenobiotics in plants are closely related to the mammalian system [72]. The main difference with animal metabolism is that plants do not usually have an excretory system. In plants, metabolites must be stored in cell walls, vacuoles, or plant tissues. The enzymes used by plants in metabolism phases I and II are generally used in the synthesis and processing of endogenous natural compounds [73]. However, the mechanisms involved in the distinction and detoxification of OP are still not enough clear. Edwards et al. [70] focused their review on the proteins responsible for the metabolism and transport of xenobiotics within plant cells, how these systems are regulated, and their relationship with functional genes involved. According to Edwards et al. [74], xenome in plants is the biosystem responsible for detecting, detoxifying, and transporting xenobiotics.

4.2.1 Phase I: Activation, Transformation, or Functionalization

The main goal in phase I is to convert nonpolar organic compounds into more polar compounds through enzymatic transformations [65, 67] in order to predispose the contaminants for the subsequent metabolism steps (phases II and III). This transformation usually involves oxidation or hydrolytic reactions [75]. Oxygenation is a common process in pesticide and herbicide metabolism. The main metabolic reactions involved in phase I are presented in Table 2 and discussed below.

However, when OPs have functional groups suitable for phase II metabolism (such as hydroxyl, phenolic, and carboxylic compounds), OPs can go directly to phase II [86].

The literature on these metabolic reactions is scarce, except in relation to herbicides. In this regard, over the last three decades, it has been established that CYP450 is responsible for the phase I metabolism of many different types of herbicides [60, 75, 87, 88]. Moreover, neomycin phosphotransferase and hygromycin phosphotransferase are known to detoxify aminoglycoside antibiotics by phosphorylation [89].

Table 2 Selected studies of oxidative metabolization of organic pollutants observed in different plants

Plant species	Initial organic compound	Culture conditions	Enzymes studied	References
Triticum aestivum	Acetaminophen	Seedlings	Peroxidase	[76]
			Superoxide dismutase	
Brassica juncea	Acetaminophen	Hydroponic	Ascorbate peroxidase	[77]
			Catalase	
			Peroxidase	
Caragana chamlagu	Bisphenol A	In vitro	Peroxidase	[78]
Typha latifolia	Carbamazepine	Hydroponic	Catalase	[79]
			Guaiacol peroxidase	
			Superoxide dismutase	
Armoracia rusticana	Diclofenac	In vitro	Monooxygenase activity	[80]
Hordeum vulgare		Hydroponic		
Populus nigra	Ibuprofen	In vitro	Lipoxygenase	[81]
Portulaca oleracea	Bisphenol A	Hydroponic	Peroxidase	[82]
Pinus sylvestris	Haloacetic acids	Seedlings	Peroxidase	[83]
Portulaca oleracea	Bisphenol A	In vitro	Polyphenol oxidase	[84]
Zea mays	Chlortetracycline	Hydroponic	Peroxidase	[85]
			Superoxide dismutase	
			Catalase	

Oxidation. These reactions are mainly catalyzed by cytochrome P450 (CYP450) associated with monooxygenases, peroxidases, and phenol oxidases [67, 75, 90]. Biotransformation reactions of CYP450 system are able to act on numerous xenobiotics due its low specificity.

Plant CYP450 proteins are encoded by very large and diverse multigene families, and they are the most important enzymes related to xenobiotic biodegradation [91]. The tolerance of plants to OPs is closely related to these enzymes because plant P450s catalyze herbicide metabolism and contribute to the activation of detoxification mechanism of other agrochemicals [92].

The most common reaction catalyzed by CYP450 is as follows:

$$\text{R-H} + \text{O}_2 + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{R-OH} + \text{H}_2\text{O}$$

where R can be any organic radical. Protons (H^+) are usually given from NADH or NADPH through specific amino acids in the CYP enzyme. All oxidation reactions

Table 3 Selected studies of conjugation of organic pollutants observed in different plants

Plant specie	Organic compound	Identified conjugates	References
Brassica juncea	Acetaminophen	Acetaminophen glycoside	[77]
		Glutathionyl-acetaminophen	
Caragana chamlagu	Bisphenol A	4-(2-Propanol)phenol	[78]
		4-Isopropenylphenol	
Lemna minor	Chlorinated phenols	Chlorinated malonyl-glucoside	[93]
		Apiosyl-glucoside	
Typha latifolia	Carbamazepine	10,11-Dihydro-10,11-epoxycarbamazepine	[79]
Eucalyptus perriniana	Bisphenol A	Glucopyranosyl conjugates	[94]
Armoracia rusticana	Acetaminophen	Acetaminophen-glutathione	[72]
Hordeum vulgare	Diclofenac	Glucopyranoside	
Arabidopsis thaliana	[UC-14C]-3,4-dichloroaniline	[14C]-DCA-N-β-D-glucoside	[95]
Glycine max		DCA-N-malonate	
Nicotiana tabacum	Bisphenol A	β-D-Glucopyranoside	[96]
Solanum nigrum	2,2′-Dichlorobiphenil	Hydroxy-methoxy-polychlorinated biphenyls	[97]
Nicotiana tabacum	Trichloroethylene	β-D-Glucoside of trichloroethanol	[98]
Populus alba	Trichloroethanol		

require NADPH and O_2. In the case of monooxygenases, CYP450 uses electrons from NADPH to activate molecular oxygen to form a molecule of water and an oxygenated product. Other enzymes that can also oxidize organic contaminants include peroxygenases, nitroreductases, and laccases.

Hydrolytic reactions. These reactions are common in plants, especially when the OP contains ester (catalyzed by esterases), amide (catalyzed by amidases), or nitrile functional groups.

Reduction. These reactions are less common than hydrolysis and oxidation. The most common reduction reaction is the reduction of nitro groups by nitroreductases to an amino group, which requires reductants such as NADPH.

Additional examples of other types of oxidative metabolization in plants are shown in Table 3.

4.2.2 Phase II: Conjugation

Conjugation reactions combine polar OPs or metabolites obtained in phase I with cell-endogenous compounds (glucuronide acids, sulfates, etc.) in order to get more polar compounds with a higher molecular weight and often higher hydrophilicity

than the initial molecules. The activated xenobiotic compounds are conjugated with glycosides, glutathione, or amino acids. These reactions are some of plants' most important pathways for avoiding or reducing plant toxicity [99].

The glycosylation of OPs obtained in phase I is mainly catalyzed by glycosyltransferases (GTs) [73]. Glutathione (GSH) and glucosides catalyzed, respectively, by glutathione S-transferases (GSTs) and glycosyltransferases are involved in the conjugation of a variety of OPs [67, 69, 100]. Other enzyme classes such as carboxylesterases, *O*-malonyltransferases, *N*-glycosyltransferases, and *N*-malonyltransferases are also associated with xenobiotic metabolism in plant cells [101]. The conjugation with GSH happens in the cytosol, but its accumulation is harmful to the plant [86] (Table 3). Conjugation common reactions in mammalians are mainly made with sulfate, amino acids, and glucuronic acid [102].

GSTs are very important to metabolize pollutants and give antioxidative protection. GST activities for different xenobiotics have been evaluated in 59 different plant species and 4 plant cell suspension cultures [103], as well as for different herbicides in *Arabidopsis* [74]. Table 3 shows other examples of organic xenobiotic conjugation evaluated in different plants and culture conditions.

GSTs were first discovered in animals and later in plants, when GST activity from maize was shown to be responsible for conjugating the chloro-*S*-triazine atrazine with GSH [104].

GSTs catalyze the general reaction as follows:

$$\text{R-X} + \text{GSH} \rightarrow \text{R-SG} + \text{HX}$$

GSTs typically catalyze the transfer of the dipeptide GSH to a substrate (R–X) containing a reactive electrophilic center forming a polar S-glutathionylated reaction product (R-SG) [105].

Many pesticides and herbicides have been metabolized to glutathione conjugates in higher plants cultivated in soil and in plant cell tissue culture [106, 107]. Glycosylation seems to be an efficient procedure for the bioremediation of environmental pollution by some plants, as bisphenol A (BPA), can be eliminated by formation of its glycosides. Conjugation with glycosylation of BPA has been studied in several plant species: (a) in soybean, wheat, foxglove, and thorn apple, three plant cell suspension cultures where BPA was glycosylated to several glycosidic compounds, highly polar compounds, or inextricable [108]; (b) in tobacco cell suspension and seedling cultures were identified as two major products BPA mono-*O*-β-D-gentiobioside and the trisaccharide BPA mono-O-β-D-glucopyranosyl-[β-D-glucopyranosyl] β-D-glucopyranoside and two minor products, mono- and di-*O*-β-D-glucopyransides [96, 109]; (c) in the aquatic plant water convolvulus where most of BPA metabolites were detected in the roots and in the stems but none in the leaves [33]; and (d) in germination and seedling hydroponic cultures of various forage grasses and horticultural crops where BPA was removed from aqueous solutions proportionally to the quantity they are exposed to [110]. Dogan et al. [111] found that wheat could tolerate the oxidative stress of BPA and tetrabromobisphenol A (TBBPA), and increases in the H_2O_2 level and lipid

peroxidation could be related to oxidative stress. In soybean seedlings grown in a greenhouse experiment, low doses of BPA improved the glutamine synthetase (GS)/glutamate synthase (GOGAT) cycle and the glutamate dehydrogenase (GDH) pathway in ammonium assimilation. The amino acid and the soluble protein content increased in higher doses, inhibiting the GS/GOGAT cycle and promoting the GDH pathway. The amino acid content increased, and the soluble protein content decreased [112]. Li et al. [113] observed that TBBPA caused stress in wheat as measured by an increased level of malondialdehyde and changes in the activity of superoxide dismutases, peroxidases, and catalases in leaves.

4.2.3 Phase III: Compartmentation

Conjugates obtained in phase II cannot usually be excreted by plants, but they can be stored in cell vacuoles as conjugates or incorporated into insoluble polymers during phase III reactions [65, 67, 75]. In this phase, xenobiotic conjugates are converted to secondary conjugates or insoluble [114] and are sequestered from sensitive cytoplasm and stored, for example, in vacuoles (soluble conjugates) or incorporated into cell wall materials (insoluble conjugates) [68, 115]. The transport to the vacuole is done by specific solute transporters in the tonoplast [68]. The effective movement is facilitated and controlled by ATP-dependent enzymes similar to a GSH conjugate pump [116]. This action is also called storage excretion [68]. Often, 70% or more of the uptaken xenobiotics can be accumulated as conjugates [64]. These conjugates may later return to the soil or enter the food chain.

Day and Saunders [93] found chlorinated malonyl-glucoside and apiosyl-glucoside conjugates stored in vacuoles and cell walls in duckweed plants. Schröder et al. [117] postulated that barley plants can stock GSH conjugates in the vacuole and that the transport is unidirectional. In contrast, studies conducted by Kotyza et al. [29] with horseradish, lupin, barley, and common reed cell cultures cultivated in a hydroponic medium suggest that acetaminophen could be stored in the vacuoles and later gradually liberated. Klein et al. [118, 119] showed that a conjugate of 17β-estradiol was transported to the vacuole of rye and barley cells by the ATP-dependent GSH conjugate pump.

Although the results obtained so far on the study of the metabolism of organic xenobiotics in plant systems are encouraging, these results also highlight the need of further research.

5 Future Developments and Research Needs

To date, the incorporation of OPs by a variety of plants in tissue, hydroponic and greenhouse experiments have been demonstrated. Nevertheless, standardization is needed to be able to compare results of different experimental setups. In fact, a

large number of variables may affect the final results and hinder the comparison thereof. Although the concentrations of OPs incorporated in plants from irrigation water are usually low, metabolites must not be neglected since mineralization is rarely achieved during wastewater treatment. Moreover, the impact of incorporated contaminants on the secondary plant metabolism is also of great interest since some OPs can mimic phytohormones and promote plant growth [120], while others can act as antagonists and inhibit it [121]. In this regard, the application of metabolomics is likewise of great interest to evaluate whether xenobiotics incorporated by plants can promote the expression of specific plant genes.

Finally, the impact of soil amendment with biochar to promote soil fertility and for carbon sequestration has some potential to restrict the bioavailability/bioaccessibility of organic contaminants from irrigation water to plants and thus deserves special attention. The higher partition coefficients of a variety of xenobiotics in soils amended with biochar suggest that the application of biochar would be beneficial to sequester OPs from soils since it degrades very slowly and has a large surface area capable of multiple interactions [122].

Acknowledgments Financial support was obtained from the Spanish Ministry of Economy and Competitiveness (CGL2011-24844).

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Irrigation with Treated Wastewater: Potential Impacts on Microbial Function and Diversity in Agricultural Soils

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Abstract The reuse of treated wastewater could be a promising measure to attenuate the water scarcity burden. In agriculture, irrigation with wastewater may contribute to improve production yields, reduce the ecological footprint and promote socioeconomic benefits. However, it cannot be considered exempt of adverse consequences in environmental and human health. Apart from the introduction of some biological and chemical hazardous agents, the disturbance of the indigenous soil microbial communities and, thus, of vital soil functions impacting soil fertility may occur. The consequences of these disturbances are still poorly understood.

This chapter summarises the physicochemical and microbiological alterations in soil resultant from irrigation with treated wastewater that are described in scientific literature. These alterations, which involve a high complexity of variables (soil,

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 105–128, DOI 10.1007/698_2015_346,

wastewater, climate, vegetal cover), may have impacts on soil quality and productivity. In addition, possible health risks may arise, in particular through the direct or indirect contamination of the food chain with micropollutants, pathogens or antibiotic resistance determinants. The current state of the art suggests that irrigation with treated wastewater may have a multitude of long-term implications on soil productivity and public health. Although further research is needed, it seems evident that the analysis of risks associated with irrigation with treated wastewater must take into account not only the quality of water, but other aspects as diverse as soil microbiota, soil type or the cultivated plant species.

Keywords Environmental contamination, Microbial communities, Public health, Sustainable reuse

Contents

Abbreviations

ASC	Australian Soil Classification
BOD	Biological oxygen demand
CFUs	Colony-forming units
COD	Chemical oxygen demand
CST	Chinese Soil Taxonomy
I	Industrial
n.a.	Not available
NR	Not reported
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
RI	Treated wastewater used in restricted irrigation
st	Secondary treated
SiBCS	Brazilian Soil Classification

tt	Tertiary treated
TSS	Total suspended solids
U	Urban
UI	Treated wastewater used in unrestricted irrigation
USDA	United States Department of Agriculture
WRB	World Reference Base for soil resources
WW	Wastewater

1 Introduction

The high demand of freshwater for anthropogenic activities sometimes exceeding the minimum recharge levels has been leading to the desiccation of water streams and depletion of groundwater [1]. The water stress index, defined as the ratio between total freshwater abstraction and total annual renewal of water (volume), is a useful indicator to seek an adequate balance between available water resources and water uses. The reuse of treated wastewater has increasingly been regarded as an important measure to attenuate the water scarcity burden, promoting an adequate balance between water resources and water uses [2, 3]. This is observed for some countries with severe water stress indexes (e.g. Spain and Israel) that already have mature wastewater reuse practices [4]. Among the activities requiring freshwater resources worldwide, irrigation consumes the highest fraction (~70%) [5]. For this reason, wastewater reuse in agriculture and landscaping has been implemented in countries such as the USA, Israel, Malta, Cyprus, France, Italy, Jordan or Spain [4, 6–8]. The reuse of treated wastewater in agriculture may contribute to improve production yields, reduce the ecological footprint and have beneficial socio-economic implications. In the socioeconomic domain, this practice can contribute to human well-being through environmental protection and economic sustainability, supporting increased production with reduced costs and fixing populations and employment in areas at risk of desertification [6, 9–11]. Additionally, it can contribute to reduce the discharges of effluents in the environment, minimising the deterioration of freshwater ecosystems through eutrophication and algal blooms [11].

The arguments presented above make the reuse of treated wastewater inevitable, at least in some world regions. However, the associated environmental and human health risks cannot be ignored. Since wastewater results from human activities, the occurrence of chemical compounds and microorganisms that can persist even after conventional and advanced wastewater treatment may be incompatible with a reuse. For instance, the occurrence of pathogens in treated domestic wastewater is well documented [12, 13]. With different ability to survive in the environment, some of these pathogens can persist and spread after treated wastewater discharge, with the possibility of infecting new hosts by direct contact or entering the food chain [14–17]. Wastewater contains also numerous recalcitrant chemical compounds, some of which are potentially toxic, teratogenic or even carcinogenic.

Table 1 Overview of physicochemical and biological properties of urban raw wastewater and the legal standards or guidelines for treated wastewater used in unrestricted (UI) and restricted irrigation (RI) (units, mg/L, unless indicated)

	Parameter	Raw WW[a]	Treated WW (UI)[b]	Treated WW (RI)[b]
Physicochemical	Chemical oxygen demand (COD)	500–1,200	10–200	60–500
	Biological oxygen demand (BOD)	230–560	10–200	10–300
	Total N	30–100	5–45	10–70
	NH_4-N	20–75	n.a.	n.a.
	Organic N	10–25	n.a.	n.a.
	NO_3-N + NO_2-N	0.1–0.5	n.a.	n.a.
	Total Kjeldahl N	30–100	n.a.	n.a.
	Total P	6–25	2–30	30
	Ortho-P	4–15	n.a.	n.a.
	Organic P	2–10	n.a.	n.a.
	Total suspended solids (TSS)	250–600	10–60	30–150
	pH	7–8	4.5–9.5	5.5–9
	Electrical conductivity (mS/m)	70–120	100–300	270
	Na adsorption ratio	n.a.	8–10	9–10
	As	n.a.	0.02–0.10	0.02–0.10
	Cl	200–600	250–350	250–350
	Cd	1–4	0.005–0.010	0.005–0.010
	Cr	10–40	0.1–0.2	0.1–0.2
	Cu	30–100	0.2–1.0	0.2–1.0
	Pb	25–80	0.1–5.0	0.1–5.0
	Mg	1–3	0.001–0.002	0.001–0.002
	Ni	10–40	0.2	0.2
	Zn	100–300	0.5–5.0	0.5–5.0
	Phenol	0.02–0.10	0.10	0.10
	PAHs	0.5–2.5	n.a.	n.a.
	Phthalates	0.1–0.3	n.a.	n.a.
Biological	Faecal coliforms (CFU/100 mL)	10^{6c}	$0–2 \times 10^4$	2×10^2 to 4×10^4
	Nematode eggs (no./L)	n.a.	0.1–1	0.1–1

The values are from [a]Henze and Comeau [25]; [b]values of legal standards from [6, 27–38]; [c]Ferreira da Silva et al. [26]
n.a. not available, *CFUs* colony-forming units

Many of these are not completely removed during wastewater treatment and are released with the final effluent [10, 18–24]. The awareness of the risks associated with these biological and chemical hazards has motivated the introduction of guidelines and legislation concerning the safe use of treated wastewater for

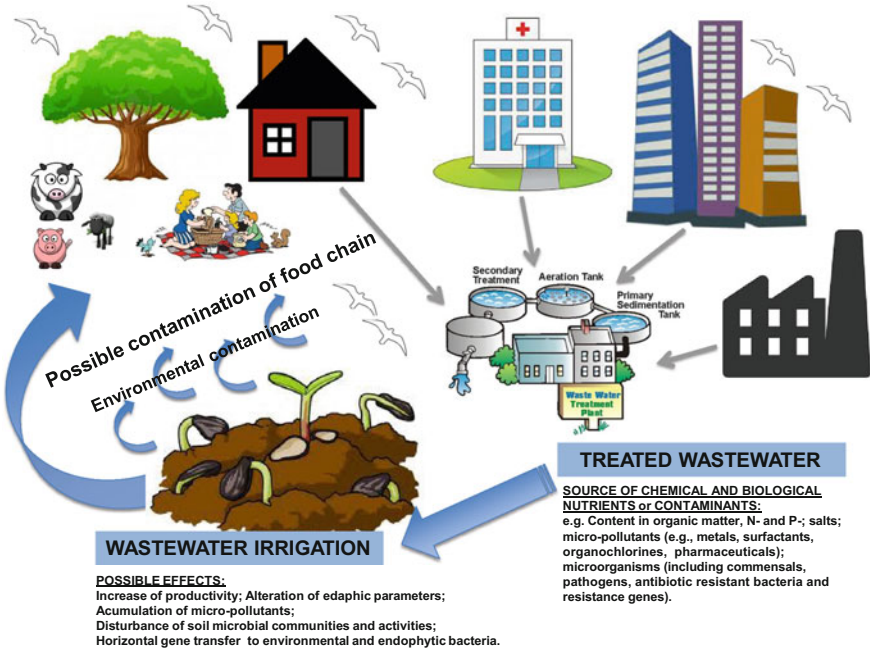

Fig. 1 Wastewater treatment and reuse for irrigation in agriculture: possible effects and human and environmental health implications

irrigation and other purposes (Table 1). However, it should be noted that some adverse effects of treated wastewater reuse cannot be evaluated based on those legal recommendations.

The microbiological risks associated with the use of treated wastewater in soil irrigation include three major lines: (1) the disturbance of indigenous microbial communities of soil, jeopardising their activity and, in turn, affecting soil health and long-term fertility; (2) the introduction of phytopathogens that may cause a reduction on either the yields or the quality of the crops or other cultivated plants; and (3) the introduction of human or animal pathogens or antimicrobial-resistant microorganisms which can be hosted by plants, contaminating the environment and/or the food chain, with implications in environmental and human health (Fig. 1). This holistic perspective of the implications of wastewater reuse involves different thematic areas such as soil microbial ecology, plant-microbe interactions and environmental-clinical microbiology. This review presents a summary of the possible direct or indirect effects of wastewater reuse on the soil microbial communities, based on studies that assessed possible alterations in soil properties after irrigation with treated wastewater. Major uncertainties, gaps of knowledge and risks associated with wastewater irrigation are discussed. The impacts of irrigation with wastewater will depend strongly on the plasticity of soil microbial

communities and on the composition of wastewater. Both microbial habitats, soil and wastewater, are briefly described in the two following sections.

2 Wastewater Composition

Urban raw wastewater usually comprises domestic, industrial and sometimes storm water. Wastewater composition is normally characterised based on few standard parameters. The chemical oxygen demand (COD), biological oxygen demand (BOD) and total suspended solids (TSS) are used to express the content of organic matter. Other parameters, such as the content in different forms of N and P and electrical conductivity, are commonly used to assess the availability of nutrients (NH_4-N, NO_3-N and P) and salinity, respectively. Avoiding the impossible task of surveying specific pathogens and parasites, the enumeration of indicators of faecal contamination, such as total and/or faecal coliforms and nematode eggs, is the standard method to assess the microbiological quality of water. A general overview of the raw wastewater composition is given in Table 1. A typical secondary treatment of urban wastewater is expected to significantly reduce the initial parameters such as BOD, TSS, total N and P contents. Nevertheless, the extent of removal depends on several factors, such as the composition of the raw wastewater and the treatment configuration and efficiency, which thus have an important influence on the characteristics of the final effluent. There is a general agreement about some standards with which treated wastewater must comply, and they are widely recommended with the aim of minimising environmental and public health negative impacts. These quality criteria are the basis for the legal standards or guidelines of treated wastewater to be discharged to surface water as well as for irrigation (Table 1). However, in the majority of the countries, routine monitoring of wastewater does not include potentially harmful agents. Although at low densities, they are inevitably present in treated effluents and may have undesirable effects on environment and human health. This is the reason why countries such as the USA, Mexico, Israel, Jordan, Oman or Italy require the determination of some trace metals and/or organic contaminants [e.g. As, Pb, Mg, Cr, Cd, phenols, polycyclic aromatic hydrocarbons (PAHs), phthalates] before the discharge of treated wastewater into the environment [6, 27–30]. However, other potentially harmful agents, such as pesticides, personal care and pharmaceutical products, including antibiotic residues, are not routinely monitored. Furthermore, treated wastewater contains antibiotic-resistant genes and bacteria with potential adverse effects on human heath [22, 39–48]. Although treatment reduces the microbial load, treated wastewater still contains a considerable diversity and number of chemicals and microorganisms (up to 10^6–10^7 CFU/100 mL) [26, 49, 50]. Among these, though not considered pathogenic, antibiotic-resistant bacteria can also negatively impact the microbiological quality of wastewater. Moreover, given the abundance of nutrients and close contact between bacteria, the occurrence of antibiotic

resistance acquisition, mainly via horizontal gene transfer, may occur either in the municipal collector or during wastewater treatment [43, 49, 51]. As an example, based on experimental data from different wastewater treatment plants, it was estimated that, irrespective of treatment type and efficiency, plant size or world region, a domestic wastewater treatment may release up to 10^9 ciprofloxacin-resistant coliforms per minute to the environment, depending on the volume and flow of water [46]. Thus, even if it can be considered with adequate quality, treated wastewater contains chemical and microbiota components which may negatively impact soil quality and characteristics.

3 Soil Holds Rich and Diverse Microbial Communities

Soil is considered the most complex and heterogeneous biomaterial on earth [52], holding structurally and metabolically diverse microbial communities [53, 54]. Due to such metabolic diversity, microbial communities are responsible for cycling abundant elements such as C and N (e.g. [55–62]) and less abundant, although essential, elements such as S and Fe (e.g. [55, 63–65]). Therefore, while each metabolic type of microorganisms has a key role in the recycling of elements, a well-balanced microbial community is essential for an adequate biogeochemical equilibrium of the soil. Microorganisms are also essential to the maintenance of soil structure, in particular soil aggregation [66–68]. Moreover, rhizosphere soil microorganisms play a key role in plants' development and health. Through the interaction with roots, microorganisms promote processes that are crucial for plant nutrition and growth (e.g. N_2 fixation, P solubilisation, siderophore production) and confer protection against phytopathogens [69–73]. Therefore, from both perspectives of soil quality and plant protection, the maintenance of the physiological and metabolic diversity of microorganisms can be considered as one of the most important determinants of soil fertility.

Other important functions are attributed to soil microorganisms. A good example is the biodegradation of several micropollutants which contribute to attenuate the negative impacts of xenobiotics or other noxious compounds discharged in soil (e.g. pesticides, organochlorides, PAHs, antibiotics, birth control and natural hormones) (e.g. [74–83]). Hence, due to biodegradation activity, soil microorganisms contribute to avoid the dissemination of micropollutants to the surrounding environment through surface run-off and leaching into aquifers. However, soil microbial communities may have a limited capacity to regenerate soils submitted to frequent discharges of xenobiotics (not naturally produced) or natural exogenous substances that will act as pollutants [84–87]. In general, it can be hypothesised that the long-term wastewater reuse, mainly if the minimal quality standards are not met, will have implications either on the turnover of some chemical components or on the adequate balance of microbial populations in soils. Both have adverse impacts on soil health and agriculture production.

4 Possible Effects of Irrigation with Treated Wastewater on the Soil Microbial Communities

The structure and function of soil microbial communities are greatly influenced by a wide variety of abiotic and biotic factors, such as soil texture, pH, organic matter content, N and P inputs, presence of different types of micropollutants, land use history, agricultural management, vegetal cover, introduction of exogenous organisms, among others (e.g. [88–96]). Considering the complex composition of treated wastewater and the myriad of factors capable of affecting soil microbial communities, it is likely that irrigation with treated wastewater disturbs the soil microbiome. Such effects may be direct, through the introduction of exogenous microorganisms, or indirect through the alteration of soil physicochemical properties resulting in a change of the microbial activities and populations. Some of these effects are illustrated by case studies assessing the effect of the reuse of treated wastewater on physicochemical and microbiological soil properties (Table 2). The studies analysed are representative of different regions (e.g. Spain, India, Mexico, France, Pakistan, Italy, China, Greece, Turkey, Brazil, Australia, Senegal, Israel, USA), type of treated wastewater reused (urban, industrial or synthetic), type of soil used (e.g. golf course, land near to a wastewater treatment plan, orchard land, agricultural, horticultural, grazed pastoral soils) and history of wastewater irrigation (from 4 months to 90 years). Most of these studies aimed to evaluate the effect of treated wastewater irrigation on soil productivity and physicochemical quality (e.g. [99, 105, 109, 116]). Other studies assessed the potential environmental impacts of metals and antibiotics introduced in soil through wastewater irrigation (e.g. [101, 107, 114, 119, 122]). The approach used in the majority of the studies involved the comparison of soil characteristics when irrigated with treated wastewater and with natural freshwater. The analysed edaphic parameters were soil pH, organic matter content, exchangeable cations, Na concentration, electrical conductivity, total available P and total N content and metal and micropollutant concentrations, including antibiotics (Table 2). The microbiological parameters included the soil biomass content, the enzymatic activity and the abundance of specific microbial groups, such as the total aerobic bacteria or fungi. Few studies focused on the diversity of specific bacterial groups, such as the ammonia-oxidising bacteria, or antibiotic-resistant bacteria and their genetic determinants.

4.1 pH

Soil pH variation, either increase or decrease, may result from irrigation with treated wastewater (Table 2). Although the analysed studies did not assess alterations in the microbial communities, both increase and decrease of pH are known to have a strong influence on the soil microbial richness (number of different species) and diversity (variety of organisms) [89, 123, 124] depending on the buffer capacity

Table 2 Case studies of potential impacts of irrigation with treated wastewater

Wastewater origin[a]	Soil description[a]/ culture/period of irrigation (years)/ country	Physicochemical changes[a]	Microbiological changes[a]	Reference
U, st	Calcisols WRB/alfalfa, maize, barley, oats/>20/Spain	↑ pH, water-soluble organic C, total available P	↑ microbial biomass, activity of beta-glucosidase, alkaline phosphatase	[9]
I (dairy)	Chromasols and tenosols ASC/grazed pastoral/> 60/Australia	↑ pH; ≡ total organic C, total N; ↓ C/N ratio; ↑ total available P, exchangeable Na, K, electrical conductivity	↑ microbial biomass C and N, soil basal respiration; ≡ metabolic quotient	[97]
U (flooding)	Typic haplustand USDA/hazel orchard/20/Italy	↑ pH, total organic C, active soil C resources, total N	↑ microbial biomass C, basal- and substrate-induced respiration; ↓ genetic diversity of the ammonia-oxidising bacteria	[98]
U	Xerofluvent USDA/grape crop/2/Spain	↑ pH; ≡ total organic C; ↑ total available P, electrical conductivity; ≡ cation exchange capacity, water holding capacity, aggregate stability	≡ activity of phosphatase, urease, beta-glucosidase	[99]
	Xerorthent USDA/grape crop/20/Spain	↓ pH; ↑ total organic C, total available P, cation exchange capacity, water holding capacity; ≡ electrical conductivity; ↓ aggregate stability	↑ activity of phosphatase, urease; ↓ activity of beta-glucosidase	
	Xerofluvent USDA/"green filter"/20/Spain	↓ pH; ↑ total organic C, total available P, cation exchange capacity, electrical conductivity, aggregate stability; ≡ water holding capacity	↑ activity of phosphatase, urease, beta-glucosidase	
	Xerorthent USDA/orange-tree orchard/40/ Spain	↓ pH; ↑ total organic C, electrical conductivity, total available P; ≡ cation exchange capacity, water holding capacity, aggregate stability	↑ activity of phosphatase; ≡ activity of urease, beta-glucosidase	

(continued)

Table 2 (continued)

Wastewater origin[a]	Soil description[a]/culture/period of irrigation (years)/country	Physicochemical changes[a]	Microbiological changes[a]	Reference
U, st	Loamy fine sand texture/alfalfa hay, sudangrass and winter grains/3, 8, 20/USA	↓ pH; ↑ organic matter content, electrical conductivity, salinity, metals (Cr, Cu, Ni and Zn)	NR	[100]
U, st	Argosols and cambosols CST/cereals and vegetables/> 40/China	≡ pH; ↑ humic acids, metals (Cd, Cr, Cu, Ni Pb, Zn)	NR	[101]
U, st	Fine texture/forage crops/2, 5, 10/Jordan	≡ pH; ↑ organic matter content, total N, total available P, K, salinity; ≡ metals (Cu, Pb, Cd)	NR	[102]
U	NR/barley, corn, cotton, alfalfa, sorghum/80/USA	≡ pH; ↑ soil compaction; ↓ Mg; ≡ total available P, electrical conductivity, metal (Zn)	NR	[103]
U, st	Fine clay and silt loam texture/corn/NR/China	↑ total organic C, total N, total available P	NR	[104]
U, st	Xerorthent USDA/orange-tree orchard/43/Spain	↑ total organic C, total available P	↑ activity of alkaline phosphatase, urease, dehydrogenase, protease, beta-glucosidase; ↓ arbuscular mycorrhizal fungi diversity	[105]
U	Vertisols WRB/cereals and vegetables/< 80/Mexico	↑ total organic C, salinisation, metals (Pb, Cd, Cu, Zn)	↑ microbial biomass, activity of dehydrogenase, denitrification activity; ↓ adenylate energy charge ratios	[106]
	Leptosols WRB/cereals and vegetables/< 80/Mexico	≡ total organic C; ↑ salinisation, metals (Pb, Cd, Cu, Zn)		
U	NR/cereals, millets, vegetable and fodder crops/5/India	↑ total organic C, metal (Fe)	NR	[107]
	NR/cereals, millets, vegetable and fodder crops/10/India	↑ total organic C, metals (Zn, Fe, Ni, Pb)		
	NR/cereals, millets, vegetable and fodder crops/20/India	↑ total organic C, metals (Zn, Cu, Fe, Ni, Pb; ↓ Mn)		

(continued)

Table 2 (continued)

Wastewater origin[a]	Soil description[a]/ culture/period of irrigation (years)/ country	Physicochemical changes[a]	Microbiological changes[a]	Reference
U, st	Vertic xerofluvent USDA/maize/ 0.25/Turkey	≡ total organic C	↑ C_{mic}/C_{org} ratio; ↓ activity of dehydrogenase, urease, alkaline phosphatase, arylsulphatase	[108]
U	Typic haplustox USDA/sugarcane/ >1/Brazil	≡ total organic C, total N; ↑ NO_3-N	NR	[109]
U, tt	Horticultural soil/ NR/1/France	≡ organic matter content	↑ activity of laccase, cellulase, protease, urease; ≡ functional diversity of soil microorganisms CLPP	[110]
I (textile)	Loamy texture/ fodder, cereals/ NR/Pakistan	↓ organic matter content, total available P, exchangeable cations; electric conductivity, total soluble salts, SO_4, NO_3-N; ↑metals (Zn, Cu, Ni, Cr)	↑ population of bacteria, vesicular arbuscular mycorrhizae, heavy metal-resistant bacterial strains	[111]
U (lagoon)	Vertic xerocrept USDA/citrus orchard/15/Italy	↑ organic N,NH_4-N, NO_3-N	≡ microbial biomass C and N; ↑ activity of hydrolase, phosphatase	[112]
Synthetic wastewater with 0 or 1.5% salinity	Sandy loam texture/mangrove swamp/ 0.25/China	≡ total N; ↑ NH_4-N, NO_3-N, total available P, metals (Cu, Zn, Cd, Mn)	↑ aerobic and anaerobic bacteria, ammonia- and nitrite-oxidising bacteria; ≡ activity of dehydrogenase, phosphatase	[113]
U	Mollic leptosol and eutric vertisol WRB/maize/5 and 90/Mexico	↑ total available P, metals (Cr, Cu, Ni, Zn, Pb)	↓ arbuscular mycorrhizal fungi free spores irrigation 90 years	[114]
U, tt	Silty sand texture/ perennial ryegrass/3/Spain	↑ Ca, Mg, salinisation	≡ microbial abundance total aerobic bacteria	[115]
U (lagoon)	Quartzarenic neosol SiBCS/ eucalyptus/5/ Brazil	↑ Na, Na adsorption ratio, exchangeable Na	NR	[116]
I (factories)	Rhizosphere soil/ wheat/~10/India	↑ metals (Fe, Cr, Zn, Pb, Ni, Cd, Cu)	↑ abundance of metal-resistant *Azotobacter chroococcum* isolates	[117]

(continued)

Table 2 (continued)

Wastewater origin[a]	Soil description[a]/ culture/period of irrigation (years)/ country	Physicochemical changes[a]	Microbiological changes[a]	Reference
I (oil refinery)	NR/agricultural/ 12/India	↑ metals (Fe, Ni, Zn)	≡ microbial dynamics viable counts of aerobic heterotrophs, actinomycetes, fungi and potentially asymbiotic diazotrophs	[118]
Synthetic industrial wastewater	NR/mangrove/ 0.5/China	↑ metals (Cd, Cr, Cu, Ni, Zn)	↓ activity of alkaline phosphatase	[119]
U and I	Silty clay loam texture/crops/ 50/China	↑ endocrine-disrupting chemicals, e.g. triclocarban, and pharmaceuticals, e.g. oxytetracycline, tetracycline	NR	[120]
U, st	Dune quartz sand/ citrus orchard lysimeter/12/ Israel	NR	≡ enumeration of antibiotic-resistant bacteria, antibiotic resistance genes	[121]
	Vertisol 60% clay/avocado orchard/12/Israel			
	Loam 20% clay/ cotton, wheat/15/ Israel			
	Vertisol 52% clay/olive trees/ 6/Israel			
U	NR/parks/ NR/China	↑ antibiotics and degradation products	↑ diversity and abundance of antibiotic resistance and integrase genes	[122]

Main alterations in physicochemical or microbiological soil parameters when irrigation with treated wastewater was compared with freshwater irrigation

[a]According to the information reported in the reference. Soil classification was used when available and indicated in parenthesis

USDA United States Department of Agriculture, *WRB* World Reference Base for soil resources, *CST* Chinese Soil Taxonomy, *ASC* Australian Soil Classification, *SiBCS* Brazilian Soil Classification, *U* urban, *I* industrial, *st* secondary treated, *tt* tertiary treated, NR not reported, ↑ increase, ↓ decrease, ≡ no variation

of the soil. In addition, pH variation can influence the solubility of different compounds, in particular metals and ionisable organic compounds and, therefore, affect the soil chemical composition [107, 111].

4.2 Organic Matter

In some studies, soil organic matter-related pools increased due to irrigation with treated wastewater (Table 2). However, through the comparison of the different studies, it is suggested that the influence of wastewater irrigation on soil properties may depend on the concentration and composition of organic matter in water as well as on the soil texture [125, 126]. In either case, variations on organic matter content and the type of organic inputs will influence the indigenous microbial communities of soil [54, 95]. Indeed, in most of the case studies in which variation in the organic matter content was reported, fluctuation was also observed in one or more microbial parameters (Table 2).

4.3 Salinisation

The increase of soil electrical conductivity/salinity (i.e. water in soil) was observed in the majority of the reviewed studies (Table 2). Soil salinity may strongly affect soil structure, and it is described as having negative impacts on soil microbial diversity, microbial biomass and activity. The hindering of functions related to C and N mineralisation had also been described [127–131]. For these reasons, salinity may reduce soil fertility and productivity.

4.4 Nutrients and Macro-elements

Wastewater has high contents of total N and P and exchangeable cations (e.g. K, Na, Mg, Ca) [25] (Table 1). This is one of the potential beneficial aspects of irrigation with wastewater, since it may supply nutrients and macro-elements, substituting synthetic fertilisation [9, 11, 132]. However, it should be noted that adverse effects can also result from the leaching of excess of available P and NO_3-N into natural waters, causing contamination [133] and eutrophication of these habitats [134]. Indeed, biological P- and N-removal technologies have been developed as a measure to reduce the impact of the introduction of these nutrients in the environment [135]. The increase of total available P content in wastewater-irrigated soils was consistently reported [9, 97, 99, 104, 105, 114], with a single exception, where the reference soil is an uncultivated land with high P content [111]. In some studies, irrigation with wastewater did not affect the soil total N content [109, 113], but in others, it led to an increase [98, 104]. Simultaneously, N-related pools were also influenced by wastewater irrigation, with the increase in NO_3-N, NH_4-N or organic N reported in different studies [109, 112, 113]. Such variation on the impact of wastewater irrigation on the soil N may be due to the presence of different N-forms and concentration both in water and soils. The increase in the content of

total available P and NO_3-N, and the simultaneous accumulation of macro-elements in soils, may contribute to change the diversity and catabolic activity of microbial communities [92, 136–139]. Whether these variations have positive or negative impacts on soil microbiota and productivity was not clear from the analysed studies. Probably because ammonia-oxidising bacteria populations do vary in response to N inputs [137], increase in the abundance of ammonia- and nitrite-oxidising bacteria was observed in soils irrigated with synthetic wastewater [113]. This is a clear example of how chemical inputs from wastewater may lead to alterations in the soil microbiota.

Wastewater irrigation influenced the abundance of exchangeable cations. However, no general trend was observed, since the abundance of exchangeable cations either decreased or increased after irrigation [97, 99, 103, 111, 115]. These observations suggest that many factors in soil and other external conditions may influence the fate of nutrients and macro-elements supplied in wastewater.

4.5 Trace Metals

Given the frequent occurrence of trace metals in wastewater (Table 1), irrigation may lead to the increase of their content in soil [100, 101, 106, 107, 111, 113, 114, 117–119]. Some of these metals, such as Fe, Zn and Cu, have a beneficial role in the functioning of biological systems when present at low concentrations [140, 141]. Others, such as Pb, Cr or Cd, may be toxic to microbes and plants, even at low concentrations. The adverse effects of metals may be aggravated by the fact that they may bioaccumulate in plants and enter the food chain [100, 101, 107, 111, 119, 142]. In soil, metal accumulation may induce changes in the soil's functional activity and in the abundance and diversity of fungi and bacteria [111, 114, 117]. Some trace metals have bacteriostatic properties and may cause cross resistance against antibiotics [143]. The selective effect of metals can be inferred from the fact that higher density of metal-resistant organisms was observed in soils with increased concentration of metals due to irrigation with wastewater than in control soils [111, 117]. The phytotoxicity of some metals and the risk of metal leaching after long periods (~20 years) of soil irrigation with wastewater [100, 119] are also important negative impacts that may result from wastewater irrigation.

4.6 Organic Micropollutants

The introduction of personal care and pharmaceutical products, including endocrine-disrupting chemicals (e.g. antibiotics, lipid regulator agents, anti-inflammatory drugs, cancer therapeutics, beta-blockers, contraceptives and other hormones), in the environment via wastewater irrigation is also a well-described problem [10, 11, 22, 41, 46, 120, 144–146]. Depending on the mobility of the

micropollutants, different risks are posed. Highly mobile micropollutants can leach into and contaminate groundwater, while those strongly adsorbing to soil particles, such as tetracycline, can accumulate in the top soil layer [147]. The contamination of the food chain, via the uptake of some pharmaceutical wastes, including antibiotics, by plants is another possible consequence of wastewater irrigation [146, 148–155]. For antibiotics, the role of these pollutants in resistance acquisition and selection cannot be ignored [122, 147]. The current state of the art shows that treated wastewater is a reservoir of antibiotic-resistant bacteria, resistance genes and mobile genetic elements [13, 43, 49, 122, 156–158 and contributions in this book]. Therefore, the hypothesis that irrigation of soils with treated wastewater is a route for resistance dissemination cannot be discarded. This is not a clear issue, since some contradictory results were found. While the discharge of treated wastewater in freshwater receiving environments is known to expand the levels of antibiotic-resistant bacteria and resistance genes, it is not clear if irrigation with treated wastewater contributes to the rise of antibiotic-resistant levels in the soil microbiome [121, 122, 159]. The possibility of occurrence of horizontal gene transfer between the exogenous bacteria (derived from wastewater) and the established soil or plant microbiota is, thus, a reason of concern.

Other organic micropollutants, such as surfactants, PAHs or polychlorinated biphenyls (PCBs), among others, may also accumulate in the soil due to long-term irrigation with wastewater. Although it is known that some micropollutants have the potential to disturb soil microbial communities [160, 161], to the best of our knowledge, studies assessing such effects due to irrigation with wastewater are not available. This is a gap of knowledge that needs to be filled.

4.7 Microbiological Parameters

Most of the analysed case studies concluded that irrigation with treated wastewater, either of urban or of industrial origin, may lead to an increase of the soil microbial biomass (Table 2) [9, 97, 98, 106, 108]. When an increase in the microbial biomass was observed, it may have been due to the supplying of additional organic C and other nutrients by wastewater [9, 106, 110]. The observed increase in the activity of different enzymes involved in the biochemical turnover of elements such as C, N and P, such as dehydrogenase, laccase, cellulase, beta-glucosidase as well as alkaline phosphatase, hydrolase, protease and urease, corroborates this [105, 106, 162–164]. The input of organic matter due to irrigation with treated wastewater may be beneficial for soil, stimulating the catabolism of not only labile compounds but also complex substrates. However, some adverse effects of excessive microbial growth can also be observed, for instance when biofilms cause the clogging of soil particles, affecting the hydraulic conductivity [165].

The biogeochemical activity of microbiota is considered the most important aspect of soil quality, with implications in soil fertility and quality of plants. One of the concerns related with irrigation with treated wastewater is the disturbance of the

soil microbiota, which may hinder the extent and rate of biogeochemical transformations. These aspects were not clearly explored in the analysed studies, although some evidences of functional redundancy were reported. Functional redundancy means that, despite the alterations on the microbial populations, the same reactions will be undertaken, involving alternative microbial groups [62, 166]. For instance, this explains why ammonia and nitrite oxidation in soils are not affected by irrigation with wastewater [98, 166]. Nevertheless, although maintaining the normal activity, functional redundancy processes may lead to a decrease in the genetic diversity. This effect was observed for ammonia-oxidising populations after a long-term (20 years) irrigation with wastewater [98]. In general, the decrease of genetic diversity may be considered an impoverishment of the soil and, thus, an undesirable effect.

5 Conclusions

Microbial communities are extremely important to assure soil quality and productivity. Both wastewater microbiological and chemical composition may have impacts on soil physicochemical properties, microbial abundance, diversity and biogeochemical activity. Although often reporting contradictory trends, the analysed case studies demonstrated changes in chemical and microbiological soil parameters due to wastewater irrigation. However, the comparison of the different studies indicates clearly that many variables influence the impact of irrigation with treated wastewater on soil. Whereas no clear predictions are possible at the moment, it seems clear that soil quality and productivity may be affected by long-term use of treated wastewater for irrigation. The factors conditioning the possible impacts may vary among different ecosystems, and there is always a degree of uncertainty regarding the preferential target populations/functional activities or the interplay between different variables. Multidisciplinary studies involving the characterisation of the system wastewater-soil-plant as a whole are necessary, supporting a deeper understanding of the impacts of irrigation with wastewater. If these studies are not possible, at least in the short term, then the precautionary principle should be applied.

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Antibiotic Resistance Elements in Wastewater Treatment Plants: Scope and Potential Impacts

Joao Gatica, Ella Kaplan, and Eddie Cytryn

Abstract Antibiotic resistance is considered to be one of the most significant public health concerns of the twenty-first century. Although traditionally the propagation of antibiotic resistance was considered to be limited to hospitals and other clinical environments, there is a growing realization that it is also associated with anthropogenically impacted environmental reservoirs. Wastewater treatment plants are considered to be significant reservoirs of antibiotic resistance because they combine extremely high levels of fecal- and environmental-derived bacteria with residual concentrations of antibiotic compounds believed to induce selection. These bacteria are primarily congregated in dense biofilms that are "hot spots" for horizontal gene transfer, which can facilitate inter- and intraspecies transfer of antibiotic genes, potentially resulting in the development of multidrug-resistant strains. Several studies have demonstrated that although wastewater treatment plants significantly reduce bacterial concentrations, relatively high levels of antibiotic-resistant bacteria and resistance genes are still present in effluents released to aquatic and soil environments and that under certain circumstances these resistance elements may persist for long periods of time in downstream

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 129–154, DOI 10.1007/698_2015_361,

environments. These elements may have significant epidemiological ramifications, especially when effluents enter drinking water and food webs; and henceforth, antibiotic resistance genes have recently been characterized as contaminants of emerging concern. This chapter summarizes current understanding of antibiotic resistance in wastewater treatment plants and downstream environments, presents knowledge gaps that need to be bridged in order to better understand the potential ramifications of this phenomenon, overviews the effect of disinfection treatments on antibiotic resistance elements, and finally discusses policy guidelines that should be implemented in the future to reduce the risks of antibiotic resistance from wastewater treatment plants.

Keywords Antibiotic resistance, Antibiotic resistance genes, Antibiotic-resistant bacteria, Horizontal gene transfer, Mobile genetic element

Contents

Abbreviations

AR	Antibiotic resistance
ARB	Antibiotic-resistant bacteria
ARGs	Antibiotic resistance genes
BHR	Broad host range
CFU	Colony-forming units
CIs	Chromosomal integrons
E-COFF	Epidemiological cutoff
ERIC	Enterobacterial repetitive intergenic consensus
ESBL	Extended spectrum beta-lactamase
GC	Gene cassettes
GFP	Green fluorescent protein
HGT	Horizontal gene transfer
IS	Insertion sequences
ISCR	Insertion sequence common regions
MAR	Multiple antibiotic resistance
MDR	Multiple drug resistance
MGEs	Mobile genetic elements

MIC	Minimal inhibitory concentration
MIs	Mobile integrons
MLST	Multilocus sequence typing
MRIs	Multidrug-resistant integrons
NGS	Next-generation sequencing
Pc	Promoter
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
RIs	Resistant integrons
TRACA	Transposon-aided capture
UV	Ultraviolet
VRE	Vancomycin-resistant *Enterococcus faecium*
WW	Wastewater
WWTPs	Wastewater treatment plants

1 Introduction

Less than 60 years after the discovery of antimicrobial agents, we have moved from an age of antibiotics to the age of antibiotic resistance (AR), which is rapidly expanding [1]. To evade the toxic effects of antibiotics, bacteria have developed an array of cellular mechanisms, including enzymatic inactivation, target modification, efflux pumps, target bypass, and noninheritable mechanisms such as persistence, biofilm production, and swarming [2, 3]. The discovery that antibiotic resistance genes (ARGs) can be transmitted between bacteria has revolutionized our understanding of ARG dynamics because horizontal (or lateral) gene transfer (HGT) of mobile genetic elements (MGEs) transcends taxonomic boarders, facilitating acquisition of ARGs by phylogenetically diverse groups of bacteria [4]. Although acquisition of ARGs through mutation or HGT is generally considered to be a neutral process, the propagation of bacteria harboring ARGs in a specific environment is strongly dictated by selective pressure conferred by antibiotic compounds.

Hospitals have long been considered the nexus of AR evolution and propagation due to selective pressure associated with extensive application of antibiotics coupled to the plethora of pathogenic bacteria that reside there. Although these conditions undoubtedly accelerate the frequency of AR in pathogens and commensals, there is a growing realization that AR originated in natural environments (i.e., in soils) and that ARGs evolved long before human use of antibiotics.

Wastewater treatment plants (WWTPs) combine high densities of bacteria that are congregated in close proximity in biofilms and flocs. These include fecal bacteria from sewage that often contain pathogen-associated ARGs, environmental bacteria that may harbor novel AR mechanisms [5], and residual concentrations of antibiotic compounds that potentially confer a selective advantage to bacteria that acquire ARGs [6]. Although WWTPs substantially reduce levels of fecal bacteria,

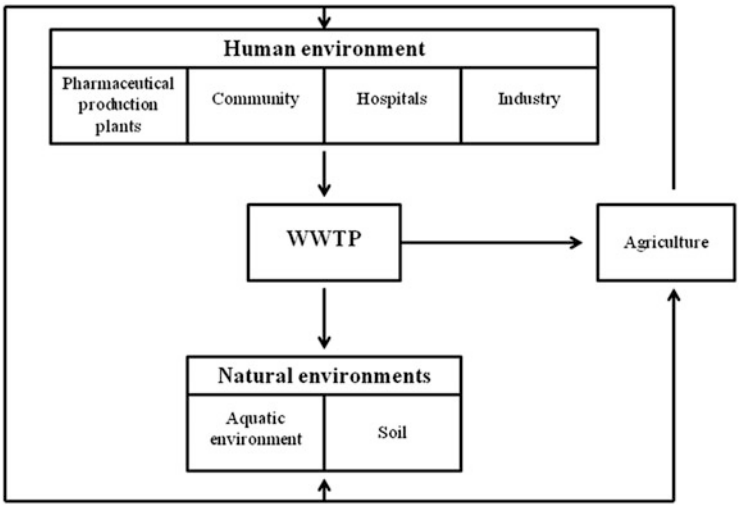

Fig. 1 Possible routes of dissemination of ARB and ARGs to and from WWTPs

they release residual concentrations of antibiotic compounds, antibiotic-resistant bacteria (ARB), and ARGs to downstream soil and aquatic ecosystems, which are believed to facilitate the transfer of ARB and ARGs through food webs where they can be ingested by humans and contribute to the global pandemic of antibiotic resistance [7–12], as summarized in the schematic diagram shown in Fig. 1.

Treated wastewater irrigation is becoming more and more prevalent in arid and semiarid regions of the world, and this trend is expected to increase given the increasing food demands and the predicted effects of global climate change [13]. Despite the obvious advantages of this process, there is a great concern regarding the potential impact of effluent-associated chemical and microbial contaminants, which have been addressed by stakeholders and regulatory bodies [14] (see also other contributions in this volume). Although regulations often address health-related factors such as heavy metals and enteric pathogens, current standards do not evaluate antibiotic compounds or ARB/ARG levels, which as discussed throughout this chapter may have significant epidemiological potential.

This chapter outlines methodological approaches that are used for assessing AR in WWTPs, summarizes the current understanding of the scope and diversity of ARB and ARGs in WWTPs, and discusses future technological and policy developments that can potentially mitigate AR from WWTPs in the future. Section 1 overviews the mechanistic aspects of MGEs, which facilitate HGT of ARGs; Sect. 2 presents methodologies that are currently applied for identifying ARB and ARGs in the environment; Sect. 3 summarizes culture- and molecular-based studies that assessed ARB and ARGs in WWTPs; Sect. 4 explores the impact of conventional wastewater disinfection processes on ARB and ARG abundance; Sect. 5 presents data on the persistence of ARB and ARGs in downstream environments; and Sect. 6 summarizes the state of the art and discusses future directions.

2 Mobile Genetic Elements

MGEs are defined as segments of DNA that encode enzymes and other proteins that mediate the movement of DNA within genomes or between bacterial cells [15]. They are transferred from one bacterium to another by means of transformation (i.e., uptake of naked DNA), conjugation (transfer of plasmids between bacteria), or transduction (viral transmission of extracellular DNA), as outlined in the schematic diagram in Fig. 2a–c [16]. Plasmids, bacteriophages, and conjugative resistance transposons can facilitate the transfer of genetic material from one bacterium to another, whereas transposons, gene cassettes, and integrons are translocated from one genetic location to another within an individual cell [17]. There is an increasing awareness that in-depth understanding of AR dynamics in the environment not only requires characterizing the function of individual ARGs that are disseminated from anthropogenic sources but also entails identifying the primary MGEs that are responsible for facilitating ARG transfer to downstream environments. This is especially true given the recent evidence that broad-host-range MGEs that harbor ARGs are disseminated from animal husbandry and

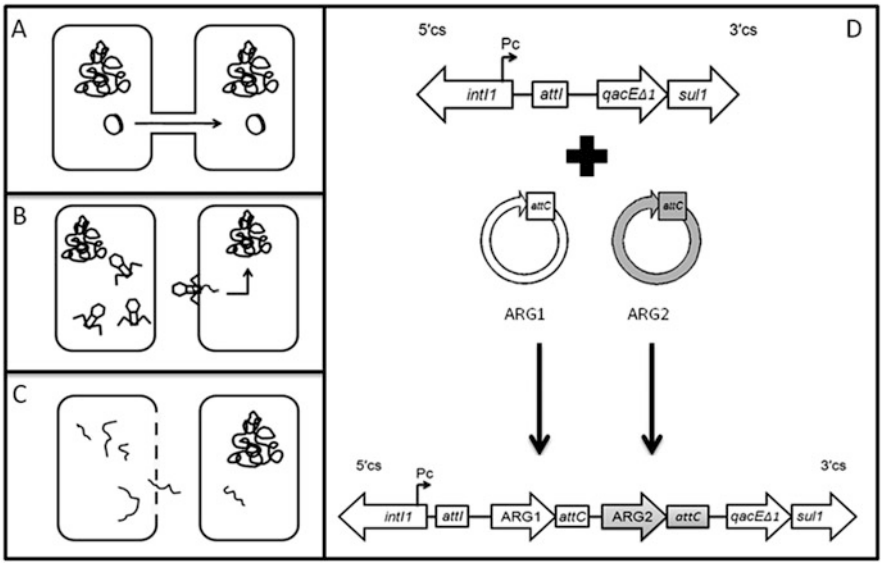

Fig. 2 Primary mechanisms of HGT among bacteria. (**a**) Conjugation: transfer of genetic material (plasmid or transposons, sometimes harboring ARGs) between bacterial cells by cell-to-cell contact. (**b**) Transduction: genetic material is transferred from one bacterial cell to another by a phage, with a subsequent incorporation of this genetic material into the chromosome of the acceptor bacterial cell. (**c**) Transformation: naked DNA is taken up by a bacterial cell, which incorporates and expresses this exogenous genetic material. (**d**) Scheme ARG integration in a class 1 integron: the *intI* gene catalyzes the incorporation of two gene cassettes harboring ARGs (ARG1 and ARG2)

aquaculture facilities and wastewater treatment plants through water and food webs into clinically relevant bacteria [3, 4]. The primary MGEs associated with horizontal transfer of ARGs and their modes of action are briefly summarized below.

Plasmids are circular extrachromosomal double-stranded DNA that replicate independently of the bacterial chromosome [15, 18]. They generally don't harbor housekeeping genes, which are responsible for the normal function of the bacterial cell, but instead carry accessory or functional genes that encode for toxins, virulence factors, specific metabolic pathways, and protective mechanisms including resistance to heavy metals and antibiotics [17]. These characteristics enable bacteria to evolve and adapt to dynamic environments without affecting their essential biochemical pathways [18]. Certain plasmids are only transferred between phylogenetically related hosts, while others, known as broad-host-range (BHR) plasmids [19–21], can be harbored by a diverse range of bacterial phyla. These plasmids are especially concerning because they can facilitate HGT on both inter- and intraspecies levels. It makes them primary drivers of AR in general and specifically of multidrug resistance in both clinical and natural environments.

Transposons are MGEs that facilitate the movement of DNA fragments from one location to another on bacterial chromosomes or plasmids [18]. They are well-structured modular systems that contain a pair of insertion sequence (IS) elements and often contain other genes that confer a selective advantage such as ARGs [17], which can transpose between bacteria chromosomes and plasmids and thereby be transferred into other cells. There are many transposons that are strongly related with AR such as Tn5 and Tn10 which encode resistance to kanamycin and neomycin, and tetracycline, respectively, in many Gram-negative bacteria [22, 23]. Tn3 confers resistance to β-lactams and Tn21 to streptomycin, spectinomycin, and sulfonamides. Both of these MGEs are frequently found in *Enterobacteriaceae* [24–26]. Recently Zhu et al. showed that manure processing in three large-scale commercial swine farms in China dramatically enriched a large fraction of ARGs and, interestingly, also transposases were enriched 1,000-fold in soil samples and even 90,000-fold in manure samples [27]. The authors reported a strong correlation between ARGs and levels of transposases, with significant associations between transposases and tetracycline and aminoglycoside resistance genes.

Integrons are two-component gene capture and dissemination elements that are frequently involved in the capture, mobilization, and spread of ARGs in Gram-negative bacteria [28]. An *intI* gene encoding for an integrase catalyzes the incorporation of gene cassettes (GCs) by site-specific recombination, directed by one or more promoters (Pc) into an integration site *attI* through recombination with a GC-associated *attC* site. A schematic description of this integration processes is shown in Fig. 2d. Integrons can be classified in two major groups: "chromosomal integrons" (CIs) and "mobile integrons" (MIs). CIs are located in the chromosome of hundreds of bacterial species and can carry up to 200 cassettes that mainly encode proteins with unknown function, whereas MIs contain a limited number of GCs, usually encoding antibiotic resistance determinants and therefore sometimes called "resistant integrons" (RIs) or "multidrug resistance integrons" (MRIs) [29]. There are three principal classes of MIs associated with AR: *class 1 integrons*,

the most ubiquitous in resistant bacteria, especially in Gram-negative bacteria of clinical interest, and are the most reported in animal and humans [30, 31], although also have been reported in nonpathogenic environmental Betaproteobacteria [32]; *class 2 integrons*, which are less prevalent and harbor a defective integrase gene resulting in a truncated and nonfunctional protein which generally produces a stable GC array mainly conferring resistance to trimethoprim, streptomycin, and spectinomycin [28, 29]; and *class 3 integrons*, which have only minor relevance in clinical settings and natural ecosystems [29]. Class 1 integrons have recently been highlighted as potential targets for source tracking of ARGs from anthropogenic environments because they are generally significantly more abundant in anthropogenic sources than in pristine environments. This was recently demonstrated in a study by Gaze et al., who showed that the relative abundance of class 1 integrons is higher in bacteria exposed to detergents and/or antibiotic residues, typically found in WWTPs, than in the non-exposed soil bacteria obtained from a farm with no known history of sludge or slurry amendment [33]. These findings should be taken with caution since a more recent study performed by Nardelli et al. found that *intI1* genes in pristine environments were not significantly more abundant in anthropogenic environments than in remote areas from urban centers [34].

Horizontal transfer of MGEs is conventionally associated with vital bacteria. However, there is increasing evidence that naked DNA can be extremely stable in the environment when attached to clay particles or organic material [35], and therefore these vectors may be naturally transformed to bacteria in downstream microbiomes. The potential for interspecies natural transformation of naked DNA harboring MGEs such as transposons, integrons, and gene cassettes between bacterial species was demonstrated by Domingues et al., who showed that acquisition of AR traits as well as entire integrons and transposons through natural transformation by environmental and clinically relevant bacterial strains occurred at high rates, in the course of a 24 h exposure period [36]. The study strongly implied that natural transformation provides a much broader capacity for horizontal acquisitions of genetic elements than previously assumed, and this may be highly relevant when assessing the potential risks of MGE-associated ARGs in wastewater effluents.

3 Methodologies for Identifying ARB and ARGs in WWTPs

Isolation of bacteria is vital for determining resistance levels and phenotypes of specific bacterial taxa, especially when evaluating pathogenic and clinically relevant commensal strains that are commonly monitored in WWTPs. Nonetheless, it is currently estimated that less than 1% of environmental bacteria can be isolated using standard microbiological procedures, and therefore, culture-dependent

methodologies are highly limited for evaluating the full scope of AR in natural environments [37].

Evaluation of AR elements in WWTPs can be addressed by application of both culture-dependent and culture-independent methodologies. Pure cultures can be screened to determine resistance profiles as well as other physiological, genetic, and biochemical characteristics. Nonetheless, because a large fraction of bacteria are unculturable, these methods undoubtedly neglect a large fraction of resistant bacteria. Molecular-based methods circumvent culturing; however, they generally can only target a limited number of ARGs and usually are not able to link detected ARGs to specific bacterial taxa. A brief outline of both of these approaches is given below.

Isolation of bacteria from WWTPs generally involves serial dilutions from selected compartments (inlet, outlet, activated sludge, etc.), using either general media that target a broad range of bacteria or selective growth media that enrich for particular groups of bacteria. At the most basic level, the relative abundance of ARB for a specific medium is estimated by dividing bacterial levels on antibiotic-amended media by the total abundance on non-amended media. In addition, minimal inhibitory concentration (MIC) and epidemiological cutoff (E-COFF) values for individual isolates can be determined using clinical and veterinary guidelines, such as EUCAST [38] and CLSI [39]. Resistant isolates are generally screened against a broad range of antibiotic compounds to assess multidrug resistance, and they may be subjected to a wide array of biochemical assays to characterize specific resistance phenotypes such as phenotypic screening of β-lactamase activity in Gram-negative bacteria [40]. Resistant isolates can be phylogenetically characterized by 16S rRNA gene analysis [41], or alternatively, strain typing can be accomplished by enterobacterial repetitive intergenic consensus (ERIC) sequence PCR [42] or more robustly by multilocus sequence typing (MLST) [43]. Once resistance phenotypes are characterized, strains can be screened for specific ARGs and MGEs using standard PCR techniques [44–47]. The extremely high throughput and economically feasibility of next-generation sequencing (NGS) platforms have revolutionized the capacity to fully sequence genomes and associated MGEs of ARB [48]. This can provide a much more comprehensive representation of bacterial resistance gene potential, and therefore, these methods are expected to replace PCR-based screening methods in the future. For example, Johnning et al. applied NGS to sequence the genome of a multidrug-resistant bacterium isolated from an antibiotic production facility and found that it contained a diverse array of MGE-associated ARGs [49], whereas Wibberg et al. used NGS to characterize a plasmid from a WWTP isolate, which was highly related to virulent plasmids from pathogenic *E. coli* isolates and contained known and putative AR and virulence genes [50].

Over the past few decades, the limitations of isolation-based methods have been circumvented by a myriad of molecular-based, culture-independent methodologies that target nucleic acids extracted directly from natural environments. It should however be noted that while molecular-based methods are highly efficient for ARG detection, these methods do not enable phenotypic analysis of antibiotic resistance

phenotypes, and therefore, optimally a combination of culture-based and culture-independent methods should be applied for comprehensive evaluation of AR in WWTPs and other environments. Currently, culture-independent quantitative PCR (qPCR) is the most widely used method for determining the relative abundance of ARGs, and these have been pivotal for source tracking of ARGs in anthropogenically impacted ecosystems [51–54]. Due to time and monetary limitations, generally only a limited amount of ARGs (out of hundreds of known genes) can be screened by qPCR, and it is therefore important to select representative ARGs that are abundant in anthropogenic point sources but not in pristine environments. As discussed below, there is a need to pinpoint selected ARGs that can be "gold standards" for use in source tracking of AR in WWTPs. Recently, commercial companies have developed platforms that enable identification and relative quantification of multiple ARGs and MGEs from individual samples in a single run [55]. Although the current cost of these platforms makes broad-scale use of them unrealistic, they can be applied to pinpoint effluent-associated ARGs, which can later be tracked using standard qPCR approaches.

The realization that ARGs themselves can be viewed as "contaminants of emerging concern" due to HGT necessitates development of risk assessment tools that can be used for tracking ARGs in WWTP and determining their fate in downstream environments. As discussed above, qPCR has become a gold standard for monitoring ARG dynamics in the environment and is valuable for source tracking studies when comparing the relative abundance of ARGs within WWTPs and in downstream environments (Table 1). Nonetheless, the lack of standardized methodologies and knowledge gaps regarding which of the hundreds of known ARGs are best suited for source tracking (i.e., genes that are highly associated with anthropogenic sources and are sparse in un-impacted environments) sometimes complicates the interpretation of qPCR data. Generally, qPCR-based studies are much more informative when they combine the quantitative gene data with conventional microbiological and chemical analyses.

Additional methods used for identifying and characterizing ARGs directly from the environment include transposon-aided capture (TRACA) [56] and functional metagenomics [57]. Both of these methods involve capturing DNA fragments in genetic vectors, transforming them to competent bacterial acceptor strains and platting transformed strains on media containing antibiotics. Since the original competent strains are sensitive to the screened antibiotics, growth of these strains indicates acquisition of a vector harboring an ARG. These vectors can then be extracted and sequenced in order to identify the gene that confers resistance. Although these methods are highly exhaustive, they enable identification of novel ARGs and they often identify flanking MGEs that are associated with the transfer of these genes.

Metagenomics, the capacity to sequence and analyze whole genomes of complex microbial communities, is a powerful tool for studying the full scope of ARGs and MGEs in the environment [58]; and the NGS revolution will inevitably facilitate a rising number of metagenomic studies specifically targeting AR dynamics in WWTPs and downstream environments. For example, Tiirik et al. characterized

Table 1 Commonly detected ARGs in WWTPs and associated MGEs downstream environments

Antibiotic/MGE class	ARGs in WWTPs	ARGs in WWTP effluents	References
Tetracyclines	*tet*(X), *tet*(G), *tet*(M), *tet*(C), *tet*(33), *tet*(36), *tet*(W), *tet*(O)	*tet*(X), *tet*(G), *tet*(M), *tet*(C), *tet*(33), *tet*(36), *tet*(W), *tet*(O), *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(H), *tet*(J), *tet*(Z), *tet*(L), *tet*(AP), *tet*(Y), *tet*(T)	[51, 54, 56, 60, 69, 92, 93, 94, 97, 98]
Sulfonamides	*sul* (I), *sul* (II)	*sul* (I), *sul* (II)	[54, 56, 60, 63, 94, 97, 98, 107, 109]
B-lactams	bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{OXA}, bla_{VEB}, bla_{VIM}, bla_{IMP}, ampC	bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}	[54, 56, 63, 64, 65]
Macrolides	*erm*F, *erm*B, *erm*A	*erm*F, *erm*B	[54, 60, 69, 94, 97]
Quinolones	*qnrA, qnrB, qnrS, qnrQ,*	*qnrA, qnrB, qnrS*	[54, 67, 101]
Amynoglicosides	*aacA, aadA, str*A, *str*B	*str*A, *str*B	[56, 60, 101]
Class I Integrons	*Intl* 1	*Intl* 1	[6, 28, 67, 68, 69, 92, 93, 94, 97, 98, 109]
Class II Integrons	*Intl* 2	*Intl* 2	[6, 68]

bacterioplankton structure and quantified ARGs in the Baltic Sea [59], whereas Wang et al. used the same platform to assess the occurrence, diversity, and abundance of ARGs and MGEs in sludge of a full-scale tannery WWTP in China [60]. Although currently metagenomic analyses are not feasible for routine monitoring of AR in downstream environments, the exponential reduction in costs coupled to increased bioinformatic capacities may facilitate cheap and rapid analysis in the future, thus enabling a holistic overview of MGEs and ARGs in effluent, upstream, and downstream ecosystems.

4 Monitoring ARB and ARGs in WWTPs

Wastewater treatment significantly reduces bacterial levels. However, substantial levels of ARB and ARGs can still be detected in effluents and therefore may contribute to AR in downstream environments. These include an array of prominent genes that confer resistance to tetracycline, sulfonamide, β-lactam, macrolide, quinolone, and aminoglycoside antibiotics. A summary of ARGs detected in WWTP isolates in the literature is summarized in Table 1, and some of these studies are detailed below.

Culture-based assessments of bacteria in WWTPs generally focus on commensal and pathogenic genera originating in the human intestinal tract such as *E. coli*, *Enterobacter*, *Enterococcus*, and *Klebsiella* [35, 61]. Although enteric bacterial levels are generally reduced by 1–4 orders of magnitude during sewage treatment, effluent levels of *Enterococci* and *E. coli* can still reach concentrations of up to 1,000 colony-forming units (CFU)/ml [62]. There is increasing evidence that WWTP processes may select for AR, and therefore, although total levels of enteric bacteria are significantly lower in effluents (relative to raw sewage levels), the relative abundance of ARB may actually increase. For example, Galvin et al. found that the relative abundance of multiple-antibiotic-resistant (MAR) *E. coli* strains harboring extended spectrum beta-lactamase (ESBL) genes was higher in WWTP effluent than in raw sewage [63]. Screening for specific ESBL genes indicated that 60.6% of the isolates encoded $bla_{CTX\text{-}M}$ group 1, 38% encoded $bla_{CTX\text{-}M}$ group 9, 23.9% encoded bla_{SHV}, and 19.7% encoded bla_{TEM} genes. This evidence is especially concerning because it indicates that effluents are point source for enterobacteria harboring clinically associated ESBLs, which can be transferred to downstream environments. Increased relative abundance of AR in WWTP effluents is supported by results of Korzeniewska et al., who assessed concentrations of *E. coli* in WWTP inlet and effluent sewage and receiving river water samples in Poland [64]. Although the WWTP reduced *E. coli* values by 99%, 2.7×10^3 CFU/ml *E. coli* still reached the receiving water. Plasmid-mediated β-lactamase genes were detected in almost 10% of the final effluent isolates, and these genes could be transferred by conjugation to *E. coli* recipient strains, demonstrating the capacity of effluent ARB to transfer AR-associated plasmids to downstream environments [65]. Luczkiewicz et al. examined the resistance profiles of 199 *Enterococcus* isolates and observed elevated levels of selected resistances and of multidrug resistance in wastewater effluents, relative to those detected within the WWTP, again suggesting that WWTPs may select for AR [66]. This was supported by work of Kaplan et al., who found that the MAR *Enterobacteriaceae* levels (resistant to more than 4 types of antibiotics) were higher in activated sludge than in raw sewage, and these isolates were more likely to harbor plasmid-mediated quinolone resistance genes [67].

Although enteric bacteria may have significant epidemiological ramifications, antibiotic resistance in WWTP has also been evaluated in other bacteria taxa, which may be significant due to their higher survival rates in natural aquatic and terrestrial environments. For example, Figueira et al. screened a collection of ciprofloxacin-resistant *Aeromonas* isolates from activated sludge and found that some of the resistant strains harbored clinically associated plasmid-mediated quinolone resistance genes *qnrS* and aac(6′)-ib-cr [68]. Additionally, the prevalence of antibiotic resistance in 366 *Acinetobacter* isolates to eight different antibiotics (including multidrug evaluation) was higher in the effluent than the observed in the influent [12]. This again indicates a potential selective advantage for antibiotic resistance strains in WWTPs.

Several studies have specifically focused on assessing MGEs in WWTPs, instead of merely analyzing presence of ARGs, due to the potential horizontal transfer of

these elements in downstream environments. For example, Pellegrini et al. showed that class 1 integrase genes were 3 times more abundant than class 2 integrase in *Enterobacteriaceae* isolates resistant to ampicillin obtained from a WWTP in L'Aquila (Italy) [6]. Class 1 integrons were also profuse in ciprofloxacin-resistant WWTP *Enterobacteriaceae*, detected in 50% and 42.7% of sludge and raw sewage isolates, respectively [66]. Ma et al. observed that class 1 integron abundance in bacterial isolates in a municipal WWTP in Jiangxinzhou (China) increased in the course of the wastewater treatment process, from 20.4% in the influent to 30.9% in the activated sludge to 38.9% in the final effluent. Moreover, 11 of the isolates contained gene cassettes conferring resistance to at least two different types of antibiotics, supporting the role of these MGEs in horizontal transfer of ARGs [69]. Collectively, these studies suggest that the relative abundance of integrons may actually increase in the course of the wastewater treatment process. To assess the genetic scope of ARGs in plasmids in a German WWTP, Szczepanowski et al. applied next-generation sequencing to a large composite sample of purified WWTP bacterial plasmids. The study revealed an array of ARGs associated with β-lactam, tetracycline, aminoglycoside, chloramphenicol, macrolide, sulfonamide, and trimethoprim antibiotics and quaternary ammonium compounds that are used as disinfectants. Furthermore, they identified several plasmids that harbored genes encoding multidrug resistance efflux systems that can confer resistance to multiple antibiotic compounds [70]. In a follow-up study, the same authors screened plasmids isolated from bacteria collected from both final effluent and activated sludge from the same WWTP and screened them by PCR using specific primers that target 192 genes, including aminoglycoside, beta-lactam, chloramphenicol, fluoroquinolone, macrolide, rifampicin, tetracycline, trimethoprim, and sulfonamide as well as multidrug efflux and small multidrug resistance genes. Almost 75% and 65% of the genes were identified in isolates from the activated sludge and final effluent, respectively, including some genes that were only recently described from clinical isolates [71]. This demonstrates the rapid genetic exchange between clinical and WWTP bacteria and demonstrates the capacity of plasmids and other MGEs to be horizontally transferred within and between environments. Furthermore, it indicates that these resistance determinants might be further disseminated in habitats downstream of the sewage plant.

As described above qPCR circumvents the need to culture bacteria from the environment and is therefore a vital tool for ARG source tracking in WWTPs. For example, Gao et al. recently measured tetracycline and sulfonamide concentrations at different WWTP stages by liquid chromatography/tandem mass spectroscopy and concomitantly used qPCR to assess the levels of tetracycline (tet(O) and tet (W)) and sulfonamide (sul(I)) resistance genes, which were normalized to total bacterial abundance by targeting bacterial 16S rRNA genes [53]. In tandem, resistant bacteria in raw influent, final effluent, and sludge samples were quantified using conventional culture-based approaches. Absolute levels of ARGs and ARB were 2–3 orders of magnitude lower in the effluents than in the influents, demonstrating the capacity of WWTPs to reduce overall levels of bacteria. Nonetheless, while the relative abundance of tet(O) and tet(W) diminished between inlet and

effluent, that of sul(I) genes remained stable throughout the treatment processes, demonstrating that certain ARGs are more persistent than others in WWTPs and therefore efforts should be made to focus on more persistent. This finding is supported by similar results previously published by several groups, including Iwane et al. and Kim et al. [72, 73], but contradicts others, who found little difference, if any, in the resistance profiles of selected bacterial groups in different stages of wastewater treatment [74].

Zhang et al. applied TRACA and next-generation sequencing to characterize plasmids from uncultured bacteria in activated sludge samples from the Shatin WWTP in Hong Kong [56]. Their results revealed high levels of ARGs encoding for tetracycline (27.2%), macrolide (25%), and multidrug (24.9%) resistances in the activated sludge and high levels of class 1 integrons harboring β-lactam (*ampC*, $bla_{VEB\text{-}3}$, $bla_{VIM\text{-}2}$, and $bla_{IMP\text{-}1}$), aminoglycoside (*aacA4*, *aadA1*, *aadA2*, *aadA2b*, and *aadA24*), sulfonamide (*sulI*), trimethoprim (*dfrA1*), and quaternary ammonium compound (*qacEΔ1*) resistance genes; additionally transposons and ISs were also detected. Interestingly, the author also observed seasonal fluctuations in tetracycline, sulfonamide, and vancomycin resistance genes. This approach may be pivotal for identifying key WWTP MGE-associated ARGs in WWTPs (which can be more robustly targeted using qPCR methods), but additional data from a larger pool of WWTPs is necessary.

Parsley et al. applied a functional metagenomic approach to identify AR determinants from bacterial chromosome, plasmid, and viral DNA from WWTP activated sludge [75]. Gene fragments transformed into *E. coli* conferred resistance to chloramphenicol, ampicillin, and kanamycin. The study demonstrated that ARGs in WWTPs are harbored on all three of the studied MGEs. Interestingly, while several known clinical-characterized genes were identified, certain genes such as those conferring resistance to chloramphenicol were not related to any known clinical genes, suggesting that the WWTP may be a source of novel ARGs.

The crucial importance of plasmids in propagation of AR has led to the development of molecular-based tools that can be applied to assess plasmid transfer dynamics in model WWTP systems. For example, Merlin et al. applied qPCR to monitor the fate of the AR plasmid pB10 and its *E. coli* DH5α donor host in microbial communities in WWTP sludge maintained in microcosms under different conditions [23]. In aerated activated sludge microcosms, pB10 did not persist because of an apparent loss of the donor bacteria. However, the persistence of the donor bacteria increased noticeably in non-aerated activated sludge microcosms when sulfamethoxazole or amoxicillin were applied at sub-inhibitory concentrations. Similar results were described by Kim et al., who found that ppb levels of tetracycline and sulfamethoxazole resulted in enhanced plasmid transfer frequencies in activated sludge [76]. Dröge et al. tested the potential of activated sludge concentrate to transfer conjugative plasmids to the 3-chlorobenzoate-degrading *Pseudomonas* sp. B13 (tagged with green fluorescent protein, GFP) recipient strain [77]. Twelve distinct tetracycline-, streptomycin-, and spectinomycin-resistant plasmids (ranging in size between 41 to 69 kb), primarily associated with the IncP incompatibility group, were identified. Seven of these were broad-host-range

plasmids displaying extremely high transfer frequencies ranging from 10^{-1} to 10^{-2} per recipient cell. Although these plasmid transfer and acquisition assays are not suitable for routine analyses of AR, they can be applied to models, which are crucial for understanding HGT dynamics in WWTPs and downstream environments.

5 Impact of Disinfection Processes on ARB and ARG Abundance

Disinfection processes are often applied to WWTP effluents for the inactivation/eradication of pathogenic organisms in order to prevent the spread of waterborne diseases to downstream users and the environment [78]. Various disinfection processes have been shown to reduce levels of *E. coli*, *Leptospira*, *Salmonella*, *Shigella*, and *Vibrio cholerae* (bacteria); *Balantidium coli*, *Cryptosporidium parvum*, *Entamoeba histolytica*, and *Giardia lamblia* (protozoa); *Ascaris lumbricoides*, *T. solium*, and *Trichuris trichiura* (helminths); and a wide range of pathogenic viruses. Although disinfection processes are generally effective for eradication of these pathogens, several studies clearly demonstrate that they do not always remove antibiotic compounds, ARB, and ARGs [12, 13, 19, 79–81]. Several frameworks are suggesting that future management guidelines for WWTP effluents should determine maximal levels for antibiotic residues, ARB, and ARGs to reduce the environmental and epidemiological risks associated with AR, in addition to current regulations that address a very narrow selection of pathogens [82]. To achieve this goal, conventional and novel disinfection processes need to be evaluated to determine which methods are best suitable for alleviating these AR elements. The impact of various disinfection processes on the diversity and abundance of ARGs and ARB is reviewed below. Sustainable solutions should focus on reducing bacterial and ARG abundance using technologies that do not generate toxic by-products of antibiotic and other micro-pollutant degradation (see other contributions in this volume).

Chlorine is the most widely used disinfectant for municipal wastewater because it destroys target organisms by oxidizing cellular material [78]. The required degree of disinfection for different systems is generally achieved by modifying the chlorine concentrations and exposure times and is most commonly evaluated by coliform plate counts. Standard protocols for chlorination of wastewater effluent apply 5–20 mg/L of chlorine, for 60 min to completely disinfect coliforms from the treated water. Unfortunately, studies have shown that other strains of ARB remain viable even after chlorination. For example, Huang et al. found that high chlorination doses resulted in enrichment of chloramphenicol-resistant bacteria in WWTP effluent, while lower doses of chlorination resulted in increased regrowth of a wider diversity of ARB, including strains resistant to ampicillin and penicillin [83].

Ultraviolet (UV) disinfection transfers energy from a mercury arc lamp at wavelengths of 250 to 270 nm that penetrate microbial cell walls and damage the

organism's genetic material (DNA and RNA), thereby destroying the cell's capacity to reproduce. The effectiveness of effluent UV disinfection depends on the characteristics of the concentration of colloidal and particulate constituents in the wastewater, the intensity of UV radiation, the amount of time the microorganisms are exposed to the radiation, and the reactor configuration [78]. Several isolation-based and culture-independent studies have assessed the effect of UV radiation on antibiotic, ARB, and ARG levels. Although comparison of results between studies is often highly ambiguous, collectively they seem to indicate that UV does not efficiently reduce ARB and ARG levels in WWTP effluent. For example, a recent study found that although combined UV and chlorination disinfection significantly reduced bacterial abundance, the percentage of the resistant bacteria, relative abundance of multidrug-resistant strains, and the detection rate of plasmid-mediated ARGs actually increased [84]. Other recent study found that UV disinfection led to enrichment of sulfadiazine-, vancomycin-, rifampicin-, tetracycline-, and chloramphenicol-resistant bacteria but reduction of isolates resistant to cephalexin, erythromycin, gentamicin, and ciprofloxacin, suggesting that the specific AR mechanisms may play a role in UV resistance either directly or through linkage to UV resistance mechanisms [85]. McKinney and Pruden investigated the potential of UV disinfection to damage four ARGs, mec(A), van(A), tet(A), and amp(C), in extracellular form and within the model bacterial pathogens – methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), *E. coli* SMS-3-5, and *Pseudomonas aeruginosa* 01 [86]. The authors found that the Gram-positive strains (MRSA and VRE) were more resistant to UV disinfection than the Gram-negative ARB (*E. coli* and *P. aeruginosa*). Interestingly, over tenfold higher UV doses were required to damage ARGs than to inactivate ARB. Furthermore, ARB with smaller genome size were less susceptible to the UV treatment. Finally, Rizzo et al. tested the effect of TiO_2 photocatalysis on the inactivation of a WWTP-derived antibiotic-resistant *E. coli* strain using different light sources and photocatalyst concentration [87]. The authors observed higher inactivation efficiency in the absence of TiO_2 when the wastewater was irradiated using a wide-spectrum 250 W lamp; but under solar simulated conditions, the highest inactivation efficiency was achieved at the lower photocatalyst levels. Interestingly, different UV and photocatalyst configurations had different effects on the AR profiles of the tested *E. coli* strain.

Ozonation is also an important disinfection methods applied in some WWTPs. The ozone applied to municipal effluents damages cell membranes, nucleic acids, and certain enzymes in microorganisms, depending on the physicochemical quality of the effluents and on the concentration of ozone applied [88]. Recently it was observed that an ozone concentration of 15.5 mg/L resulted in a 99% reduction of total coliforms, fecal coliforms, and helminth eggs [89]. Despite these promising effects, more studies are required to confirm that ozonation is antagonistic toward other bacteria and to determine the impact of ozonation on the stability of ARGs.

During wastewater treatment, and specifically during tertiary disinfection processes, a large portion of the microbiota is lysed resulting in the release of large

quantities of naked DNA. Given the relatively organic content in WWTP effluents, it is probable that high quantities of stable naked ARG-harboring MGEs from lysed cells are released in the effluents and these elements may be associated with natural transformation (Fig. 2c) of bacteria in downstream environments. This was supported by a study conducted by Hong et al., who measured the persistence of several ARGs before and after being discharged into the environment and found some of the ARGs tested still detectable even 16 months after discharge [90]. Other studies showed that transformation efficiency is determined by the concentration of naked DNA and potential acceptor cells, as well as by the natural composition of the soil or sediments, the sorption of DNA to organic and clay particles in the environment (thus protecting it from DNase-1), and the silica and organic matter composition of the sediment itself [91].

Additional wastewater treatment methodologies that specifically target ARGs should also be explored. For example, thermophilic anaerobic digestion at temperatures ranging from 37 to 55°C was found to remove 99.9% of class 1 integrons and have a significant impact in the reduction of *tet* genes encoding resistance to tetracycline antibiotics, and therefore may be pivotal for reducing AR contamination [92, 93], although other studies have found that this process may not efficiently eradicate all ARGs and MGEs [94]. Breazeal et al. examined the potential for membrane treatment of microconstituent ARGs and the effect of colloids present in the WW on the scope of their removal; ARG levels were significantly reduced in membranes of 100 kDa and smaller, and the presence of wastewater colloids enhanced ARG removal [95]. Furthermore, alumina membranes reduced wastewater-derived ARGs more than polyvinylidene fluoride (PVDF) membranes of the same pore size (0.1 μm). Nonetheless, Yang et al. found that ARGs are horizontally transferred in membrane bioreactors due to the high density of bacterial cells, biofilms, and the presence of ARB and ARGs, suggesting that they may indirectly promote ARG propagation [96].

In certain cases less sophisticated methods may be even more efficient in removing ARB and ARGs than advanced methods, and these should not be overlooked, especially in more rural areas or in developing countries that do not have resources for advanced disinfection processes. For example, Burch et al. found that aerobic digestion reduced ARG abundance in municipal biosolids [97], and Chen and Zhang found that constructed wetlands were more efficient in reducing relative abundances of ARGs than more sophisticated technologies such as ultraviolet disinfection [98].

Collectively, these studies indicate that conventional disinfection processes do not efficiently alleviate ARG and ARGs from wastewater effluent, although a broader spectrum of analyses are required to verify this preliminary findings. Therefore, future studies should focus on improving current processes and developing novel disinfection methods such as advanced oxidation processes and DNA-binding elements that specifically focus on reduction of ARGs and MGEs.

6 Impact of WWTP Effluents in Downstream Environments

Following secondary or tertiary treatment, wastewater effluents are generally either discharged into freshwater or marine water bodies or used for irrigation. The understanding that WWTP effluents contain significant levels of both ARB and ARGs (even following tertiary treatment and various disinfection schemes) and that MGEs harboring ARGs can vertically and horizontally spread from WWTPs to environmental microbiomes is highly concerning because of the potential dissemination of ARGs through water and food webs into clinically relevant bacteria, supporting their recent classification as contaminants of emerging concern [27, 99]. In this section we review the current knowledge regarding the scope and dynamics of AR elements in downstream environments and explore the potential impact of these elements on public health and the environment.

Effluents from most large-scale WWTPs in temperate climates are released into the rivers, streams, and lakes. These water bodies are often used for recreation, irrigation, and even drinking water, and therefore, it is crucial to understand their full microbial epidemiological potential, including the scope and intensity of AR. As described above, ARB and ARGs are crucial for developing dispersion and risk assessment models; however, as discussed above, it is necessary to apply appropriate genetic and bacterial markers that are highly abundant in effluents and are not present in pristine natural environments. Slekovec et al. determined that MDR *Pseudomonas aeruginosa* levels were significantly higher in effluent-receiving river water than in upstream samples, indicating that these multidrug-resistant opportunistic pathogens may be a good marker for anthropogenic contamination [100]. *Enterobacteriaceae* such as *E. coli* are often characterized by poor survival in natural environments, and therefore, the selection of non-enteric strains such as *Pseudomonas* that are known to persist longer in natural environments may be advantageous for source tracking experiments.

Based on the current state of the art, it seems most logical that qPCR-based analyses that target ARGs and MGEs will be the primary tool for monitoring AR potential from anthropogenic sources in the future. Nonetheless, the unfathomable array of ARGs necessitates selection of specific markers that can be used as reliable indicators of AR contamination in WWTP effluents. As discussed above these "select" indicators should be chosen based on their presence in a broad range of hosts, their ubiquitous occurrence in WWTP effluents, their stability, and their absence in pristine environments. Based on current studies, we can begin to assemble lists of ARGs and MGEs that meet these criteria. LaPara et al. applied qPCR to examine the presence and the abundance of effluent-associated genes encoding tetracycline resistance (tet(A), tet(X), and tet(W)) and intI1 (the type-1 integron integrase) in 13 locations in Duluth-Superior Harbor including a point adjacent to WWTP effluent in the harbor, a point along the St. Louis River (upstream from the WWTP), and from Lake Superior (downstream from the WWTP). Levels of tet(A), tet(X), tet(W), and intI1 were 20-fold higher in the

tertiary-treated wastewater than the surface water samples; and a positive correlation between proximity to the point of effluent discharge and tet(W) gene abundance was detected, suggesting that this gene may be a prime indicator for future source tracking studies. However, tet(W) is generally associated with Gram-positive bacteria, and therefore, genes with broader host range or genes primarily associated with Gram-negative bacteria should also be identified [80].

The prevalence of *qnrS*, *blaTEM*, *bla CTX-M*, *bla SHV*, erm(B), sul(I), sul(II), tet(O), and tet(W) in both biofilms and sediment samples before and after effluent discharge in the Ter River in Spain was evaluated using qPCR [54]; thus, although several of the genes were detected in upstream biofilms suggesting native AR or contamination from other anthropogenic sources, a significant increase in the relative abundance of almost all of the analyzed ARGs was detected in the biofilm samples proximal to the effluent discharge. Higher relative abundance of sul(1) and sul(2) genes in sediments proximal to WWTP effluent (relative to distant sediment levels) was also detected in a study by Czekalski et al. who applied qPCR to target these sulfonamide resistance genes in Vidy Bay, Lake Geneva [79]. Collectively, these two studies indicate that ARGs mitigate from the water column to biofilms and sediments, suggesting that these static substrates may be better than water column samples for determining the long-term impact of effluent discharge on AR in downstream aquatic environments. Furthermore, they suggest that sul(1) and sul (2) may be good candidates for source tracking of ARGs in aquatic ecosystems.

As discussed above, MGE capture technologies enable identification of mobile ARGs that may have significant epidemiological potential. Akiyama et al. applied such a plasmid capture assay to assess the type and frequency of BHR plasmids associated with incompatibility groups IncA/C, IncN, IncP, and IncW in two WWTP effluents and effluent-receiving streams in Northwest Arkansas [19]. The authors detected IncP plasmid amplicons in effluent and downstream sites in both streams analyzed, while IncN and IncW plasmid amplicons were detected in effluent and downstream but not upstream, and IncA/C plasmid amplicons were detected at all sites, including most upstream samples. This may suggest that IncN and IncW may be functional markers for source tracking of mobile ARGs from WWTPs.

Although currently not feasible for routine monitoring, high-throughput sequencing-based metagenomic approaches can provide a broad picture of effluent-derived ARGs and MGEs in effluent, upstream, and downstream environments. This comprehensive approach can identify prime ARG candidates for source tracking markers, which can be used by stakeholders in routine monitoring schemes. Kristiansson et al. applied culture-independent shotgun metagenomics to compare upstream and downstream microbiomes in river sediments adjacent to a pharmaceutical WWTP in India and in a municipal WWTP in Sweden [101]. The researchers found significantly higher abundances of sulfonamide, fluoroquinolone, and aminoglycoside resistance genes in the antibiotic production facility-contaminated river sediment, where downstream ARG levels were significantly higher than those measured upstream. For example, the levels of *strA* and *strB* were 22 and 54 times higher than upstream levels, and 6.7 times more copies of class

1 integrases and 24 times higher levels of transposases associated with insertion sequence common regions (ISCRs) of class 2 integrons were found in the downstream sediments, strongly suggesting that the elevated AR levels were linked to effluents from the antibiotic production facility. It should be noted that the antibiotic concentrations in the Indian WWTP were orders of magnitude higher than in conventional municipal WWTP effluents, and ARGs and MGEs were rarely detected in the Swedish sediments. Nonetheless, this study was conducted using the 454 pyrosequencing approach and current NGS platforms that provide significantly higher sequencing depth and should provide more insight into ARGs and MGEs in upstream and downstream sediments adjacent to municipal WWTP discharge.

The effect of WWTP effluents on AR in soil environments has received far less attention than downstream aquatic environments and appears to be much more complex, seemingly due to the intricate nature of the soil microbiome. Negreanu et al. assessed the impact of TWW irrigation on ARB and ARG abundance in irrigation water and four different agricultural soils [13]. While ARB and ARG levels were substantially higher in treated effluent that in freshwater irrigation water, ARB and ARG abundances in the irrigated soils were never higher in treated wastewater irrigated soils. Surprisingly, on several occasions, AR levels were actually higher in freshwater-irrigated soils. Levels of sul(1), sul(2), and erm (B) that appear to be reliable for source tracking of ARGs in aquatic environments showed identical levels in treated wastewater- and freshwater-irrigated soils, indicating high natural AR levels in soil microbiomes, regardless of the irrigation water type used. The presence of ARB and ARG in pristine soils has been well documented, and there is strong evidence that many clinically associated resistance elements are found in soil microbiomes [102–104]. As stated before, there is a need to identify ARGs that are abundant in WWTP effluents but are not profuse in soils for monitoring discharge of ARs in effluents. McLain and Williams studied AR patterns in *Enterococcus* isolated from water storage basins in central Arizona containing either reclaimed water or groundwater. Similar to the study above, they found that MDR levels were actually higher in the sediments of groundwater reservoirs than in sediments containing reclaimed wastewater [105]. Although these two studies are cause for cautious optimism regarding the use of TWW irrigation, they demonstrate that the soil microbiome is characterized by extremely high native AR levels, which may mask effluent-associated ARGs in soil. This reinforces the necessity for development and application of WWTP effluent-associated AR markers that are not abundant in native soil microbiomes, which can be used to track mobile ARGs.

Activated sludge biosolids are frequently amended to soils following compostation or other stabilization processes to enhance physicochemical soil properties. These biosolids contain residual concentrations of antibiotic compounds, especially hydrophobic compounds such as fluoroquinolones [106], ARB, and ARGs [107], which can potentially be transported to amended soils. To assess the impact of biosolid application on ARG levels in amended soil, Munir and Xagoraraki applied qPCR to measure the relative abundance of tetracycline and

sulfonamide resistance genes in two different soils with and without biosolid amendment and found that while in one of the soils biosolid amendment resulted in higher ARG levels, ARG levels in the other soil were similar to non-amended soil levels [108]. Similar to the treated wastewater study above, the observed discrepancy is most likely associated with the high natural AR in the pretreated soil resistome, again establishing the need for reliable effluent-specific AR markers.

7 Summary and Future Directions

The past decade has witnessed a large number of scientific studies that have assessed AR in wastewater treatment facilities. Collectively, these studies indicate that conventional WWTP processes may select for AR and that WWTP effluents contain significant levels of ARB and ARG. Application of standard disinfection processes does not remove these materials; in fact they may thoroughly select for certain resistant strains and generate unknown transformation products. Research has shown that WWTP-derived AR elements are often stably transferred to downstream environments, demonstrating the epidemiological ramifications of this process but also underlining the complexity of monitoring AR elements released from WWTPs in receiving aquatic and terrestrial environments. Despite the current state of the art, a comprehensive understanding of the abundance, diversity, and mobility of ARB and ARGs in sewage effluents and their impact on downstream environments is still lacking. Analytical methods for identification and quantification of these markers need to be standardized, so they can be used for comparative studies between environments and applied to routine monitoring protocols in the future. Furthermore, there is currently a lack of available data regarding the correlations between ARB and ARG levels and WWTP parameters such as antibiotic concentrations, treatment processes, and climatic conditions. There is a need for collaborations that can better link such datasets and for development of publically available databases that can integrate the data with epidemiological and toxicological data in order to develop models and risk assessment projections.

Concomitant to elucidating the scope and epidemiological impact of effluent-associated AR elements, there is a need for novel technologies and management options for reducing the spread of antibiotics and antibiotic resistance determinants from WWTPs. Certainly, more research is required to clarify the real efficiency, of different technologies, for reduction of ARG and MGE levels in the different steps of wastewater treatment; and the decision of which of these technologies need to be applied in each situation needs to be determined. The characterization of ARGs as contaminants of emerging concern could promote the development of new approaches in technologies for risk reduction, which added to national policies and regulations could reduce significantly both the impact of ARGs into natural environments and the impact on human health. This undoubtedly needs to be coupled to additional measures such as more prudent use of antibiotics in humans and animals and development and selection of antibiotic compounds that do not persist for long times in the environment.

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Safe Drinking Water? Effect of Wastewater Inputs and Source Water Impairment and Implications for Water Reuse

Susan D. Richardson and Cristina Postigo

Abstract The elimination of contaminants of emerging concern (CECs) during conventional wastewater treatment is not complete, and therefore, different amounts of these compounds are continuously released via wastewater effluents into the aquatic environment. This constitutes a major issue for water reuse, because these compounds can undergo transformation in the environment or during disinfection if reclaimed water is used for drinking water production. Different emerging contaminants, e.g., perfluorinated compounds, pharmaceuticals, antibacterials, plasticizers, and preservatives, and transformation products, which are in some cases more toxic than original compounds, have been occasionally found in finished drinking waters. The present chapter reviews the CECs detected in drinking water and the disinfection by-products generated by different CECs present in the aquatic environment. Moreover, the potential toxicologic effects that these pollutants and their transformation products pose for human health are also reviewed. Levels of these compounds in treated waters, and therefore exposure, could be reduced by the use of advanced removal technologies.

Keywords Chlorination, Contaminants of emerging concern, DBPs, De facto reuse, Disinfection by-products, Drinking water, Water reuse

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 155–182, DOI 10.1007/698_2015_371,

Contents

Abbreviations

BDCM	Bromodichloromethane
BPA	Bisphenol A
CCL	Contaminant candidate list
CEC	Contaminants of emerging concern
DBP	Disinfection by-product
DDT	Dichlorodiphenyltrichloroethane
DOC	Dissolved organic carbon
E2	17β-Estradiol
EC50	Half maximal effective concentration
EDDP	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EE2	17α-Ethinyl estradiol
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
GSTT1	Glutathione *S*-transferase theta-1
GSTZ1	Glutathione *S*-transferase zeta-1
GWRS	Groundwater Replenishment System
HAA	Haloacetic acid
LDPE	Low density polyethylene
LOEC	Lowest observed effect concentration
MDA	3,4-Methylenedioxyamphetamine
MDEA	3,4-Methylenedioxyethylamphetamine
MDMA	3,4-Methylenedioxymethamphetamine or Ecstasy
MF	Microfiltration
MTBE	Methyl *tert*-butyl ether
MX	Mutagen X (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone)

N-DBPs	Nitrogen containing disinfection by-products
NDMA	*N*-Nitrosodimethylamine
NF	Nanofiltration
PET	Polyethylene terephthalate
PFCs	Perfluorinated compounds
PFCAs	Perfluoroalkyl carboxylates
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonate
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFPeA	Perfluoropentanoic acid
PFSA	Perfluoroalkyl sulfonate
PTFE	Polytetrafluoroethylene or Teflon®
PVC	Polyvinylchloride
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
RO	Reverse osmosis
TCA	1,1,1-Trichloroethane
THC	(±)-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol
THM	Trihalomethane

1 Introduction

More than four billion people in the world live in regions where scarcity of freshwater directly threatens human water security [1, 2]. As populations continue to grow and droughts continue to become more frequent, alternative sources of water are being sought. Of the different potential sources, reuse of domestic wastewater is one of the most energy-efficient, sustainable options, if compared to interbasin transfer of water and desalination of seawater [1]. Treated wastewater has been reused for several decades for industrial applications, agriculture, landscaping, habitat restoration, and recreational lakes and as a barrier to prevent seawater intrusion to groundwater [3–5] and is now used in more than 50 countries [6]. Of these countries, the USA is first in total volume of water reused [5]. Notably, 73% of Israel's municipal wastewater is treated and reused for agricultural irrigation [7].

Potable reuse of reclaimed wastewater is also now a reality in many locations. Advanced treatment methods are typically used, and the treated water can either be used directly (direct potable reuse) or indirectly by holding the water for a time in groundwater or surface-water reservoirs (indirect potable reuse) [1]. The longest running example of direct potable reuse is in Windhoek, Namibia, where recycled wastewater has been added to the drinking water distribution system since the late 1960s [1]. The world's largest indirect potable reuse system is the Groundwater Replenishment System (GWRS) in Orange County, CA, which uses conventional

treatment (primary and secondary sewage treatment) followed by advanced treatment using microfiltration (MF), reverse osmosis (RO), and UV-C/H_2O_2 [8].

In addition to planned reuse, many regions of the USA (and the world) have de facto reuse, where treated wastewater constitutes a substantial portion of the potable water supply [9]. In times of low rainfall in the Western USA, wastewater effluents can make up to 90–100% of the river's flow. For example, the Santa Ana River in Southern California typically consists of >90% wastewater effluent from upstream communities during the dry season (April through October) [5], and the Trinity River, which flows south of Dallas/Fort Worth, consists almost entirely of wastewater effluent under base flow conditions [10].

A major issue with water reuse is that many of the chemicals present in wastewater are not fully removed in conventional wastewater treatment. As a result, many wastewater contaminants can enter ecosystems and drinking water supplies [11–13]. Moreover, these chemicals can often transform in the environment or during treatment of drinking water or wastewater to form new products, which can have greater toxicity than the parent compounds [13–15].

Treated wastewater can also impact source waters with increased nitrogen, which can include ammonia, nitrite, nitrate, amino acids, nitrogen-containing pharmaceuticals, pesticides, or other constituents of personal care products [16–18]. Chlorination of these waters in drinking water treatment can result in the formation of nitrogen-containing disinfection by-products (the so-called N-DBPs), which are more genotoxic and cytotoxic than disinfection by-products (DBPs) that do not contain nitrogen [19–21]. These N-DBPs include nitrosamines, haloacetonitriles, halonitromethanes, haloamides, and cyanogen halides [16, 18, 21–23]. A large occurrence study of 16 drinking water treatment plants in the USA focused on treated water impacted by wastewater and/or algae and found high levels of N-DBPs when the wastewater contained high levels of inorganic nitrogen and dissolved organic nitrogen [17].

Increased energy extraction activities, including shale gas extraction and conventional oil and gas extraction, are also contributing to impaired waters, resulting in high releases of bromide (and potentially iodide) as well as other mostly unknown other/organic chemicals to US surface waters [24–26]. New pollution controls being installed at coal-fired power plants are also contributing high releases of bromide [27–29]. These activities are presenting new issues for human health because when these high-bromide/iodide waters are chlorinated, they can result in the formation of highly toxic brominated and/or iodinated DBPs, several of which are genotoxic or carcinogenic [21]. The levels of bromide being released to the environment are unprecedented, and new regions of the USA which have not had these high-bromide levels before are now being exposed to high levels of brominated DBPs [30], most of which have not been characterized.

Increased nitrogen and other nutrients from treated wastewater can also result in increased algal growth and an accompanying increased incidence of shellfish poisoning, large fish kills, and deaths of livestock and wildlife, as well as illness and death in humans [23, 31, 32]. Toxins produced by these algae have been implicated in the adverse effects. The most commonly occurring algal toxins are

microcystins, nodularins, anatoxins, cylindrospermopsin, and saxitoxins. "Red tide" toxins are also often found in coastal waters. Nearly every part of the world that uses surface water as a drinking water source has encountered problems with cyanobacteria and their toxins. Several countries, including Australia, Brazil, Canada, France, Italy, Poland, and New Zealand, have guideline values for microcystins, anatoxin-a, and cylindrospermopsin (ranging from 1.0 to 1.5 μg/L). An excellent review on the occurrence and management of harmful cyanobacterial blooms and their toxins in surface water and drinking water was recently published by Merel et al. [32].

2 DBPs of Emerging Concern in Drinking Water

Currently, 11 DBPs are regulated in the USA: 4 trihalomethanes (THMs), 5 haloacetic acids (HAAs), bromate, and chlorite [33]. However, DBPs of emerging concern beyond those that are currently regulated are becoming important. In general, brominated DBPs are now being recognized as toxicologically important because there is indication that brominated DBPs may be more carcinogenic than their chlorinated analogues, and new studies are indicating that iodinated compounds may be even more toxic than their brominated analogues [20, 34, 35]. Brominated and iodinated DBPs form due to the reaction of the disinfectant (such as chlorine) with natural bromide or iodide present in source waters. Coastal cities, whose groundwaters and surface waters can be impacted by salt water intrusion, and some inland locations, whose surface waters can be impacted by natural salt deposits from ancient seas or oil-field brines, are examples of locations that can have high-bromide and iodide levels. A significant proportion of the US population and several other countries now live in coastal regions that are impacted by bromide and iodide; therefore, exposures to brominated and iodinated DBPs can be important. And, as mentioned earlier, there are now new inputs of bromide (and potentially iodide) from energy extraction and utilization activities that are resulting in the change in speciation from primarily chlorine-containing DBPs to predominantly bromine-containing DBPs, which are more toxic. This is now happening in regions of the USA located away from the coast (e.g., in Pennsylvania), which generally would have near non-detectable bromide and very low brominated DBPs.

Early evidence in epidemiologic studies also gives indication that brominated DBPs may be associated with the new reproductive and developmental problems [36, 37], as well as cancer effects. Specific DBPs that are of current interest include iodo-acids, bromonitromethanes, iodo-THMs, haloamides, halofuranones, halopyrroles, haloquinones, haloaldehydes, halonitriles, and nitrosamines. In particular, *N*-nitrosodimethylamine (NDMA) and other nitrosamines are known carcinogens; 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone, better known as "mutagen X" (MX), is also an animal carcinogen; and more recently, iodoacetic acid was shown to be tumorigenic in mice [38].

Many of these were part of a nationwide occurrence study in the USA, which reported the most extensive quantitative occurrence of priority, unregulated DBPs [39, 40]. In addition, many of these are N-DBPs, which are generally more genotoxic and cytotoxic than those without nitrogen [20]. As mentioned earlier, increased nitrogen inputs from treated wastewater can cause increased formation of these more toxic N-DBPs.

3 Contaminants of Emerging Concern (CECs) in Finished Drinking Water

Several CECs from treated wastewater have been present at high enough levels in wastewater effluents that they have been detected in source waters and in finished drinking water. These include perfluorinated compounds (PFCs), pharmaceuticals, antibacterials, hormones, bisphenol A, benzotriazoles, dioxane, perchlorate, and algal toxins [13, 41, 42]. Several of these CECs were recently recommended for monitoring in potable water reuse by a Science Advisory Panel convened by the State of California [43] (Table 1). In this effort, environmental concentrations were considered together with toxicity, and chemicals prioritized for study had measured environmental concentrations greater than their monitoring trigger levels, which were based on toxicity.

3.1 Perfluorinated Compounds (PFCs)

PFCs have been manufactured for more than 50 years and have been used to make stain repellents, e.g., polytetrafluoroethylene (PTFE or Teflon®), that are widely applied to fabrics and carpets. They are also used in the manufacture of paints, adhesives, waxes, polishes, metals, electronics, fire-fighting foams, and caulks, as well as grease-proof coatings for food packaging (e.g., microwave popcorn bags, French fry boxes, hamburger wrappers, etc.). PFCs are unusual chemically, in that

Table 1 Priority CECs recommended for monitoring in potable water reuse [43]

Analyte	Compound use
17α-Ethinyl estradiol (EE2)	Pharmaceutical (synthetic hormone)
17α-Estradiol	Pharmaceutical
17β-Estradiol (E2)	Hormone
Erythromycin	Antibiotic
Estrone	Hormone
N-Nitrosodimethylamine (NDMA)	Disinfection by-product (DBP)
Perfluorooctanoic acid (PFOA)	Industrial chemical
Perfluorooctane sulfonate (PFOS)	Industrial chemical

they are both hydrophobic (repel water) and lipophobic (repel lipids/grease), and they contain one of the strongest chemical covalent bonds known (C–F). Due to these properties, they are highly stable in the environment (and in biological samples) and have unique profiles of distribution in the body. Two of these PFCs, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have received the most attention because they are generally found the most often and at the highest levels in the environment. Potential health concerns include developmental toxicity, cancer, and bioaccumulation. The US Environmental Protection Agency (EPA) has listed PFOA and PFOS on the new Contaminant Candidate List (CCL-3) [44]. PFOA was also voluntarily reduced in emissions and product content by 95% (2010) and is being phased out in 2015 [45]. In Europe, the European Food Safety Authority (EFSA) has established tolerable daily intakes for PFOA and PFOS [46], and there are new restrictions on the use of PFOS as part of the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program [47], and environmental quality standards have been also set for this compound in water and biota [48].

PFCs have been widely found in environmental waters, drinking water, and biota [23, 49]. One of the first studies of PFCs in drinking water was conducted in Germany, in which 12 PFCs were measured in drinking waters and surface waters [50]. A relatively high maximum concentration of PFCs was found in drinking water (598 ng/L), with PFOA being the major component (519 ng/L). Since this early study, there have been numerous detections of PFCs in drinking water from several countries [42, 51–57].

An occurrence study carried out in Australia found PFOS and PFOA in 49% and 44% of the drinking water samples collected, respectively [53]. In a French drinking water study conducted by Boiteux et al. [55], 331 source water and 110 finished drinking water samples were collected from several regions in France, representing 20% of the national water supply. Of the ten PFCs measured, PFOS, perfluorohexane sulfonate (PFHxS), PFOA, and perfluorohexanoic acid (PFHxA) predominated in the source waters (detected in 27%, 13%, 11%, and 7% of the samples, respectively). In finished drinking water, short-chain perfluoroalkyl carboxylates (PFCAs) predominated, suggesting a relative effectiveness of certain water treatments in removing perfluoroalkyl sulfonates (PFSAs) but also the potential for degradation of PFCA precursors by water treatment processes. A particularly interesting discovery was that eight of these drinking water treatment plants actually had higher levels of some PFCs, PFBA, perfluoropentanoic acid (PFPeA), PFHxA, and perfluoroheptanoic acid (PFHpA), in the finished water vs. the raw source waters. Normally, levels would be expected to be lower in the finished water vs. the source water, due to some partial removal, dilution, or degradation. In total, seven of these eight plants used activated carbon to treat raw water, and results suggest release of PFCs from saturated activated carbon or degradation of precursors during the treatment process. PFHxA was found at the highest levels in finished drinking water, up to 125 ng/L. And as expected, areas with higher population densities showed higher levels of PFCs in their finished drinking water.

PFCs have also been followed along the whole water cycle (wastewater, river water, tap water, and mineral bottled water) in a large occurrence study of several cities in Germany and Spain [56]. In this study, 21 PFCs were measured, and perfluorocarboxylic acids were found most often in drinking water, with 54% of the tap water samples containing perfluorobutanoic acid at levels up to 27 ng/L and PFHpA, PFOA, and PFOS up to 53, 35, and 258 ng/L, respectively.

3.2 Pharmaceuticals, Antibacterials, and Hormones

Pharmaceuticals, antibacterials, and hormones have become important CECs, due to their ubiquitous presence in environmental waters, threat to drinking water, and potential estrogenic and other effects to ecosystems and humans [58, 59]. A major concern also includes the development of bacterial resistance from the release of antibiotics and antibacterials, such as triclosan, to the environment [60]. Pharmaceuticals are introduced not only by humans but also through veterinary use for livestock, poultry, and fish farming. Various drugs are commonly given to farm animals to prevent illness and disease and to increase the size of the animals. One lingering question has been whether the relative low environmental concentration levels of pharmaceuticals (generally ng/L range) would cause adverse effects in humans or wildlife. It is estimated that approximately 3,000 different substances are used as pharmaceutical ingredients, including painkillers, antibiotics, antidiabetics, betablockers, contraceptives, lipid regulators, antidepressants, and impotence drugs. However, only a very small subset of these compounds has been investigated in environmental studies so far. Three pharmaceuticals – erythromycin, nitroglycerin, and 17α-ethinyl estradiol (EE2) – are included as priority drinking water contaminants on EPA's CCL-3 list [44]. In Europe, the pharmaceuticals diclofenac and EE2, following proposal for their consideration as EU priority substances, have been recently included in the first watch list in order to gather additional monitoring data to facilitate the determination of appropriate measures to address their potential environmental risk [61].

While many pharmaceuticals can have an acute or chronic effect on aquatic or other organisms, most of the lowest observed effect concentrations (LOECs) are substantially above environmental concentrations. However, there are a few notable exceptions, where toxicity LOECs approach concentrations observed in environmental waters or wastewater effluents. These include ciprofloxacin, the synthetic hormone EE2, salicylic acid, diclofenac, propranolol, clofibric acid, carbamazepine, and fluoxetine [13]. Two compelling studies highlight the potential adverse effect of pharmaceuticals on wildlife. In the first study, residues from the veterinary use of diclofenac were implicated in the death of approximately 40 million vultures in Pakistan (more than 95% of the vulture population) [62]. This incident is being referred to as the "worst case of wildlife poisoning ever," far eclipsing the numbers of birds affected by dichlorodiphenyltrichloroethane (DDT) a few decades ago. In the second study, EE2 was shown to feminize male fish and

cause complete collapse of a wild fish population [63]. This 7-year study involved dosing of 5–6 ng/L of EE2 to a lake in the experimental lake area of Ontario, Canada, in which chronic exposure of the fathead minnow led to production of vitellogenin mRNA and protein and impacts on gonadal development in males and altered oogenesis in females, ultimately leading to a near extinction of this native species in the lake due to lack of reproduction. These two studies highlight the fact that low, environmentally relevant doses of pharmaceuticals can adversely impact wildlife.

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is an important antibacterial, as it is commonly used in many hand soaps and is one of the most common personal care products found in the environment [64]. Its use as a preservative in cosmetic products has been recently restricted in Europe [65], and the ban of its use as disinfectant and algaecide, film preservative, and fiber, leather, rubber, and polymerized material preservative is still under discussion. In the Kolpin et al. study of wastewater-impacted streams and rivers in the USA, triclosan was detected in 58% of the locations sampled [12]. There is concern that levels found in the environment are contributing to antibiotic resistance; in fact, many triclosan-resistant bacteria have already been found [64]. Triclosan is also toxic to aquatic organisms, such as fish, crustaceans, and algae, with half maximal effective concentrations (EC50) close to environmental concentrations observed, and it has cytotoxic, genotoxic, and endocrine disrupting effects [64]. Moreover, triclosan can transform into potentially more toxic compounds in wastewater and drinking water treatment (as discussed later under Sect. 4 on Pollutant DBPs).

Parabens are a group of substances (alkyl esters of *p*-hydroxybenzoic acid) with bactericidal and fungicidal properties that are widely used as preservatives in personal care products, pharmaceuticals, and food. Similarly as for triclosan, their use as preservatives in cosmetic products is also restricted in Europe [65]. To date, only a few studies have investigated their presence in the aquatic environment. Parabens have been found in surface waters at levels as high as 3,142 ng/L [66, 67]. Methylparaben and propylparaben, which are the most commonly detected parabens in waters (in agreement with their extensive use in cosmetic formulations), have been quantified in tap water at levels up to 40 [68, 69] and 135 ng/L [70], respectively. The main concerns regarding the presence of parabens in the environment arise from their endocrine disrupting potential and their possible involvement in the process of carcinogenesis, both of which are currently under investigation [67].

Natural and synthetic hormones can have inputs from wastewater and agriculture, and they are often not completely removed in wastewater treatment, such that they have the potential to enter drinking water sources. There is concern due to potential estrogenic and androgenic effects, but mostly for wildlife, and not for human health [71]. Nine natural and synthetic hormones (EE2, 17α-estradiol, 17-β-estradiol (E2), equilenin, equilin, estriol, estrone, mestranol, and norethindrone) are included on the US EPA's CCL-3 [44] as priority drinking water contaminants. Two hormones E2 and EE2 are included in the first EU watch list for future consideration as EU priority substances [61].

Pharmaceuticals, antibacterials, and hormones have been reported in finished drinking water from several countries [57, 70, 72–76]; these are typically ones that are present at highest levels in wastewater and are not well removed in wastewater treatment. Drinking water systems that treat surface waters generally have the highest levels in their drinking water, with ibuprofen, triclosan, carbamazepine, phenazone, clofibric acid, gemfibrozil, and acetaminophen found most often, with levels up to high ng/L [73, 77].

Recent "source-to-tap" studies have reported the fate of pharmaceuticals over the cycle from wastewaters to river waters, to source waters, and to finished drinking water. Due to some removal during wastewater treatment followed by some removal/degradation in river waters and further removal/transformation in drinking water treatment, pharmaceuticals are only occasionally reported in finished drinking water. For example, in a study carried out in Canada by Metcalfe et al., antidepressants and their metabolites were removed in wastewater treatment by ~40%, with two (venlafaxine and bupropion) detected in untreated drinking water source water, but none detected in finished drinking water [74]. Several of these compounds persisted in river water collected several kilometers downstream of the wastewater treatment plants, and modest accumulation factors (<100) were observed in caged fathead minnows downstream of the plants. In another study, Watkinson et al. followed the occurrence and fate of 28 antibiotics from three hospital effluents, five wastewater treatment plants, six rivers, and a drinking water storage catchment in Southeast Queensland, Australia [78]. Most antibiotics were detected at least once and were up to 14.5 μg/L in hospital effluents, up to 64 μg/L in wastewater influents, up to 3.4 μg/L in wastewater effluents, and up to 2 μg/L in surface waters, but they were not detected in finished drinking waters. On the other hand, Benotti et al. reported measureable levels of pharmaceuticals in finished drinking water in a study of 20 pharmaceuticals and other contaminants in 19 drinking water treatment plants from the USA[11]. The 11 most frequently detected compounds were atenolol, atrazine, carbamazepine, estrone, gemfibrozil, meprobamate, naproxen, phenytoin, sulfamethoxazole, tris(1,3-dichloro-2-propyl)phosphate, and trimethoprim. Maximum pharmaceutical levels observed were 110, 42, and 40 ng/L for source waters, finished drinking water, and distribution system tap water, respectively. The occurrence in finished drinking water was controlled by the type of disinfectant (ozone or chlorine) used at each plant.

Most pharmaceuticals, antibacterials, and hormones are "removed" in drinking water treatment, such that the parent chemicals are no longer detected following disinfection, filtration, and other treatments, but as discussed later, DBPs can be formed by them, which often have unknown properties, fate, and toxicity.

3.3 Illicit Drugs and Their Human Metabolites

Illicit drugs have been detected in different environmental matrices [79]. The investigation of this class of CECs in the aquatic environment has a double

objective: (1) to increase the knowledge on their environmental levels to evaluate their potential environmental risk and (2) to back-calculate illicit drug use at the community level [80]. Cocaine, methadone, and their respective metabolic by-products, benzoylecgonine and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), are the most ubiquitous compounds in tap waters [81]. Illicit drugs and metabolite levels in drinking waters are at the low ng/L, with 15 ng/L of benzoylecgonine the highest value reported [81]. The low concentrations found in drinking waters can be attributed to removal and transformation of these compounds during water treatment processes, because slightly higher concentrations of these compounds are frequently found in surface waters, and they can reach the μg/L level in wastewaters [82].

3.4 Bisphenol A

Bisphenol A (BPA) is widely used in the manufacture of many consumer products, as a monomer in the synthesis of polycarbonates and as a polymerization inhibitor in polyvinylchloride (PVC) pipes. BPA is commonly found in wastewater effluents and in surface waters and has also been observed in drinking water [41, 73, 83–85]. Levels as high as 41 μg/L have been reported in surface waters in the USA [12], and BPA levels in the μg/L range have been also detected in rivers from Spain and China [86]. In a study of 17 drinking water systems in Ontario, Canada, BPA was among the contaminants most frequently measured in finished drinking water, with levels up to 420 ng/L [73]. Concerns surround BPA due to its estrogenicity.

3.5 Benzotriazoles

Benzotriazoles are complexing agents widely used as anticorrosives (e.g., in engine coolants, aircraft deicers, or antifreeze liquids) and for silver protection in dishwashing liquids. Benzotriazoles are soluble in water, resistant to biodegradation, and only partially removed in wastewater treatment. There is evidence for estrogenic effects in vitro [87] and in vivo, as observed in recent fish studies [88]. While reports of benzotriazoles are fairly recent, studies indicate that they are likely ubiquitous environmental contaminants. Janna et al. reported an interesting study entitled “From dishwasher to tap? Xenobiotic substances benzotriazole and tolyltriazole in the environment” [89]. This study demonstrated their presence in UK wastewaters, rivers, and drinking water and suggested that their use as silver polishing agents in dishwasher tablets and powders may account for a significant proportion of inputs to wastewaters. Benzotriazole and tolyltriazole ranged from 840 to 3,605 ng/L and 2,685 to 5,700 ng/L, respectively, in sewage effluents and from 0.6 to 79.4 ng/L and <0.5 to 69.8 ng/L, respectively, in drinking water. More effective removal of tolyltriazole by activated carbon was suggested as the reason

for its lower levels in finished drinking water vs. river water [89]. Also, in a multicountry-European study by Loos et al., 1*H*-benzotriazole and methylbenzotriazole were found in >50% of the groundwaters sampled, up to 1.03 and 0.52 μg/L, respectively [90]. Maximum concentrations of 8 and 20 μg/L in surface waters for benzotriazole and tolyltriazole, respectively, were observed in a study carried out in rivers from 27 European countries, where these compounds were among the most ubiquitous and abundant polar pollutants investigated [91].

3.6 Dioxane

1,4-Dioxane is a widespread industrial contaminant in environmental waters (often exceeding water quality criteria and guidelines) and has been found in contaminated groundwater up to 2,800 μg/L [92], as well as in drinking water [13, 93, 94]. Dioxane is a high production chemical used as a solvent stabilizer in the manufacture and processing of paper, cotton, textile products, automotive coolants, cosmetics, and shampoos, as well as a stabilizer in 1,1,1-trichloroethane (TCA), a popular degreasing solvent. It has been classified as a probable human carcinogen and is currently listed on the US EPA's CCL-3 [44]. Dioxane is considered nonbiodegradable and is difficult to remove from water. A recent study in Germany found it to be up to 62,260 ng/L in wastewater effluents, 2,200 ng/L in river water, and 600 ng/L in finished drinking water, which was above the precautionary guideline limit of 100 ng/L [93]. Interestingly, wastewater effluent levels from one plant were higher than wastewater influents due to dioxane impurities in the methanol used in the postanoxic denitrification process.

3.7 Perchlorate

Recent studies have found perchlorate in finished water, with median levels up to 1.2 μg/L [95]. Using individual tap water consumption data and body weight, the median perchlorate dose attributable to tap water was 9.1 ng/kg-day. Perchlorate was also measured in tap water and bottled water from China in another recent study, which found perchlorate in 86% of the samples and mean levels of 2.5 and 0.22 μg/L, respectively [96]. Perchlorate is a widespread contaminant in surface waters, and it results from the use of perchlorate in rockets, missiles, fireworks, and highway flares, as well as in fertilizers [13]. It can also be a contaminant in sodium hypochlorite (liquid bleach) that is used in drinking water treatment. Perchlorate is not removed by conventional water treatment processes, so human exposure can also occur through drinking water. Health concerns arise from perchlorate's ability to displace iodide in the thyroid gland, which can affect metabolism, growth, and development. The US EPA has recently decided to regulate perchlorate in drinking water, and a new regulation is currently under development [23].

3.8 Antimony

Antimony can leach into bottled drinking water from polyethylene terephthalate (PET) plastic water bottles, producing the highest levels of human exposure to antimony, close to 10 μg/L [97]. Antimony trioxide is used as a catalyst in the manufacture of PET plastics, and it can contain >100 mg/kg of antimony. Highest levels of antimony can leach from these plastic bottles over prolonged storage and especially at warm temperatures [98]. This is a concern because of the growing popularity of bottled water. Compared to PET bottles, low density polyethylene (LDPE) bottles contain much lower levels (~1%) of antimony [99].

3.9 Algal Toxins

Algal toxins or cyanotoxins (i.e., hepatotoxins, neurotoxins, and dermatotoxins) are harmful metabolites synthesized by certain species of cyanobacteria. The hepatotoxins microcystins, nodularins, and cylindrospermopsin and the neurotoxins, anatoxins, and saxitoxins are considered as priority hazards to human and animal health. To date, microcystins are the most investigated cyanotoxins, since they are also the most widespread. The presence of microcystins is regulated in many countries at a maximum level of 1 μg/L in drinking water [32, 100]. The reported total microcystin (intracellular plus dissolved) levels in surface waters vary from trace to several mg/L; however, the dissolved fraction usually does not comprise more than 10% of the total. This is not the case for cylindrospermopsin, which is often found at higher levels in the dissolved form than within the cells [101]. In fact, this cyanotoxin has been commonly detected in drinking waters from Taiwan, reaching levels as high as 8.6 μg/L [102]. Microcystin-LR has been measured in tap waters from Serbia and China at a concentration of 2.5 μg/L [103] and 1.3 μg/L [104], respectively. Intracellular cyanotoxins are also released following oxidation of cyanobacterial cells. This could result in higher cyanotoxins in oxidant treated waters if the oxidative treatment applied presents low reactivity to the metabolites released [105].

4 Pollutant DBPs

Just as natural organic matter can react with disinfectants to form DBPs in drinking water, many pollutants which have activated benzene rings, phenol groups, amine groups, or double bonds can also react with disinfectants to form DBPs. As such, DBPs have been reported for pharmaceuticals, illicit drugs, antibacterial agents, estrogens, BPA, pesticides, textile dyes, parabens, alkylphenol ethoxylate surfactants, musks, and even algal toxins like microcystins or cylindrospermopsin, as illustrated in Fig. 1.

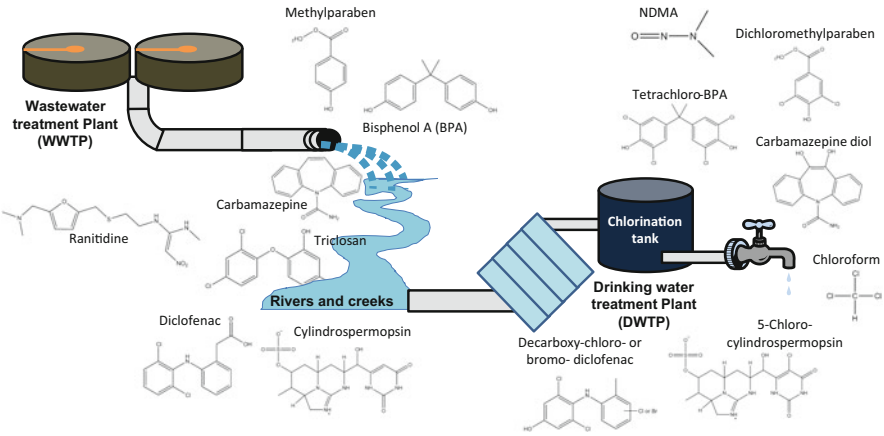

Fig. 1 Transformation of not fully eliminated CECs in WWTPs during disinfection in a chlorination system to produce safe drinking water

For example, a microbial transformation product of the fungicide tolylfluanide (*N*,*N*-dimethylsulfamide) can react with ozone to form NDMA [106]. This was discovered after high ng/L levels of NDMA were observed in ozonated drinking water from Germany and came as a surprise because ozone does not form NDMA by reaction with natural organic matter. However, the $(CH_3)_2$-N group of tolylfluanide is highly reactive with ozone to produce stoichiometric levels of NDMA. Moreover, the presence of bromide was later shown to catalyze this formation [107], and as mentioned earlier, wastewater inputs can increase bromide levels, which could result in increased formation of NDMA.

An initial electrophilic attack of chorine on the aromatic ring or amine group of diclofenac produces up to four major DBPs: chloro-diclofenac, bromo-diclofenac, decarboxy-diclofenac, and chloro-decarboxy-diclofenac [108, 109]. Reaction of ozone with this compound generates several hydroxylated and dechlorinated products [110].

The antiepileptic drug carbamazepine, one of the most frequently detected pharmaceuticals in drinking water systems in Europe and in the USA, rapidly reacts with ozone, producing three DBPs: 1-(2-benzaldehyde)-4-hydro-(1*H*,3*H*)-quinazoline-2-one, 1-(2-benzaldehyde)-(1*H*,3*H*)-quinazoline-2,4-dione, and 1-(2-benzoic acid)-(1*H*,3*H*)-quinazoline-2,4-dione [111]. Oxidation of carbamazepine at chlorination conditions commonly used in water treatment systems was observed to be low. However, reaction of this pharmaceutical with chlorine formed monohydroxylated, epoxide, diol, and monohydroxylated chlorinated derivatives and carbamazepine chloramide [112].

Pharmaceuticals used for medical imaging can also react with disinfectants to form DBPs. For example, the X-ray contrast media iopamidol can react with chlorine or chloramine to form highly toxic iodo-THM and iodo-acid DBPs, e.g., dichloroiodomethane and iodoacetic acid [14]. The parent X-ray contrast media

compounds are not toxic and are used in high doses (200 g/person/day) for medical imaging; however, iopamidol can transform in treatment to form the most genotoxic DBPs identified to date. The mechanism of reaction is not yet known and is currently under investigation. Chloraminated and chlorinated source waters with iopamidol were genotoxic and cytotoxic in mammalian cells. This is in agreement with the previously reported high genotoxicity and cytotoxicity of the iodo-acids and iodo-THMs [34, 35]. Reactivity of amphetamine-like compounds, cocaine, and the cannabis metabolite (±)-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) with chlorine has been also reported in the peer-reviewed literature [81, 113, 114]. 3,4-Methylenedioxymethamphetamine (MDMA, also known as Ecstasy), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxyethylamphetamine (MDEA) react with chlorine to form a highly stable product, 3-chlorocatechol, in the case of MDMA, and 3-chlorobenzo-1,3-dioxole, in the case of MDA and MDEA [115]. In the case of cocaine, four transformation products, i.e., benzoylecgonine, norcocaine, norbenzoylecgonine, and N-formylnorcocaine, were formed via hydrolytic dealkylation of the ester group and chlorine attack on the amine group, leading to *N*-dealkylation and, to a minor extent, to amide formation [113]. Chlorination DBPs of THC-COOH were formed by electrophilic substitution of hydrogen per chlorine (or bromine) in the aromatic ring and via additional hydration and/or halogenation reactions of the C–C bond conjugated with the carbonyl moiety of the THC-COOH molecule [114].

NDMA, a DBP commonly found in chloraminated drinking water, can be formed from contaminants that contain dimethylamine groups [13]. Among different pharmaceuticals containing dimethylamine groups, the antacid ranitidine showed the strongest potential to form NDMA [116, 117].

The antibacterial triclosan can react with chlorine or chloramine to form chloroform, 5,6-dichloro-2-(2,4-dichlorophenoxy)phenol, 4,5-dichloro-2-(2,4-dichlorophenoxy)phenol, 4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol [118]. The reaction of triclosan with monochloramine is slow, however, compared to chlorine [119]. The chlorophenoxyphenols are formed via electrophilic substitution of triclosan. In the presence of iodide, iodo-phenols can form [120].

Levels of free chlorine usually present in tap waters are sufficient to form mono- and dihalogenated by-products of parabens, with brominated species dominating when trace amounts of bromide are present [121]. Di-chlorinated forms of methylparaben and propylparaben were found to be recalcitrant to further chlorine oxidation, and therefore, they are likely to be found in environmental waters [121]. In fact, these compounds, together with monochlorobenzylparaben, were the only paraben DBPs out of the 14 investigated monochloro- and dichloro-parabens found in chlorinated swimming pool waters [122], and they have been also reported to be present in surface waters [123]. Ozonation of parabens in aqueous solutions produced paraben DBPs mainly through hydroxylation of their aromatic ring and/or their ester chain [124].

The plasticizer BPA also has phenolic groups that can readily react with chlorine, forming monochloro-, dichloro-, trichloro-, and tetrachloro-derivatives [125]. These reactions also occur by electrophilic substitution. When iodide is present in source waters, it can react with aqueous chlorine to form HOI, which reacts with the phenolic groups of BPA to form iodo-phenol derivatives [120]. As mentioned earlier, iodo-DBPs are generally much more toxic than chloro-DBPs [34]. BPA can also react with ozone to form catechol, *ortho*-quinone, muconic acid derivatives of BPA, benzoquinone, and 2-(4-hydroxyphenyl)-propan-2-ol [126].

Estrogens can also react with chlorine and ozone to form by-products [76]. Structures of 46 natural estrogen and synthetic estrogen (EE2) DBPs have been proposed, along with the oxidation/disinfection processes that give rise to them. The reaction of estrogens, i.e., estrone, E2, and EE2, with free chlorine occurs mainly via an electrophilic substitution at the *ortho* and *para* positions, which results eventually in cleavage of the aromatic structure. Several authors have reported that dichlorinated derivatives present less estrogenic activity than monochlorinated derivatives, and in most cases, estrogen DBPs are less potent in terms of estrogenicity than the parent compounds. Molecular ozone can react with double bonds, activated aromatic structures, or heteroatoms, but it can also form highly reactive and nonselective free radicals, e.g., $HO^{\bullet}$. As a result, some of the estrogen DBPs generated during the ozonation of estradiol water solutions are common to those formed during diverse photocatalytic processes (O_3/UV, TiO_2/UV, and photo-Fenton) [15]. In addition to forming hydroxylated derivatives from estrogens, ozone can also form dicarboxylic acids via the opening of an aromatic ring. This transformation route was also identified during the heterogeneous photocatalysis with TiO_2 of estradiol [76].

The algal toxins microcystins, nodularins, cylindrospermopsin, and saxitoxins are highly reactive to chlorine, but this is not the case for anatoxin-a. Overall, reaction of algal toxins with monochloramine and chlorine dioxide is slower than with free chlorine, and therefore, these disinfectants are not as efficient for compound removal in water treatment processes [32]. As a consequence, most research on DBP formation from algal toxins has been performed with chlorine. The reaction of cylindrospermopsin with free chlorine leads to the formation of three DBPs: 5-chloro-cylindrospermopsin, cylindrospermopsic acid, and an unnamed by-product with *m*/*z* 375.097 ($C_{13}H_{18}N_4O_7S$) [127]. In the case of microcystins, up to six chlorination DBPs and their respective isomers have been identified: dihydroxy-microcystin, monochloro-microcystin, monochloro-hydroxy-microcystin, monochloro-dihydroxy-microcystin, dichloro-dihydroxy-microcystin, and trichloro-hydroxy-microcystin. Ozonation of microcystins transforms these molecules through initial $HO^{\bullet}$ attack on the conjugated diene and cleavage of the Adda amino acid that leads to the opening of the peptide ring [32]. In the case of microcystins and saxitoxins, the toxicity of the mixture after chlorination and ozonation was decreased.

5 Human Exposure to CECs and DBPs

Human exposure to contaminants through drinking water includes ingestion, inhalation, and dermal adsorption routes. Exposure through ingestion includes not only contaminants in drinking water but also when tap water is used to make other drinks, such as coffee and tea, and when tap water is used in the cooking of foods. Foods can adsorb and concentrate DBPs and other contaminants (like solid-phase extraction materials) [128], and they can also be a source of precursors to form additional DBPs [129, 130].

Inhalation and dermal exposure to DBPs and CECs can occur during showering, bathing, and swimming in chlorinated swimming pools [131–148]. THMs, HAAs, and haloketones have been measured in human blood, urine, or exhaled breath after showering, bathing, or swimming [131, 133, 135, 136, 142, 143, 146, 149]. Inhalation can sometimes give much higher exposure to volatile chemicals. For example, inhalation during a 10-min shower has been shown to produce twice the level of THMs in blood compared to drinking 1 L of water [150]. Dermal exposure can also result in higher blood levels than ingestion for some chemicals and can result in different blood concentration profiles [133, 150–153]. A human exposure study involving ^{13}C-labeled bromodichloromethane (BDCM) showed that blood levels of BDCM from 1-h dermal exposures were 25–130 times higher than from oral exposures. Moreover, BDCM remained in the blood much longer following dermal exposure vs. oral ingestion, such that BDCM was still detectable in the blood 24 h after dermal exposure, whereas BDCM returned to preexposure levels within 4 h after oral ingestion.

New epidemiologic studies suggest that exposure to chlorinated water through dermal and inhalation routes may contribute to bladder cancer [154], and bromine-containing THMs (or co-occurring iodo-DBPs) may be a significant contributing factor to populations with certain genotypes [133, 143, 155, 156]. There is also new evidence that genetic susceptibility may play a role in bladder cancer. A recent epidemiologic study conducted in Spain revealed that people who carry a particular glutathione *S*-transferase zeta-1 (GSTZ1) polymorphism and are missing one or both copies of glutathione *S*-transferase theta-1 (GSTT1) were particularly susceptible to bladder cancer when exposed to >49 μg/L THMs in drinking water [155]. Approximately 29% of the Spanish study population had this genetic susceptibility, and approximately 25% of the US population would also have this genetic susceptibility.

These three exposure routes contribute to the overall dose of contaminants and DBPs through drinking water, and as highlighted earlier, water reuse has the potential of increasing human exposure to CECs and to DBPs. Because DBPs are ubiquitous whenever chemical disinfectants are used, they may impact human health more than other contaminants that are generally found at lower levels in finished drinking water.

6 Potential Removal Technologies

Some advanced removal technologies are effective for removing many of these CECs. For example, ketoprofen, diclofenac, sulfamethoxazole, carbamazepine, hydrochlorothiazide, propyphenazone, glibenclamide, sotalol, and metoprolol can be removed to a large extent (>85%) with the use of nanofiltration (NF) and RO membranes [157]. X-ray contrast media and perfluorinated compounds are also well removed (<90%) by RO membranes [158]. However, lower molecular weight compounds, such as NDMA, methyl *tert*-butyl ether (MTBE), gemfibrozil, and mefenamic acid, are not as effectively removed by these membranes (e.g., almost no removal to 70% removal) [157, 159]. For compounds with molecular weights between 100 and 200 Da, there is a large range of rejection values depending on the membrane type [158]. Membrane feed temperature, permeate flux, feed solution pH, and ionic strength can affect removals by RO membranes, causing disparate results for NDMA in the literature [159]. It has been suggested that while NF and RO are effective for removing many micropollutants, it cannot serve as an absolute barrier, and additional treatment technologies, such as ozonation or activated carbon adsorption, could be combined with RO or NF to ensure complete removal [160].

For example, ozone is effective for removing many CECs whose structures contain activated aromatic rings, amine groups, or double bonds, such as sulfamethoxazole, diclofenac, and carbamazepine, which could be removed during wastewater treatment to below detection (<25 ng/L in most cases) with ozone concentrations of 0.47 g O_3/g dissolved organic carbon (DOC) [161]. More resistant compounds, such as atenolol and benzotriazole could be removed by >85% with an increased ozone dose of 0.6 g O_3/g DOC, which is a concentration still relevant to real-world wastewater treatment. In a study of 220 micropollutants, only a few contaminants, including X-ray contrast media and triazine herbicides, were not effectively removed by ozonation [161]. Some compounds that were formed by ozonation (e.g., NDMA and bromate) were at concentrations lower than drinking water standards. Further, it was possible to remove biodegradable compounds by biological sand filtration, such that NDMA could be removed by 50%.

While advanced treatment using membranes, ozonation, and filtration have been shown to be effective for removing many CECs in laboratory- or pilot plant-based studies, the situation can be somewhat different at full-scale advanced wastewater recycling plants. For example, in a recent study by Linge et al., full-scale plants in Perth, Australia, had several DBPs (THMs, dihalomethanes, HAAs, haloacetonitriles, and haloketones) in their MF/RO effluents that were not otherwise present in the incoming secondary wastewater or were initially present at significantly lower levels [162]. This is because MF/RO treatment typically includes chloramination of wastewater before MF to minimize RO membrane fouling [162, 163], and thus, chloramination DBPs can form. The majority of DBPs are typically small, neutral molecules that show intermediate or poor RO rejection. Plant residence time played an important role in the levels of DBPs observed, which

resulted in greater frequency of detections at the full-scale plants vs. pilot-scale plants (which have smaller residence times). An unusual finding was the consistent detection of two dihalomethanes: dibromomethane and bromochloromethane, which are not routinely monitored and may be more toxicologically important than the regulated THMs [162]. It was suggested that DBP precursor removal should be optimized in secondary wastewater, such as implementing advanced biological treatment upstream of MF and RO processes.

7 Conclusions

In conclusion, population increases and climate change are resulting in increased complexity of chemicals present in environmental waters and in drinking water. CECs, such as pharmaceuticals, antibacterials, PFCs, hormones, BPA, benzotriazoles, dioxane, perchlorate, antimony, and algal toxins, are not completely removed by wastewater treatment and are entering drinking water supplies, where they can either contaminate finished drinking water directly or become transformed by disinfectants into DBPs. Increased nitrogen and bromide are also entering from wastewater, agriculture, and energy extraction and utilization activities, and they are resulting in the formation of more toxic nitrogen- and bromine-containing DBPs. While some contaminants can be effectively removed by advanced treatment (e.g., RO, UV, and ozonation), others are not removed completely even by these advanced technologies. Moreover, when chloramine is used to prevent fouling of RO membranes, DBPs (including unregulated ones) can form that are not completely removed by the RO membranes. These new DBPs have unknown properties and toxicity. As a result, it is wise to look beyond the chemicals that are regulated in drinking water and consider these CECs and their potential transformation products, especially when assessing the safety of impaired waters for potable use.

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Design of Water Recovery System with Process Integration

Dominic C.Y. Foo

Abstract Water is commonly used in the process industries as raw material and utility systems as well as for washing operations. In recent years, stricter environmental regulations and water scarcity issues have led to the growing need for better water management. Concurrently, the development of various *process integration* tools for resource conservation has become very established in recent years. This chapter presents one of the important process integration tools, known as *water pinch analysis*, for the design of a *water recovery system*. A water recovery case study of a steel plant is used for illustration.

Keywords Pinch analysis, Process design, Process synthesis, Targeting, Water reuse/recycle

Contents

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 183–192, DOI 10.1007/698_2014_322,

Abbreviations

MCA Material cascade analysis
NNA Nearest neighbour algorithm
RCN Resource conservation network

1 Introduction

A recent report by the United Nations revealed that global water demand (in terms of water withdrawals) is projected to increase by approximately 55% by year 2050 [1]. This is due to the growing needs from several sectors such as manufacturing (400%), electricity generation (140%) and domestic use (130%). The net effect of this trend is the freshwater scarcity situation. It is expected that more than 40% of the global population will be living in areas of severe water stress through 2050 [1]. The report also mentioned that groundwater supplies are diminishing, with approximately 20% of the world's aquifers being overexploited. Apart from this mismanagement of water resources, the rising of population growth, water pollution problems and climate change also increase water stress [2, 3]. In the process industry, there is a growing need for better water management to secure sustainable development.

In the past three decades, process integration techniques such as *pinch analysis* and *mathematical optimisation* have been developed to address various resource conservation issues, ranging from energy, material and more specifically water recovery. To date, process integration techniques are documented in various textbooks [4–10] and review papers [11–15]. One of the widely accepted definitions for process integration is given as *a holistic approach to process design, retrofitting and operation which emphasises the unity of the process* [8].

In the following section, conceptual understanding of process integration for resource conservation is first given. Process integration tools based on pinch analysis techniques are next illustrated, followed by a case study on water recovery in a steel plant.

2 Conceptual Understanding

In the past two decades, generic process integration tools were developed for various material *resource conservation networks* (RCNs) including *water minimisation*, *gas recovery* and *property integration* [10]. Different strategies for RCN are formally defined from the perspective of process integration, i.e. *direct reuse/recycle* and *regeneration reuse/recycle*. Direct reuse refers to the scheme where a process effluent is sent to other processes and does not re-enter its original

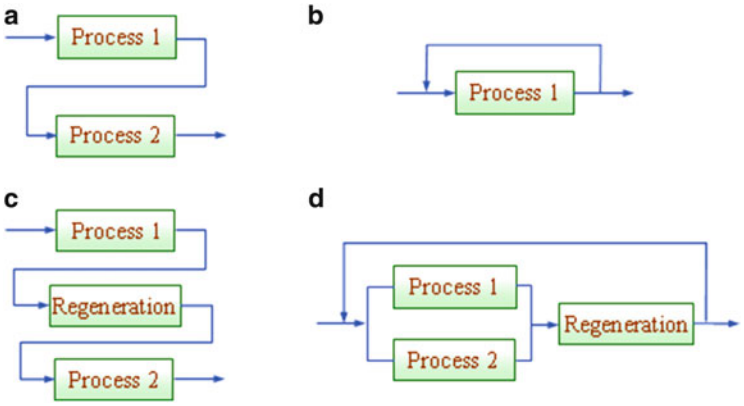

Fig. 1 Strategies for an RCN: (**a**) direct reuse, (**b**) direct recycle, (**c**) regeneration reuse, (**d**) regeneration recycle [10, 16]

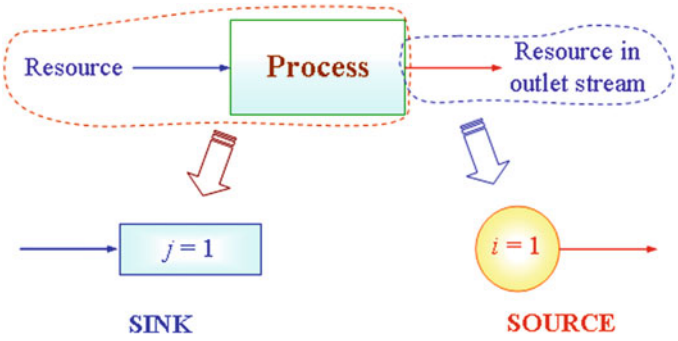

Fig. 2 Conceptual understanding of process source and sink [10]

process. On the other hand, direct recycle refers to the recovery scheme where the process effluent re-enters its original process. A process effluent may be partially purified in an *interception unit* to improve its quality prior to reuse/recycle; these are known as regeneration reuse and regeneration recycle. Figure 1 shows the recovery schemes for an RCN. For most cases, the priority is given to direct reuse/recycle scheme, as it involves lowest investment cost and ease of implementation.

To better understand an RCN problem, it is important to understand the concept of *process sink* and *source*. Source refers to a process stream that can be recycled (normally the outlet) where material recovery is to be performed. On the other hand, sink refers to a process unit where a resource (typically a fresh material) is needed (Fig. 2).

3 Process Integration Tools for Direct Water Reuse/Recycle

Different variants of pinch analysis tools are eligible for use to design a water recovery system. Typically, pinch analysis involves a two-step design stage. In step 1, targeting tool is used to perform benchmarking to identify the maximum recovery targets, which corresponds to the minimum freshwater and wastewater flowrates for an RCN. For this stage, various graphical or algebraic tools such as *limiting composite curve* [16] and *material recovery pinch diagram* [17, 18] may be used. For step 2, the RCN is designed to match the targets identified in step 1. For this step, one may utilise tools such as *sink-source mapping diagram* [7] or *nearest neighbour algorithm* (NNA) [18]. Due to space constraint, only the targeting and design for direct reuse/recycle scheme will be illustrated here. Readers may refer to the review paper [14] to understand the strength and weakness of the various targeting and design tools.

3.1 Algebraic Targeting Tool

The algebraic targeting tool has the advantage of identifying accurate RCN targets, overcoming the cumbersome problems of the graphical tools. One of such tool is the *material cascade analysis* (MCA) technique, with the general framework given in Table 1 [19].

Flowrates of the process sinks (F_{SKj}) and sources (F_{SRi}) are located at their quality levels in the first three columns of Table 1, in which the quality levels (q_k) are arranged in descending order. At each quality level k, the total flowrate of the process sink(s) is deducted from that of the process source(s), with the *net flowrate* given in column 4. In the following column, net flowrate is cascaded down the quality levels to yield the *cumulative flowrate* ($F_{C,\,k}$). The first entry of this column corresponds to the fresh resource (i.e. water) consumption for the RCN (F_R), which is first assumed to be zero, i.e. $F_R = 0$. The last entry in this column is the minimum waste (i.e. wastewater) discharged from the RCN (F_D). In column 6, the impurity/property load in each quality interval (Δm_k) is calculated, given by the product of the cumulative flowrate ($F_{C,\,k}$, column 5) with the difference across two quality levels ($q_{k+1} - q_k$). The load values are cascaded down the quality levels to yield the cumulative load (Cum. Δm_k) in column 7 of Table 1. If negative Cum. Δm_k values are observed, the *interval fresh resource flowrate* ($F_{R,\,k}$) is calculated for each quality level in column 8, by dividing the cumulative loads (Cum. Δm_k, column 7) by the difference between the quality levels of interest (q_k) with that of the fresh resource (q_R), given by Eq. (1):

Table 1 General framework for MCA

q_k	$\Sigma_j F_{SKj}$	$\Sigma_i F_{SRi}$	$\Sigma_i F_{SRi} - \Sigma_j F_{SKj}$	$F_{C,\,k}$	Δm_k	Cum. Δm_k	$F_{R,\,k}$
				$\boldsymbol{F_R}$			
q_k	$(\Sigma_j F_{SKj})_1$	$(\Sigma_i F_{SRi})_1$	$(\Sigma_i F_{SRi} - \Sigma_j F_{SKj})_1$	⇩			
				$F_{C,\,k}$	Δm_k		
q_{k+1}	$(\Sigma_j F_{SKj})_{k+1}$	$(\Sigma_i F_{SRi})_{k+1}$	$(\Sigma_i F_{SRi} - \Sigma_j F_{SKj})_{k+1}$	⇩		Cum. Δm_{k+1}	$F_{R,\,k+1}$
				$F_{C,\,k+1}$	Δm_{k+1}	⇩	
...	...	...	...	...	...	...	...
q_{n-2}	$(\Sigma_j F_{SKj})_{n-2}$	$(\Sigma_i F_{SRi})_{n-2}$	$(\Sigma_i F_{SRi} - \Sigma_j F_{SKj})_{n-2}$	⇩		Cum. Δm_{n-2}	
				$F_{C,\,n-2}$	Δm_{n-2}		
q_{n-1}	$(\Sigma_j F_{SKj})_{n-1}$	$(\Sigma_i F_{SRi})_{n-1}$	$(\Sigma_i F_{SRi} - \Sigma_j F_{SKj})_{n-1}$	⇩		Cum. Δm_{n-1}	$F_{R,\,n-1}$
				$F_{C,\,n-1} = \boldsymbol{F_D}$	Δm_{n-1}	⇩	
q_n						Cum. Δm_n	$F_{R,\,n}$

$$F_{\mathrm{R},\,k} = \frac{\Delta m_k}{(q_k - q_{\mathrm{R}})}. \tag{1}$$

The absolute value of the largest negative $F_{\mathrm{R},\,k}$ in column 8 is identified as the *minimum fresh resource consumption* (F_{R}) of the network. This value is then used as the first entry in column 5, and all calculations in columns 5–7 are repeated. The quality level where zero cumulative load value (Cum. Δm_k, column 7) is found indicates the *pinch quality*.

3.2 Network Design Technique

One of the useful tools to design an RCN that achieves the flowrate targets (identified in step 1) is the NNA [18]. To utilise NNA to design a water recovery network, two important criteria are to be met by all process sinks, i.e. *flowrate* ($F_{\mathrm{SK}j}$) and *load* ($m_{\mathrm{SK}j}$) requirements. The latter is given by the product of its flowrate and quality index (i.e. $m_{\mathrm{SK}j} = F_{\mathrm{SK}j}\, q_{\mathrm{SK}j}$). In most cases, the quality index for a water recovery network is the concentration of the main impurity. The detailed design steps of NNA are given as follows [18]:

1. Arrange all material sinks and the sources in descending order of quality levels, respectively (i.e. ascending order of impurity concentration). Note that the sources should include the external fresh(water) resource, with their respective flowrates obtained in the targeting stage. Start the design from sink with highest quality index ($q_{\mathrm{SK}j}$).
2. Match the selected sink SK_j with source(s) SR_i of the same quality level, if any are found.
3. Mix two source candidates SR_i (with flowrate $F_{\mathrm{SR}i}$ and quality $q_{\mathrm{SR}i}$) and SR_{i+1} (with flowrate $F_{\mathrm{SR}i+1}$ and quality $q_{\mathrm{SR}i+1}$) to fulfil the flowrate and load requirements of sink SK_j. Note that the source candidates SR_i and SR_{i+1} are the nearest available 'neighbours' to the sink SK_j, with quality levels just lower and just higher than that of the sink, i.e. $q_{\mathrm{SR}i} < q_{\mathrm{SK}j} < q_{\mathrm{SR}i+1}$. The respective flowrate between the source and the sink is calculated via the mass balance Eqs. (2) and (3):

$$F_{\mathrm{SR}i,\ \mathrm{SK}j} + F_{\mathrm{SR}i+1,\mathrm{SK}j} = F_{\mathrm{SK}j}, \tag{2}$$

$$F_{\mathrm{SR}i,\mathrm{SK}j} q_{\mathrm{SR}i} + F_{\mathrm{SR}i+1,\ \mathrm{SK}j} q_{\mathrm{SR}i+1} = F_{\mathrm{SK}j} q_{\mathrm{SK}j}, \tag{3}$$

   where $F_{\mathrm{SR}i,\ \mathrm{SK}j}$ is the allocation flowrate sent from SR_i to SK_j. If SR_i has sufficient flowrate to be allocated to SK_j, i.e. $F_{\mathrm{SR}i} \geq F_{\mathrm{SR}i,\ \mathrm{SK}j}$, go to step 5, else to step 4.
4. If the source has insufficient flowrate to be used as the allocation flowrate, i.e. $F_{\mathrm{SR}i,\ \mathrm{SK}j} > F_{\mathrm{SR}i}$, then whatever is available of that source is used completely. A new pair of neighbour candidates is considered to satisfy the sink.
5. Repeat steps 2–4 for all other sinks. Once all sinks are fulfilled, the unutilised source(s) are discharged as waste.

4 Case Study: Water Recovery for a Steel Plant

In this section, a water recovery case study in a steel plant [20] is illustrated. The limiting water data is shown in Table 2. The impurity in concern for water recovery is identified as the chlorine content. From Table 2, it is observed that the freshwater and wastewater flowrates for the base case design are identified as 5,280 and 3,720 t/d, respectively, given by the summation of the individual flowrates of sinks and sources. In order to minimise freshwater and wastewater flowrates for the process, direct reuse/recycle scheme is explored. For this case, freshwater has an impurity (chlorine) content of 20 mg/L.

Step 1 of water pinch analysis is first carried out using the MCA. Following the MCA procedure, the feasible cascade table (i.e. no negative Cum. Δm_k is observed in column 7) is shown in Table 3. The MCA identifies that the minimum freshwater (F_{FW}) and wastewater (F_{WW}) flowrates for the direct reuse/recycle scheme are 2,234.21 and 674.21 t/d, respectively. This corresponds to a reduction of 57.7% and 81.8% of freshwater and wastewater flowrates in the base case design.

Table 2 Limiting water data for steel plant case study

j	Sinks, SK_j	$F_{\mathrm{SK}j}$ (t/d)	$C_{\mathrm{SK}j}$ (mg/L)	i	Sources, SR_i	$F_{\mathrm{SR}i}$ (t/d)	$C_{\mathrm{SR}i}$ (mg/L)
1	Hot-air furnace	1,680	90	1	Hot-air furnace	960	400
2	Blast furnace	1,920	75	2	Blast furnace	1,440	100
3	Power plant	1,680	40	3	Power plant	1,320	45

Table 3 Feasible cascade table for steel plant case study

C_k (mg/L)	$\Sigma_j F_{\mathrm{SK}j}$ (t/d)	$\Sigma_i F_{\mathrm{SR}i}$ (t/d)	$\Sigma_i F_{\mathrm{SR}i} - \Sigma_j F_{\mathrm{SK}j}$ (t/d)	$F_{\mathrm{C},\,k}$ (t/d)	Δm_k (g/d)	Cum. Δm_k (g/d)
				$\boldsymbol{F_{\mathrm{FW}} = 2{,}234.21}$		
20						
	20			2,234.21	44.68	
40		1,680				44.68
	5			554.21	2.77	
45			1,320			47.46
	30			1,874.21	56.23	
75		1,920				103.68
	15			−45.79	−0.69	
90		1,680				102.99
	10			−1,725.79	−17.26	
100			1,440			85.74
	300			−285.79	−85.74	
400			960			**0**
	999,600			$\boldsymbol{F_{\mathrm{WW}} = 674.21}$	673,940.84	**(Pinch)**
1,000,000						673,940.84

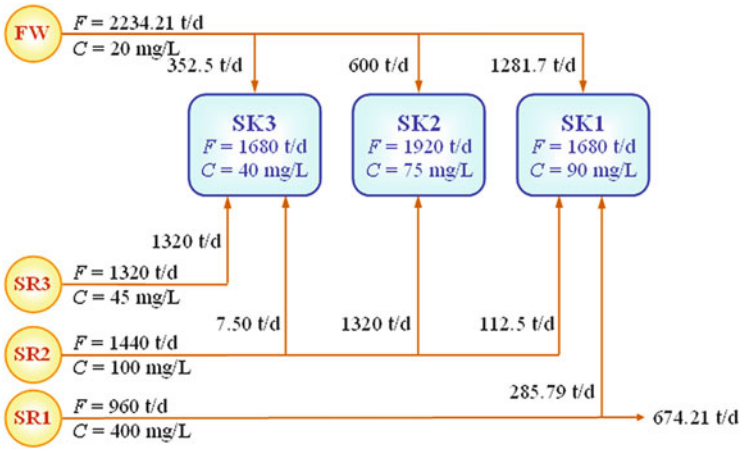

Fig. 3 Water recovery network for steel plant case study

Next, the NNA is used to design the water recovery network. Note that the sinks are arranged from lowest to highest concentration order. For SK3, we first identified freshwater (FW) and SR3 as its neighbour candidates. However, since SR3 has lower flowrate than the allocated flowrate as determined by Eqs. (2) and (3), the entire SR is sent to SK3, in which FW and SR2 are identified as the new pair of neighbour candidates for SK3. The design then proceeds to SK2 and SK1. Note that SK1 has the same situation as SK3, where SR2 is fully allocated before FW and SR1 are being identified as new neighbour candidates. The unutilised water from SR1 is sent for wastewater treatment. A complete water recovery network is shown in Fig. 3. Note that the design achieves the minimum freshwater and wastewater flowrates identified using the MCA in step 1.

5 Conclusion

This chapter presents process integration techniques to design a water recovery system. The technique is based on pinch analysis technique, which is divided into a two-step approach. In step 1, the minimum flowrates of freshwater and wastewater are first identified. In step 2, the water recovery network is designed to match the identified water flowrate targets. Other industrial case studies for water recovery may be found in literatures [8,10].

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Wastewater Use in Agriculture and Relevance of Micropollutants in North African Countries

Olfa Mahjoub

Abstract Irrigation is the largest practice of water reuse worldwide. In North African countries, both formal and informal uses of wastewater were practiced for a long time thus exposing users and consumers to microbiological and chemical health risks. Negative environmental impacts are also of concern because secondary biological treatment is not effective in removing ubiquitous and persistent contaminants like some emerging micropollutants. Based on research findings during the last decades, the presence of micropollutants in reclaimed water has gained interest in developed countries, and the release of some of them into water bodies has been regulated. In North African countries, in view of the prevailing quality of reclaimed water and its current usage for growing crops, the occurrence of such contaminants has recently raised concern with an increasing number of research works and publications. However, it remains challenging to identify, quantify, and prioritize the most relevant to be regulated. This paper aims at shedding light on the usage of reclaimed water for irrigation in Algeria, Egypt, Libya, Morocco, and Tunisia while pinpointing the potential sources of micropollutants in wastewater. It discusses the extent to which some micropollutants could be relevant and challenging to public health and environmental quality.

Keywords Agriculture, Micropollutants, North Africa, Reuse, Wastewater

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 193–212, DOI 10.1007/698_2015_337,

Contents

Abbreviations

BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
E1	Estrone
E2	17β-estradiol
E3	Estriol
EC	Electrical conductivity
EE2	17α-ethinylestradiol
FAO	Food and Agriculture Organization
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PPCP	Pharmaceuticals and personal care products
TDS	Total dissolved solids
US EPA	United States Environmental Protection Agency
WFD	Water Framework Directive
WHO	World Health Organization

1 Introduction

Wastewater is recognized as the main source of large classes of organic micropollutants to natural water resources. More than 65 million chemicals are identified and currently listed in SciFinder. About 100,000 are frequently used including a large class of synthetic organic compounds. A great share of these chemicals is emitted into the environment resulting in adverse effects on ecosystems and human health [1]. Considering the efficiency of the existing conventional treatment technologies, micropollutants are not expected to be completely removed. Hormones (natural and synthetic), pharmaceuticals and personal care products (PPCPs), detergents, plasticizers, pesticides, etc., are all frequently detected at trace level in treated wastewater.

Reclaimed (waste) water or recycled water is treated wastewater that can be officially used under controlled conditions for beneficial purposes like irrigation. In North African countries, reuse encompasses secondary, primary, and partially treated and untreated wastewater. By combining this range of effluent qualities and the regular types of reuse as direct and indirect and planned and unplanned [2], various schemas of wastewater reuse are observed.

Environmental and health impacts of reuse are assessed based on a certain number of actual data and assumptions for risk assessment. A recent review attempted to address the impact of the repeated release of treated wastewater for reuse applications in respect to the wastewater residual load of heavy metals in soil, plants, and their edible parts [3]. The occurrence of emerging micropollutants like endocrine disrupters, PPCPs, drugs' metabolites, illicit drugs, transformation products, etc. in wastewater is clearly highlighted when in case of reuse and is becoming relevant to the agricultural environment [3]. However, to decide on the relevance of a contaminant to a given milieu, studies should be carried out. For instance, in the United States, the determination of the concentrations of 48 high-priority substances, including pharmaceuticals, hormones, degradation products of surfactants (alkylphenols), bisphenol A, and perfluorinated compounds in 50 treatment plants by the US EPA, has led to the conclusion that the risk related to the prevalence of these substances in wastewater is low for humans [4]. The increasing interest to micropollutants in wastewater and their transfer to the agroecological environment through reuse in the developing countries should raise the following questions: Are micropollutants relevant to these countries or is it just an emerging topic? Do we know already enough? Which countries might be the more concerned? What should be the rationale behind prioritization, if needed, and what are the challenges?

In an attempt to answer these questions, available water resources (quantity and quality), wastewater status and reuse practices, potential micropollutants occurring in agricultural environment, and potential environmental and health issues related to micropollutants in North African region are the focus of the present paper. The emphasis is put on the four following countries of the region: Algeria, Egypt, Morocco, and Tunisia. Examples from Libya are given when proper information is available.

2 Wastewater Status

Since micropollutants are detected in surface waters [5], reclaimed water-irrigated soils [6], and groundwater [7], a brief overview on the current status of wastewater management in various countries is described in this section.

Egypt is the largest producer of wastewater; it generates about 5,400 million m^3/year [8]. About 40% is not treated [9] because of the lack of connection to improved sanitation, especially in the rural areas. Along the Nile River, 79% of the existing industrial facilities discharge more than four billion m^3/year of wastewater, directly and indirectly [10]. Agricultural drainage water represents the largest amount of wastewater in Egypt flowing into the Nile River. It represents 4.9% of the total water resources. Drainage water and wastewater of domestic, industrial, and commercial activities are blended in the agricultural drains and reused for irrigation [11]. In Tunisia, 240 million m^3/year of secondary treated wastewater is produced in 110 treatment plants. Many are outdated representing a real threat for the environment, in case of both reuse and disposal in the receiving environment. This volume is expected to reach 10% of the available resources by the year 2021. Wastewater is 80% domestic, 15% industrial, and 5% from the tourism sector. Areas affected by industrial pollution are generally located on the coastline of Tunis, Sfax, Bizerte, Sousse, Nabeul, and Gabes [12]. Morocco is producing 700 million m^3/year of domestic wastewater. However, only 25% is treated in 62 sewage treatment plants; 90% are primary and secondarily treated [8]. Textile and tannery activities generate ten million m^3/year of wastewater. The domestic and industrial sewage from Fez City is directly discharged into the Sebou River [13]. The volume of wastewater is expected to reach 900 million m^3 by 2020, representing about 4% of the total water resources [14]. About 660 million m^3 of wastewater was discharged in the water bodies in 1985 in Algeria. Around 85% is released from the industrial sector which is discharged without appropriate treatment. The agro-food and textile sector effluents represent 55 and 22% of the total produced wastewater, respectively [10]. The number of treatment plants has raised to 123 in 2011 with a total treatment capacity of 700 million m^3/year. By 2020, 1.2 billion m^3/year is expected [15]. To prevent industrial pollution, 158 projects were launched for the construction of infrastructure for sewage network. In Libya, wastewater hardly represents 1% of the total water resources [16]. Wastewater is disposed off partially treated or untreated. In Al Jabal Al Akhdar, nine treatment plants treating 5,000–15,000 population equivalents were planned for 2009. Currently, the political unrest is preventing from obtaining any update on the situation of wastewater status in the country [17].

3 Wastewater Reuse in Agriculture

Wastewater reuse is the most suitable solution to fill the increasing gap between the limited water resources and the growing demand. North African countries are producing around 6,300 billion m^3 of wastewater. Only 15% is reused chiefly in agricultural irrigation (Table 1). Based on the different types of wastewater and categories of reuse [20], there is a clear trend toward the direct use of treated wastewater spread in all the countries and the indirect use of treated and untreated wastewater [21]. In this regard, North African countries have evolved with different perspectives toward reuse of wastewater. While in all the countries, reuse of raw wastewater for irrigation of eaten-raw vegetables is forbidden by law, in Algeria eaten-raw crops are allowed to be irrigated by treated wastewater if they comply with the standards [22]. Regulations, when they do exist, allow fruit tree, forest, forage, and urban irrigation. As per the revised WHO guidelines, they are not well understood by the stakeholders and thereby are not adjusted to the local needs [23] to prevent risks related to irrigation.

Egypt is the largest wastewater user in the Arab countries as a whole. Primary treated wastewater has been used since 1911 in agriculture in El Gabal el Asfar and Abou Rawash, near Cairo, to irrigate about 1,260 ha. Unofficial wastewater reuse is significant and planned, and regulated types of reuse are still limited. Up to four billion m^3/year are used thus posing threats to human health and environment and hampering the implementation of governmental plans and strategies [24]. About 5.5 to 6.5 billion m^3/year of wastewater was produced in 2011. The sewage water drained to the agricultural canals is reused after blending with less polluted water downstream [11]. The total amount of official drainage reuse reached around seven billion m^3 in 2010 [19]. Egypt is developing reuse in afforestation projects in the desert and establishing greenbelt around the capital [25]. Nowadays, 63 forests and about 5,040 ha (12,000 feddan) are cultivated with sunflower, *Jatropha*, casuarinas, etc. [11].

Tunisia is the most advanced country in North Africa. Reuse of treated wastewater has been practiced since the 1960s for the irrigation of citrus in the northeast of the country, and it has become an integral part of the National Water Resources Strategy since the 1990s [26]. Reuse was developed within the National Strategy for

Table 1 Produced, treated, and reused wastewater in North African countries in 2009 [11, 14, 18, 19]

Country	Produced (million m^3/year)	Treated (million m^3/year)	Reused (million m^3/year)	Ratio reused to produced (%)
Algeria	820	700	51	6
Egypt	7,600	2,971	700	19
Libya	546	40	40	7
Morocco	700	177	80	11
Tunisia	461	240	68	15
Total	10,127	4,128	939	15

Wastewater Reuse, as well, to reach the rate of 35%. In Tunisia, the national standards are limiting the range of crops allowed to be irrigated with secondary treated wastewater [8] causing some reluctance. Irrigated areas cover around 8,100 ha of fruit trees and fodder crops and 1,490 ha of landscape [27]. For the future, it is planned to transfer 135 million m^3 of reclaimed water from Grand Tunis area in the northeast to water-short area after complementary treatment.

In Morocco, 45% of the treated wastewater is reused in agriculture, green spaces, groundwater recharge, and industry [14]. About 80 million m^3/year of untreated wastewater is reused. Raw wastewater is sometimes mixed with water from wadis. The irrigated area covers around 7,000 ha located mainly in Marrakech (2,000 ha), Meknes (1,400 ha), and Oujda (1,175 ha). Recent pilot projects were implemented in Fez, El Attaouia, and Drarga including the construction of innovative wastewater treatment plants [21]. The national program "Plan National d'Assainissement Liquide et d'Epuration des Eaux" has focused on the depollution of the river Sebou and building of treatment plants for all populated centers on the Mediterranean coast. Comparatively, Algeria has a very low rate of reuse (3.2%) [28] due to the malfunctioning state of the park of treatment plants. Since 2005, a remarkable progress has been made within the National Water Plan [29]. The decree regulating reuse was enacted in 2012 [22]. Nowadays, 15,770 ha is irrigated [19], and by 2020, 1,200 million m^3/year is expected to irrigate 100,000 ha. As for Libya, in 1999, a volume of 546 million m^3 of wastewater was produced, but only 40 million m^3 was treated and reused [28]. Tripoli and Benghazi were the main areas of reuse with 6,000 ha with crops limited to fruit trees and animal fodders [30].

4 Challenging Risks Related to Micropollutants

Risk management related to reuse of reclaimed wastewater in agriculture is challenging in the North African region. Few initiatives have been taken within the water policy components and regulations which are still not sufficiently enforced for that purpose. When it comes to micropollutants and to their long-term impacts on soils and plants, trace metals are deemed to be relatively well addressed.

4.1 Reclaimed Water Reuse and Environment-Related Risks

Contamination of water resources and soil is the major environmental risk ensuing from the reuse of reclaimed water. The presence of toxic chemicals in raw wastewater could be due to the illegal discharge of industrial effluents in the sewer system which can disrupt the treatment process at the facility and result in the release of more toxic compounds as metabolites.

Mixing effluents of different types and origins may result in the transfer of chemical substances and their potential accumulation in soil and the irrigated crops or their migration to groundwater [2]. From the WHO and reuse experts' perspective, heavy metals are the compounds to consider in priority because of their toxicity under specific conditions [23]. For instance, wastewater used for irrigation in Marrakech City was shown to be polluted with heavy metals such as Cd, Pb, and Cr. Craft industries using chemical products in the treatment of wool and leather are probably responsible for their high content in wastewater [31] and deleterious effects.

From an environmental standpoint, it is widely recognized that spreading domestic wastewater on soil through irrigation would result in less polluting burden than discharging it directly into the water bodies [23]. Irrigation of crops using raw or treated wastewater was identified as the main route of contamination of the environment in both developed and developing countries. Cities with large historical background in wastewater treatment and reuse like Braunschweig in Germany [32], Mezquital Valley in Mexico City [33], and others have evidenced the presence of some organic micropollutants in the agricultural environment after a long time of reuse; hormones and pharmaceuticals were detected in groundwater. Similarly, the impact of wastewater discharge in the North African countries has been tackled by the scientific community during these recent years. Studies on the aquatic environment have demonstrated the effects of wastewater release on aquatic organisms with several cases of loss of aquatic life in addition to the acute and chronic toxicities detected by using bioassays and various bioindicators [34, 35]. In over and above organic compounds, heavy metals like Cd, Hg, Pb, and some of their compounds are recognized as micropollutants of concern [36].

4.2 Reclaimed Water Reuse and Health-Related Risks

Environmental health risks associated with the presence of emerging micropollutants in wastewater used for irrigation were considered negligible because of the limited instrumental capabilities. Nowadays, they have become more obvious but still not well evidenced. In developed countries, human health risks are mitigated by conducting epidemiological studies and setting regulations. Indeed, the Water Framework Directive (WFD) limited the discharge of 45 chemical compounds to preserve the ecological and chemical status of water and human health [20]. In North African countries, and considering the routes of exposure, limited observations have been made on the impacts of wastewater on human health, like the increase of the prevalence of some types of cancers. In Morocco, the detection of Pb and Al in infant's hair was extremely alarming and clearly linked to their exposure to sewage water in suburban area [37]. A significantly high correlation was previously found between Pb concentrations in hair of children whose parents were exposed to wastewater through agricultural activity. Family occupation and

direct contact with wastewater, in addition to customs and food habits, were significant factors influencing the metal content of children's hair [38].

Based on this review, to the best of our knowledge, no epidemiological studies were carried out, or at least published, in the North African region linking the occurrence of micropollutants in wastewater reused for irrigation to adverse health effects, except for some regulated heavy metals. Even for regulated toxic heavy metals, risk communication is strictly linked to risk management strategies which depend on decision-making. For organic micropollutants potentially transferred to wastewater-irrigated crops, the long-term impacts on consumers' health are not under investigation yet because risk assessment, which should be performed by the scientific communities, is still not established.

As for animals, exposure to heavy metals through feeding crops irrigated with wastewater has been evidenced in Morocco. Toxic trace metals were detected in the muscles, bones, liver, and kidney of bovine grazing on the municipal wastewater spreading field of Marrakech City. High concentrations of Cd in the liver (5.1 μg/g) and kidney (10.3 μg/g) resulted in a reduction in Zn and Cu concentrations as essential elements. Since livestock feed is based on wastewater-irrigated lucerne and corn leaves, the concentration factor (concentration in animal tissues/concentration in plants) was greater than 3. The bioaccumulation of Cd is calling for an extensive epidemiological study of the population consuming wastewater-irrigated products like garden market crops and meat produced in the area of Marrakech [39]. The direct use of the wastewater for animal watering has also significantly increased the frequency of genetic damage in the animals' white blood cells induced by exposure to pollution, and a serious genotoxic risk was identified. Some authors suggested in previous work to use herbivore mammals (sheep, dairy cows) as the most suitable "bioindicators" to assess risks for human health [40].

In view of the current status of knowledge and agricultural practices in North African countries, more research is needed in order to address long-term health risks related to (organic) micropollutants transferred to crops during irrigation with (treated) wastewater.

5 Relevant Emerging Micropollutants in Reclaimed Water

In Europe, limiting the load of micropollutants in wastewater is intended for protecting water resources. Since wastewater reuse is still optional in large number of European countries, setting threshold values for reclaimed water is not a priority. The situation is quite different in North African countries and is worth thorough study.

5.1 Heavy Metals

The reuse of treated wastewater contributes to the contamination of irrigated soils by toxic heavy metals like Cd, Cr, Ni, Hg, Pb, and Zn [41]. In view of the large scientific knowledge acquired on the subject, the objective of this section is not to showcase the range of concentrations encountered in the different environmental compartments in the various countries but rather to highlight the most relevant elements based on the pollution sources, the type of reuse, the prevailing quality of wastewater, and the irrigation practices under the local conditions. The presence of toxic metallic elements in wastewater has been largely studied since the 1980s. However, long-term studies are usually missing to reflect on the risks incurred by living organisms and natural resources. In almost all the North African countries, heavy metals are the main micropollutants whose concentrations in effluents are regulated. Table 2 depicts the allowed concentrations for some of them in treated wastewater used for agricultural irrigation. Morocco and Tunisia have almost the same threshold concentrations adapted in major part by the WHO guidelines

Table 2 Quality standards for pH, EC, and some heavy metals applied to treated wastewater for reuse in agriculture in the North African countries

Parameter/Country	Algeria (1)	Egypt (2)	Morocco (3)	Tunisia	WHO
pH	6.5–8.5	7–8.5	6.5–8.4	6.5–8.5	6.5–8
EC (mS/cm)	3	500 mg/L as TDS[a]	12	5	0.7–3[b]
Al (mg/L)	20	–	5	2–5[c]	5
As (mg/L)	–	–	0.1	0.1	0.1
Be (mg/L)	0.5	–	0.1	0.1	0.1
B (mg/L)	2	–	3	2	–
Cd (mg/L)	0.05	0.01[a], 0.01	0.01	0.01	0.1
Co (mg/L)	5	–	0.05	0.05	0.05
Cr (mg/L)	1	–	0.1	0.1	0.1
Cu (mg/L)	5	1[a], 0.2	0.2 (1)	0.2	0.2
F (mg/L)	15	0.5[a]	1	1–1.5[c]	1
Hg (mg/L)	–	0.001[a]	0.001	–	–
Mn (mg/L)	10	–	0.2	0.2	0.2
Mo (mg/L)	0.05	–	0.01	0.01	0.01
Ni (mg/L)	2	0.2	0.2	0.2	0.2
Pb (mg/L)	–	5	5	5	5
Se (mg/L)	0.02	–	0.02	0.05	0.02
Zn (mg/L)	10	1[a]	2	2	2

(1): [42]
(2): [43]
(3): [44] for Morocco, figure between brackets is the new threshold value for the revised version
[a]Standards for mixing drainage water with canal water for reuse [11]
[b]Depends on sodium adsorption ratio
[c]For category III (recharge of aquifer in which water is used for irrigation)

[23]. As mentioned earlier, the use of raw wastewater in Morocco is among the most threatening practices not only in terms of type of pollutant [45] but also in terms of irrigated crops. In the 1990s, it was found that the concentrations of Cu, Zn, Pb, and Cd in the plots and irrigated crops (broad bean, carrot pea, lettuce, common wheat, and oats) with raw wastewater for more than 30 years are significantly higher than those observed in the control [46]. It was previously noticed in the area of Marrakech that the population has the highest prevalence of Cd and Pb, exceeding the recommended threshold values by the WHO [47]. Indeed, plants were further tested for their capacity to accumulate heavy metals as a technique of phytoremediation [48] of soils.

In Egypt, the code of practice for the reuse of wastewater for agricultural purposes and for mixing drainage water with canal water shows that values for Pb and Cd are similar to those recommended by the WHO. The presence of heavy metals in wastewater, soil, and irrigated products was extensively studied. Long-term irrigation in El Gabal El Asfar showed that Cd accumulated (0.8–3 mg/kg) with Pb, while Ni did not reach hazardous levels in the soil top layer [49]. In Katta-ElKheel, the concentrations of Mn, Cr, and Co in soil exceeded the limit values recommended by FAO (1976). Irrigation with wastewater has also caused the accumulation of Co, Cr, Pb, Zn, and Mn in soil and alfalfa plants [50]. Health risk assessment of heavy metals in products irrigated from Bahr el-Baqar drain showed that Al, B, Co, Cr, Cu, Mn, Mo, Ni, Sr, and Zn exceeded the limits allowed by WHO/FAO standards. Hence, consumption of vegetables was of high risk for the human health for Cu, Mn, Mo, and Ni [51].

In Tunisia, the impact of irrigation with treated wastewater on the soils was studied since the 1980s [52]. For the first time in 2006, the short- and long-term impact of wastewater use in agriculture on one of the most important areas in Tunisia (El Hajeb, Sfax) that received wastewater for more than 20 years showed no significant accumulation of heavy metal in soil despite the concentrations of Cr in the irrigation water were exceeding the limit (0.11–0.17 mg/L) [53]. Likely, furrow irrigation and applied cropping system contributed to the leaching of element. Ben Fredj [54] also concluded that the accumulation is less likely to occur in the top layer of soil irrigated for 20 years than in soil irrigated for 12 years. The concentrations of Co, Cr, Ni, Pb, and Zn were detected at 20 and 40 cm depth inducing toxicity to cells in in vitro bioassays. The absence of heavy metals would be the result of leaching to groundwater, especially in sandy soils.

In Algeria, the concentrations of Al, Mn, Pb, Hg, and Zn allowed in effluents used for irrigation are very high compared to those recommended by the WHO. Very high concentrations of Cd, reaching more than 12 mg/kg at the 30 cm horizon of soil, were found in a soil irrigated with wastewater while the limit is set at 2 mg/kg. Concentrations in plants' roots and shoots (maize) were excessive with 100-fold the allowed concentration [55]. In another study, Cu was identified as the main source of contamination of soils in agricultural environment [56]. Cu, Cd, and Zn are known to show high concentrations in agricultural soils due to the use of fertilizers and pesticides.

5.2 Pesticides

In North African countries, pesticides are among the most occurring pollutants responsible for water resource degradation in agricultural areas. However, in the context of wastewater reuse for irrigation, their contribution is not assessed if we exclude run-off as a source. In Egypt, reuse of drainage water contaminated with pesticides is a real threat for health since it is reused for the irrigation of market garden crops. Numerous studies were carried out on pesticides in the 1980s. Chlorinated insecticides were found in municipal water in Alexandria City, but the concentrations were not threatening [57]. Leptophos, a stable organophosphorus pesticide, was detected in water samples from Nile River water and drainage water [58]. Surface water and groundwater were contaminated by 18 organochlorine pesticides in El Rahawy area. α-HCH, γ-HCH, heptachlor, heptachlor epoxide, endosulfan I, endosulfan II, p,p′-DDE, p,p′-DDD, and endrin were present in surface water with a total concentration of 0.34–2.16 μg/L. In groundwater, almost all compounds were below the detection limits (0.01 ng/L). A seasonal trend was observed for endosulfan I with 0.021–0.375 μg/L and 0.08–0.82 μg/L in dry and wet seasons, respectively [59]. Chlorinated pesticides were also detected in the Nile River tributaries and canals after a long period of their ban [60, 61]. Organophosphorus compounds were also investigated in drainage water from canals. Chlorpyrifos-methyl and prothiphos were detected at 30.0 and 41.5 ng/L, respectively [62]. Several studies have evidenced organochlorine and organophosphorus pesticides in drainage water either from irrigation or drainage canals. Chlorpyriphos, dimethoate, parathion, endosulfan, carbosulfan, carbaryl, and aldicab were ranging between 3.4 and 290 μg/L [63]. In the new Damietta drainage canal, chlorpyriphos and malathion were found at concentrations exceeding 300 μg/L [64]. Chlorinated compounds DDT, γ-HCH, and HCB, in addition to PAHs and organotin compounds, are of the highest concern in water resources [65]. In spite of the several cases of contamination observed for waters, the contamination of crops through the use of wastewater for irrigation is not well studied yet.

In Tunisia, the list of domestic and agricultural pesticides existing in the market includes some substances suspected for their endocrine disrupting potency like cypermethrin, permethrin, glyphosate, malathion, mancozeb, maneb, methomyl, metribuzin, trifluralin, and ziram. Pesticide residue occurrence in water bodies and sewerage network is regulated by the National Standards NT 106.002 (1989) under the term "pesticides and similar substances." It includes insecticides (organophosphorus and carbamates), herbicides, and fungicides. Their total concentration is limited to 0.001 mg/L in water bodies, 0.005 mg/L in marine environment, and 0.01 mg/L in public sewerage system. This term is vague and needs profound revision and precision of the type of molecules.

In Tunisia, studies about pesticide detection in water resources date back to the 1980s, but they are related to wastewater discharge rather than reuse [66]. Organophosphorus pesticides in surface and tap waters were also studied [67]. Water and sediment contamination by organochlorine, organophosphorus, carbamates,

phytohormones, and synthetic pyrethroids was evidenced for the first time in 1994 in the protected area of Ichkeul Lake, north of Tunisia [68]. In sediments from the Bizerte lagoon, p,p′-DDT was detected showing recent inputs in the environment from run-off; HCB and DDT concentrations were moderate, while high ratios of ΣPCBs/ΣDDTs indicated predominant industrial origin [69] and contamination through effluent discharge. Direct exposure to pesticides through ingestion, inhalation, or skin contact is the main direct route of contamination of humans rather than through reuse of wastewater. However, mobility, behavior, fate of pesticides in soil, and transfer to groundwater should be considered during reuse because the organic load of wastewater can interfere.

5.3 Estrogenic and Endocrine Disrupting Compounds

In North Africa, Tunisia was the most advanced in addressing the emerging topic of estrogenic compounds in water resources. Estrogenicity of wastewater was evidenced in 2004 [70]. Later, estrogenic compounds in natural and sewage waters were studied [71]. Currently, estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethinylestradiol (EE2) and their metabolites are investigated at a larger scale in Tunisian effluents [72]. E3 was detected at 300 ng/L in influent and 36 ng/L in effluent with a removal rate of 85% by biological treatment, while E2, E1, and EE2 were removed at a lower rate (less than 75%) [73]. Different types of treatment processes were assessed for their efficiency in removing estrogens from domestic effluents. In two sewage treatment plants, one touristic and one domestic, the anoxic/aerobic activated sludge process with a high hydraulic retention time (40 h) resulted in more than 97 and 77% of removal of E1 and EE2, respectively. This study investigated the removal, the diurnal trends, and the daily loads of estrogens [74]. However, research was based on assumptions and estimations. Data on the consumption and discharge of contraceptive pills, for instance, was not provided for the estimation of the EE2 load as the latter has a lower rate of degradation. Qualitative studies on estrogens were made possible, thanks to the introduction of new tools like in vitro bioassay systems and the use of biomarkers [75]. Indeed, in Tunisia E2 was detected in streams [71, 75]. In Egypt, the first study on E2 in Bahr el-Baqar (receiving raw wastewater) and surrounding ecosystem showed significantly high concentrations (1,029 μg/L) [76] indicating the relevance of the estrogenic compounds if they are not degraded. The estrogenic potency of nonylphenols was first evidenced through their induction of morphological disorders in toads in the Nile River [77].

Dyes released by textile industries may be also estrogenic. Some of the blue dyes are classified as mutagenic, associated with bladder cancer development. In Tunisia, the low removal rate of dyes from industrial textile facilities is widely recognized. Their endocrine disrupting effects were studied recently. A weak estrogenic but significant antiestrogenic effect was measured for 23 dye types after the release of blue jeans textile effluent [78]. In Morocco, where leather and textile industries

are very well developed, the topic is still emerging and no studies were carried out for our knowledge.

At the Arab level, not only North African, the environmental regulations limiting the discharge of harmful compounds are not sufficiently enforced. Raw and treated wastewater represents a route of contamination of soil and groundwater by estrogenic compounds [79]. The relevance of estrogens and estrogenic compounds to the agro-environmental environment is still not well recognized, and the occurrence of estrogenic compounds in soils and groundwater is not well studied yet. Estrogen-like compounds in treated wastewater used for irrigation, groundwater, and soils were studied in Tunisia in 2005 for the first time [80]. In 2010, evidences were given of the implication of heavy metals in the estrogenic activity of domestic and industrial influents/effluents, and treated wastewater used for irrigation. Estrogenic activity in groundwater used for irrigation and contaminated by effluents was also studied [80]. Estrogenic activity may disappear in soil irrigated with treated effluents, in spite of the high activity observed in the irrigation water [54]. Other chemicals with endocrine disrupting activity, in addition to dioxin-like compounds, were also investigated in wastewater, irrigated soils, and groundwater in an area that have received wastewater for more than 30 years [81].

In view of these preliminary results, the estrogenic chemicals could be challenging for aquatic organisms. When wastewater is used for irrigation, more studies are needed because it is merely unclear whether they are harmful to soil quality. Transfer to groundwater would be more problematic if its usage extends to potable purposes.

5.4 Pharmaceuticals and Personal Care Products

Studies carried out on the occurrence of pharmaceuticals and personal care products (PPCPs) in water resources date back to the 1970s [82] driven by their detection in and aquatic environment in developed countries [83]. The interest shown to transformation products excreted or produced after structural change in the treatment plants is more recent because of the tedious analytical procedures required for metabolites. These metabolites may have different properties than the parent compounds; thereby, different fates and behaviors are expected. For their removal and degradation, many advanced technologies were tested in case of reuse [84–86]. Till now, accumulation of parent compounds and metabolites in soils and plants requires more studies even in developed countries.

Tunisia was the first North African country where pharmaceuticals have been studied and detected in wastewater and irrigated soils. Carbamazepine, a persistent antiepileptic drug, and four of its major metabolites were identified for the first time in wastewater and groundwater in the area of Nabeul where reuse is practiced since the 1980s. Concentrations from 0.28 to 0.94 ng/g dw were observed at the top soil [87]. The load of carbamazepine in influents was also predicted based on sales in various countries. Egypt and Morocco had almost twice the concentration predicted

for Tunisia with 650, 667, and 1,187 ng/L, respectively, while Europe was a hot spot with concentrations above 1,000 and 2,000 ng/L [88]. In Korba (Tunisia) where wastewater was used for 4 years for an aquifer recharge, a combination of carbamazepine and isotope tracers was used to assess the impact of this practice on the coastal aquifer contamination [89]. Since carbamazepine was found to migrate in plants to reach leaves and fruits [90, 91], it is highly relevant to assess health risks related to indirect reuse in this context.

Studies on natural and advanced treatment technologies are currently carried out at laboratory scale. In Tunisia, the use of olive cake, as an agricultural by-product, was tested after transformation into active carbon to remove ketoprofen, ibuprofen, naproxen, and diclofenac [85]. Similar experiments were implemented based on the properties of the chemicals, like the ability to degrade or produce persistent metabolites. However, very few of them refer to the actual concentrations in wastewater produced in the treatment plants by taking into account consumption, degradation, and kinetics in the local conditions. In Egypt, clofibric acid, the active metabolite of clofibrate, was detected in El Gabal El Asfar area at 40–75 ng/L in groundwater as a result of raw wastewater use for more than 80 years [92]. Few are research works dealing with the removal of some compounds from wastewater [93–95], and results on the occurrence of pharmaceuticals in irrigated areas are still very scarce. In Algeria, research studies are still carried out at laboratory experimental scale. A case study on effluents from the treatment plant of Boumerdes has investigated the impact of doxycycline (antibiotic), ketoconazole (antifungal), and loratadine (histamine antagonist) on the degradation of the organic load as BOD and COD. An effect on organic matter was observed and sludge process was affected [96].

During the last decade, antibiotics were pinpointed as the chemicals of main environmental concern and health risks [97] because they may result in the development of bacterial and gene resistance.

6 Conclusion

Wastewater reuse in agriculture has considerably progressed in North African countries. The assessment of risks related to the current farmers' practices combined with the quality delivered/available for irrigation shows that reuse objectives are not fully achieved in terms of environment preservation and human health protection. The relevance of micropollutants and their occurrence in wastewater, water resources, and soils does need more attention because the issue remains exclusively raised by scientific research communities; the topic is neither well understood/perceived by farmers and stakeholders nor does represent a priority of decision-/policy-makers for setting appropriate policies and regulations.

The state of knowledge on the occurrence of emerging micropollutants in wastewater used for irrigation and in the agroecological environment in the special context of the North African region varies among the countries. Countries lagging

behind like Libya might be the most exposed to a certain type of contaminants, since research results and data are not available. Egypt, as the largest platform of unplanned and informal reuse for irrigation, still needs to tackle the impacts of this activity on the aquatic environment and public health. In Morocco, the most challenging and serious risk is related to heavy metals. The relevance of organic molecules is worth thorough studies under local conditions. Serious measures have to be taken to limit mixing industrial and domestic effluents and enforce the ban of raw wastewater use. In Tunisia, a pace was made forward with significant advancement in research. The critical environmental status observed these recent years has to be closely examined to monitor some micropollutants issued from illegal industrial discharges. In Algeria, the disposal of treated and untreated effluents directly in the receiving environment is of high concern when the reuse is not regularly practiced. Observations on the contamination of crops and soils have become alarming, pushing toward more studies for setting and enforcing regulations.

In view of the current situation, it is the role of the scientific communities gathered into consortia and multidisciplinary teams to take up the challenge for identifying and to monitoring some relevant micropollutants from quantitative (concentration) and qualitative (type of pollutant) point of view taking into account the different types of reuse in agriculture in the region. Research results should be brought to the large audience to raise awareness among wastewater end users and decision-makers. This would help taking the appropriate actions upstream the treatment plant to reduce the load of pollutants before they reach the food chain.

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Water Reuse Within the Paper Industry

Angeles Blanco, Daphne Hermosilla, and Carlos Negro

Abstract Pulp and paper industry is still an intensive water consumer, although fresh water use by this sector has decreased by 90% along the last three decades, which currently shows its long water reuse tradition. Sustainable water management has been achieved by following the principle of water fit for use, which has mainly been developed through the optimization of water circuits, the cascade use of water, the implementation of internal water treatments, the optimal treatment of effluents to be reused and the use of alternative water sources, such as reclaimed water from municipal wastewater treatment plants. In fact, this sector is nowadays regarded as a reference for water reuse. Paper mills need to use fresh water to compensate evaporation losses and in critical applications. In addition, the final degree of circuit closure depends on the quality of the final product. For example, whereas unbleached paper grade mills may work with highly closed circuits, this is not usually possible for virgin pulp and bleached paper grade mills. Filtration and dissolved air flotation are the most common treatments applied to internal water reuse. Otherwise, the combination of physicochemical, biological and filtration technologies is generally considered to enable the reuse of mill effluents. Finally, tertiary effluent from municipal wastewater treatment plants must be further treated by filtration technologies and disinfection stages to be finally reused within the papermaking process safely.

Keywords Effluents, Integrated water management, Paper, Reclamation, Water reuse

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 213–238, DOI 10.1007/698_2015_360,

Contents

Abbreviations

AOX	Adsorbable organic halogens
BAT	Best available technologies
BOD	Biochemical oxygen demand
CIP	Cleaning-in-place
COD	Chemical oxygen demand
DAF	Dissolved air flotation
EFC	Elemental chlorine-free
EGSB	Expanded granular sludge blanket
IC	Internal circulation
MBRs	Membrane bioreactors
MF	Microfiltration
MWWTPs	Municipal wastewater treatment plants
NF	Nanofiltration
RO	Reverse osmosis
TCF	Totally chlorine-free
TMP	Transmembrane pressure
TOC	Total organic carbon
UASB	Upflow anaerobic sludge blanket
UF	Ultrafiltration

1 Introduction

Pulp and paper industry provides an essential commodity with more than 500 different uses. Paper is primarily a sheet of cellulose fibres combined with a certain amount of fillers and additives that are designed to provide the final product with the quality that is demanded by its designed use. Pulp for papermaking is mainly produced from wood or recycled paper, and it may be bleached (for white grades) or not (brown grades). There are many different paper grades, which may be manufactured either in integrated mills (pulp + paper) or nonintegrated mills. Similar products can be made from different fibre mixes and processes, but these combination alternatives generate different emissions [1].

The paper sector was considered one of the heaviest water-consuming and polluting sectors 30 years ago, whereas it enjoys great recognition for its good water-use practices nowadays. In fact, it is considered as a reference model for water reuse applications in industry. Although pulp and paper industry is still an intensive water user, only a 5–8% of the water that is used in the process is actually consumed, whereas the remaining 92–95% returns back to water streams or networks after its proper treatment [2, 3]. Organic matter, solids content and pH are the main pollution parameters to consider in the effluents from pulp and paper mills. In addition, adsorbable organic halogens (AOX), colour and metals (mainly zinc, iron and manganese) content may be also of concern when virgin fibre is produced (directly from wood) and then processed [4]. In general, the presence of toxic substances (mainly AOX and chlorinated dioxins) has been reduced by 95% down to a level of $\leq$0.1 kg AOX $\cdot$ t^{-1} of pulp since 1990, mainly thanks to the substitution of chlorine gas by chlorine dioxide in elemental chlorine-free (ECF) pulps or by oxygen, ozone and hydrogen peroxide in totally chlorine-free (TCF) pulps [2, 5]. The amount of chemical oxygen demand (COD) discharged in the pulping process is basically inversely proportional to the pulp yield, resulting that high-yield pulping, such as mechanical pulping, produces less COD than low-yield chemical pulping. In turn, there is no significant trend regarding the COD load of the wastewater that is generated by the paper manufacturing process of any of the possible different types of paper products because the yield is similar; but there is however a direct relationship between product quality specifications, internal process-water reuse and the COD loads in these effluents. Therefore, board or packaging paper grades may be produced with closed or nearly closed water circuits, which implies low water consumption (just the amount devoted to compensate water evaporation during the process, which is $\approx$1.5 m^3 t^{-1} of paper), whereas the production of white paper grades generally requires more open systems. In addition, paper mills processing recycled paper show higher concentrations of dissolved organic substances in their process water.

Over recent decades, the trend to minimize water consumption per production unit has been driven by three main factors: environmental legislation in force, the cost of energy and certain water-use issues:

- Before 1990, the pulp and paper industry mainly focused efforts on water conservation by reducing water demand in their different units and closing water circuits without affecting the process. The developed programmes for water reduction firstly aimed to optimize the most significant volumetric discharges; these were due to paper manufacturing (35%) and bleaching (33%) processes in integrated mills [4]. The initial concept of a zero effluent mill was next conceived, and the separation of water loops began to be adopted in the mills. In addition, since retention is a preponderant aspect of controlling the level of materials build-up under conditions of low water use, new retention control strategies for closed systems were implemented.

- The development of non-polluting papermaking processes represented the main challenge for the sector during the 1990s. Therefore, the accumulation of contaminants in closed water circuits was thoroughly monitored, their sources identified and the processes wisely modified to become more environmentally friendly. As a result, many mills partially reopened their water circuits due to severe operational and quality problems [6, 7]. The new set of objectives included (1) improving the prediction of the level of contaminant build-up as a function of process effluent discharge, (2) identifying optimum water consumption for different types of paper products approaching the zero liquid effluent production as far as possible without reaching that point at which operational and product quality problems may occur, (3) implementing internal treatments to clean up process water and achieve further closure of the water circuits and (4) integrating energy consumption issues. On the other hand, external treatments were also applied to reduce the contaminant load of the final discharge, thus minimizing the overall environmental impact of the mills.
- In the 2000s, papermakers implemented best available technologies (BAT) to their processes and developed the concept of "more from less" by considering an integrated approach aiming to optimize the combination of internal and external technologies to both improve the efficiency of resources use (fibres, fillers, chemicals, water and energy) and minimize environmentally related capital and operating costs, especially considering the increase of the cost in energy. In this sense, the concept of "water fit for use" was further developed adapting water quality use to the specifications of each of the different processes that are run in the mills aiming to minimize the cost of treatment, as well as considering the use of new alternative water sources [8]. In addition, emerging technologies were validated at industrial sites serving as actual demonstration cases.

In any case, these efforts have not resulted enough to solve the problem because water scarcity has re-emerged as one of the most serious natural resource concerns the world is currently facing. Water is no longer just a consumable or a utility, but a highly valuable asset. As a consequence, water use has also become an important strategic issue for the pulp and paper industry potentially affecting the ability of a large number of mills located worldwide to remain in operation. Achieving an integrated responsible and sustainable water use therefore represents a new challenging aim for this industrial sector. In this context, a better understanding of the environmental impacts of water use, considering water availability and quality at both local and regional levels, is a key factor for a sustainable paper industry. Accordingly, best practices and tools for assessing water sustainable use, water footprinting and its disclosure, are being used in the paper sector to identify and manage business risks related to water use.

Further reduction of water demand may only be achieved by radical innovations of the pulp and paper processes – developing lower water demanding ones or improving effluent treatment to open new opportunities for internal and external water reuse. As the access to water resources is an issue of global concern, it is critical to recognize local site-specific resources, including alternative water sources, in regions suffering water scarcity. In particular, reclaimed water from municipal wastewater treatment plants (MWWTPs) may be used to substitute fresh water intake [9]. In this case, innovative integrated water management practices must be adopted to save high-quality water use and avoid mill shutdowns during severe drought episodes.

2 Water Uses in the Pulp and Paper Industry

Among its many functions and roles within the pulp and paper mill processes, water is also one of the key components of pulp and paper manufacturing itself, as well as it is used to transport raw materials and additives through the different stages of the process (woodyard, cooking, pulping, bleaching, deinking, washing, refining, cleaning and paper forming). In addition, it is also used for dilution, to prepare chemical additives and filler suspensions, in showers for cleaning forming and press fabrics; to perform process cooling; to clean equipment, as a sealant in vacuum systems; to generate steam; etc. [1, 3]. If fresh water is used for all these purposes without considering recovery and reuse possibilities, a huge volume of water would be needed ($>200\ m^3\ t^{-1}$). However, the closure of water circuits has significantly reduced water consumption in the last decades. It can be estimated that an overall 90% water reduction has globally been achieved during the last three decades; but these water savings depend on process-related factors, such as the type of process, the particular paper or pulp grade being produced, the used raw materials, the age of the mill and its optimization level. In addition, general local conditions such as wastewater discharge requirements, water scarcity and environmental awareness tradition in each region [3] are also other contributing factors. Particularly, it has been reported that effluent volumes have decreased by 78% (from 46 to $10\ m^3\ t^{-1}$ of paper) in Germany's pulp and paper industry between 1974 and 2007 [1]. Moreover, water consumption decreased by 45% in Europe between 1990 and 2012 [2] and about 70% in North America between 1960 and 2010.

Actual fresh water consumption figures in European mills span from 9 to $90\ m^3\ t^{-1}$ of pulp (average of $30\ m^3\ t^{-1}$) and from <1.5 to $65\ m^3\ t^{-1}$ of paper (average of $10\ m^3\ t^{-1}$) [1, 5] (Fig. 1). The highest water consumption values are generally related to the production of bleached kraft pulp. Water use is also high for mills producing specialty papers, mainly because of the high-quality requirements

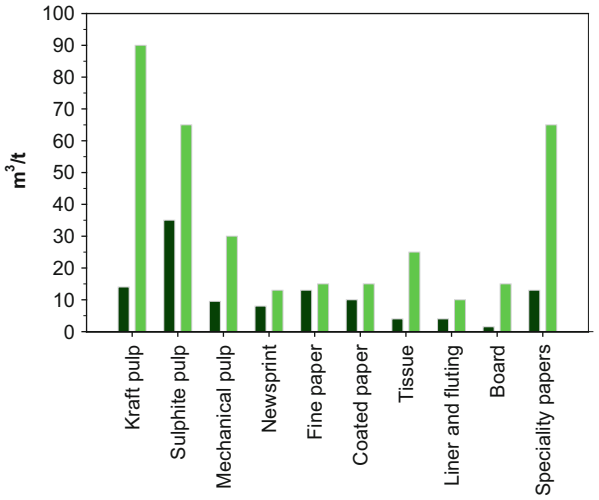

Fig. 1 Maximum and minimum fresh water consumption values in pulp and paper mills [1]

of the products and the high number of grade changes, which in many cases require cleaning the machine. The lowest figures are currently provided by mills producing packaging paper. Some of these mills have almost closed water circuits, resulting in water consumptions of 1.0–1.8 $m^3\ t^{-1}$ of paper product and zero effluent production [10]. Fresh water must generally be used in processes demanding high water quality [11]: mainly showers, chemical preparation, sealing circuits, steam production, cooling and pulp washing. In addition, minor quantities of fresh water may also be used in other applications such as cutting the web. In addition, water from the above-mentioned processes is also further reused in cascade in other pulp and papermaking processes.

Fresh water must meet several different quality standards depending on its use and the paper grade to be manufactured (Table 1) [15]. In addition, these requirements are further tightened in order to satisfy the guaranties that are agreed with equipment suppliers. In this respect, hardness and alkalinity are among the most critical parameters to consider because they may produce scaling in machinery and water circuits, as well as they may promote the formation of aggregates and deposits with organic colloids present in pulp suspensions. Furthermore, silica may also produce scaling and irreversible fouling in membranes; and some metals (Fe, Al or Mn), chloride and sulphate are highly corrosive and their presence may likewise cause scaling and odour. In addition, the presence of colloidal material may produce deposits and product quality losses. Finally, the presence of microorganisms may produce biofilms and odour troubles [15, 18, 19].

Table 1 Limit values that water must fulfil for its use in different pulp and paper mill processes

Parameter	Cooling [12]	Boiler[a] [13]	Sealing [14]	Pulp and paper grades [15–17]			
				Mechanical pulping	Paper (high pressure showers)	Pulp and paper bleached	Chemical pulp unbleached
pH	6.9–9.0	8.5–9.5	>7.0	6–10	6.5–7.5	6–10	6–10
TSS (mg L^{-1})	100	–	–	40	5	10	10
TDS (mg L^{-1})	500	–	1000	250–1,000	300	300	300
Conductivity (mS cm^{-1})	–	–	2.0	–	0.5	–	–
Cl^- (mg L^{-1})		–		1,000		200	200
Turbidity (NTU)	50	–	–	70	–	40	40
Color (PCU)	–	–	–	30	30	10	30
COD (mg L^{-1})	75	–	–	–	5	–	–
BOD_5 (mg L^{-1})	25	–	–	–	–	–	–
TOC (mg L^{-1})	–	–	–	–	–	–	–
Hardness (mg$CaCO_3$ L^{-1})	650	0.3–0.0	200	100–200	200	100	100
Alkalinity (mg$CaCO_3$ L^{-1})	350	–	–	75–150	100	75	75
Ammonia-N (mg L^{-1})	1.0	–	–	–	0.5	–	–
PO_4^{3-} (mg L^{-1})	4.0	–	–	–	–	–	–
HCO_3^- (mg L^{-1})	24	–	–	–	–	–	–
NO_3^- (mg L^{-1})	–	–	–	–	–	–	–
Si (mgSiO_2 L^{-1})	50	–	–	50	5	50	50

(continued)

Table 1 (continued)

Parameter	Cooling [12]	Boiler[a] [13]	Sealing [14]	Pulp and paper grades [15–17]			
				Mechanical pulping	Paper (high pressure showers)	Pulp and paper bleached	Chemical pulp unbleached
Cu (mg L^{-1})	–	0.05–0.01	–	–	–	–	–
Fe (mg L^{-1})	–	0.10–0.01	–	0.3	0.1	0.1	1
Mn (mg L^{-1})	–	–	–	0.1	0.05	0.05	0.5

[a]These values depend on drum pressure. The most restrictive ones are for 15–20 10^7 Pa

3 Internal Water Reuse

The primary water circuit of paper mills consists of a short loop enabling a direct reuse of white water after draining from the wire section (Fig. 2). This holds the largest volumetric flow, and its main purpose is diluting the stock in the approach system to about 1%. Performance conditions on the wire section, such as retention, dewatering and additive performance, are decisive providing water characteristics. The excess of water from the wire and press sections is clarified by filtration (e.g. disc filters) or by dissolved air flotation (DAF); and clarified water may be reused in the process (e.g. consistency control and machine showers). Internal treatments (e.g. ultrafiltration, biological treatment, evaporation and ozonation) may be used to further close the water circuit producing the high water quality that is demanded for certain applications, such as in the showers. Finally, the excess of water is recirculated to the secondary circuit to be used in the pulping process; and the excess of water from the stock preparation is sent to the wastewater treatment plant, which is generally based on a primary and secondary treatment combination. The tertiary circuit includes the recirculation of the treated effluent back to the process. In this case, the effluent can actually be recirculated from the primary, secondary or tertiary treatments. In some cases, a double-membrane tertiary treatment plus disinfection is included for applications requiring very high water quality.

Facing the fact that process-water reuse is limited by the accumulation of dissolved matter from raw materials (wood or recycled paper and fillers), and chemicals entering the process, is nowadays among the key challenges of pulp and paper mill management [20]. Table 2 shows the main advantages and disadvantages of closing water circuits in the paper industry. Some of the problems that are associated with the accumulation of contaminants are deposition and scaling, foaming, corrosion, low efficiency of chemicals, etc., which may produce operational problems and degradation of the quality of the final product. For example, Fig. 3 represents the predicted accumulation of contaminants when fresh water consumption is reduced in the production of recycled newsprint paper, showing that

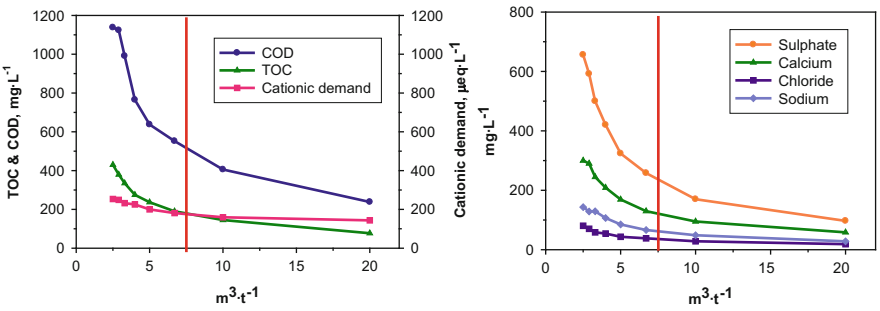

Fig. 2 Accumulation of dissolved COD, TOC, cationic demand and salts for the production of recycled newsprint paper

Table 2 Advantages and disadvantages of closing water circuits in the paper industry [21]

Advantages	Disadvantages
Increase in suspended solids	
Reduction in raw material losses	Plugging of pipes and showers
Less production of sludge	Dirt and spots in the final product
	Deposit formation
	Abrasion
	Fabric life reduction
	Increase of fines
	Modification of the drainage capacity
	Loss of tensile strength
Increase in dissolved solids	
Increased retention of dissolved material	Scaling
	Formation of deposits
	Increase of biological activity
	Corrosion
	Colour
	Bad odour in the process and product
	Reduction in brightness
	Less stability in the wet-end
Higher temperature	
Better drainage processes	Sizing problems
Energy savings	Reduction of the vacuum pump efficiency
	Increase and/or alteration of the microbiological activity

further closure of the circuits beyond 7–8 $m^3\ t^{-1}$ would produce an exponential accumulation of dissolved and colloidal contaminants in the process water. Moreover, some contaminants are less accumulated and easily removed in the product than others, such as cationic demand (amount of cationic polymer required to neutralize the anionic charge of water) versus COD or TOC or sulphates versus chlorides [20].

Wastewater from washing represents one of the main water flows in pulp mills, and it would also be a highly loaded effluent to manage if it cannot be integrated in the chemical recovery system of the plant, which is composed of multiple evaporators and black liquor concentrators. In addition, the implementation of more efficient washing equipment and the use of the condensate from evaporation are effective procedures to reduce water consumption. Moreover, press washing at the ultimate stage would be able to reduce the amount of water from 6–10 to 2–3 $m^3\ t^{-1}$, thereby increasing the amount of chemicals and contaminants that are burnt in the recovery boiler, that is, further reducing the contamination load of final effluents. Furthermore, dry debarking, recirculation of alkaline or ozone bleaching

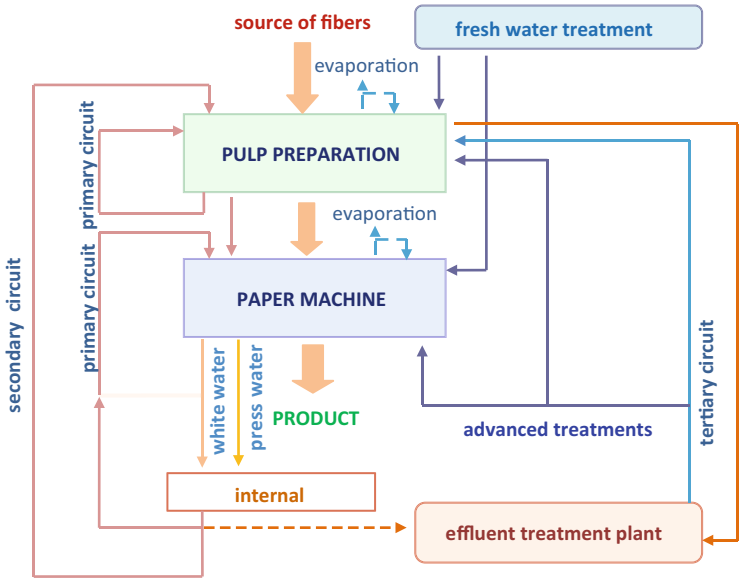

Fig. 3 Simplified outline of water circuits in a pulp/paper mill

filtrates and ECF bleaching are other alternatives to additionally reduce water consumption [1]. A strict separation of water loops is always recommended to maintain the paper machine as clean as possible. In particular, the pulp is thickened up to 30% before leaving the stock preparation stage. In this way, detrimental substances are retained in the stock preparation water loop.

As it has already been mentioned, the quality of reused water is critical when it is intended to be used back in paper mills. The values of key water quality parameters are included in Table 3 [11]. COD, cationic demand, conductivity and pH have a high influence on critical processes such as the wet-end or the operation of internal treatments. Nutrients are of crucial importance because they will determine bacterial growth. In addition, other parameters may also be relevant in certain specific cases, such as pathogens content if reclaimed or post-biological treated water is reused in the mill.

The types of contaminants that are present in wastewater are basically determined by the type of raw materials and chemicals that are used in the processes. For example, natural plant constituents (such as hemicelluloses, pectin, lipophilic extractives like resin acids, lignin, lignin-related substances, terpenes, catechol, hydroxybenzaldehyde, carbohydrates and carboxylic acids in small quantities, such as acetic and formic) are the main expected contaminants in mills using virgin fibres [22–24], whereas starch-related contaminants are more present in wastewater from recycled paper mills [25], resulting in a much more biodegradable pollutant matrix [26].

Table 3 Limit values of process water quality for different paper grades [11]

Parameter	Unit	Process water PM loop	Graphic paper			Packaging paper	Sanitary paper (virgin fibre)	Sanitary paper (recovered fibre)
			1st loop DIP	2nd loop DIP	Mechanical pulp	Process water PM loop	Process water PM loop	Process water PM loop
pH	–	7.0–8.5	7.5–8.5	7.5–8.5	6.0–7.0	(5.9) 6.3–7.3 (7.5)	7.1–8.1	7.1–8.3
Temperature	°C	40–60	40–60	40–60	60–80	35–48 (48)	36–44	30–46
Conductivity, 25°C	mg L^{-1}	1,000	3,500	2,000	1,500–2,000	2,000–6,000 (13,000)	500–1,700 (3,500)	1,000–2,500 (4,000)
COD	mg L^{-1}	1,000	3,500	2,000	2,000–3,000 (10,500)	1,000–8,000 (25,000)	150–450 (1,000)	500–1,500 (2,500)
Cationic demand	μeq L^{-1}	25	200	125	–	–	–	–
Cl^-	mg L^{-1}	100	n.a.	n.a.	–	100–700 (1,200)	50–350 (700)	60–250 (700)
SO_4^{2-}	mg L^{-1}	100	300	200	–	200–900 (1,600)	80–200 (900)	150–350 (600)
Hardness	mg L^{-1} ($CaCO_3$)	50	200	150	–	–	–-	–
Ca^{2+}	mg L^{-1}	–	–	–	–	200–1,200 (3,800)	50–400 (1,800)	80–250 (330)
N-NH_4^+	mg L^{-1}	1	1	1	–	–	–	–
N-NO_3^-	mg L^{-1}	1	1	1	–	–	–	–
N-NO_2^-	mg L^{-1}	1	1	1	–	–	–	–
Total P	mg L^{-1}	1	1	1	–	–	–	–
Fungi	UFC·ml^{-1}	2	2	2	–	–	–	–
Algae	UFC·ml^{-1}	2	2	2	–	–	–	–
Microorganisms	per ml	10	10	10	–	–	–	–
LSI	–	–	–	–	–	<0.5		<0.5

Furthermore, wastewater characteristics are also different depending on pulp yield and the type of bleaching process. For example, different toxic chlorinated organic compounds (e.g. chlorinated catechols, dehydroabietic acid, guaiacols and syringols), dioxins and furans can be found in low proportions in ECF pulp mill wastewater [24, 27, 28], whereas an additional load of 5–15 kg t^{-1} of BOD_7 and 15–40 kg t^{-1} of COD is expected in effluents from TCF processes due to the use of hydrogen peroxide and at the same time that its content of hemicelluloses is lower and the presence of pectin and aliphatic carboxylic acids is higher. Additionally, the papermaking operation also introduces several chemical compounds to the wastewater stream, such as different additives, fillers (e.g. $CaCO_3$, kaolin, clay, talc), whiteners (e.g. diaminostilbene sulphonate derivatives), dyes (e.g. direct dyes having a highly conjugated, planar structure and an anionic charge due to the presence of sulphonate groups; they may be modified with amine groups to be cationic), defoamers (e.g. esters or amides of fatty acids and polyethylene glycols), dispersion/antiscaling agents (polyphosphates, hydroxyl ethyl diphosphate, poly(acrylic acids) and relatively low-molecular-weight polymers containing carboxylic, phosphonic, phosphoric and other functional groups), surfactants (e.g. fatty acid soaps, polyethylene oxide alkyl ethers), biocides and slimicides (e.g. quaternary ammonium compounds, glutaraldehyde, halogenated hydantoin, 2-bromo-2-nitropropane-1,3-diol, 5-chloro-2-methyl-4-isothiazolin-3-one, 2,2-ibromo-2-cyanoacetamide) and complexing agents (e.g. ethylenedia minetetraacetic acid, diethylene triamine pentaacetic acid), among others [29]. Moreover, all these products may incorporate other compounds to the flow, such as endocrine disruptors like bisphenol A and phthalates, which may come from softeners, additives, glues and printing inks; alkylphenolic constituents of some defoamers, cleaners and emulsifiers; pentachlorophenol, which is a major component of some biocides; complexing agents; photoinitiators like acetyloxytrimethylbicycloheptanedione; and adsorbents like acetylmorpholine [24, 30].

The main general purification strategies that are implemented in modern mills aiming for reducing fresh water consumption and optimizing water circuit closure are listed in Fig. 4. A particular good strategy for systems of separated loops is the one based on calculating the K values defined by Kappen and Wilderer, which actually compares COD figures at different locations of the production process [31]. $K1$ evaluates the efficiency of fresh water use in the paper machine by calculating the ratio between COD values (in filtered samples) of the effluent and white water 1 (water from the forming wire and press section in the paper machine), whereas $K2$ is an indicator estimating the concentration ratios in the water loops of stock preparation and paper machine:

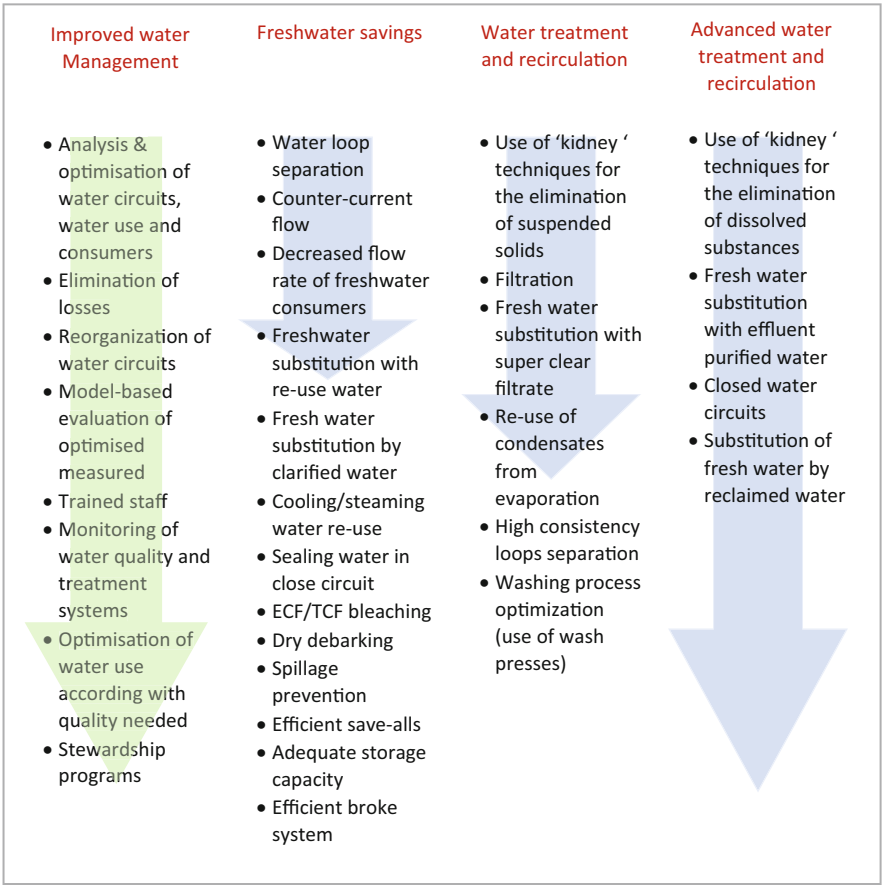

Fig. 4 General purification strategies aiming to reduce fresh water consumption in pulp and paper mills (adapted from [1]). *Green*: management. *Blue*: technical solutions

$$K1 = \frac{\mathrm{COD}_{\mathrm{Effluent}}}{\mathrm{COD}_{\mathrm{White\ water\ 1}}} \qquad K2 = \frac{\mathrm{COD}_{\mathrm{Stock\ preparation}}}{\mathrm{COD}_{\mathrm{White\ water\ 1}}}$$

If $K1$ is lower than 1, water has not been used efficiently and $K2$ must be greater than 1, which means that the contamination load is partially being accumulated in the stock relieving the paper machine. For simple systems just bearing one stock preparation system and one paper machine, the $K1/K2$ ratio further allows assessing the design of the circuits of the water loops in the stock preparation stage and the paper machine. In fact, $K1/K2 = 1$ indicates good countercurrent arrangement, that is, wastewater is mainly being discharged from the section holding the highest COD load (Fig. 5) [32]:

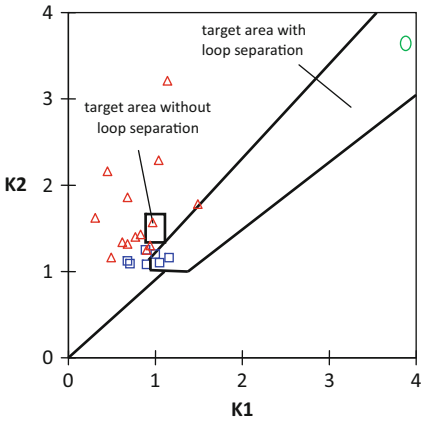

Fig. 5 Representation of $K1$ and $K2$ values in different European paper mills. Note: Δ represent values for coated paperboard, *square* for packaging board and *circle* for a highly optimized graphic paper mill (adapted from [31])

$$\frac{K1}{K2} = \frac{\mathrm{COD}_{\mathrm{Effluent}}}{\mathrm{COD}_{\mathrm{Stock\ preparation}}}$$

3.1 Internal Treatment Technologies

Figure 6 shows potential treatments that may be applied to remove COD, bacteria, suspended solids and salts from wastewater of pulp and paper mills. DAF and filtration are the mostly used technologies for internal water treatment, although other processes may be used to some extent, namely, micro-/ultrafiltration; anaerobic and/or aerobic biological treatments aiming for removing dissolved organic substances to minimize odour problems in the final product; ozone treatment to further reduce organic matter, colour and odour or achieve disinfection; or enzymatic treatments devoted to decolourize, degrade lignin compounds and reduce xenobiotic compounds [33]. Electrodialysis and ionic exchange may also be used to separate and/or recover some ions.

Particularly, DAF is a really cost-effective treatment for large water flows transporting a wide range of solids content (300–5,000 mg L^{-1}), so much so that it is possible to implement up to five DAF units (first loop, second loop, paper machine loop, sludge treatment and effluent treatment) in recycled paper mills, which may efficiently remove 80–98% of the suspended solids, as well as a wide variety of contaminants such as ink particles and lipophilic extractives. Furthermore, it is possible to efficiently remove finely dispersed and colloidal organic particles (>0.2 μm) using appropriate coagulants and flocculants. On the other hand, there is a limit to about 20% of the COD for the reduction of organics [34]. Finally, sludge from DAF units may be jointly treated in some mills with sludge flowing out the biological wastewater treatment plant.

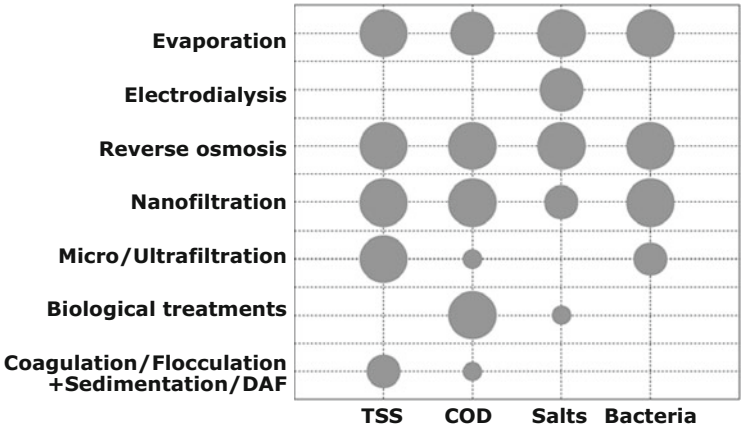

Fig. 6 Qualitative efficiency of the removal of contaminants by different treatments

Although membrane technologies may consume more energy than other processes, they are able to significantly improve water quality fulfilling all required standards and reducing the emission of contaminants of emerging concern [30]. Furthermore, they may also be easily installed close to the treatment location, such as an additional treatment for clear filtrate devoted to obtain high-quality water for wire section showers [35]. The development of new membrane filtration systems and membrane materials aiming for reducing its fouling has much extended the implementation of this technology in the paper sector, although fouling and erosion of the active layer are still the critical factors limiting its further application [36].

As mentioned above, the reuse of condensate (about 8–10 m^3 t^{-1}) from the chemical recovery system is a key issue to reduce water consumption in pulp mills. These condensates include a high amount of organic compounds (10–20 kg COD m^{-3}) that may be reused (e.g. ethanol) after treating these streams by stripping, which also subsequently produces a water free of metals content that could be reused in different applications, contributing to further close water circuits (e.g. in the bleaching plant, liquor scrubbing, in lime kilns or as white liquor make-up water) [1].

4 Reusing Mill Wastewater: Towards a Zero Liquid Effluent

Several different alternative systems may be operated in paper mills for the treatment of their final effluents depending on the types of mill and load that is present in the wastewater to be regenerated (Fig. 7). In general, an equalization of the flow is usually performed before the removal of solids (>90%) is addressed by

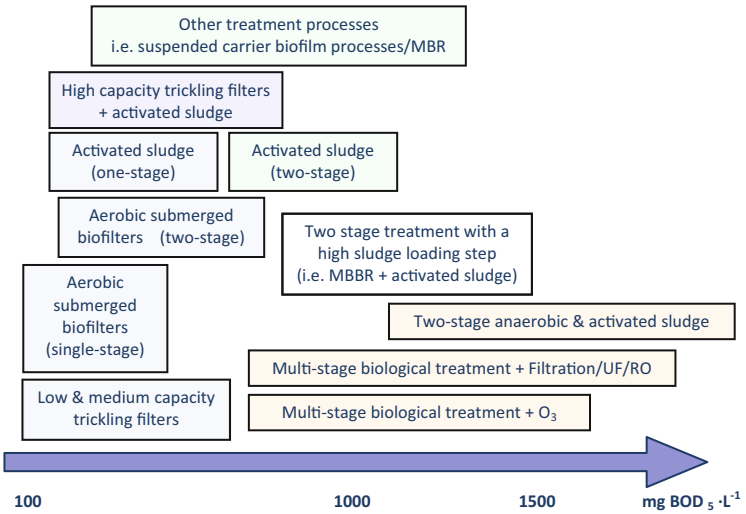

Fig. 7 Water treatment technologies applied to the treatment of effluents of the pulp and paper mills in the function of the organic load of the effluent (adapted from [1])

physical or physicochemical procedures. If necessary, biodegradable organics may be removed by means of a biological treatment implementation. The selection of this treatment depends on the paper grade being produced [1]. Activated sludge processes are the most commonly used for this purpose, followed by trickling filters and moving beds bioreactors, all of them resulting in a BOD removal of about 80–95% at a retention time of <1 day. In addition, aerated oxidation lagoons, despite requiring a large space for its implementation, are also still being used in Northern Europe and North America, resulting in a 60–80% BOD removal after a dwell time of 3–10 days. Finally, a final clarification step is applied to separate sludge from water, which is partially returned back to the process. Sludge bulking has been one of the major problems affecting activated sludge performance under specific conditions, so flotation has therefore been preferred over sedimentation in several paper mills. Additionally, filtration or chemical precipitation has also been implemented in some cases as a tertiary treatment aiming for removing nondegradable COD, nutrients (mainly phosphorus) and suspended solids.

Nevertheless, anaerobic digestion is nowadays taking over in detriment of aerobic treatment, mainly due to its complementary capacity to produce energy and its much lower sludge production [35]. The efficiency of anaerobic treatment widely depends on the volumetric organic load, temperature and the characteristics of the wastewater to be treated (e.g. alkalinity). Particularly, organic loads in the range of 5–15 kg COD m^{-3} day^{-1} usually provide the best results; and mesophilic conditions (20–45°C) favour the stability of the process, although thermophilic ones (45–120°C) have been assessed to potentially improve treatment efficiency [33, 35, 37]. Particularly, the production of methane will be reduced when the inflow especially contains a significant quantity of inorganic sulphur (COD/SO_4^{2-}

<7.5), and the resulting sulphide content will cause biogas management problems [37]. Particularly, UASB (upflow anaerobic sludge blanket) reactors have been widely and successfully applied in the pulp and paper industry [37]. EGSB (expanded granular sludge blanket) and IC (internal circulation) reactors, which are actually the evolution of the UASB type, have also already been implemented improving digestion rate and gas yield.

Moreover, anaerobic processes may be combined with aerobic ones to improve BOD removal and the oxidation of some inorganics such as hydrogen sulphide. The resulting treated water may finally be subjected to sedimentation, flotation and sand filtration before being reused as low-quality water, but taking into account that the potential presence of bacteria should be reported within the health and safety assessments of the mills. This combination of treatments has successfully been implemented in different European mills such as Smurfit Kappa Zülpich Paper (Germany), AssiDomän Packaging in Lecoursonnois (France), Papierfabrik Julius Schulte Söhne in Düsseldorf (Germany), Stora Enso Sachsen (Germany) and VPK (Belgium) [38–42] – all of them producing different grades of paperboard.

In addition, the implementation of advanced treatments will be necessary when the effluent is going to be reused as high-quality water. In this case, the key contents to remove are salts (e.g. sulphate, carbonate and silica), in order to avoid scaling and corrosion, and nutrients (mainly nitrogen, phosphorous and carbon), aiming to prevent biogrowth and the presence of pathogens for safety control. Furthermore, soluble organic matter must especially be removed to a higher extent than 95% to control biofouling [35, 37]. Effluents from biological treatments of paper mills are particularly characterized by their high concentration of solids, including fibres and bacterial flocs, among other production residues. Therefore, microfiltration (MF) or ultrafiltration (UF) is a necessary pretreatment for this wastewater that will be inflowing a final nanofiltration (NF) or reverse osmosis (RO) unit aiming for removing its salts content [43].

Although MF is suitable for removing suspended solids, including larger microorganisms like protozoa and bacteria, UF may even remove viruses and organic macromolecules down to approx. 0.02 μm. In general, UF has intensively been used in treatment plants reclaiming wastewater worldwide. MBRs (membrane bioreactors) are currently gaining popularity for different urban and industrial applications. Therefore, and although encased dead-end-mode UF systems may imply a lower operational cost [9, 44], MBRs are able to operate in a submerged design, thus requiring to work at low values of transmembrane pressure (TMP), which minimize fouling effects. Moreover, as MF or UF membranes are installed to separate sludge, MBR technology will not show problems associated with filamentous bulking, which may occur when sedimentation or flotation is implemented instead. Furthermore, incorporating membrane treatment to biological processing makes reactors run with a higher dry solids concentration (8–15 g L^{-1}) than conventional activated sludge (3–5 g L^{-1}), therefore producing less biological sludge. These properties also lead to require lower hydraulic retention time and/or volumes to perform the biological treatment in MBRs [45]. In any case, it should be considered that encased dead-end-mode UF systems may entail lower operational cost [9, 44].

RO systems and disinfection are recommended as the final steps joining any treatment train aiming for reducing electrical conductivity and pathogens content up to potable water values, which ensures a final water quality that is safe for operating workers and guarantees very stable operational conditions. Nevertheless, scaling and fouling phenomena may however cause water production rate to decline, a lower permeate quality, unsteady-state operation conditions and severe damage to the integrity of membranes in these systems [46–49]. Furthermore, the management of the generated rejects must also be considered. In short, these are the main actual bottlenecks that paper mill managers must carefully deal with, mainly scaling associated with silica and calcium compounds contents.

Advanced oxidation processes (mainly ozone, which is already used for bleaching in some paper mills) have already also been used in some cases aiming for removing bio-recalcitrant organic compounds, odour and colour and to provide disinfection – all in order to meet the limits that may be imposed for the characteristics of the effluent. In addition, AOPs have likewise been combined with biological treatment to allow water reuse [50], although oxidation may also generate by-products of toxicological concern that may limit the posterior biological stage. The content of these bio-recalcitrant compounds will have more importance in the presence of chlorinated compounds, which are usually produced during chlorinated bleaching processes. Although scientific research regarding the effects of these compounds in pulp and paper industrial wastewater is still limited, the determination of its presence and concentration is every day becoming more important in relation to reducing the emission of contaminants of emerging concern [51]. Finally, algae, fungal and enzymatic treatments are actually being assessed, mainly at a small scale, as emerging environmentally friendly treatment alternatives.

There are only a few examples of the full-scale application of the above-mentioned technologies. For example, different UF, NF and RO membranes were comparatively assessed at Stora Enso Kotka's mill (Finland) aiming for the treatment of part of the effluent, although RO permeability was as low as 2.5 L m^{-2} h^{-1}·bar^{-1} [19]. McKinley Paper Mill (New Mexico, USA), which produces linerboard from 100% recycled board and old corrugated containers, is already operating an MF + RO system that recycles all the effluent within the mill. This paper mill is currently consuming just 1.2 m^3 of fresh water per ton of produced paper, a volume that is mainly devoted to compensate evaporation losses during paperboard drying [18]. In addition, Mondi Paper Mill (Piet Retief, South Africa) has successfully reported reusing up to 1,700 m^3 d^{-1} of black liquor after its treatment with tubular UF, ion exchange and RO. Finally, more recent pilot trials have been performed at Holmen Paper's newsprint paper mill in Madrid (Spain), consisting of a treatment train integrating an anaerobic biological stage followed by another aerobic one, UF and RO membrane filtration. This system was able to produce water fulfilling the quality parameters that are required to substitute fresh water use in some critical applications of the paper machine, such as its high-pressure showers, although permeate recovery is limited by the high silica content that is typical in deinked paper mill effluents [44].

5 Reusing Reclaimed Water from Municipal Wastewater Treatment Plants

Municipal wastewater reclamation, that is, treating and reusing effluents from MWWTP, represents a viable alternative to water shortage and contributes to integral sustainable water management, representing an important alternative water source for many regions worldwide [15]. The most viable treatment train to purify these effluents will depend on the final use of water, the legislation in force, the particular requirements that would be allowed, the level of water availability, its geographical situation, stakeholders' acceptance and the economic figures of implementation and operation.

The occurrence of potential health hazards is one of the most important issues to consider when assessing the use of MWWTP reclaimed water as a possibility for fresh water substitution. This is, in fact, the main question that is highlighted within all available legislations in force regulating this particular application [12, 17, 52–54]. The removal of pathogens (bacteria, helminths, protozoa and enteric viruses) must be primarily ensured as mandatory by the processes that would be applied to reclaim water [55]. Moreover, the control of the presence of microorganisms will also aid limiting biofilm growth, scale and corrosion, which are actually associated with their activity. Additionally, the removal of salts should likewise serve to avoid clogging and scaling problems, especially in high-pressure showers [9, 19]. Besides, it would complementarily be necessary to also remove those compounds that may affect product quality, for example, providing colour to white paper grades. Furthermore, contaminants of emerging concern must be removed to avoid their accumulation in the process.

Table 4 includes the summary of the removal efficiencies that are expected to be achieved by applying different technological alternatives to reclaim water from MWWTPs [9, 15, 46]. Conventional tertiary treatment (flocculation + clarification + filtration + disinfection) is usually applied when reclaimed water is going to be used for less stringent uses, whereas membrane filtration is required for more exigent applications to avoid potential health hazards. In this sense, MF and UF are generally adopted as the preferred processes for the retention of microbial and suspended solids and as best suitable pretreatments for posterior NF or RO stages, which are able to generate process water of a very high quality standard, even drinking water [56, 57].

Although almost any membrane design can be applied to the treatment of wastewater with low suspended solids content, only specifically designed modules with suitable operation modes would be able to handle effluents carrying high amounts of solids, bacteria and/or organic pollutants, which are very frequent in effluents from MWWTPs. In these cases, higher cross-flow velocities or submerged systems may be a good option, including MBRs [15, 45]. In addition, the optimization of the operating cost must be mainly limited by technical considerations. Therefore, while pressurized systems run at higher pressure thresholds, which implies a greater cost associated with pumping, submerged systems require a

Table 4 Removal efficiencies (%) achieved by different treatments applied to reclaim municipal sewage.

Parameter	CAS[a]	CAS + filtration	CAS +BNR[b]	CAS + BNR + filtration	MBR	MBR +IE	CAS +MF/ UF +RO MBR+RO
TSS (mg L^{-1})	96–94	98	95–96	99	>98	>98	>99
TDS (mg L^{-1})	0	0–19	0–19	0–19	0–19	–	85–98
VOCs (μm)	90	90	90–95	90–95	90–95	90-95	>99
COD (mg L^{-1})	84–90	88–91	92–95	92–96	>96	>96	96–99
BOD_5 (mg L^{-1})	93–95	94–95	95–96	98–99	>99	>99	>99
TOC (mg L^{-1})	85–88	88–90	90–92	98–99	>98	>98	99.0–99.9
Total nitrogen (mg L^{-1})	25–50	25–50	85–89	90–93	>86[c]	>80	>95
Total phosphorous (mg L^{-1})	0–17	0–33	75–83	>83	58–93[d]	>80	>86
Metals (mg L^{-1})	33–40	33–40	33–40	33–40	Trace	Trace	–
Total coliforms (CFU·100 mL^{-1})	99.0–99.9	>99.9	99.0–99.9	99.0–99.9	>99.9	>99.9	~100
Protozoan cysts and oocysts (CFU·100 mL^{-1})	0–99.9	>99.9	>99.9	>99.9	>99.9	>99.9	~100
Viruses (PFU·100 mL^{-1})	0–90.0	0–99.9	0–90.0	0–90.0	>90	>90	~100

[a]CAS: conventional activated sludge+nitrification
[b]BNR: biological nutrient (N and P) removal
[c]With anoxic stage
[d]With coagulant addition
Adapted from [12]

greater investment in aeration application, and thus recovery rates are lower as well. The cost assessment for this application also includes the consideration of other factors, such as water quality, operating flux, recovery rate of the systems, type of pretreatment and the costs of labour and materials.

One of the main challenges for the viability of this technology is minimizing the occurrence of fouling. In general, the content of dissolved organic matter that is typically present in effluents from MWWTPs (TOC ≈ 5–20 mg L^{-1}; BOD_5 ≈ 3–10 mg L^{-1}), together with the presence of other colloidal matter, may produce membrane fouling. Furthermore, although the salinity of these effluents is much lower than the figures in seawater (≈1,500 versus 38,000 mg L^{-1}, respectively), scaling may also occur, particularly when the MWWTP receives a large amount of industrial wastewater [9]. In this case, special attention must be paid to industrial cleaning processes, which may lead to periods of time in which residual chemicals will create membrane fouling in the treatment train of the MWWTP. In order to minimize fouling problems, membrane surface may be modified to further enhance its antifouling behaviour [48]. Another alternative strategy consists in the installation of aeration systems, mainly in MF and UF modules, aiming to enhance surface

membrane shear, but it highly increases the cost of treatment [45]. The selection of the best cleaning strategy (type of chemical, cleaning conditions and frequency) for backwash and cleaning-in-place (CIP) operations is a key to achieve both a constant membrane system performance and the lowest possible contribution to the cost of operation [58]. Furthermore, the removal of micropollutants would be another challenge to face.

Finally, the management of the rejects that are produced in membrane technology applications must be focused on finding direct applications for them, that is, addressing its recycling as much as possible. Besides, several initiatives have been reported regarding the removal of hazardous components from concentrated streams [59]. Although some compounds are effectively removed, others equally dangerous do remain, so special attention should be paid in the future to detecting and treating the accumulation of new contaminants of emerging concern.

5.1 Industrial Case Studies

The substitution of process water by reclaimed wastewater has not yet been widely applied in the paper industry. Only some pulp and paper mills located in the USA (e.g. Simpson Paper and Garden State Paper in California, Bronx Community Paper in New York, Blue Heron Paper in Georgia and SCA Tissue, Flagstaff, Arizona) and South Africa (Mondi Paper Mill in Durban, Sappi Enstra, Sappi Cape Mill and Sappi Fine Paper, Port Elizabeth) currently use reclaimed water from MWWTPs, although these are not applying a final membrane treatment [60]. For example, Durban's water reclamation plant particularly supplies 47,000 m^3 day^{-1} of tertiary treated water (sedimentation + ozonation + activated carbon filtration + chlorination) to Mondi Paper Mill [61, 62]. In Europe, Holmen Paper Madrid (Spain) has totally substituted fresh water use by reclaimed water since 2013. The reclamation treatment train consists of a combination of pressurized UF and RO systems that are applied after a conventional tertiary treatment [9]. This is the first paper mill in Europe of such characteristics using the 100% of reclaimed water. After one year from the implementation of this initiative, no runnability issues have been reported to date or any effect on the quality of the final product that could be associated with this use.

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Reusing Landfill Leachate Within the Framework of a Proper Management of Municipal Landfills

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Abstract The possibility of reusing leachate substances for agronomical purposes might be of interest, especially in arid areas when used in addition to the leachate water content. This study presents a simple procedure for the revegetation of the walls of closed landfills, reusing the leachate as a fertigant. The results demonstrated the real possibility of employing blended leachate as a fertigant for the revegetation of the walls of closed landfills. The native plants *Lepidium sativum*, *Lactuca sativa* and *Atriplex halimus,* which suit the local climate, were chosen for this study in Southern Italy. The methodology was structured into three phases: (i) early-stage toxicity assessment phase (apical root length and germination tests), (ii) adult plant resistance assessment phase and (iii) soil properties verification phase. The rationale of the proposed approach was first to look at the distinctive qualities and the potential toxicity in landfill leachates for fertigation purposes. Afterwards, through specific tests, the plants used were ranked in terms of resistance to the aqueous solution that contained leachate. Finally, after long-term irrigation, any possible worsening of soil properties was evaluated. In particular, the plants maintained good health when leachate was blended at concentrations of lower than 25% and 5%, respectively, for *Atriplex halimus* and *Lepidium sativum*. Irrigation tests showed good resistance of the plants, even at dosages of 112 and 133.5 mm/m^2, at maximum concentrations of 25% and 5%, respectively, for *Atriplex halimus* and *Lepidium sativum*. The analysis of the total chlorophyll content and of aerial parts dried weight confirmed the results reported above.

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 239–256, DOI 10.1007/698_2014_325,

Keywords *Atriplex halimus*, Closed landfill, Fertigation, *Lactuca sativa*, Landfill leachate, *Lepidium sativum*

Contents

Abbreviations

ANOVA	Analysis of variance
AOP	Advanced oxidation process
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
DMSO	Dimethylsulfoxide
DOC	Dissolved organic carbon
EB	Electron beam
EC_{50}	Half maximal effective concentration
ECP	Electrochemical peroxidation
GI	Germination index
LOEC	Lowest observed effect concentration
MATC	Maximum acceptable toxic concentration
NOEC	Not observed effect concentration
PEC	Photoelectrochemical
TSS	Total suspended solid
US	Ultrasound
UV	Ultraviolet
VSS	Volatile suspended solid

1 Introduction

A landfill site is a large area of ground where waste materials are dumped or disposed of. This method is widely used because of its cost-effectiveness. One of the most important problems with designing and maintaining a landfill is managing

the leachate that is generated when water passes through wastes. Therefore, leachate is the aqueous effluent, generated by rainwater percolation through wastes, biochemical processes in waste cells and the inherent water content of the waste itself [1–4].

The chemical composition of landfill leachate is influenced by a number of factors including seasonal precipitation, waste composition and the age of the landfill [5]. The age of the landfill site is one of the main variables that affect leachate characteristics [4, 6]. Usually, young landfill leachates contain large amounts of biodegradable organic matter (i.e. volatile fatty acids) that decreases with increasing landfill age as a result of anaerobic decomposition that takes place in landfill site. As volatile fatty acid content decreases, organic matter in the leachates becomes dominated by refractory compounds, such as humic-like compounds and fulvic acid-like substances with consequent reduction of BOD/COD ratio [7]. Also, ammonia concentration increases with the increase of landfill age as a result of the fermentation of protein-containing organic matter, being typical concentration values higher than 2 g/L in old landfill leachates. Therefore, stabilised landfill leachates are much more difficult to treat as compared to young ones. Although leachate composition may vary widely within the successive aerobic, acetogenic, methanogenic stabilisation stages of the waste evolution, in general, three types of leachates can be defined according to landfill age, namely, recent, intermediate and old.

According to widely employed regulations, landfill leachate must be properly treated before its disposal of to receiving water bodies. The most common practice to avoid environmental risks is to pump and discharge leachate into conventional wastewater treatment plants [8]. However, landfill leachate is very difficult to treat biologically, due to the presence of recalcitrant compounds and high concentration of ammonia. Therefore, new technologies and new treatment combinations are required [9]. Selection of treatment must also be cost-effective, allowing compliance with local discharge standards at the lowest cost [10].

One method of leachate management that is more common in uncontained sites was leachate recirculation in which leachate is collected and reinjected into the waste mass. This process greatly accelerates decomposition and therefore gas production and has the impact of converting some leachate volume into landfill gas and reducing the overall volume of leachate for disposal. However, it also leads to substantial increase of the concentrations of recalcitrant compounds making it a more difficult waste to be treated [11].

Conventional landfill leachate treatments can be classified into three major groups: (a) leachate transfer, i.e. recycling and combined treatment with domestic sewage; (b) biodegradation, aerobic and anaerobic processes; and (c) chemical and physical methods, i.e. chemical oxidation, adsorption, chemical precipitation, coagulation/flocculation, sedimentation/flotation and air stripping [4].

Examples of the most used physicochemical processes for stabilised leachate treatment also include electro-oxidation processes, Fenton reaction, ozonation, ion exchange, coagulation/flocculation, adsorption, air stripping or combinations of two processes or more.

Biological treatments of landfill leachate are more attractive, and they are, probably, the most efficient and cheapest processes to reduce the chemical oxygen demand (COD) and nitrogen from leachate. These biological treatment processes are quite effective for leachate generated in the early stage with a high BOD_5/COD; however, they generally fail to treat a landfill leachate with a rather low BOD_5/COD ratio [12–16]. Some recent breakthroughs in the membrane filtration industry have now made possible the employment of some previously difficult separation applications. Nowadays, by the use of open high turbulence membrane modules that are resistant to fouling and plugging, membranes are becoming one of the most used options for treating landfill leachate. Microfiltration, ultrafiltration, nanofiltration and reverse osmosis are the main membrane processes applied in landfill leachates treatment [17–21].

On the other hand, advanced oxidation processes (AOP) are able to decompose a great number of organic compounds. These processes are characterised by the transformation of a large number of organic pollutants into carbon dioxide, water and inorganic anions through degradation reactions involving oxidative transitory species, mainly the hydroxyl radical ($HO^{\bullet}$) [22]. AOPs have been demonstrated to oxidise organic substances to their highest stable oxidation states being carbon dioxide and water (i.e. to reach complete mineralization) or to improve the biodegradability of recalcitrant organic pollutants up to a value compatible with subsequent economical biological treatment. Most of the AOPs, except simple ozonation (O_3), use a combination of strong oxidants, e.g. O_3 and H_2O_2; irradiation, e.g. ultraviolet (UV), ultrasound (US) or electron beam (EB); and catalysts, e.g. transition metal ions or photocatalysts.

AOPs, such as electrochemical oxidation, Fenton oxidation, electro-Fenton oxidation, photoelectro-Fenton, photoelectrochemical (PEC), electrochemical peroxidation (ECP), etc., have been proved highly capable and efficient in reducing refractory organic substance and colour as well as in oxidising ammonia from raw and pretreated landfill leachate [23]. In any case, AOPs remain an expensive way to deal with leachate management.

However, it would be also desirable to reuse the leachate. The composition of leachate is characterised by a high organic load, a high concentration of a lot of elements and important macro- and micronutrients for plants, namely, N, K, Mg, Ca, Zn and B [7].

The possibility of reusing leachate substances for agronomical purposes might be of interest, especially in arid areas when used in addition to the leachate water content. There is even a possibility of reusing leachate as a fertigant for many crops which are not for human consumption [24]. There have been several studies on the possibility of using leachate for irrigation purposes. There are papers focused on soil properties related to leachate irrigation [25–28], on using pretreated leachate [24] and on fertigation of plants for energy productions purpose [29]. But, more importantly, it would be beneficial to apply in situ procedures using leachate for fertigating the walls of the same landfill.

If we look at landfills where solid waste has reached its maximum available load and therefore the waste can no longer be disposed of (i.e. has reached the end of its

life cycle), then a new perspective can be proposed. In situations such as these, it is necessary to ensure that the landfill is maintained in a safe condition after its closure and that it can also be adaptable for future use. Governments have started converting closed landfills into recreational facilities such as playgrounds, sports facilities and parks, after suitable restoration. One of the main issues of the management of closed landfills is the disposal of leachate which still continues to be produced for a long time after the closure of the landfill. Such leachate could be thought to be employed for irrigation of vegetation that covers closed landfills. The use of leachate as a fertigant could therefore lead to added value which otherwise would be lost, contributing to a substantial reduction of disposal operating costs. The employment of leachate as a fertigant for the revegetation of the walls of closed landfills could prove an attractive proposition. Assessing the opportunity for the revegetation of the walls of closed landfills employing the leachate as a fertigant requires a specific plant choice in order to overcome the problems such as water stress, methane exhalation and relatively high soil temperatures. The plant species should be chosen from native species.

The procedure proposed here includes a set of experimental tests aimed at assessing leachate toxicity, plant sensitivity and soil degradation. These three tests provided information about the real possibility of using a particular leachate with respect to the resistance capability of the chosen set of plant species and finally the impact of leachate on soil matrix. In fact, the procedure gives the manager the information about leachate dilution so that it can be suitable for the growth of specific plant species minimising the negative impact on soil. It supports the manager in selecting plant species most suitable for the specific landfill and leachate, providing a viable option for environmentally sustainable management.

2 Employed Leachate and Procedure

Raw leachate was sampled from a medium-aged (5 years) municipal landfill located in Apulia, Southern Italy. The landfill contains nonhazardous waste including municipal solid waste. In the present study, leachate was characterised according to standard methods [30]. The obtained chemical and physical properties are listed in Table 1.

The procedure that was used consisted of three phases: (i) early-stage toxicity assessment (apical root length and germination tests), (ii) adult phase plant resistance assessment (irrigation trials) and (iii) soil degradation assessment. Phytotoxicity of the leachate was determined by calculating the germination index of *Lepidium sativum* Linnaeus and *Lactuca sativa* Linnaeus seeds. The plant species used for the irrigation trials were *L. sativum* and *Atriplex halimus* Linnaeus. The latter is one of the most tolerant species to leachate [31, 32] and among the most popular in the area of the selected landfill. Finally, at the end of the test, pH and electric conductivity were measured on growth substrate extracts.

Table 1 Municipal landfill leachate composition range (used in this study)

Parameter	Unit	Value range
COD	g L^{-1}	2.8–3.6
BOD_5/COD		0.2–0.3
DOC	g L^{-1}	0.9–1.2
NH_4–N	g L^{-1}	1.5–2.0
pH		7.8–8.3
P_{tot}	mg L^{-1}	4–6
TSS	mg L^{-1}	150–300
VSS	mg L^{-1}	120–230
Chlorides	g L^{-1}	3.0–4.0
Conductivity	mS cm^{-1}	16–22
Sulfates	g L^{-1}	1.0–1.5
Na	g L^{-1}	1.5–2.0
K	g L^{-1}	1.2–1.6
Mg	g L^{-1}	0.2–0.4
Cr	mg L^{-1}	<0.1
Ni	mg L^{-1}	0.5–1
Mn	mg L^{-1}	<0.02
Fe	mg L^{-1}	1–1.5
Zn	mg L^{-1}	<0.01
Cu	mg L^{-1}	0.01–0.2

3 Phytotoxicity Bioassay

Germination tests of *L. sativum* were carried out according to 850.4200 EPA method [33] using cress (*L. sativum*) and lettuce (*L. sativa*) seeds. The composite germination index (GI) was determined according to the following formula [34]: $GI = Gs \cdot Ls/Gc \cdot Lc$ where Gs and Ls are seed germination (%) and root elongation (cm) for the sample, respectively, while Gc and Lc are the corresponding control values. To facilitate comparison between different tests, GI was expressed as a percentage of the GI of control. Raw leachate was diluted by Milli-Q water to the desired concentration (see Fig. 1).

Ten healthy seeds of each species were placed randomly in a Petri dish (9 cm diameter) lined with Whatman no. 1 filter paper which was moistened with 5 mL of diluted leachate. Distilled water was used as a control and five replicates were made for each used dilution (Fig. 1). Germinated seeds were counted and the primary root length was measured (rounding at the nearest cm) after 2 days for *L. sativum* seeds and 5 days for *L. sativa*.

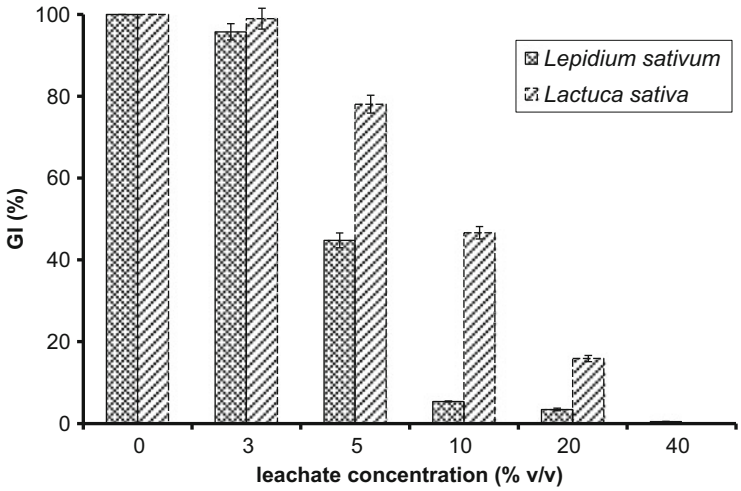

Fig. 1 Profiles of germination index (SE) for *L. sativa* and *L. sativum*. Error bars deriving from standard error of 50 measurements are also reported

4 Irrigation Trials

L. sativum and *A. halimus* were used. The hostile environment of the walls of the closed landfill caused by water stress, methane emission and relatively high soil temperatures were taken into consideration. The plants were chosen using native species, which suited the South Italian climate, as this is where the investigations were carried out. The selection of the plants was made by taking into account the plants' ability to engraft themselves and grow on the landfill final coverage layer resisting to leachate stress [35, 36]. Furthermore, these species tolerate harsh conditions such as salinity, light stress and drought [37–39].

For each plant the experimental design included five different concentrations (% v/v) of raw leachate to be used, consisting of 0% (i.e. irrigation with tap water as a control), and 5, 25, 50 and 100 (%), respectively. For each concentration, 15 replicates were prepared leading to a total of 150 plant samples for both species. They were arranged according to a randomised block design in a covered and ventilated structure to avoid any interference from rain. The only water supply was the one used throughout the test. Through one growing season (January–July), the plants were irrigated according to their water needs (as to keep the soil moist) with known volumes of diluted and undiluted leachate specified as aqueous solution dosage (mm/m^2). Plants were left to grow in pots with a diameter of 14 cm (*L. sativum*) and 16 cm (*A. halimus*) filled with peat as the growth substrate. Several solution dosages were used (Table 2) to evaluate the plant growth in terms of height and leaf chlorophyll content.

Plant height was measured manually with a measuring tape. Determination of leaf chlorophyll content was carried out by sampling three leaves from each of the

Table 2 Amount of aqueous solution dosed while checking the health of the plant

Lepidium sativum	*Atriplex halimus*
Leachate dosage (mm/m^2)	
0	0
58.8	59.7
133.3	112
–	248.8

15 plant replicates. 100 mg of leaf tissue was placed in a glass centrifuge vial containing 7 mL of dimethylsulfoxide (DMSO). Chlorophyll was extracted by heating the sampled leaves in a water bath at 65°C for 30 min. The extract was then transferred into a graduated tube and diluted with DMSO up to 10 mL. The chlorophyll extract was then transferred to a 10 mm cuvette, and the absorbance at 645 and 663 nm was measured using a spectrophotometer against a DMSO blank. The content of chlorophyll a and b was determined according to the following equations [40]:

$$\text{Chla}\left(\text{g L}^{-1}\right) = 0.0127 \times \text{A663} - \text{A645} \times 0.00269,$$
$$\text{Chlb}\left(\text{g L}^{-1}\right) = 0.0229 \times \text{A663} - \text{A645} \times 0.00468.$$

The total chlorophyll content was obtained by totalling the two contributions (Chla + Chlb). At the end of the test, the aerial parts were collected and dried in an oven at 40°C for 4 days and then weighed.

5 Possible Soil Degradation Assessment

Soil pH, in deionised water and in KCl 1 M, and electrical conductivity were measured in order to verify any possible accumulation of salts and pH changes due to increasing leachate dosage. The pH in water estimates the H^+ ions concentration in the soil circulating solution while in KCl estimates H^+ ions both in the circulating solution and those adsorbed on the exchange complex. Determination of pH and conductivity were carried out according to EN 13037 method [41] in soil extracts using growth substrate sieved to 2 mm, with a solid phase: liquid phase ratio equal to 1:50. The electrical conductivity was measured on the same aqueous extracts according to EN 13038 method [42] using a conductivity metre equipped with a thermometer for temperature compensation.

6 Data Testing

The GI was analysed using one-way analysis of variance (ANOVA) to identify significant effects with a type I error rate (α) of 0.05. To test the assumptions of ANOVA, the data set was subjected to an analysis of residual error for the end point to ensure that errors were independent, homogeneous and randomly distributed. Kolmogorov–Smirnov test [43, 44] was used to determine if raw observations followed a normal distribution. When a normal distribution was not observed, the end point was subjected to a Box-Cox transformation. Bartlett test and Levene test were applied on raw data to check the homogeneity of variance. Finally, Anscombe-Tukey test [45] was applied to check for the presence of outliers. After applying ANOVA, groups were analysed by means of Fisher-LSD test ($\alpha = 0.05$) which allows for unplanned multiple comparisons between all means and the control. This procedure allows obtaining values of NOEC (not observed effect concentration), LOEC (lowest observed effect concentration), MATC (maximum acceptable toxic concentration) and EC_{50} (half maximal effective concentration).

7 Toxicity Assessment at Early Stages (Apical Root Length and Germination Tests)

The two end point values, namely, the germinated seeds percentage and the apical root average length for both investigated plant species, are listed in Table 3.

Results show that, for both investigated species, using leachate concentrations at $\leq$10%, the percentage values of germinated seeds were identical, within the experimental error, to the values obtained when leachate was absent. When using a higher leachate dosage, the number of germinated seeds drastically decreased leading to an absence of germination at leachate concentration of 40%. Average length of apical root showed that for both species there was similar behaviour to that of the germinated seeds percentage. Using *L. sativum* with leachate concentrations lower than 5%, results show values comparable, within the experimental error, to those obtained when leachate was absent. When leachate concentrations were higher than 5%, the average length of apical roots decreased down to 0.5 ± 0.1 cm, corresponding to a length reduction of 72%. Results obtained for *L. sativa*, with leachate concentrations of lower than 10%, were shown to be comparable to those obtained when leachate was absent. It was again found that at higher leachate dosages the average length of apical roots decreased reaching 0.4 ± 0.2 cm at the concentration of 20%, corresponding to a 78% reduction compared with the plant irrigated without leachate. The obtained results for both species therefore showed that the response of the average length of apical roots was an order of magnitude higher than that of germinated seeds percentage. It follows that average length of apical roots was more sensitive to toxicity of the leachate, since the response

Table 3 Percentage of germinated seeds and average length of apical roots during irrigation with landfill leachate at several dilution rates

Leachate concentration (% v/v)	*Lepidium sativum*		*Lactuca sativa*	
	Germinated seeds (%)	Average length of apical roots (cm)	Germinated seeds (%)	Average length of apical roots (cm)
0	98.0 ± 4.5	1.8 ± 0.4	94.0 ± 5.5	1.8 ± 0.9
3	96.0 ± 5.5	1.8 ± 0.2	90.0 ± 7.1	1.9 ± 0.2
5	92.0 ± 8.4	0.9 ± 0.2	86.0 ± 8.9	1.6 ± 0.2
10	92.0 ± 8.4	0.5 ± 0.2	82.0 ± 10.1	1.0 ± 0.1
20	62.0 ± 3.1	0.5 ± 0.1	68.0 ± 7.9	0.4 ± 0.2
40	2.0 ± 4.5	0.5 ± 0.1	n.g.	n.g.
60	n.g.	n.g.	n.g.	n.g.
80	n.g.	n.g.	n.g.	n.g.
100	n.g.	n.g.	n.g.	n.g.

n.g. not germinated

Table 4 Relevant toxicity parameters, namely, NOEC, LOEC, MATC and EC_{50}, calculated by ANOVA as a consequence of the presence of leachate (percentage added to the irrigation water) on *Lepidium sativum* and *Lactuca sativa*

Species	NOEC (%)	LOEC (%)	MATC (%)	EC_{50} (%)
Lepidium sativum	3	5	3.8	6
Lactuca sativa	5	10	7.1	10.6

change rate of the average length of apical roots was greater than that of germinated seed percentage. Indeed, such a trend was more evident at lower leachate dosages. At the same leachate concentration, the values of germinated seeds percentage were comparable, while values of the average length of apical roots were significantly lower for the *L. sativum*. This suggests a greater sensitivity of *L. sativum* than *L. sativa* to the leachate. The two end points were then merged to create a single germination index (GI) in order to better assess the effect of the toxicity of leachate on the two investigated species. The GI vs leachate dosage for the two species is shown in Fig. 1. GI values for *L. sativa* are always greater than for *L. sativum*, suggesting that the latter species was the most sensitive to the toxicity of leachate. Furthermore, the obtained values of LOEC and NOEC for the two investigated species (Table 4) showed greater sensitivity of *L. sativum* to the leachate treatment.

Irrigation water containing 5% of leachate concentration did not lead to an observable toxic effect for *L. sativa*, while toxic effect was obtained for *L. sativum* at a leachate dosage of 3%. At the same time, the calculated values of MATC (Table 4) were 3.8 and 7.1 (%), respectively. From GI profiles it was also calculated that EC_{50} was 6 and 10.6 (%) for *L. sativum* and *L. sativa*, respectively. The high toxicity values are fully justified by the landfill-impacted environment. In fact, as landfill age increases, the organic fraction in the leachate becomes dominated by refractory compounds, such as humic substances; moreover, the ammonia concentration increases as a result of the fermentation of organic matter containing

proteins [4]. According to the age of studied landfill (5 years), the values shown in Fig. 1 and Table 4 are not surprising. Nevertheless, even by using solutions with low leachate content, its reuse is possible and could be included within a proper management protocol of closed landfills especially for irrigation of plant species on both top cover and side slopes of landfills.

8 Resistance of Adult Plants (Irrigation Trials)

The influence of leachate concentration on the average height of *L. sativum* and *A. halimus* is shown in Table 5.

Results showed that the plants were able to grow even when high concentrations of leachate were used. As for *L. sativum*, Table 5 showed that using leachate concentrations at low aqueous solution dosages tended to give rise to the same statistically average height plants, but they differed at higher concentrations. At the dosage of 58.5 mm/m^2, leachate concentration ranging from 0 to 25% had the same effect on plant growth. At the two greater dilutions, however, average plant heights were statistically different and also lower than the values obtained at higher leachate concentrations. At the aqueous solution dosage of 133.3 mm/m^2, a similar trend to the lower dosage was obtained; the higher the leachate concentration is, the lower the average plant height. However, a greater differential between adjacent leachate concentrations was obtained, and the average heights were always much higher than those obtained at the dosage of 58.5 mm/m^2 except for 100% leachate concentration where no increase in average height was found. Results obtained by *A. halimus* were similar to those of *L. sativum* and showed an increase of the average height at greater aqueous solution dosages, even though such an increase was much more limited. On the other hand, the results obtained were shown to be very different when considering the average heights obtained within a fixed dosage of aqueous solution. Results revealed that the effects of different leachate concentrations on the average height were negligible, statistically not different, up to a dosage of 112 mm/m^2. Also, at 248 mm/m^2 dosage, the effect of leachate concentration on the plants' average height began to be evident. Overall, both species showed a degree of adaptability to the leachate which was more pronounced for *A. halimus* which gave lower differences to the leachate concentration within each investigated aqueous solution dosage. Thus, the *A. halimus* was more 'suitable for purpose' than *L. sativum*. The former plant, even at the highest investigated aqueous solution dosage (248 mm/m^2), could be fertigated with a 25% leachate solution without any detriment to the plant growth.

The influence of leachate concentration on total chlorophyll (a plus b) of the investigated plants is shown in Table 6.

Results show that for both *L. sativum* and *A. halimus*, increasing the dosage of the irrigation solution led to a slight decrease of total chlorophyll content which was more evident at higher concentration of leachate. The same trend was evident within each aqueous solution dosage. This suggests that the general trend was

Table 5 Average height of *L. sativum* and *A. halimus* as a function of aqueous solution dosage at several leachate dosages

Lepidium sativum	Dosage (m/m^2)		
	58.5	133.3	
Leachate concentration (%)	Average height (cm)		
0	47 ± 6.5	89 ± 9	
5	43 ± 5.2	79 ± 6.7	
25	35 ± 5.1	61 ± 5.9	
50	33 ± 2.7	41 ± 5.5	
100	27 ± 2.3	28 ± 1.5	
Atriplex halimus	Dosage (m/m^2)		
	59.7	112	248.8
Leachate concentration (%)	Average height (cm)		
0	42 ± 3.7	45 ± 3.9	70 ± 3.5
5	43 ± 4.9	49 ± 6.1	63 ± 3.3
25	42 ± 4.2	52 ± 6.3	60 ± 3.2
50	43 ± 6.0	49 ± 5.8	52 ± 3.5
100	42 ± 5.6	45 ± 5.5	49.2 ± 2.3

Table 6 Total chlorophyll (a plus b) of *L. sativum* and *A. halimus* during irrigation with aqueous solutions containing landfill leachate

Lepidium sativum	Dosage (m/m^2)		
	58.5	133.3	
Leachate concentration (%)	Total chlorophyll ($mg/g_{wet\ weight}$)		
0	1.7 ± 0.06	1.5 ± 0.06	
5	1.7 ± 0.03	1.6 ± 0.03	
25	1.3 ± 0.23	1.2 ± 0.21	
50	1.3 ± 0.25	1.1 ± 0.22	
100	0.8 ± 0.19	0.6 ± 0.23	
Atriplex halimus	Dosage (m/m^2)		
	59.7	112	248.8
Leachate concentration (%)	Total chlorophyll ($mg/g_{wet\ weight}$)		
0	2.1 ± 0.09	2.1 ± 0.10	2.1 ± 0.09
5	1.9 ± 0.14	1.8 ± 0.15	1.6 ± 0.14
25	1.4 ± 0.06	1.3 ± 0.18	1.1 ± 0.06
50	1.4 ± 0.09	1.2 ± 0.27	0.9 ± 0.09
100	1.3 ± 0.09	1.2 ± 0.32	0.8 ± 0.09

that the higher the absolute amount of leachate within the aqueous solution, the lower the total chlorophyll content of plants. Specifically, for the *L. sativum*, the results showed that for several levels of leachate concentrations, statistically identical results were obtained. Interestingly, at the aqueous solution dosage of 133.3 mm/m^2, the measured value at leachate concentration of 5% was higher

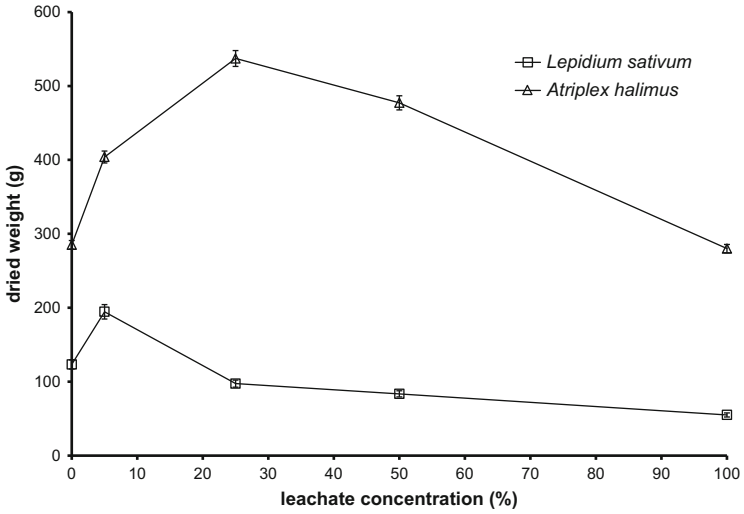

Fig. 2 Dried weight of aerial parts of *L. sativum* and *A. halimus* at the end of irrigation trials (i.e. at maximum dosage for each concentration)

than that obtained without any leachate suggesting a fertilising effect of the leachate at such a percentage. *A. halimus* shows similar results to *L. sativum*, with a general trend characterised by lower total chlorophyll content at higher leachate concentration (Table 6). At the end of the irrigation trials (i.e. at maximum dosage for each concentration), the dried weight of plant aerial parts were also measured. This was a typical measurement of plant biomass and was aimed at assessing the status of plant biology and growth, thus evaluating the impact of leachate solutions at different investigated concentrations. The results obtained for *L. sativum* and *A. halimus* are displayed in Fig. 2 and show that both plants had a similar bell-like trend.

The maximum dried weight was obtained at two different leachate concentrations for the two plant species, namely, at 5% and 25% for *L. sativum* and *A. halimus*, respectively. Results depicted in Fig. 2 suggest that the leachate has two opposite effects on the plants, namely, a fertilising effect and a toxic effect. The fertilising effect was evident at low leachate concentrations which gave rise to a higher dried weight of the plants irrigated without any leachate. The toxic effect was evident at high leachate concentrations where lower values of dried weight were measured. Therefore, the trends reflected a balance between the two aforementioned opposite effects. It follows that for both plants a threshold value of certain leachate concentration was obtained, and above that threshold value the progressive increase of leachate concentration led to a drop in dried weight.

The calculated threshold concentration of leachate was quite different between *L. sativum* and *A. halimus* due to the strength of each plant species. Height values greater than the observed control can be explained knowing that some substances, although toxic at higher doses, can be stimulatory or even beneficial at low doses. This biphasic dose–response phenomenon is commonly termed hormesis. However, hormetic effects are not necessarily entirely beneficial for an organism, as, for example, increased shoot elongation at the cost of stem robustness may lead to more fragile plants, or increased biomass growth at the expense of pathogen defence compounds could make treated plants more vulnerable to diseases [46]. In fact, in the case at hand, the increase in average height was accompanied by a decrease of chlorophyll contents.

9 Soil Worsening Assessment After Plant Growth

In order to check for a possible worsening of the soil characteristics, several parameters could be measured. As the proposed method was thought to be fast and economical at the same time, the choice was to balance the obtained information amount and the considered number of parameters. The pH and electrical conductivity were selected, because indirectly, these parameters provide information on microbial communities structure, biogeochemical cycles, solubility equilibrium and precipitation of the elements as well as information on the speciation and toxicity potential of some elements [47]. The pH (in both water and KCl) and electrical conductivity were measured on the substrate used for plant growth (peat) at the end of irrigation tests (Fig. 3).

It was evident that for *L. sativum* the pH (in both water and KCl) did not change significantly by increasing the leachate concentration. For *A. halimus*, instead, a slight increase of pH was found at higher leachate concentrations. The increase was higher in KCl than in water, being 1.4 with respect to 0.5 pH unit. It is also worth noting that the pH measured for *L. sativum* was always higher than that found for *A. halimus*. Conductivity results showed quite different behaviour, the higher the leachate concentration, the (much) higher the conductivity. For *L. sativum*, conductivity increased from 276 up to 2,253 μS cm^{-1}, while for *A. halimus* a much higher increase was measured, namely, from 188 to 4,140 μS cm^{-1}. Irrigation with the leachate solutions did not significantly affect the pH of substrate used for the plant growth, possibly due to the buffering capacity of the soil. On the other hand, under the experimental conditions that were characterised by the absence of rain leaching, the irrigation with the leachate solutions led to an accumulation of soluble salts leading ultimately to an evident increase of electrical conductivity. This phenomenon would generate negative effects on plant growth, by affecting the root absorption of nutrients, ultimately causing nutritional deficiency in plants [48]. However, from the results that were obtained, it can be concluded that, for both investigated plant species, at low leachate concentrations (5–25%), the soil quality did not become compromised as a result of the irrigation process.

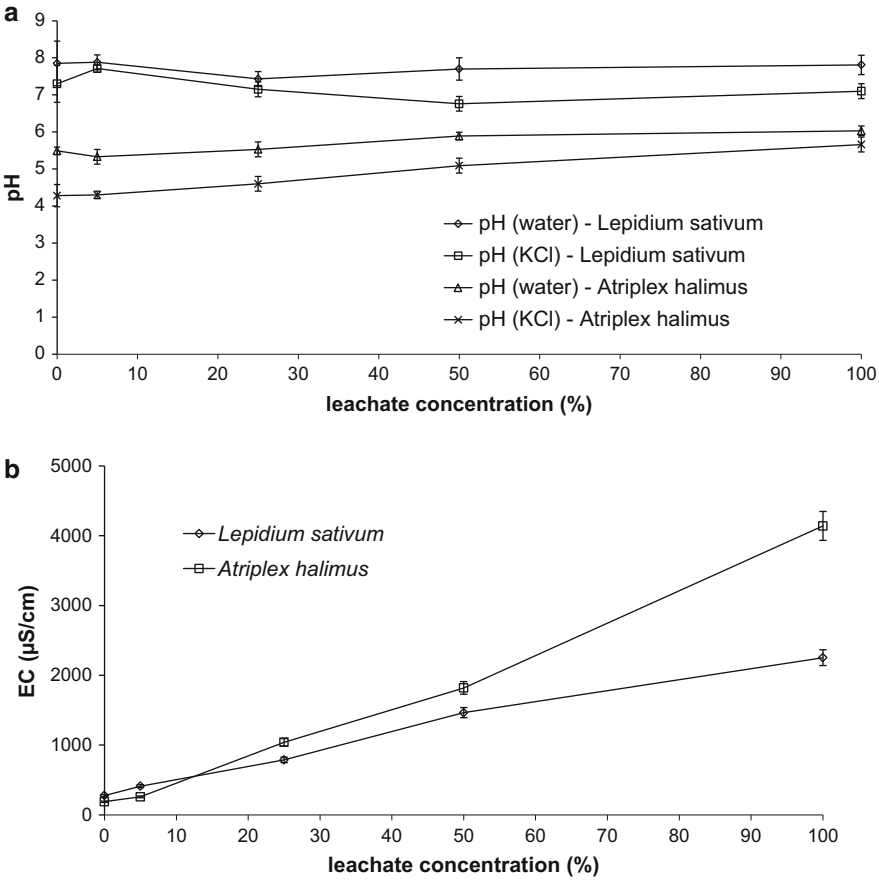

Fig. 3 pH (**a**), in water and KCl, and electrical conductivity (**b**) measured on the substrate employed for plant growth (peat) at the end of irrigation tests

10 Conclusions

The investigations as set out in this work have demonstrated that a speedy, economical methodology for the possible revegetation of the walls of closed landfills, employing the leachate as a fertigant, is potentially available. This method could be of importance to decision makers seeking to switch from standard landfill management mode to a more environmentally sustainable one. The methodology was structured into three phases: (i) early-stage toxicity assessment phase (apical root length and germination tests), (ii) adult plant resistance assessment phase and (iii) verification phase of possible worsening of the soil characteristics. The rationale of the proposed approach was firstly to identify the potential degree of toxicity in landfill leachate for fertigation purposes. Secondly, through specific tests, the

chosen plants were ranked in terms of their resistance to the aqueous solution that contains leachate. Finally, after a long-term irrigation programme and investigation, the possible worsening of soil properties was evaluated. By using such an approach, it was found that a leachate characterised by high concentration of $N–NH_4$ and COD could be used for fertigation purposes up to a dosage of 112 and 133.5 mm/m^2, at 25% and 5% concentration for *A. halimus* and *L. sativum*, respectively. The proposed procedure was applied to a specific leachate, and the obtained results appeared able to be realistically extended to a wider range of cases. In fact, the landfill average age and intrinsic characteristics of leachate seem to be truly representative and can be found in a wide class of real-world situations [4, 49]. The purpose is the application of this procedure in different situations in order to collect the widest possible cases (different leachates, different plant species and different climatic conditions) until to formalise a semiautomatic tool of wide application. The correct use of the proposed procedure can lead to the solution of two important problems: the recovery of an exhausted landfill and the disposal of leachate through recirculation. Further study would be needed, however, in order to understand whether, and to what extent, very long-term use for irrigation of such a saline water matrix could affect the electrical conductivity of the soil and thus adversely affect and cause deterioration of its fertility.

Acknowledgements The experimental work was performed within the strategic project PS_057 "Ottimizzazione e recupero ambientale di discariche da RSU", co-funded by Apulia Region and the European Commission under the POR Puglia 2000-2006 Misura 3.13 "Ricerca e Sviluppo Tecnologico".

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D. Fatta-Kassinos et al. (eds.), *Wastewater Reuse and Current Challenges*,
Hdb Env Chem (2016) 44: 257–262, DOI 10.1007/978-3-319-23892-0,