

Subsurface flow constructed wetlands for the removal of arsenic and metals from contaminated water

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MONASH University

Department of Civil Engineering

Monash University

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Abstract

The presence of arsenic (As) in aquatic environments is a worldwide concern due to its toxicity and chronic effects. In many cases, the choice of treatment technologies is limited due to the isolated location of the water source and the high cost of conventional treatment technologies. In addition, other pollutants are often found alongside As, such as iron (Fe) and boron (B). Constructed wetlands have shown capability to remove As and metals. However, few experimental studies have been undertaken to investigate As removal in wetland systems, leaving understanding of their removal mechanisms and performance wanting.

This thesis has investigated the As, Fe and B removal capabilities of vertical and horizontal flow wetlands, using conventional and alternative media. Laboratory studies were performed with the objective of gaining further understanding of the processes that remove these pollutants.

In vertical flow wetlands, alternative wetland media -limestone, zeolite and cocopeat- proved to be more effective than conventional gravel media. In horizontal flow wetlands, the zeolite media achieved high As and Fe removal rates. Wetlands made up of a sequential arrangement of limestone and cocopeat achieved similar As removal rates, higher B removal rates, but slightly lower Fe removal rates. Target pollutants were mainly retained in the wetland media instead of wetland plants, thus confirming the key role of wetland media in removing As, Fe and B.

The main recommendation from this research is that subsurface flow wetlands should use alternative wetland media to enhance As, Fe and B removal from acidic water, and ideally they should be operated under continuous horizontal flow. Wetland media able to provide alkalinity and sorption sites, such as limestone and zeolite, are recommended, since they enhance As coprecipitation with Fe, and As and Fe sorption, respectively. Organic wetland media, such as cocopeat, have potential to enhance B removal by sorption. Continuous horizontal flow maximises contact time for sorption onto wetland media and also favours anaerobic processes such as precipitation of sulfides, which can also enhance As and Fe removal.

This research has advanced understanding of the removal of arsenic, boron and iron in vertical and horizontal flow wetlands, offering recommendations to improve wetland design, with the ultimate aim to develop cost-effective technologies that can provide reliable water treatment for the protection of human health and aquatic ecosystems.

Declaration

This statement is to certify that, to the best of the candidate's knowledge, the thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other institution, and that the thesis contains no material previously published or written by any other person, except where due reference is made in the text of the thesis. The length of this thesis is less than 100,000 words, exclusive of figures, tables and references.



Katherine Lizama Allende

June 2013

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I have been fortunate to have guidance, assistance and friendship from many people. As such, I managed to obtain not only this thesis, but also an improved version of myself.

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My husband Javier, for his unconditional love, patience and understanding all these years, for being always there providing caring support, encouragement and wise advice, thanks very much for being such an important part of this adventure. I love you honey.

List of publications

The following publications have resulted from the studies undertaken for this degree. The four journal papers lead by the candidate formed the core of the thesis:

Refereed Journal Papers

Lizama A., K., Fletcher, T.D. & Sun, G. 2011. Removal processes for arsenic in constructed wetlands. *Chemosphere*, 84 (8), 1032-1043.

Lizama Allende, K., Fletcher, T. D. & Sun, G. 2011. Enhancing the removal of arsenic, boron and heavy metals in subsurface flow constructed wetlands using different supporting media. *Water Science and Technology*, 63 (11), 2612-2618.

Lizama Allende, K., Fletcher, T. D. & Sun, G. 2012. The effect of substrate media on the removal of arsenic, boron and iron from an acidic wastewater in planted column reactors. *Chemical Engineering Journal*, 179, 119-130.

Lizama Allende, K., McCarthy D.T. & Fletcher, T.D. (submitted). The removal of arsenic, boron and iron from acidic wastewater using horizontal flow constructed wetlands with different wetland media. *Submitted to Water Research*.

Conference Papers

Lizama Allende., K., Fletcher, T. D. & Sun, G. Enhancing the removal of arsenic and heavy metals in subsurface flow wetlands using different supporting media. *12th International Conference on Wetland Systems for Water Pollution Control*, 2012, Venice, Italy, pp 591-599.

Lizama Allende, K., Fletcher, T. D. & Sun, G. Subsurface flow constructed wetlands for the removal of arsenic and metals from acidic contaminated water. *7th International Conference on Water Sensitive Urban Design*, WSUD 2012, Melbourne, VIC, paper 210.

Lizama Allende, K., Fletcher, T. D. & Sun, G. Removal of arsenic by coprecipitation with iron in vertical flow wetland columns. In: SORIAL, G. A. & HONG, J. (eds.) *Environmental Science and Technology 2012 Volume 2*. Houston, USA: American Science Press.

Lizama Allende, K., Fletcher, T. D. & Sun, G. Arsenic removal by subsurface flow constructed wetlands. *4th International Congress on Arsenic in the Environment*, As 2012, Cairns, QLD, pp 278-279.

Preface

This thesis presents the results of this research mainly in the form of four journal papers (three published and one submitted), all of them with the candidate as the main author. The introduction and literature review focus on the current state of the use of constructed wetlands for removing arsenic, iron and boron and the main knowledge gaps that impede their widespread adoption for this purpose. Key sections of the first paper (*Removal processes for arsenic in constructed wetlands, published in Chemosphere*) were extracted, so as to present an integrated literature review on the problems related to the presence of arsenic, iron and boron in aquatic environments, and the capability of constructed wetlands in removing these pollutants. This paper, which focuses mainly on the mechanisms that remove As in constructed wetlands, is presented at the end of the literature review. The second paper (*Enhancing the removal of arsenic, boron and heavy metals in subsurface flow constructed wetlands using different supporting media, published in Water Science and Technology*) examines the performance of vertical flow wetlands using a range of wetland media. Most sections of the paper were incorporated in Chapter 3. This Chapter also presents additional analyses that were not included in the paper, alongside with a review of media used to enhance arsenic and metal removal. This paper is presented at the end of Chapter 3. The third paper (*The effect of substrate media on the removal of arsenic, boron and iron from an acidic wastewater in planted column reactors, published in Chemical Engineering Journal*) investigates the effect of different media on the performance of vertical flow wetlands. This paper is the core of Chapter 4, as such it is accompanied by introduction, additional context on the presence of As in acidic waters, and conclusions. The fourth paper (*The removal of arsenic, boron and iron from acidic wastewater using horizontal flow constructed wetlands with different wetland media, submitted to Water Research*) assesses the effectiveness of alternative wetland media in horizontal flow wetlands. This paper is presented in Chapter 5, accompanied by introduction, additional information about bacterial communities found in these wetlands, and conclusions. Finally, concluding remarks are presented and future work is suggested.

Declaration of publications and authorship

In accordance with Monash University Doctorate Regulation 17/ Doctor of Philosophy and Master of Philosophy (MPhil) regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

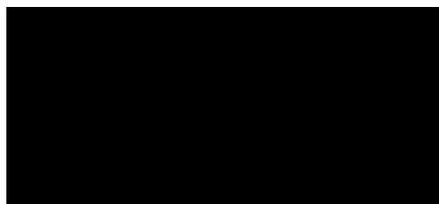
This thesis includes four original papers: three published and one submitted for publication in a peer-reviewed journal. The core theme of this thesis is subsurface flow constructed wetlands for the removal of arsenic and metals from contaminated water. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the Department of Civil Engineering under the joint supervision of Dr. Guangzhi Sun and Professor Timothy Fletcher during the first years of my studies, and the joint supervision of Professor Timothy Fletcher and Dr. David T. McCarthy during the last years.

Chapter 2 was written considering the most important sections of the review paper co-authored with Dr. Guangzhi Sun and Professor Timothy Fletcher and adding additional background information on constructed wetlands. Similarly, Chapter 3 was written based on the results presented in the journal paper co-authored with Dr. Guangzhi Sun and Professor Timothy Fletcher, also presenting a review of wetland media, together with additional analyses that were not included in that paper. In both chapters, the papers are attached at the end. Chapter 4 presents a journal paper co-authored with Dr. Guangzhi Sun and Professor Timothy Fletcher. Chapter 5 presents a journal paper co-authored with Dr. David T. McCarthy and Professor Timothy Fletcher. Appendix A encloses three conference papers. In each paper, my contribution to the work as a first author involved planning and initiation of the study, acquisition and analyses of experimental data, discussion with my co-authors, and writing up the papers. As such, my contribution to the work developed in the papers involved the following:

Chapter	Publication title	Publication status	Nature and extent of candidate's contribution
2	Removal processes for arsenic in constructed wetlands	Published <i>Chemosphere</i>	Review of literature and write up 85%
3	Enhancing the removal of arsenic, boron and heavy metals in subsurface flow constructed wetlands using different supporting media	Published <i>Water Science and Technology</i>	Initiation, ideas, experimental design and works, data analysis, write up 80%

Chapter	Publication title	Publication status	Nature and extent of candidate's contribution
4	The effect of substrate media on the removal of arsenic, boron and iron from an acidic wastewater in planted column reactors	Published <i>Chemical Engineering Journal</i>	Initiation, ideas, experimental design and works, data analysis, write up 75%
5	The removal of arsenic, boron and iron from acidic wastewater using horizontal flow constructed wetlands with different wetland media	Submitted <i>Water Research</i>	Initiation, ideas, experimental design and works, data analysis, write up 75%

I hereby declare the statement of candidate's contribution to be true and correct.



Katherine Lizama Allende
June 2013

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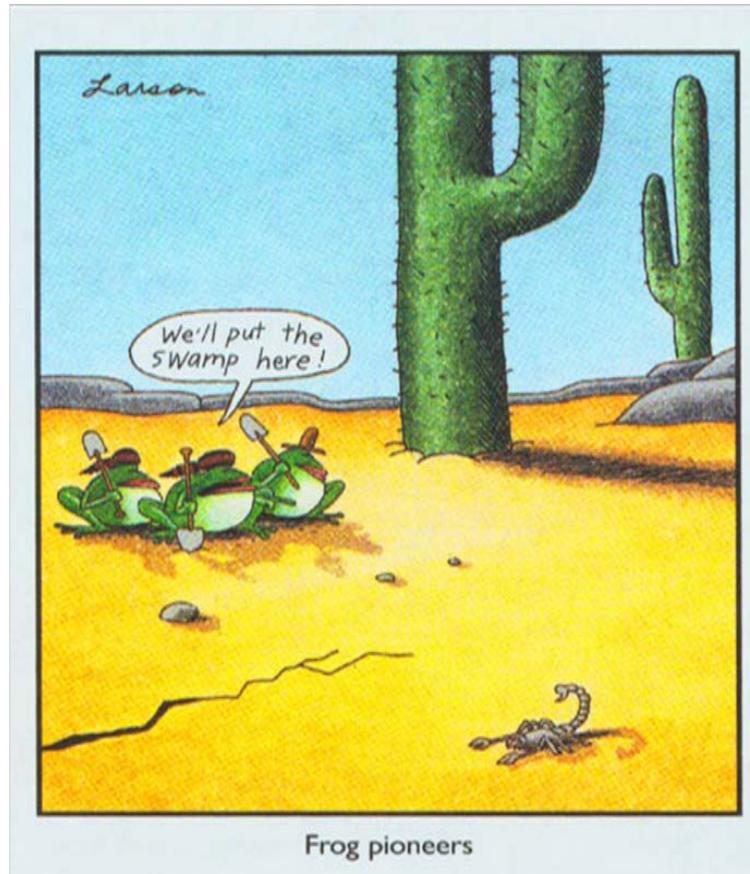
List of abbreviations

Al	Aluminium
AMD	Acid mine drainage
ANOVA	Analysis of variance
As	Arsenic
Au	Gold
B	Boron
Ca	Calcium
Cd	Cadmium
Cr	Chromium
Cu	Copper
CW	Constructed wetland
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
EDS	Energy Dispersive X-Ray Spectrometer
Eh	Oxidation reduction potential
EPA	Environmental Protection Agency
Fe	Iron
Hg	Mercury
HSSF	Horizontal subsurface flow
ICP	Inductively coupled plasma

K	Potassium
M	Molar
Mg	Magnesium
Mn	Manganese
MS	Mass spectrometry
N	Nitrogen
NATA	National Association of Testing Authorities, Australia
NH ₄	Ammonium
Ni	Nickel
OES	Optical emission spectrometry
ORP	Oxidation reduction potential
P	Phosphorus
Pb	Lead
PCR	Polymerase chain reaction
PO ₄	Phosphate
Pt	Platinum
qPCR	Real-time polymerase chain reaction
RNA	Ribonucleic acid
S	Sulfur
Se	Selenium
SEM	Scanning electron microscopy
SF	Surface flow

SO ₄	Sulfate
SRB	Sulfate-reducing bacteria
SSF	Subsurface flow
TOC	Total organic carbon
UV/VIS	Ultraviolet visible
VSSF	Vertical subsurface flow
WHO	World Health Organisation
XRD	X-ray diffraction
Zn	Zinc

Chapter 1: Introduction



Frog pioneers

(Kadlec and Knight, 2006)



Azufre River, Chile

1.1. Introduction

This thesis investigates the removal of arsenic, boron and iron by subsurface flow constructed wetlands. It examines the treatment capabilities of different wetland media and design configurations, and the factors that influence their treatment efficiency. To examine the pollutant removal processes, laboratory studies were performed. Key findings from each study are presented to provide recommendations to enhance the performance of constructed wetlands for As, B and Fe removal, with the ultimate aim of protecting environmental and human health.

1.2. Background

The presence of arsenic and metals in water sources is a worldwide problem. Different treatment technologies have been studied, both conventional and emergent, to mitigate this issue. However, none are completely applicable in all cases, due to one or more drawbacks, limiting their application (Mohan and Pittman Jr., 2007).

Indeed, to date, treatment of arsenic and metal-polluted water has been performed primarily by conventional technologies, such as chemical precipitation. However, these technologies are not well suited to the treatment of effluents from mining sites or other watercourses located in isolated areas, since the often-remote location prohibits the transportation and continuous supply of chemicals that are needed for conventional treatment. In addition, it is well known that conventional treatment systems for metal removal are commonly expensive and present problems due to the elevated amounts of sludge generated and the associated disposal (Cohen, 2006, Kosolapov et al., 2004, Nelson et al., 2006). As such, it is critical to develop onsite treatment systems that have low maintenance requirements and operating costs, to remove arsenic and metals from polluted waters and protect surrounding waterways.

Constructed wetlands are natural treatment systems that have been increasingly applied in wastewater treatment since the mid-1980s, having the advantage of low energy consumption and operating costs (Sun and Saeed, 2009). Although their main

application is the treatment of domestic sewage, their application in the treatment of other effluents such as urban runoff, agricultural and industrial waste is steadily developing (Sun and Saeed, 2009).

Constructed wetlands are engineered systems designed to treat water using largely natural treatment processes (Nuttal et al., 1995). There are two types of constructed wetlands (Wallace and Knight, 2006): surface flow wetlands, in which wastewater flows above the supporting medium (and where the sediment-water interface becomes important), and subsurface flow wetlands, in which wastewater flows through the supporting medium (thus increasing the degree of sediment-water contact), and this flow can be vertical or horizontal. Hybrid wetland systems consist of different wetland types combined to maximise removal. Currently, most hybrid systems employ combinations of horizontal and vertical flow wetland cells (Wallace and Knight, 2006).

Constructed wetlands have the potential to remove metals and metalloids, including arsenic. Current knowledge of metal removal in wetlands has been obtained from studies on acid mine drainage (AMD), where the main pollutants are sulfate, iron and manganese (Mn) (Wallace and Knight, 2006). For this purpose, surface flow wetlands have been most commonly employed and little is therefore known about the performance of subsurface flow wetlands in such applications.

In the USA, wetlands have been an important part of passive treatment systems, providing reliable low cost and low maintenance mine water treatment in remote locations over the past 20 years (Brodie, 1993). The Tennessee Valley Authority has constructed 14 wetland systems to treat acid drainage with successful results. However, due to the interaction between different factors such as pollutant concentrations, substrate, vegetation, etc. design guidelines are difficult to propose (Skousen and Ziemkiewicz, 2005).

Unfortunately, constructed wetlands have not been commonly applied for arsenic removal, due to a lack of understanding on arsenic removal mechanisms (Singhakant et al., 2009). As such, substantial further research is needed before this technology can be confidently applied for this purpose.

The overall objective of this project is therefore to quantify the potential of subsurface flow constructed wetlands to remove arsenic and metals from contaminated water, and to shed light on the removal processes occurring in such systems.

1.3. Research objectives and scope

The main aim of this study is to obtain further understanding of the removal mechanisms for arsenic, boron and iron in subsurface flow constructed wetlands, so as to gain insights into ways of improving treatment efficiency.

This research focuses on the removal of arsenic, boron and iron in subsurface flow wetlands. Whilst the presence of other pollutants such as nutrients and pathogens is also an issue, this thesis is focused on the key factors involved in the removal of these three target pollutants. Furthermore, despite the fact that other heavy metals (copper, manganese and zinc) are targeted in the first experimental stage of the thesis, they are not considered in the rest of the study. The pollutants and their target concentrations were based on a case study, the Azufre River in Chile, in order to ensure a reality and representativeness of the testing. Only subsurface flow wetlands are investigated, since their potential in the removal of metals and metalloids has been barely explored. Related issues to the application of wetlands for this purpose, such as clogging of the media and effect of hydraulic parameters on the wetland performance cannot be tackled in this research, since the main focus is the understanding of the As, B and Fe removal mechanisms. Only one vegetation type -*Phragmites australis*- will be used throughout the thesis, given its worldwide distribution and tolerance to a wide pH range (Wallace and Knight, 2006). This thesis uses water quality analysis to identify treatment processes occurring within subsurface flow constructed wetlands. Since plant uptake plays a minor role in As and metal removal in constructed wetlands (García et al., 2010), chemical analysis of As, B and Fe in plant tissues was only performed in the last experimental stage, so as to verify its role in the specific context of the target pollutants and wetland media being used.

1.4. Thesis outline

The structure of the thesis is presented in Figure 1.1. Chapter 2 presents a review of the literature, identifying current knowledge gaps. This Chapter begins with an overview of the worldwide problem of the contamination of water resources by arsenic, boron and iron, providing relevant information about these pollutants. Then, the concept of constructed wetlands and their application for arsenic and metals removal is presented. A review of the processes that remove arsenic, iron and boron in constructed wetlands is conducted. The long term performance for arsenic and metal removal is discussed. Key knowledge gaps addressed in this thesis are identified and objectives of the research are provided. Finally, conclusions from the literature review are drawn.

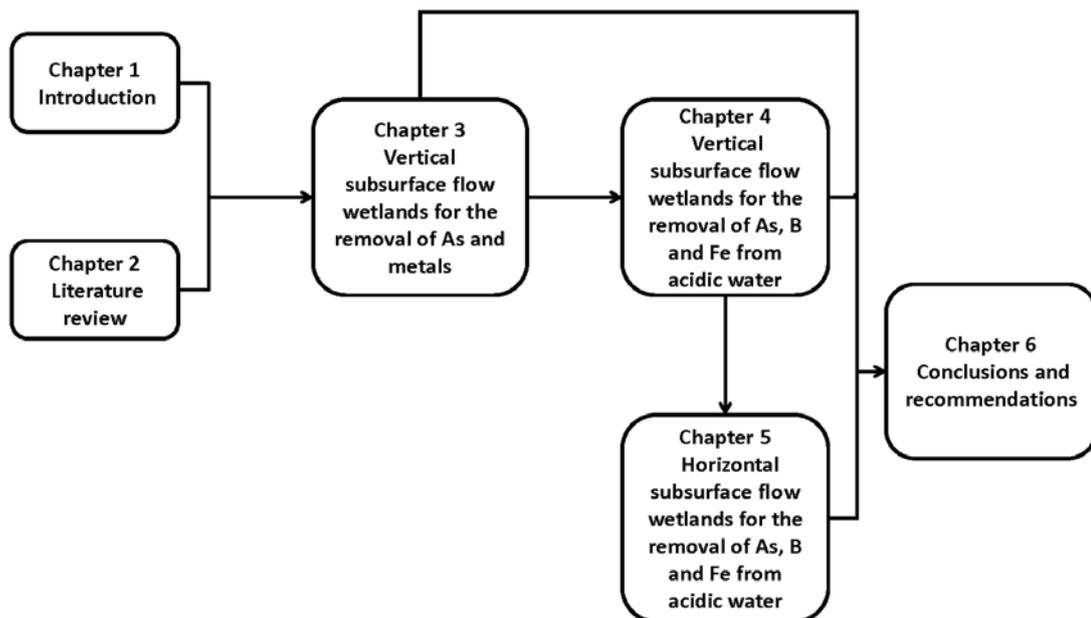


Figure 1.1. The thesis structure.

Chapter 3 reports on the performance of vertical subsurface flow constructed wetlands using different wetland media. Laboratory-scale wetlands were built and operated with the aim of removing arsenic and metals. Water quality monitoring together with

chemical analysis were performed, to examine the pollutant removal efficiencies and the main factors that impact those efficiencies.

In Chapter 4, similar experiments to those in Chapter 3 were performed, but using a different type of water: highly polluted, acidic wastewater, simulating the Azufre River, the case study chosen for this research. The capability of vertical flow constructed wetlands, using different wetland media, to remove arsenic, boron and iron from this water was assessed.

The results obtained in Chapter 4 identified the most effective wetland media to remove As, B and Fe from acidic water. Using these as the main media, horizontal subsurface flow wetlands were constructed and tested, so as to understand the removal of the target pollutants by using effective media when applied in these systems. The results are provided in Chapter 5. Thus, Chapter 5 reports on the performance of horizontal flow wetlands with effective, alternative wetland media previously tested in vertical flow wetlands.

In Chapter 6, the findings from the laboratory studies are drawn together, alongside strengths and weaknesses, to provide key lessons learnt and identify remaining knowledge gaps. Conclusions and future research needs are presented.

1.5. References

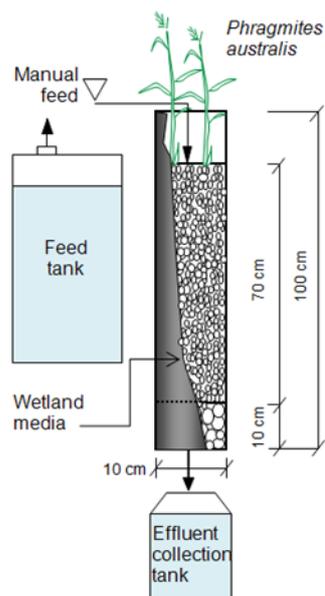
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Chapter 3: Vertical subsurface flow wetlands for the removal of As and metals



DECLARATION FOR THESIS CHAPTER 3

Declaration by candidate

In the case of Section 3.8, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Initiation, ideas, experimental design and works, data analysis, write up	80

The following co-authors contributed to the work:

Name	Nature of contribution	Extent of contribution (%)
Guangzhi Sun	Ideas and reviewing	10
Tim D. Fletcher	Ideas and reviewing	10

**Candidate's
Signature**

	Date 01/02/2013
--	---------------------------

Declaration by co-authors

The undersigned hereby certify that:

- (1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
- (2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
- (3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility
- (4) there are no other authors of the publication according to these criteria;
- (5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
- (6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location

School of Engineering & Physical Sciences, James Cook University

Signature
Guangzhi Sun



Date
01/02/2013

Location

Waterway Ecosystem Research Group. Melbourne School of Land & Environment. The University of Melbourne

Signature
Tim D. Fletcher



Date
01/02/2013

3.1. Introduction

As presented in the literature review (Chapter 2), although constructed wetlands are commonly used to treat an array of heavy metals, there is little reported literature on the specific use of wetlands for As removal. There are some studies on the use of surface flow wetlands for As removal, but few using subsurface flow wetlands. In subsurface flow wetlands, water flows through the supporting media ('substrate'), allowing extensive contact between the water and the media. Furthermore, the media can provide reactants to facilitate particular removal processes (Kadlec and Wallace, 2009), thus enhancing the overall performance.

Since the use of alternative media has been proposed, but not well investigated, the first objective of this research was to study the effect of four different wetland media on the removal of six target pollutants: As, Fe, B, Cu, Mn and Zn. A review of suitable supporting media was conducted (Section 3.2), and based on this review, four media were selected and compared. In particular, the effect of alkalinity, ion exchange and organic matter of limestone, zeolite and cocopeat were compared with a conventional gravel substrate.

The principal research questions and hypotheses are:

Q1: What is the role of the supporting media in subsurface flow wetlands in the removal of the target pollutants?

The type of wetland media is a main factor affecting the removal of the target pollutants

Q2: Can alternative media enhance arsenic and metal removal?

Alternative media, such as those enhanced using organic matter, or pH buffering material, can be more effective than conventional gravel since they can promote certain reactions that will retain pollutants in the wetland matrix

Q3: Which are the key removal mechanisms involved?

Arsenic removal mechanisms are different to those of the other target pollutants, given the chemical reactivity of arsenic

A laboratory-scale wetland system was built and operated in order to answer these questions. The main results of these experiments were presented at the 12th *International Conference on Wetland Systems for Water Pollution Control* and this paper was selected for publication in *Water Science and Technology*. Most sections in this paper have been integrated into this chapter. However, the complete paper is also included at the end of the chapter (Section 3.7). This chapter also presents additional analyses that were not included in that paper. The chapter concludes with summary of the findings and implications for the next experimental stage of the thesis.

3.2. Review of supporting media for the removal of arsenic and metals

Four different media have been chosen for this experimental stage due to their particular characteristics. This section discusses their role in the removal of arsenic and metals and thus the rationale for their selection. These materials were prioritised against slags and peat (described in Chapter 2) due to their potential for arsenic and metal removal, availability and easy adoption.

3.2.1 Gravel

Gravel is rock material of a given size range; according to the Wentworth scale, fine gravel has 4-8 mm diameter, coarse gravel 16-32 mm (Wentworth, 1922). Gravel is the most common supporting media used in constructed wetlands. Furthermore, it has been used for the removal of metals (Dunbabin and Bowmer, 1992), including for the removal of As (Buddhawong et al., 2005, Singhakant et al., 2009). Buddhawong et al.

(2005) used grain size 0.2-0.6 cm, whilst Singhakant et al. (2009) employed 1.25-2.5 cm (small), 2.5-5 cm (large), and sand.

However, Kadlec and Wallace (2009) did not recommend the use of subsurface sand/gravel wetlands for the removal of metals, due to their limited sorption capacity and the inability to form new storage sediments without clogging the bed. Moreover, they recommended the use of peat for metal removal, based on the study of Sartaj et al. (1999) (section 3.2.2). In addition, Buddhawong et al. (2005) also reported that the As adsorption capacity of gravel was very low, and therefore other processes must have been responsible for the removal of As in gravel bed systems (Buddhawong et al., 2005). They attributed the high removal in the gravel systems to the presence of Fe on the surface of the gravel, which could have directly sorbed all the As.

This evidence suggests that gravel can be used for the removal of metals, but it may not be as effective as other supporting media, which could enhance other removal processes. However, gravel provides surface sites for the attachment of particles, minerals and biofilms. Furthermore, gravel has significant advantages in terms of low cost (Kadlec and Wallace, 2009), large pore spaces (Steiner and Freeman, 1989) and excellent structural stability, making it worthy of consideration, at least as the structural base of any substrate mix. In fact, gravel is commonly recommended for subsurface flow wetlands (Steiner and Freeman, 1989).

3.2.2 Peat

Peat is partly fossilised plant organic matter, usually of a dark brown colour. Peat has a pH around 4 due to the presence of humic acids. It degrades over pH 9, and its chelation properties decrease under pH 3 (Couillard, 1994).

Several authors have reported the use of peat as a sorbent for the removal of metals, such as Cu, Zn, Fe, Mn, Pb, Cr, Al and Hg (Snoeyink and Jenkins, 1980, Couillard, 1994, Brown et al., 2000). Even though peat has been mostly used as a sorbent medium, it has been used as a substrate in constructed wetlands for the removal of Cu

from acid mine drainage (Sobolewski, 1996) and B from landfill leachate (Sartaj et al., 1999). It has been found that peat is able to retain As (Cloy et al., 2009), but it appears that it has not been employed in constructed wetlands to enhance As removal.

The removal of Cu from mine drainage depends on the nature of the mine drainage: for low strength mine drainage (0.1-3 ppm Cu, pH 6-8), Cu is mostly removed as a sulfide, whilst for high strength mine drainage (40-50 ppm Cu, pH 3), Cu is mainly organically bound (Sobolewski, 1996). Sobolewski (1996) concluded that peat-based wetlands may not be effective in treating acidic mine drainage, probably because it is generally accepted that sulfide precipitation is a long term removal mechanism in constructed wetlands (Lett and Fletcher, 1980, Gammons and Fradsen, 2001), whilst organic-binding is a finite process, reducing once all available binding sites become occupied (Sobolewski, 1996). It has been found that for low strength mine drainage, the removal of Cu by peat exceeded 98%, and this value was not affected by spikes in influent Cu up to 10 ppm (Sobolewski, 1996). On the other hand, for high strength mine drainage, the removal of Cu was effective only for the first 6-8 weeks, and after that the removal decreased markedly in the autumn months. The author argued that it is difficult to determine whether this decrease was due to the strength of the drainage or to the effect of cold conditions on biological activity. The author used SEM-EDS to analyse samples of the peat substrate and found evidence that suggested the formation of chalcopyrite CuFeS_2 . This was interesting because the conventional view is that this mineral is formed over geological time, under high temperature and pressure conditions (Sobolewski, 1996). As such, Sobolewski (1996) suggested studying the formation process of this mineral because it could be possible that a rapid formation of chalcopyrite occur in experimental wetlands with a peat substrate.

As discussed above, the metal removal mechanisms of peat are not completely understood. However, it is commonly believed that metals react with the carboxylic and phenolic acid groups of the fulvic and humic acids (Brown et al., 2000).

Peat has been shown to have a great affinity for boron (Sartaj et al., 1999, Sartaj and Fernandes, 2005). Sartaj et al. (1999) reported B removal from 15 mg/L to 1.34 mg/L on average, in a peat filter receiving landfill leachate. This filter was the first cell of an

engineered wetland, and it served as a vertical flow subsurface system. However, this cell was a filter and not a wetland cell, because no vegetation was present. Furthermore, batch adsorption tests were performed to find out the adsorption capacity of peat, so as to estimate the peat requirement. It was found from batch adsorption tests that 1 gram of peat was able to remove at least 0.1 mg of B, Fe and Pb (i.e. adsorption capacity higher than 0.1 mg/g). This fact could indicate that the authors considered adsorption as the primary removal mechanism in the system.

Sartaj and Fernandes (2005) concluded that the presence of organic matter contributed to B adsorption in soils, but there is not much information on the adsorption of B by organic soils such as peat. These authors studied the adsorption of B by peat in batch and columns experiments -not as a substrate in constructed wetlands- and they found that the main factor that affected B adsorption was the pH. Moreover, they suggested the addition of lime to peat to increase the pH (Sartaj and Fernandes, 2005), based on the results of Sartaj and Fernandes (2000). In this study, the adsorption capacity of compost and peat was compared, and lime was added to both of them up to pH 9, which increased the adsorption capacity (Sartaj and Fernandes, 2000).

From the literature presented above, it could be asserted that the main boron removal mechanism in peat substrates is sorption, enhanced by high pH.

Even though peat seems to be readily available in Europe and North America, in Australia there are problems in terms of supply availability, due to restrictions on peat mining in protected areas (Morrish and Hofstede, 2000). Other alternatives to peat are coconut husk carbon and coconut peat. Coconut husk carbon was found to effectively remove As(III) (Manju et al., 1998), whilst coconut peat has not been employed for metal removal apart from this study. Other organic substrates such as wooden mulch have been used to provide a carbon source in wetlands (Saeed and Sun, 2011). Due to their organic matter content, coconut peat could be hypothesised to have a similar effect to that of peat on the removal of metals. In addition, organic substrates provide a carbon source for heterotrophic bacteria such as sulfate-reducing bacteria and metal oxidising bacteria, which as discussed in Chapter 2, can play a key role in the removal of As and metals.

This research will thus investigate the use of cocopeat instead of peat, taking into account the potential loss of diverse wildlife habitats of rare or endangered plants and animal species caused by the extraction of peat (Morrish and Hofstede, 2000). We thus work on the hypothesis that cocopeat has similar key properties to peat, mainly due to the content of organic carbon: peat has between 12 and 60% of organic carbon (Lucas, 1982), whereas cocopeat has 64% organic carbon (643 g/kg, as reported by the laboratory analysis).

3.2.3 Zeolite

Zeolites are aluminosilicates which can occur as a natural mineral or can be synthesised artificially (Sakadevan and Bavor, 1998). Zeolites have affinity for ammonium and other cations, mostly because of their high ion exchange capacity (Yalcuk and Ugurlu, 2009). They are selective with certain metal ions such as Cd, Fe, Mn, Pb, Ni and Zn (Yalcuk and Ugurlu, 2009). Furthermore, natural zeolites, especially clinoptilolite, have been studied for the removal of metals due to their low cost and availability (Pitcher et al., 2004).

The most common application of zeolite is as a filter medium, for example in stormwater biofiltration (Kandra et al., 2012) and wastewater treatment (Wang and Peng, 2010, Tchobanoglous et al., 2003). However, a few researchers have studied it as a constructed wetland medium. Yalcuk and Ugurlu (2009) used zeolite (clinoptilolite) in a vertical subsurface constructed wetland for the treatment of landfill leachate (Yalcuk and Ugurlu, 2009). The use of zeolite decreased $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, Fe and Pb concentrations, but Cr and Zn concentrations were higher in the outflow. The authors concluded that Cr and Zn were washed out from the system, but no further explanation was provided. On the other hand, Sarafraz et al. (2009) showed that the use of zeolite in a horizontal subsurface enhanced Zn sorption, and proposed zeolite as an alternative to sand and gravel (Sarafraz et al., 2009).

Natural and synthetic zeolites have been found to remove As in batch reactors, but not in constructed wetlands. Elizalde-González et al. (2001) found that three natural zeolites -clinoptilolite, erionite and modernite, and synthetic mordenite- all removed arsenite and arsenate. Furthermore, the natural zeolites oxidised arsenite to arsenate, probably due to the sorption of O₂ and to the presence of Fe⁺³ and Mn⁺⁴ (Elizalde-González et al., 2001). Chutia et al. (2009) studied the removal of arsenate using two different synthetic zeolites. According to the authors, the removal capacity of zeolites is due to exchange between aluminol or silanol hydroxyl groups and adsorbate anionic species (Chutia et al., 2009). Payne and Abdel-Fattah (2005) used natural (chabazite and clinoptilolite) and synthetic zeolites; all of them coated with Fe (iron-treated), to study the removal of arsenite and arsenate (Payne and Abdel-Fattah, 2005). The authors believed that the removal mechanism was surface complexation between Fe sites and arsenite (arsenate) species rather than solid precipitates. This hypothesis was supported by XRD (x-ray diffraction) and FTIR (Fourier transform infrared spectroscopy) measurements and by the stable removal results obtained under a wide pH range. If the removal mechanism were electrostatic, the removal would have been unstable (Payne and Abdel-Fattah, 2005). However, the authors found that the removal was higher at a higher pH -between 7 and 11- for both arsenite and arsenate, and they attributed it to the presence of Fe hydroxides attached to the surface of the zeolites. Above pH 3, these hydroxides are present as Fe(OH)₂⁺ and arsenate is present as anions (as discussed in Chapter 2), whilst alkaline conditions are required for arsenite removal (above pH 7) (Payne and Abdel-Fattah, 2005).

From the literature, it is apparent that zeolite has not been used as a supporting medium in constructed wetlands aiming to remove As. However, there is enough evidence to hypothesise that the removal of As could be enhanced using zeolite as the supporting medium.

3.2.4 Limestone

Limestone is a sedimentary rock composed largely of carbonate minerals, especially carbonates of calcium and magnesium (Oxford University Press, 1990). In wetlands,

limestone is mostly used for the treatment of acid mine drainage, as a preliminary system for raising pH (Wallace and Knight, 2006). Anoxic limestone drains or permeable reactive barriers containing limestone are used for that purpose, required for the removal of Fe and Mn (Wallace and Knight, 2006). However, the removal of other metals requires a high pH as well. In particular, the use of limestone beds for the removal of zinc from alkaline mine waters has been studied (Younger, 2000, Nuttall and Younger, 2000). However, these systems cannot be considered as wetlands due to the lack of vegetation.

Apparently, limestone has been tested only as part of the supporting medium in constructed wetlands for the removal of metals, but not as the only material in the medium. Groudev et al. (2008) reported the use of crushed limestone in a mixture with soil, silt, compost, cow manure and sand as the supporting medium for four constructed wetlands, designed for the treatment of acid mine drainage generated in a uranium deposit (Groudev et al., 2008). Haffner (1992) mixed mushroom compost, peat and limestone in sixteen wetland cells, designed for the removal of N, Mn and Zn. Duncan (2002) employed crushed limestone and wood pulp in three surface flow wetlands, designed for the removal of As, Cd and Zn. Unfortunately, none of these studies give further information on the role of the limestone in the supporting media. Ye et al. (2003) suggested the use of crushed limestone because it increased the removal of metals from acidic mine waste. However, the authors based this claim on the study of Stark et al. (1996), in which only the removal of Mn was studied. In this study, the performance of crushed limestone and spent mushroom compost was compared, and the removal of Mn was higher in the crushed limestone wetland, due to the higher pH achieved in this wetland (Stark et al., 1996).

There are, however, some possible disadvantages in the use of limestone. In wetlands, it has been found that limestone can cause chlorosis in newly planted vegetation (Nuttall et al., 1995), and the surface of limestone gravel can foul with Al or Fe precipitates (Wallace and Knight, 2006). Moreover, Groudev et al. (2008) reported that the limestone was disabled due to the Fe precipitates on its surface, and therefore the generation of alkalinity decreased steadily. It should be noted that the authors only referred to the role of limestone in permeable reactive barriers and not in constructed

wetlands. Furthermore, they mentioned sulfate microbial reduction and solubilisation of acid-consuming minerals as the main sources of alkalinity in the constructed wetlands, rather than limestone. A possible explanation is that the effect of these sources on the generation of alkalinity is greater than that of the limestone, and perhaps the amount of limestone in the mixture employed as a medium was small compared to that of the other components.

According to the literature, it is likely that limestone provides a good way to enhance the removal of metals by increasing the pH if used as a wetland supporting medium.

In this study, the performance of gravel will be compared with a range of other supporting media, tested in isolation. While in practice media are more likely to be used in combinations, testing them individually allows their performance and behaviour to be understood, prior to considering optimal mixes of media (in Chapter 4).

This research will therefore use the following as main supporting media in vertical flow wetlands:

- White/grey pebbles obtained from a local distributor (Monash Garden Gear), 7 mm median size
- Coir husk chips obtained from a local supplier (Ratoonmat), 4-6 mm median size
- Commercial zeolite filter media (Zeolite Australia Pty Ltd.), 6 mm median size
- High calcium limestone chips donated by a local producer (Unimin Australia Limited), size range: 1-3 mm

3.3. Materials and methods¹

3.3.1 The wetland system

Laboratory-scale wetlands were constructed, consisting of twelve subsurface vertical flow wetland columns that were built using stormwater PVC pipes. Each column had 1 m height and 100 mm internal diameter and was installed in a greenhouse. The wetland columns were divided into four groups, namely group G -employing gravel as main substrate, Z -zeolite as the main substrate, C -cocopeat as the main substrate, and L -crushed limestone as main substrate; each group had three replicate columns. Each column had a drainage layer of 20-40 mm diameter pebbles to a depth of 100 mm at the base. The drainage layer was topped with a layer of main substrates (G, Z, C, or L) to a depth of 700 mm. In each wetland column, common reed (*Phragmites australis*) was planted. The *Phragmites* were given two months to adapt to their new growth environment prior to the experiment.

3.3.2 Operation of the wetland system

Synthetic wastewater was prepared to simulate the concentration of the target pollutants in polluted surface waters in Chile (Dirección General de Aguas, 2008, Romero et al., 2003). The synthetic wastewater was prepared using tap water, with the following reagents added per litre of water: 1 mL 1,000 mg/L arsenic standard solution (As_2O_5 in H_2O), 0.025 mL 10,000 mg/L boron standard solution (H_3BO_3 in H_2O), 125 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 7.2 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 3.9 mg $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$, 4.4 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.7 mg $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$. The total concentration of the metals were (average \pm standard deviation): 0.89 ± 0.05 mg/L As, 24.0 ± 0.0 mg/L B, 1.43 ± 0.40 mg/L Cu, 21.0 ± 1.4 mg/L Fe, 2.38 ± 0.82 mg/L Mn and 1.25 ± 0.44 mg/L Zn. An agitated feed tank stored the wastewater during the experiment. From it, two litres of synthetic wastewater were taken and dosed manually in each wetland, three times per week in the first month and twice per week in the second month. These dosing rates fell in the range of hydraulic

¹ Section 3.3.1 to 3.3.3 were mainly extracted from Lizama Allende et al. (2011)

loading rates used in practice (7-500 mm/d) (Water Pollution Control Federation, 1990).

3.3.3 Sampling and analysis

After each dosing, water samples from the outlet of each wetland column and from the influent feed tank were collected and acidified with nitric acid (HNO₃) to pH < 2 for total and dissolved metals analysis. For dissolved analyses, approximately 100 ml of each sample was filtered through 0.45 µm cellulose acetate filters. Weekly composite samples were prepared, adding an equal volume of each corresponding daily sample for every week. Metal concentrations in both total and dissolved composite samples for weeks 1, 3, 5 and 7 were determined by ICP-MS in a NATA accredited laboratory (4 values per column plus the inflow). *In-situ* parameters were also monitored after each dosing (20 values per column plus the inflow). Dissolved oxygen (DO) was measured using HACH 51970 probe, whilst for pH and conductivity HACH 51910 and 51975 probes were used, respectively. All these probes were connected to a Sension 378 meter. An ORP Testr10 probe was used to measure redox potential (Eh). Sulfate (SO₄) was measured using HACH DR5000 UV/VIS spectrophotometer based on an adapted standard method (APHA et al., 2005) equivalent to US EPA procedure 375.4 for wastewater. Alkalinity was quantified using a HACH alkalinity test kit, low range (5-100 mg/L) and high range (20-400 mg/L) tests.

3.3.4 Statistical analysis

To evaluate the role of the different wetland media in the removal of the target pollutants, statistical tests were performed using PASW Statistics 19 (IBM Corporation, 2011) with a significance level of $\alpha=0.05$ adopted. Prior to this testing, Kolmogorov-Smirnov test was performed to check data normality, with non-parametric tests applied in the case where normality was not satisfied (see details below).

The effect of different wetland media on the treatment was statistically assessed by one-way ANOVA to compare the mean concentrations of target pollutants in gravel, cocopeat, zeolite and limestone wetland columns, as well as the environmental parameters in the columns. When significant difference caused by the media was found ($p_A < 0.05$), multiple comparison post-hoc tests were performed to distinguish which wetland media were significantly different: Tukey's test was applied when the assumption of homogeneity of variances was satisfied (as determined by Levene's test), or Games-Howell's test when this assumption was not satisfied (notation pT and pGW, respectively).

If the assumption of normality was not met, Kruskal-Wallis analysis was performed instead of ANOVA. When significant difference was found ($p_{KW} < 0.05$), Mann-Whitney post hoc tests were performed to distinguish the mean differences which were significantly different ($p_{MW} < 0.05$).

To assess a possible joint removal of As and Fe, Pearson's correlation coefficient was calculated for outflow concentrations of As and Fe in the same wetland group. Pearson's coefficient was used given the normality of the corresponding set of data (Table 3.1).

Table 3.1. Kolmogorov-Smirnov test results. Bold type denotes significant difference.

p value K-S test	Gravel	Cocopeat	Zeolite	Limestone
As total	0.988	0.184	0.888	0.419
Fe total	0.454	0.454	0.983	0.901

3.4. Results

3.4.1 Overall performance²

As shown in Table 3.2, the average concentration of all the target pollutants in the outflow from the gravel wetland columns was higher than that from other columns, demonstrating that the three types of alternative wetland substrate had greater pollutant removal efficiencies than the traditional gravel substrate. Furthermore, gravel appeared to have limited capability to remove As, Fe, Cu and Zn, and almost no capability to remove B or Mn.

Table 3.2. Mean inflow and outflow concentrations of the target pollutants

	Mean inflow concentration (mg/L) [CV*]		Mean outflow concentration (mg/L) [CV*]							
	dissolved	total	G - gravel		C - cocopeat		Z - zeolite		L – limestone	
			dissolved	total	dissolved	total	dissolved	total	dissolved	total
As	0.001 [0.952]	0.890 [0.062]	0.001 [1.13]	0.253 [0.685]	0.011 [0.149]	0.013 [0.121]	0.008 [0.347]	0.011 [0.431]	0.006 [0.145]	0.009 [0.409]
B	23.5 [0.04]	24.0 [0.0]	23.5 [0.06]	24.3 [0.06]	22.0 [0.27]	22.17 [0.28]	21.33 [0.12]	21.67 [0.09]	21.67 [0.11]	21.92 [0.11]
Cu	0.047 [0.699]	1.425 [0.283]	0.34 [0.75]	0.462 [0.69]	0.012 [0.37]	0.014 [0.386]	0.007 [0.271]	0.009 [0.29]	0.013 [0.513]	0.024 [0.375]
Fe	UD**	21.0 [0.07]	0.08 [0.44]	6.7 [0.59]	0.095 [0.47]	0.238 [0.281]	0.047 [0.686]	0.246 [0.615]	0.023 [1.457]	0.205 [0.575]
Mn	2.275 [0.33]	2.30 [0.32]	2.21 [0.24]	2.233 [0.25]	0.044 [0.73]	0.046 [0.761]	0.007 [0.286]	0.016 [0.466]	0.131 [0.627]	0.143 [0.576]
Zn	0.723 [0.473]	1.245 [0.353]	0.043 [1.44]	0.664 [0.57]	0.012 [0.19]	0.013 [0.18]	0.008 [0.216]	0.01 [0.273]	0.003 [0.425]	0.008 [0.364]

*CV=coefficient of variation= σ/μ **UD: undetectable. For samples under the detection limit (0.001 mg/L for As and 0.02 mg/L for Fe), half of that value was considered for the calculations.

² Mainly extracted from Lizama Allende et al. (2011)

The variability in the monitored parameters is presented in Table 3.3.

Table 3.3. Mean inflow and outflow levels of monitored water quality parameters

Mean value in the inflow [CV*]		Mean value in the outflow [CV]				
Parameter	Unit	Inflow value	Gravel	Cocopeat	Zeolite	Limestone
SO ₄	mg/L	74.7 [0.083]	67.3 [0.136]	65.7 [0.07]	56.5 [0.087]	56.2 [0.098]
pH	-	5.77 [0.976]	4.95 [0.936]	5.67 [0.995]	6.13 [1.025]	7.95 [1.001]
DO	mg/L	6.3 [0.205]	6.3 [0.217]	6.1 [0.2]	6.2 [0.192]	5.9 [0.215]
T	°C	24.2 [0.121]	24.4 [0.139]	24.4 [0.133]	24.7 [0.125]	25 [0.124]
Alkalinity	mg/L CaCO ₃	14.3 [0.172]	9.3 [0.27]	11.4 [0.243]	10.8 [0.189]	155.4 [0.619]
Eh	mV	195.2 [0.082]	225.4 [0.15]	235.2 [0.137]	233.4 [0.129]	181.5 [0.143]
Conductivity	µS/cm	198.6 [0.068]	217.7 [0.112]	176.5 [0.103]	185.2 [0.459]	290.8 [0.041]

*For the pH values, the CV was calculated using $CV = -\log\{\sigma\} / -\log\{\mu\}$, where σ is the average of $\{H^+\}$ and μ is the standard deviation of $\{H^+\}$

3.4.2 Removal trends³

3.4.2.1 Arsenic

Cocopeat, zeolite and limestone wetlands presented high As removal rates (average above 98%) for the entire experimental period, whereas for gravel wetlands the rates decreased over time, both in percentage terms and as daily mass removed per volume. In addition, the removal of As in terms of mass was almost the same for the alternative

³ Graphs adapted from Lizama Allende et al. (2011)

wetland media, and it only decreased when the inflow concentration decreased (Figure 3.1a).

Significant differences were found for both dissolved and total As concentrations in the inflow and the outflow ($pA < 0.001$). As presented in Table 3.4, the levels of As were significantly higher in the outflow from gravel wetlands than from any of the other media. A significant difference in dissolved As levels was found between the two most promising media (limestone and zeolite), but this was not observed for total As. Cocopeat wetlands presented significantly higher concentrations than limestone for both total and dissolved As, but not compared to zeolite.

Table 3.4. Comparison of dissolved and total As concentration in the inflow of the wetland system and the outflow of each wetland group using Games-Howell post hoc tests. Bold type denotes significant difference.

	Inflow		Gravel		Cocopeat		Zeolite	
	As dissolved (mg/L)	As total (mg/L)	As dissolved (mg/L)	As total (mg/L)	As dissolved (mg/L)	As total (mg/L)	As dissolved (mg/L)	As total (mg/L)
Gravel	0.998	<0.001	-	-	-	-	-	-
Cocopeat	<0.001	<0.001	<0.001	0.004	-	-	-	-
Zeolite	<0.001	<0.001	<0.001	0.004	0.111	0.312	-	-
Limestone	0.011	<0.001	<0.001	0.004	<0.001	0.027	0.046	0.954

3.4.2.2 Iron

The removal of Fe presented a very similar trend to that of As: cocopeat, zeolite and limestone wetlands removed around 99%, but removal in the gravel wetlands decreased with time (Figure 3.1b).

Even though the type of substrate significantly affected the total concentration of Fe ($p_A < 0.001$), no significant differences were found between the alternative media (Table 3.5), with gravel being the only substrate which was significantly different to the others ($p_{GW}=0.001$ for cocopeat, zeolite and limestone).

Table 3.5. Comparison of total Fe concentration in the inflow of the wetland system and the outflow of each wetland group using Games-Howell post hoc tests. Bold type denotes significant difference.

	Inflow	Gravel	Cocopeat	Zeolite
	Fe total (mg/L)	Fe total (mg/L)	Fe total (mg/L)	Fe total (mg/L)
Gravel	<0.001	-	-	-
Cocopeat	<0.001	0.001	-	-
Zeolite	<0.001	0.001	1	-
Limestone	<0.001	0.001	0.956	0.963

3.4.2.3 Boron

Boron was removed by cocopeat, zeolite, limestone and gravel wetlands at the beginning of the experiment, but was then leached out by cocopeat and gravel wetlands (Figure 3.2a). Despite this, no significant differences were detected between the inflow and outflow concentrations of B ($p_{KW}=0.358$ for total B and $p_{KW}=0.905$ for dissolved B).

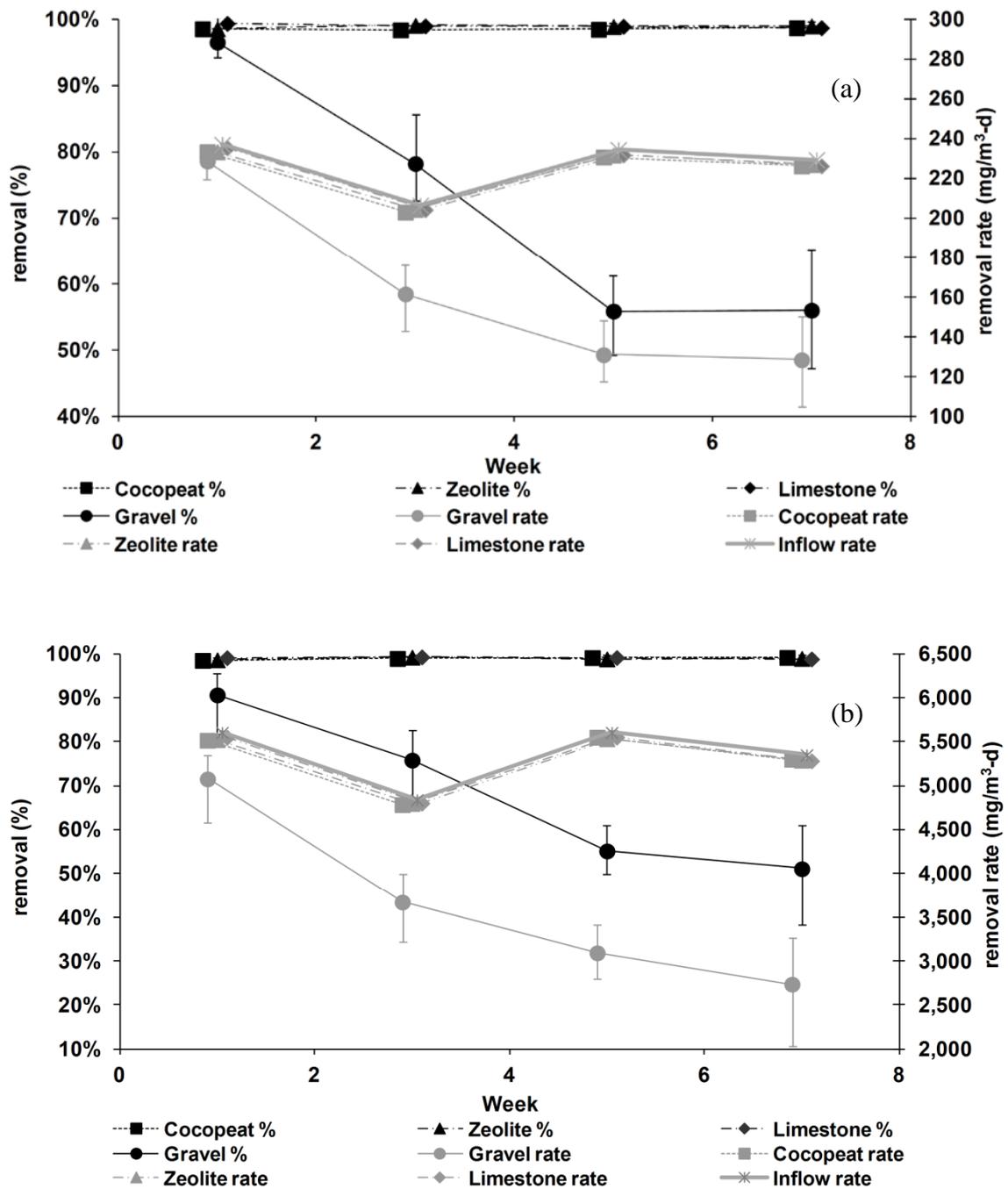


Figure 3.1. Mean removal rates of total As (a) and Fe (b) over time, expressed as percentage (principal axis) and as daily mass removed per unit of volume of wetland (secondary axis). Error bars indicate the corresponding minimum and maximum values of the three replicates.

3.4.2.4 Manganese

Gravel wetlands barely removed Mn, and often presented negative removal. As shown in Figure 3.2b; cocopeat, zeolite and limestone wetlands removed around 94% total Mn on average ($pKW < 0.001$; Table 3.6), suggesting that the wetland media played a significant role. Furthermore, both total and dissolved concentrations of Mn in the outflow from each wetland group were significantly different to those from the others.

Table 3.6. Comparison of dissolved and total Mn concentration in the inflow of the wetland system and the outflow of each wetland group using Mann-Whitney post hoc tests. Bold type denotes significant difference.

	Inflow		Gravel		Cocopeat		Zeolite	
	Mn dissolved (mg/L)	Mn total (mg/L)	Mn dissolved (mg/L)	Mn total (mg/L)	Mn dissolved (mg/L)	Mn total (mg/L)	Mn dissolved (mg/L)	Mn total (mg/L)
Gravel	0.684	0.770	-	-	-	-	-	-
Cocopeat	0.004	0.004	<0.001	<0.001	-	-	-	-
Zeolite	0.003	0.004	<0.001	<0.001	<0.001	<0.001	-	-
Limestone	0.004	0.004	<0.001	<0.001	0.019	0.002	<0.001	<0.001

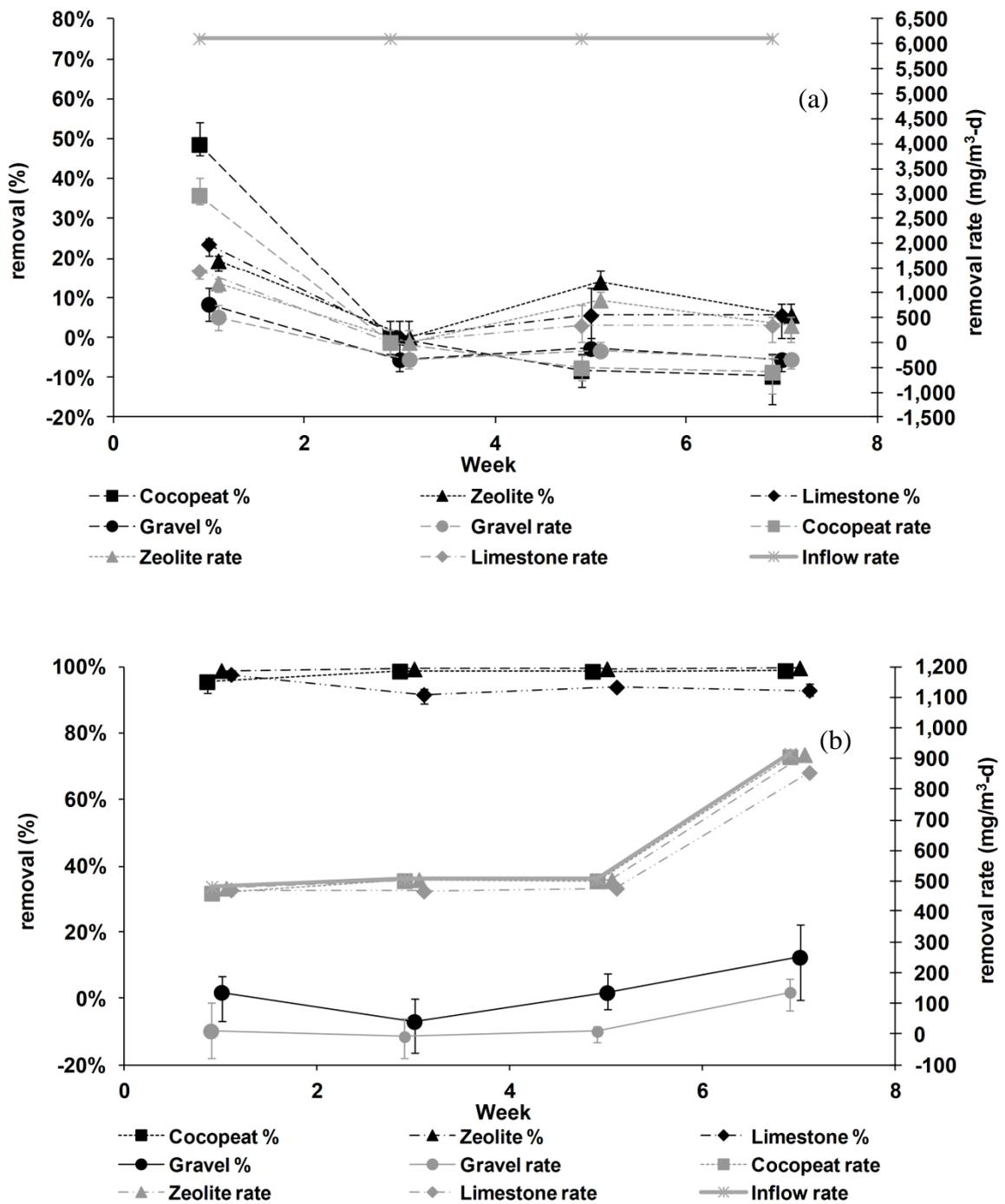


Figure 3.2. Mean removal rates of total B (a) and Mn (b) over time, expressed as percentage (principal axis) and as daily mass removed per unit of volume of wetland (secondary axis). Error bars indicate the corresponding minimum and maximum values of the three replicates.

3.4.2.5 Copper

High removal rates were observed in cocopeat, zeolite and limestone wetlands for Cu, regardless of fluctuations in inflow concentration (Figures 3.3a). In contrast, decreasing removal rates over time were observed in gravel wetlands, despite the fact that the daily mass removal rate increased when the inflow concentration increased.

The wetland media had a significant effect on the removal of Cu ($pA < 0.001$), since each type of wetland had a particular effect on the levels of Cu in the outflow (Table 3.7). Even though zeolite wetlands presented the lowest levels in the outflow (Table 3.2), the total concentration of Cu was not significantly different to the one from cocopeat wetlands, which presented the second lowest concentration.

Table 3.7. Comparison of dissolved and total Cu concentration in the inflow of the wetland system and the outflow of each wetland group using Games-Howell post hoc tests. Bold type denotes significant difference.

	Inflow		Gravel		Cocopeat		Zeolite	
	Cu dissolved (mg/L)	Cu total (mg/L)						
Gravel	0.019	0.046	-	-	-	-	-	-
Cocopeat	0.399	0.005	0.009	0.005	-	-	-	-
Zeolite	0.307	0.004	0.008	0.004	0.011	0.086	-	-
Limestone	0.414	0.006	0.009	0.006	0.998	0.031	0.067	0.001

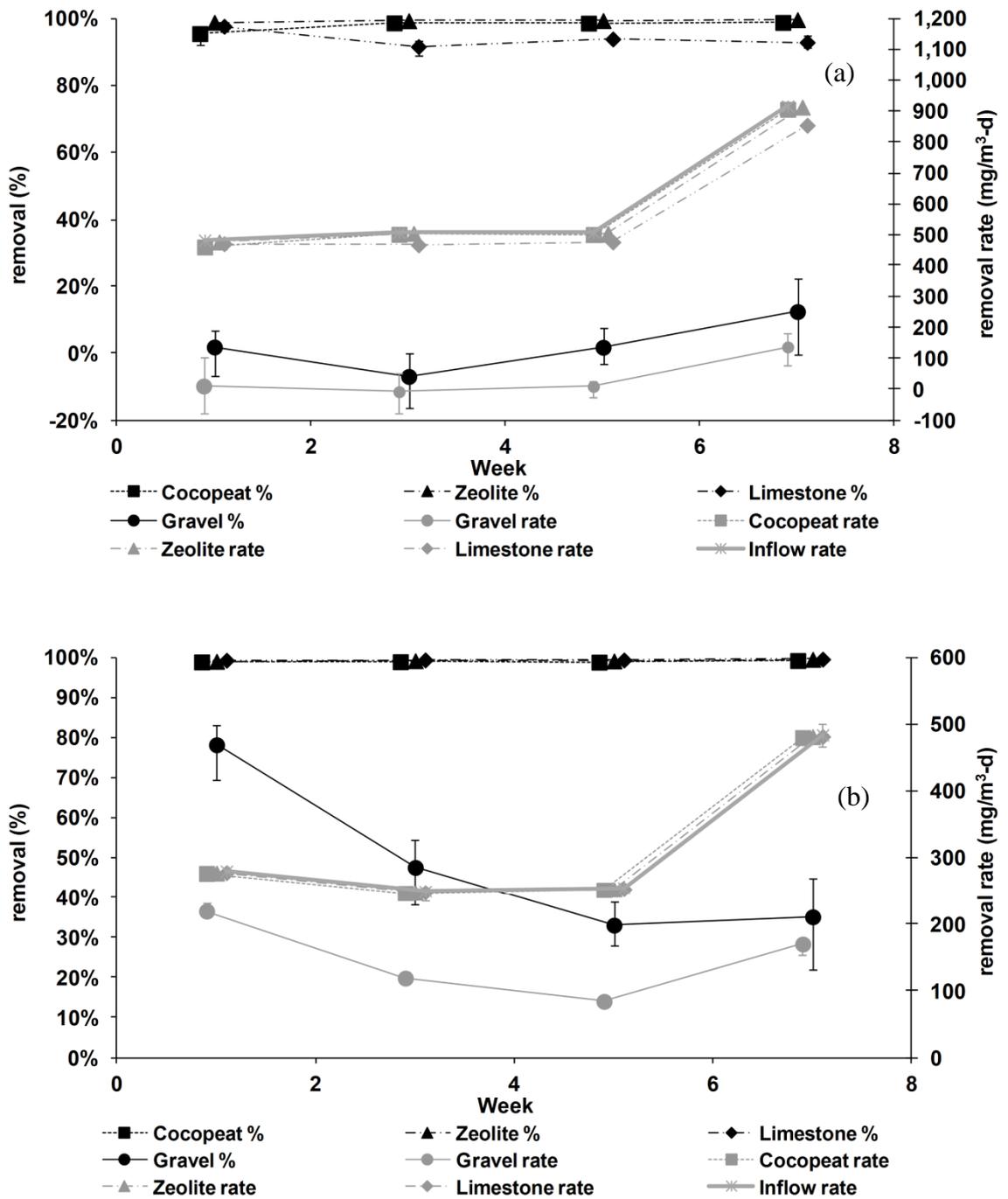


Figure 3.3. Mean removal rates of total Cu (a) and Zn (b) over time, expressed as percentage (principal axis) and as daily mass removed per unit of volume of wetland (secondary axis). Error bars indicate the corresponding minimum and maximum values of the three replicates.

3.4.2.6 Zinc

Similarly to the case of Cu, high removal rates were observed in cocopeat, zeolite and limestone wetlands for Zn, regardless of fluctuations in inflow concentration (Figures 3.3b). In gravel wetlands, Zn removal rates also decreased over time.

The type of media affected significantly the removal of Zn ($p < 0.001$). However, in this case, gravel wetlands did not affect the removal significantly (Table 3.8). Limestone wetlands, although they presented the lowest concentration of Zn in the outflow (Table 3.2), were not significantly different to cocopeat and zeolite wetlands.

Table 3.8. Comparison of dissolved and total Zn concentration in the inflow of the wetland system and the outflow of each wetland group using Games-Howell post hoc tests. Bold type denotes significant difference.

	Inflow		Gravel		Cocopeat		Zeolite	
	Zn dissolved (mg/L)	Zn total (mg/L)						
Gravel	0.972	0.285	-	-	-	-	-	-
Cocopeat	0.094	0.043	0.001	0.002	-	-	-	-
Zeolite	0.093	0.043	0.001	0.002	0.001	0.060	-	-
Limestone	0.091	0.043	0.001	0.002	<0.001	0.001	<0.001	0.267

3.4.3 Correlation between the removal of arsenic and iron

Figure 3.4 presents the total As concentrations versus total Fe concentrations in the outflow from gravel, zeolite and limestone wetlands. In gravel wetlands, the outflow concentrations of total As and total Fe were significantly correlated ($p < 0.001$, Pearson $\rho=0.988$). In addition, significant correlation were also found in zeolite ($p=0.006$) and limestone wetlands ($p=0.001$), Pearson ρ being 0.739 and 0.827 respectively.

Conversely, in cocopeat wetlands no significant correlation was found between As and Fe ($p=0.196$, Pearson $\rho=0.401$).

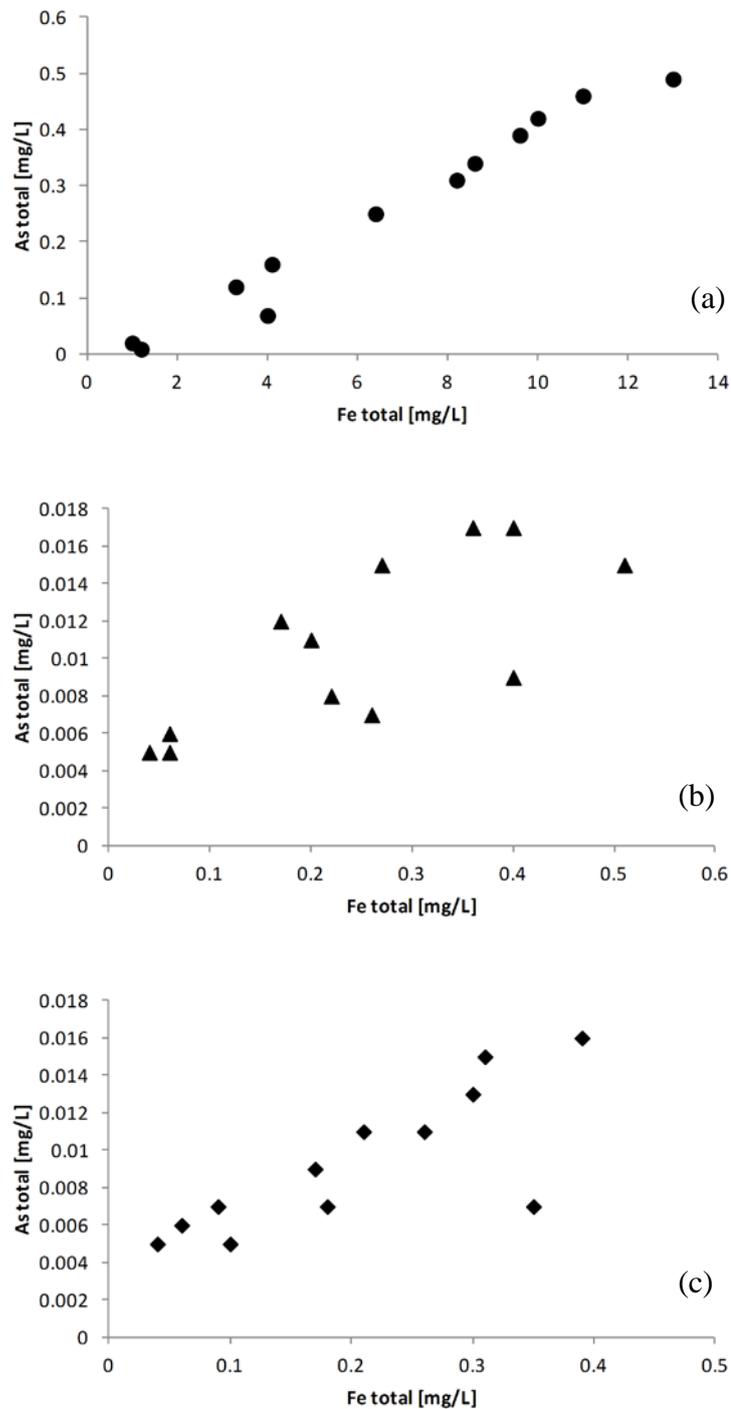


Figure 3.4. Total arsenic concentration vs total iron concentration in the effluent from (a) Gravel wetlands (b) Zeolite wetlands (c) Limestone wetlands.

3.4.4 Effect of wetland media on environmental parameters

The type of substrate also affected key parameters such as pH ($pKW < 0.001$). Almost every wetland group significantly changed the pH ($pMW < 0.001$ for gravel, $pMW=0.031$ for cocopeat, $pMW < 0.001$ for limestone), except the zeolite group ($pMW=0.397$).

Electrical conductivity was also significantly affected ($pKW < 0.001$). Each type of wetland had a particular effect on the electrical conductivity as the outflow levels from every wetland group was significantly different to those in the inflow ($pMW < 0.001$), and to each wetland group ($pMW < 0.001$) with the exception of cocopeat and zeolite wetlands ($pMW=0.133$).

Gravel, cocopeat and zeolite wetlands raised the redox potential significantly ($pA < 0.001$), but the three of them had a similar effect on the outflow ORP levels ($pGH > 0.05$ when comparing between them), whereas limestone wetlands did not have any significant effect ($pGH=0.053$ when comparing to the inflow ORP levels).

On the other hand, dissolved oxygen was not significantly affected by any wetland group ($pA=0.382$).

3.5. Discussion⁴

3.5.1. The effect of wetland media on pollutants removal

The difference in removal rates suggests that the removal of the target pollutants in vertical flow wetlands was primarily affected by the type of substrate. Gravel presented limited removal of As, compared to all of the alternative media (average removal percentage over 98%). Others have also found sands and gravels to have limited As removal capacity. For example, Singhakant et al. (2009) reported that the efficiency of As removal decreased over time in their sand/gravel constructed wetlands. This

⁴ Mainly extracted from Lizama Allende et al. (2011)

limitation was also observed in the performance of the gravel wetlands to remove the other five target pollutants. Furthermore, the use of sand/gravel media in subsurface flow wetlands is not recommended for the removal of metals due to limited sorption capacity and inability to form new storage sediments without clogging the wetland matrix (Kadlec and Wallace, 2009). As such, the results suggest that gravel is unsuited to be employed as wetland media for the removal of As -and metals- rich water. On the other hand, the lack of similar studies prevents comparison of the performance of cocopeat, zeolite and limestone as alternative media. However, these materials have been employed in some extent to remove various combinations of the target pollutants. The exception is cocopeat (chosen as an alternative to peat), which apparently has not been studied for metal removal.

3.5.2. Pollutant removal mechanisms and related factors

The removal of heavy metals can be achieved via different processes depending on whether the metals are in dissolved or particulate form. The principal process that removes heavy metals in natural and constructed wetlands is sedimentation; however, other processes such as precipitation must occur first since sedimentation only removes particulate metals (Sheoran and Sheoran, 2006). Looking at the speciation of As, Fe and Cu in the inflow, the three of them were mostly particulate (Table 3.2). Therefore, physical processes such as filtration and sedimentation would be sufficient to remove these pollutants. However, this situation may not be realistic for some polluted waters in Chile. According to Table 3.2, As and Fe in the outflow from gravel wetlands were also mainly particulate. The significant correlation between As and Fe supports the hypothesis that in gravel, zeolite and limestone wetlands, sorption/coprecipitation of As by Fe oxides was the main removal mechanism. Similar findings were reported by Buddhawong et al. (2005), who concluded that As binding with the Fe content of the gravel media was responsible for As removal. The sorption of metals on oxides is widely known (Stumm and Morgan, 1996). Different authors attribute this property as an important source of metal removal in constructed wetlands if Fe/Mn oxides are present (Sjöblom, 2003). Arsenate sorption onto most metals (hydr)oxides (Inskeep et al., 2002), but specially onto Fe and Mn oxyhydroxides has been reported by a number

of researchers (Kneebone et al., 2002, Pastén et al., 2006). Thus, Fe oxides containing As were filtered in gravel wetlands, but given the median gravel size (7 mm), filtration capability was limited.

Conversely, in cocopeat wetlands, sorption/coprecipitation of As by Fe oxides may not be the main removal mechanism. Organic matter present in this medium may be contributing to As removal by sorption, as organic matter is able to sorb arsenic (Redman et al., 2002). However, given that cocopeat performance is similar to that of zeolite, further evidence is required to ascertain with confidence which is the key removal mechanism that removes As more efficiently: sorption by cocopeat (organic matter) or sorption by zeolite (ion exchange capacity). Sorption efficiency is affected by environmental conditions (see Chapter 2). On the other hand, no significant correlation was found between As and Mn concentrations in the outflow from any wetland group (Figure 3.5 and 3.6). Since Mn was mainly dissolved and As was mainly particulate (Section 3.4.1), As and Mn may be removed via different mechanisms. Manganese is soluble at acidic pH, so it is not possible to precipitate Mn in acidic waters (Kadlec and Wallace, 2009). In addition, the presence of Fe may inhibit the oxidation (and therefore precipitation) of Mn because Fe exerts a preferential claim on available oxygen (Hedin and Nairn, 1993).

The total concentration of Cu was always lower in the outflow from gravel wetlands than in the inflow, but the dissolved concentration was on average higher in the outflow than in the inflow (Table 3.2). This could be explained by a limited Cu removal capacity, so once the maximum removal was reached, Cu started to leach. In addition, Zn was mainly particulate in the outflow from gravel wetlands, whereas it was mostly dissolved in the inflow (Table 3.2). However, the dissolved concentration of Zn was lower in the outflow than in the inflow. Therefore, gravel wetlands filtered total As, Fe, Cu and Zn; released dissolved Cu, and removed dissolved Zn, but due to their limited removal capacity the total concentration of Zn increased consistently in the outflow.

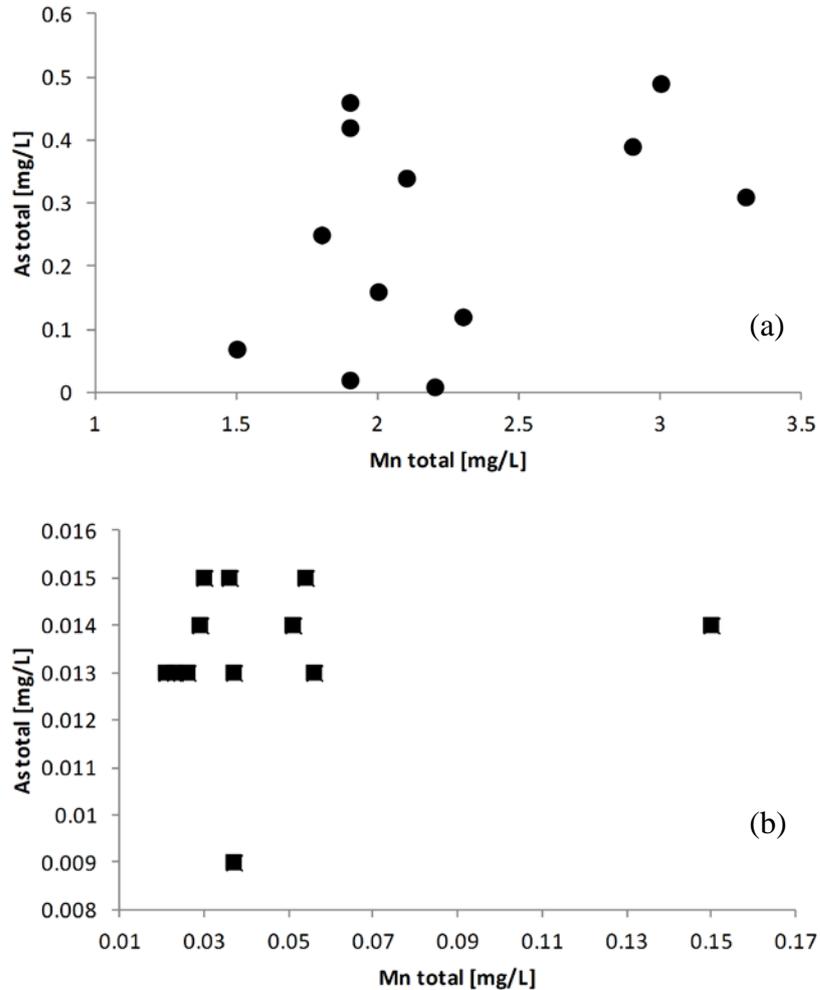


Figure 3.5. Total arsenic concentration vs total manganese concentration in the effluent from (a) Gravel wetlands (b) Cocopeat wetlands.

Both Cu and Zn can be removed by Fe oxides (Kröpfelová et al., 2009), but because conflicting information exists regarding competitive sorption of Cu and Zn on Fe oxides (Covelo et al., 2007, Violante et al., 2003), their effect on Cu and Zn removal is not clear. In cocopeat, zeolite and limestone wetlands, dissolved Cu and Zn were removed; whereas dissolved As and Fe were not (Table 3.2). This shows that these wetlands were able not only to retain particulate metals, but also to remove/release dissolved metals. Different authors have proposed that the main mechanism for mobilisation of As sorbed on Fe oxides is reductive dissolution (Mukherjee et al., 2009). Since mostly aerobic conditions were found (Table 3.3) and no significant differences were found between ORP levels in gravel, cocopeat and zeolite wetlands, plus the fact that limestone wetlands did not affect these levels (Section 3.4.4), further

experimental evidence is required to understand the As and Fe retention/mobilisation mechanisms.

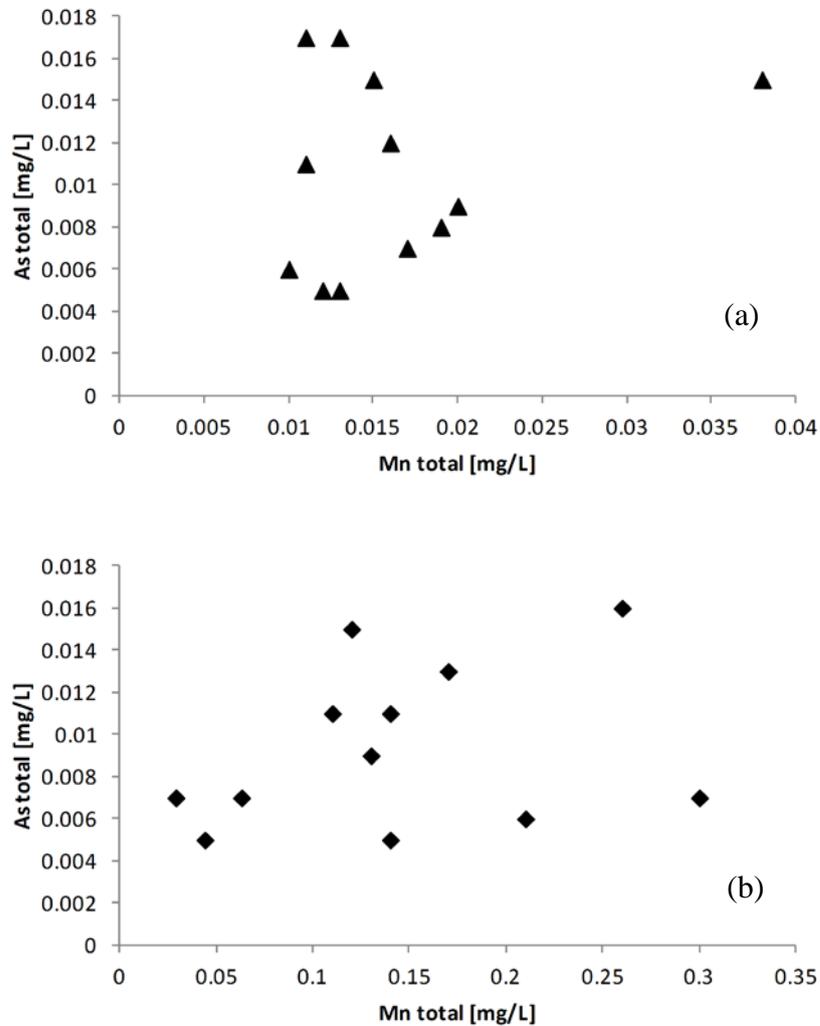


Figure 3.6. Total arsenic concentration vs total manganese concentration in the effluent from (a) Zeolite wetlands (b) Limestone wetlands

Boron was mostly dissolved in the inflow (98%) and the outflow from gravel (97%), cocopeat, zeolite and limestone wetlands (99%), whereas Mn was mostly dissolved in the inflow (99%) and the outflow from gravel (99%), cocopeat (97%) and limestone wetlands (91%); but not in the outflow from zeolite wetlands (43%) (Table 3.2). This may indicate that the main B removal process is sorption, mainly when it is present as borate $B(OH)_4^-$. In addition, the presence of organic matter contributes to the adsorption

of B in soils (Sartaj and Fernandes, 2005). The good performance of cocopeat wetlands at the beginning of the experimental period only can be explained by a limited sorption capacity of this substrate, as reported by Sartaj et al. (1999). In addition, the adsorption of boron on soils depends on the pH of the solution (Kot, 2009); so the lower the pH, the lower the adsorption. Low pH could explain the low removal rates in gravel wetlands, whilst high pH could explain the highest removal rates in zeolite and limestone wetlands (Table 3.3).

As such, it could be asserted that the wetland media and pH were the key factors affecting the removal of the target pollutants. Other environmental factors may also be important (dissolved oxygen, ORP), but in this case, looking at the changes in monitored water quality parameters (Table 3.3), and the differences between wetland groups (Section 3.4.4), both appear to confirm that for the particular conditions of this experiment, wetland substrate and pH were the most important factors.

There remain, however some important questions. For example, while these results show the performance of wetland substrates in isolation, they do not give an indication of the likely results when different media are combined. It is also possible that the performance of media varies with different influent water quality (concentrations, pH, etc.). Lastly, it is possible that the removal processes involve microbial interactions.

Finally, the outlet in all wetland columns was non-restricted. As such, the flow rates were determined by the characteristics of the media. Since gravel had the largest size, it is probably that the detention time in the gravel wetlands was the lowest. This fact could have contributed to the lower removal rates in these columns, as it has been proposed that the detention time is a critical factor affecting metal removal efficiency in wetlands (Cohen and Staub, 1992).

3.5.3. Replicability

It should be considered that only 12 set of data on pollutants concentration could be obtained for each group of wetlands (3 replicate columns x 4 weeks), and therefore

statistical analysis and significance must be interpreted in this context. For the environmental parameters, 20 sets of data were obtained. Future experiments will consider a larger number of replicates and also a longer experimental period in order to assess the performance of the system confidently (Chapter 4).

3.6. Conclusions

Vertical flow wetlands with alternative wetland media (coccopeat, zeolite and limestone) presented higher efficiency than wetlands with conventional gravel media in the removal of all the target pollutants. The results support the hypothesis H1 (the media plays an important role in the removal of the target pollutants), since the wetlands having alternative media performed significantly better than wetlands having gravel media in the removal of As, Fe, Cu, Mn and Zn. The exception was for removal of B, with no significant differences occurring between media types. Since B is mainly removed by sorption at high pH, probably the slightly acidic pH -together with the presence of the other pollutants- affected the removal efficiency of B.

The most promising wetland media for As removal appear to be limestone and zeolite; whereas for Fe removal they appear to be limestone and cocopeat. Despite the fact that the three alternative wetland media presented no significant difference in outflow Fe levels, these levels were the lowest in limestone wetlands, followed by cocopeat wetlands. On the other hand, removal of Mn varied between media, with zeolite and cocopeat being the most efficient. These two wetlands groups were also the most efficient in the removal of Cu and Zn, however no statistically significant differences were found between the wetland types.

The effect of raising the pH in limestone columns supports hypothesis H2 (the alternative wetland media can be effective due to the promotion of reactions which result in pollutant retention); in this case the capability of limestone of providing alkalinity to buffer pH makes it a promising medium to remove Fe by precipitation and As by coprecipitation. Likewise, the cation-exchange capability of zeolite and the supply of carbon in cocopeat are characteristics that enhance As and heavy metal

removal; these are characteristics that cannot be provided by conventional gravel substrates. Although limestone wetlands raised the pH significantly (Section 3.4.4), this fact did not affect the removal of Fe as strongly as expected (Section 3.4.2.2), since Fe levels in limestone wetlands were the lowest among the different wetland groups but they were not significantly different to those of the other wetlands groups. The high removal capability in all three alternative media wetlands probably impeded distinguishing between their performances. Other factors may also be considered, such as detection limits issues and differences in outflow rates.

The relationship between the removal of As and that of Fe (Section 3.4.3) supports the hypothesis that they may be removed via the same mechanism and/or that the removal of Fe facilitates the removal of As. On the other hand, no significant correlation was found between the outflow concentration of As and Mn. In addition, the effect of wetland media on the removal of As was different to that on the removal of Mn. These observations suggest that As and Mn were removed via different routes, and therefore they support hypothesis H3 (arsenic removal mechanisms are different to those of the other metals, due to the high reactivity of arsenic). Moreover, the most efficient wetland media in the removal of As did not coincide with those in the other pollutants, probably because they were being removed via different processes. Unfortunately, as arsenic was mainly particulate in the inflow, the capability of the media of removing As in the dissolved phase could not be tested.

The following experimental stage of this study will test the performance of the same four alternative media, this time in the removal of As from more realistically contaminated water (Chapter 4). This water will mimic a natural contaminated water resource, the Azufre River in Chile, where the main pollutants are As, B and Fe under highly acidic conditions. Therefore, As, B and Fe will be mainly in the dissolved phase. The analysis of this particular case-study will enable us to focus on the removal of the target pollutants when present under high concentrations and as dissolved species, which are the conditions commonly found in acid mine drainage.

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3.8. Journal article “Enhancing the removal of arsenic, boron and heavy metals in subsurface flow constructed wetlands using different supporting media”

Enhancing the removal of arsenic, boron and heavy metals in subsurface flow constructed wetlands using different supporting media

K. Lizama Allende, T. D. Fletcher and G. Sun

ABSTRACT

The presence of arsenic and heavy metals in drinking water sources poses a serious health risk due to chronic toxicological effects. Constructed wetlands have the potential to remove arsenic and heavy metals, but little is known about pollutant removal efficiency and reliability of wetlands for this task. This lab-scale study investigated the use of vertical subsurface flow constructed wetlands for removing arsenic, boron, copper, zinc, iron and manganese from synthetic wastewater. Gravel, limestone, zeolite and cocopeat were employed as wetland media. Conventional gravel media only showed limited capability in removing arsenic, iron, copper and zinc; and it showed virtually no capability in removing manganese and boron. In contrast, alternative wetland media: cocopeat, zeolite and limestone, demonstrated significant efficiencies – in terms of percentage removal and mass rate per m³ of wetland volume – for removing arsenic, iron, manganese, copper and zinc; their ability to remove boron, in terms of mass removal rate, was also higher than that of the gravel media. The overall results demonstrated the potential of using vertical flow wetlands to remove arsenic and metals from contaminated water, having cocopeat, zeolite or limestone as supporting media.

Key words | arsenic removal, boron removal, heavy metal removal, subsurface flow constructed wetlands, supporting media

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INTRODUCTION

Arsenic is well known for its chronic toxicity, particularly when exposure occurs over prolonged periods. Arsenic pollution in natural waters has been reported in different countries, such as Bangladesh, USA, China, India and Chile. About 100 million people are currently drinking water with As concentrations up to 100 times 10 µg/L which is the World Health Organisation guideline (Mohan & Pittman 2007). In Chile, the Loa River has As and boron (B) concentrations around 1,400 and 21,000 µg/L, respectively (Romero *et al.* 2005). This river is a major waterway in Antofagasta Region and the main source of drinking and irrigation for several populated areas (Landrum *et al.* 2009). The presence of As and B in the Loa River is due to the El Tatio Geysir Field geothermal field, which forms the headwaters of the Salado River – a tributary to the Loa River – and these headwaters have As concentrations around 33,700 µg/L (Landrum *et al.* 2009).

In addition, metals/metalloids such as boron, iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) can also limit

the use and reuse of water resources, either by natural or anthropogenic pollution. Boron contamination in the water environment is causing increasing concern (Xu & Jiang 2008). In many cases, treatment of contaminated water is limited due to isolated location of the water streams and the elevated investment and operation costs of conventional technologies.

Constructed wetlands are known to be effective in removing several trace metals from contaminated water (Kadlec & Wallace 2009). A number of studies have been carried out to investigate their metal removal efficiency (Kleinmann & Girts 1987; Richards 1992; Sobolewski 1999; Sjöblom 2005). Most of these studies have focused on acid mine drainage treatment, primarily to remove sulfate, Fe and Mn (Wallace 2006) by surface flow wetlands. Little is known about subsurface flow wetlands, and few literature reports are available on the performance of wetlands for the removal of As and B. Furthermore, the mechanisms of As removal have not been elaborated (Singhankant *et al.* 2009).

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The use of alternative media in constructed wetlands has been suggested by different researchers, with the aim of improving removal performance. For example, Sarafraz *et al.* (2009) showed that the use of zeolite in a horizontal subsurface constructed wetland enhanced Zn sorption, and proposed zeolite as an alternative to sand and gravel. Nevertheless, little research in this area has been conducted. This study investigated the performance of four different supporting media: gravel, cocopeat, zeolite and limestone in the removal of six target pollutants: As, B, Fe, Mn, Cu and Zn using vertical subsurface flow constructed wetlands.

MATERIALS AND METHODS

The wetland system

The lab-scale wetlands consisted of twelve subsurface vertical flow wetland columns that were built using stormwater PVC pipes. Each column had 1 m height and 100 mm internal diameter and was installed in a greenhouse. The wetland columns were divided into four groups, namely group G – employing gravel as main substrate, Z – zeolite as main substrate, C – cocopeat as main substrate, and L – crushed limestone as main substrate; each group had three replicate columns. Each column had a drainage layer of 20–40 mm cream pebbles at the base which was 0.1 m deep. The drainage layer was topped with a layer of main substrates (G, Z, C or L) that was 0.7 m deep. In each wetland column, common reed (*Phragmites australis*) was planted. The *Phragmites* were given two months to adapt to their new growth environment prior to the experiment.

Operation of the wetland system

Synthetic wastewater was prepared to simulate the concentration of the target pollutants in polluted surface waters in Chile. The synthetic wastewater was prepared using tap water, with the following reagents added per litre of water: 1 mL 1,000 mg/L arsenic standard solution (As_2O_5 in H_2O), 0.025 mL 10,000 mg/L boron standard solution (H_3BO_3 in H_2O), 125 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 7.2 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 3.9 mg $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$, 4.4 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.7 mg $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$. The total concentration of the metals were (average \pm standard deviation): 0.89 \pm 0.05 mg/L As, 24.0 \pm 0.0 mg/L B, 1.43 \pm 0.40 mg/L Cu, 21.0 \pm 1.4 mg/L Fe, 2.38 \pm 0.82 mg/L Mn and 1.25 \pm 0.44 mg/L Zn. An agitated feed tank stored the wastewater during the experiment. From it, 2 L of synthetic wastewater were taken and

dosed manually in each wetland, three times per week in the first month and twice per week in the second month.

Sampling and analysis

After each dosing, two types of water samples from each wetland column and from the feed tank were collected and acidified with nitric acid (HNO_3) to pH <2 for total and dissolved metals analysis. For the latter, the samples were filtered through 0.45 μm cellulose acetate filters. Weekly composite samples were prepared adding an equal volume of each corresponding daily sample for every week. Metal concentrations in these composite samples for weeks 1, 3, 5 and 7 were determined by ICP-MS in a NATA accredited laboratory (4 values per column plus the inflow). *In-situ* parameters were also monitored after each dosing (20 values per column plus the inflow). Dissolved oxygen (DO) was measured using 51,970 probe, whilst for pH and conductivity 51,910 and 51,975 probes were used, respectively. All these probes were connected to a Sension 378 meter. An ORP Testr10 probe was used to measure redox potential (Eh). Sulfate (SO_4) was measured using DR5000 UV/VIS spectrophotometer based on an adapted standard method (APHA/AWWA/WEF 2005). Alkalinity was quantified using a HACH alkalinity test kit, low range and high range tests.

RESULTS

Overall performance

Table 1 presents the average performance of the system during the operation period, for each group of wetland columns. As shown in Table 1, the concentration of all the target pollutants in the outflow from the gravel wetland columns was higher than that from other columns, demonstrating that the three types of alternative wetland substrate had greater removal pollutant removal efficiencies than the traditional gravel substrate. Furthermore, gravel appeared to have limited capability to remove As, Fe, Cu and Zn; and almost null capability to remove B and Mn.

The changes in the monitored parameters are presented in Table 2.

The removal of As and Fe

Cocopeat, zeolite and limestone wetlands presented high As removal rates (average above 98%) for the entire

Table 1 | Mean inflow and outflow concentrations of the target pollutants

	Mean inflow concentration (mg/L) [CV]		Mean outflow concentration (mg/L) [CV]							
	Dissolved	Total	G – gravel		C – cocopeat		Z – zeolite		L – limestone	
			Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved	Total
As	0.001 [0.952]	0.890 [0.062]	0.001 [1.13]	0.253 [0.685]	0.011 [0.149]	0.013 [0.121]	0.008 [0.347]	0.011 [0.451]	0.006 [0.145]	0.009 [0.409]
B	23.5 [0.04]	24.0 [0.0]	23.5 [0.06]	24.3 [0.06]	22.0 [0.27]	22.17 [0.28]	21.33 [0.12]	21.67 [0.09]	21.67 [0.11]	21.92 [0.11]
Cu	0.047 [0.699]	1.425 [0.283]	0.34 [0.75]	0.462 [0.69]	0.012 [0.37]	0.014 [0.386]	0.007 [0.271]	0.009 [0.29]	0.013 [0.513]	0.024 [0.375]
Fe	UD	21.0 [0.07]	0.08 [0.44]	6.7 [0.59]	0.095 [0.47]	0.238 [0.281]	0.047 [0.686]	0.246 [0.615]	0.023 [1.457]	0.205 [0.575]
Mn	2.275 [0.33]	2.50 [0.52]	2.21 [0.24]	2.253 [0.25]	0.044 [0.73]	0.046 [0.761]	0.007 [0.286]	0.016 [0.466]	0.131 [0.627]	0.143 [0.576]
Zn	0.725 [0.473]	1.245 [0.353]	0.043 [1.44]	0.664 [0.57]	0.012 [0.19]	0.013 [0.18]	0.008 [0.216]	0.01 [0.273]	0.003 [0.425]	0.008 [0.364]

CV – coefficient of variation – σ/μ , UD: undetectable, where σ is the average of (H^+) and μ is the standard deviation of (H^+) .
For samples under the detection limit (0.001 mg/L for As and 0.02 mg/L for Fe), half of that value was considered for the calculations.

Table 2 | Mean inflow and outflow levels of monitored water quality parameters

Parameter	Unit	Mean value in the outflow [CV]				
		Inflow value	Gravel	Cocopeat	Zeolite	Limestone
SO ₄	mg/L	74.7 [0.085]	67.3 [0.136]	65.7 [0.07]	56.5 [0.087]	56.2 [0.098]
pH	–	5.77 [0.976]	4.95 [0.936]	5.67 [0.995]	6.13 [1.025]	7.95 [1.001]
DO	mg/L	6.3 [0.205]	6.5 [0.217]	6.1 [0.2]	6.2 [0.192]	5.9 [0.215]
T	°C	24.2 [0.121]	24.4 [0.139]	24.4 [0.133]	24.7 [0.125]	25 [0.124]
Alkalinity	mg/L CaCO ₃	14.3 [0.172]	9.3 [0.27]	11.4 [0.245]	10.8 [0.189]	155.4 [0.619]
E _h	mV	195.2 [0.082]	225.4 [0.15]	235.2 [0.137]	233.4 [0.129]	181.5 [0.143]
Conductivity	µS/cm	198.6 [0.068]	217.7 [0.112]	176.5 [0.103]	185.2 [0.459]	290.8 [0.041]

^aFor the pH values, the CV was calculated using $CV = -\log(\sigma)/-\log(\mu)$, where σ is the average of (H^+) and μ is the standard deviation of (H^+) .

experimental period, whereas for gravel wetlands the rates decreased over time, both as percentage and as daily mass removed per volume. In addition, the removal of As in terms of mass was almost the same for the alternative wetland media, and it only decreased when the inflow concentration decreased (Figure 1(a)). The removal of Fe presented a very similar trend: cocopeat, zeolite and limestone wetlands removal was around 99%, but gravel wetlands removal decreased with time (Figure 1(b)).

The removal of B and Mn

Boron was removed by cocopeat, zeolite, limestone and gravel wetlands at the beginning of the experiment, but then it was leached out by cocopeat and gravel wetlands

(Figure 2(a)). In addition, gravel wetlands barely removed Mn. As shown in Figure 2(b); cocopeat, zeolite and limestone wetlands removed around 94% total Mn on average, whereas gravel wetlands even presented negative removal.

The removal of Cu and Zn

High removal rates were observed in cocopeat, zeolite and limestone wetlands for these metals, regardless of fluctuations in inflow concentration (Figure 3(a) and 3(b)). In contrast, decreasing removal rates over time were observed in gravel wetlands, despite the fact that the daily mass removal rate increased when the inflow concentration increased.

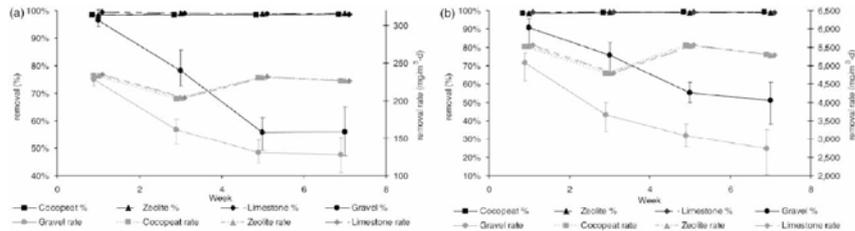


Figure 1 | Mean removal rates of total As (a) and Fe (b) over time, expressed as percentage (principal axis) and as daily mass removed per unit of volume of wetland (secondary axis). Error bars indicate the corresponding minimum and maximum values.

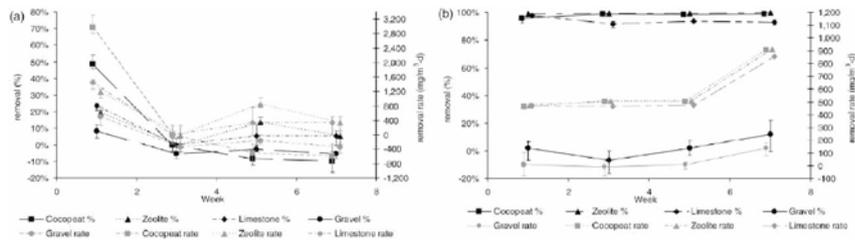


Figure 2 | Mean removal rates of total Bi (a) and Mn (b) over time, expressed as percentage (principal axis) and as daily mass removed per unit of volume of wetland (secondary axis). Error bars indicate the corresponding minimum and maximum values.

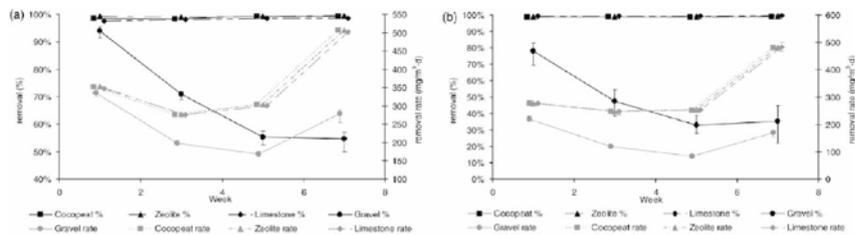


Figure 3 | Mean removal rates of total Cu (a) and Zn (b) over time, expressed as percentage (principal axis) and as daily mass removed per unit of volume of wetland (secondary axis). Error bars indicate the corresponding minimum and maximum values.

DISCUSSION

The difference in the removal rates suggests that the removal of the target pollutants in vertical flow wetlands was primarily affected by the type of substrate. Gravel presented limited removal of As, whereas all the alternative media exhibited excess capability to remove As (average removal percentage over 98%). This supports Singhankant *et al.*'s (2009) study, who reported that the efficiency of As removal decreased over time in their sand/gravel constructed wetlands. This limitation was also observed in the performance of the

gravel wetlands to remove the other five target pollutants. Furthermore, the use of sand/gravel media in subsurface flow wetlands is not recommended for the removal of metals due to limited sorption capacity and inability to form new storage sediments without clogging the wetland matrix (Kadlec & Wallace 2009). As such, the results suggest that gravel is unsuited to be employed as wetland media for the removal of As – and metals-rich wastewater. On the other hand, the lack of similar studies prevents comparison of the performance of cocopeat, zeolite and limestone as alternative media. However, these materials have been

employed to some extent to remove various of the target pollutants. The exception is cocopeat (chosen as an alternative to peat) which apparently has not been studied for metals removal.

The removal of heavy metals can be achieved via different processes depending on whether they are dissolved or particulated. The principle process that removes heavy metals in natural and constructed wetlands is sedimentation; however, other processes such as precipitation must occur first since sedimentation only removes particulated metals (Sheoran & Sheoran 2006). Looking at the speciation of As, Fe and Cu in the inflow, the three of them were mostly particulated (Table 1). Therefore, physical processes such as filtration and sedimentation would be enough to remove them. According to Table 1, As and Fe in the outflow from gravel wetlands were also mainly particulated. Moreover, given the correlation between total As and Fe outflow concentration ($R^2 = 0.98$) and As and Fe removal rate ($R^2 = 0.97$), it can be asserted that Fe oxides trapped As by coprecipitation/sorption. Similar findings were reported by Buddhawong *et al.* (2005), who concluded that As binding with the Fe content of the gravel media was responsible for As removal. The sorption of metals on oxides is widely known (Stumm & Morgan 1996). Different authors attribute this property as an important source of metal removal in constructed wetlands if Fe/Mn oxides are present (Sjöblom 2005). Arsenate sorption onto most metals (hydr)oxides (Inskip *et al.* 2002), but specially onto Fe and Mn oxyhydroxides has been reported by a number of researchers (Kneebone *et al.* 2002; Pastén *et al.* 2006). Thus, Fe oxides containing As were filtered in gravel wetlands, but given the medium gravel size (7 mm), filtration capability was limited.

In contrast, the total concentration of Cu was always lower in the outflow from gravel wetlands than in the inflow, but the dissolved concentration was on average higher in the outflow than in the inflow (Table 1). In addition, Zn was mainly particulated in the outflow from gravel wetlands, whereas it was mostly dissolved in the inflow (Table 1). However, the dissolved concentration of Zn was lower in the outflow than in the inflow. Therefore, gravel wetlands filtered total As, Fe, Cu and Zn; released dissolved Cu, and removed dissolved Zn, but due to their limited removal capacity the total concentration of Zn increased consistently in the outflow. Both Cu and Zn can be removed by Fe oxides (Kröpfelová *et al.* 2009), but because conflicting information exists regarding competitive sorption of Cu and Zn on Fe oxides (Violante *et al.* 2003; Covelto *et al.* 2007), their effect on Cu and Zn removal is

not clear. In cocopeat, zeolite and limestone wetlands, dissolved Cu and Zn were removed; whereas dissolved As and Fe were not (Table 1). This shows that these wetlands were able not only to retain particulated metals, but also to remove/release dissolved metals. Different authors have proposed that the main mechanism for mobilisation of As sorbed on Fe oxides is reductive dissolution (Mukherjee *et al.* 2009). Since mostly aerobic conditions were found (Table 2), further experimental evidence is required to understand the As and Fe retention/mobilisation mechanisms.

Boron was mostly dissolved in the inflow (98%) and the outflow from gravel (97%), cocopeat, zeolite and limestone wetlands (99%), whereas Mn was mostly dissolved in the inflow (99%) and the outflow from gravel (99%), cocopeat (97%) and limestone wetlands (91%); but not in the outflow from zeolite wetlands (43%) (Table 1). This may indicate that the main B removal process is sorption, mainly when it is present as borate $B(OH)_4^-$. In addition, the presence of organic matter contributes to the adsorption of B in soils (Sartaj & Fernandes 2005). The good performance of cocopeat wetlands only at the beginning of the experimental period can be explained by a limited sorption capacity of this substrate, as reported by Sartaj *et al.* (1999) for a peat filter. In addition, the adsorption of boron on soils depends on the pH of the solution (Kot 2009); so the lower the pH, the lower the adsorption. Low pH could explain the low removal rates in gravel wetlands, whilst high pH could explain the highest removal rates in zeolite and limestone wetlands (Table 2).

The speciation of metals is the main factor that determines their bioavailability. Dissolved metals represent the most bioavailable form, especially when the metal is present as ionic or weakly complexed species (Cooper *et al.* 1996). Most metal removal studies only report total concentrations, however the dissolved fraction should be reported since some guidelines (such as US EPA National Water Quality Criteria (US EPA 2009)) do consider it. Dissolved As(V), as a highly reactive metalloid, may have different routes, As(III) being the most toxic species. Due to the complexity of the wetland environment and the reactivity of the pollutants with media, vegetation and microorganisms, different solid and soluble species can be found in the wetland system. They may be modelled using an aqueous geochemical program, and identified in the solid phase using advanced techniques such as X-ray diffraction. Future stages of this investigation will consider the use of these tools to provide knowledge in the performance of constructed wetlands.

Further research is currently being undertaken to remove As efficiently in subsurface constructed wetlands, particularly aiming to understand the removal mechanisms. Apparently, plant uptake plays a minor role in As removal (García et al. 2010). Future work will investigate the role of vegetation and microorganisms in the removal of the target pollutants.

CONCLUSIONS

This experimental study showed that cocopeat, zeolite and limestone can be used as the main media in vertical flow wetlands, to enhance the removal of As, B, Fe, Mn, Cu and Zn. In addition to providing filtration capability to remove particulated pollutants, these alternative substrates were able to provide different factors, such as organic matter (cocopeat), ion exchange sites (zeolite) and alkalinity (crushed limestone), which all contributed to the removal of the target pollutants of this study. In comparison, gravel wetlands only showed limited ability to remove As, Fe, Cu and Zn, and virtually no capability to remove B and Mn.

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Chapter 2: Literature review



*"Sure, kid. You start by working for the ecosystem,
but pretty soon you figure out how to get the ecosystem
working for you!"*

(Kadlec and Knight, 2006)



DECLARATION FOR THESIS CHAPTER 2

Declaration by candidate

In the case of Section 2.8, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Review of literature and write-up	80

The following co-authors contributed to the work:

Name	Nature of contribution	Extent of contribution (%)
Guangzhi Sun	Ideas and reviewing	10
Tim D. Fletcher	Ideas and reviewing	10

**Candidate's
Signature**

	Date 01/02/2013
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Declaration by co-authors

The undersigned hereby certify that:

- (1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
- (2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
- (3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility
- (4) there are no other authors of the publication according to these criteria;
- (5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
- (6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location

School of Engineering & Physical Sciences, James Cook University

Signature
Guangzhi Sun



Date
01/02/2013

Location

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2.1. Introduction

The occurrence of metals and metalloids in water environments is a great concern. Use of water contaminated with heavy metals poses a risk to the health of humans, fauna, vegetation and microorganisms (Kadlec and Knight, 1996), depending on the chemical speciation of the metals, which determines their bioavailability (Dunbabin and Bowmer, 1992, Morel and Hering, 1993, Manahan, 1994). The occurrence of metals has both natural and anthropogenic sources. Metals can reach water streams by point-source or diffuse pollution of effluents from surface runoff or mining activities, since they can be released from soils and/or solids.

This chapter provides background information on arsenic (As), boron (B) and iron (Fe) and their presence in aquatic environments. An overview of constructed wetland systems and their application for the removal of arsenic and metals is presented. The removal mechanisms for arsenic in constructed wetlands are reviewed and this review was published as a paper in the journal *Chemosphere*, which is presented in Section 2.8, so as to provide the reader with detailed information regarding arsenic removal mechanisms in wetlands. In this review, a poor understanding of these mechanisms within wetlands was identified as a critical knowledge gap. Therefore, the main focus of this thesis is to provide insights into these mechanisms, and into the performance of constructed wetlands for arsenic and metal removal. Wetland design criteria for arsenic and metal removal are critically reviewed, before an overall summary of knowledge gaps and directions for future research is presented.

2.2. The presence of arsenic, iron and boron in aquatic environments

The biogeochemical cycles of arsenic and iron are coupled in natural systems (Kneebone et al., 2002), to the extent that the dynamics of Fe is an important control of As mobility, particularly in acid mine waters ($\text{pH} < 4$) (Williams, 2001). As such, it is reasonable to look at their chemistry and interactions as a whole. Despite the fact that the presence of boron is not often associated to that of arsenic and iron, in some cases such as effluents produced in electricity generating facilities, arsenic and boron are

found together (Ye et al., 2003). Since Fe oxides and oxyhydroxides play a role in regulating dissolved B in estuaries and oceans (Kot, 2009), and considering that in the case-study of this research (Azufre River, Lluta River catchment, Northern Chile) these three pollutants, As, Fe and B are found together, they will be described as such in the following section.

2.2.1 Chemistry

Arsenic is a highly reactive metalloid and is most commonly found in oxidation states -3, 0, +3 and +5. In natural waters, arsenic occurs as arsenite (AsO_3^{-3}) and arsenate (AsO_4^{-3}), referred to as As(III) and As(V). As(III) mostly exists in reducing groundwaters and hydrothermal waters, whilst As(V) is more often present in surface waters and oxidising groundwaters (Henken, 2009a).

Arsenic is known for its toxicity, which depends on its speciation. The main factors that control arsenic speciation are pH and redox potential (Cheng et al., 2009). In particular, arsenite is many times more toxic than arsenate (APHA et al., 2005), and at the same time uncharged species such as arsenite are harder to remove compared to charged species such as arsenate. In natural waters, under normal pH conditions (6-9), arsenite is mostly found as uncharged species (H_3AsO_3), and only at high pH (>9), arsenite is found as negatively charged species (H_2AsO_3^- , HAsO_3^{-2} and AsO_3^{-3}). On the other hand, arsenate is commonly found as negatively charged species (H_2AsO_4^- and HAsO_4^{-2}), and this charge increases as pH increases (Henken and Hutchison, 2009). Therefore, under natural conditions, arsenate is easier to remove than arsenite since the charges in arsenate species allow them to be removed by different processes such as sorption and anion exchange (Henken and Hutchison, 2009). Figure 2.1 presents the speciation of arsenite (a) and arsenate (b) as function of pH, whereas Figure 2.2 shows the Eh-pH diagram (a), considering also the presence of sulfur (b).

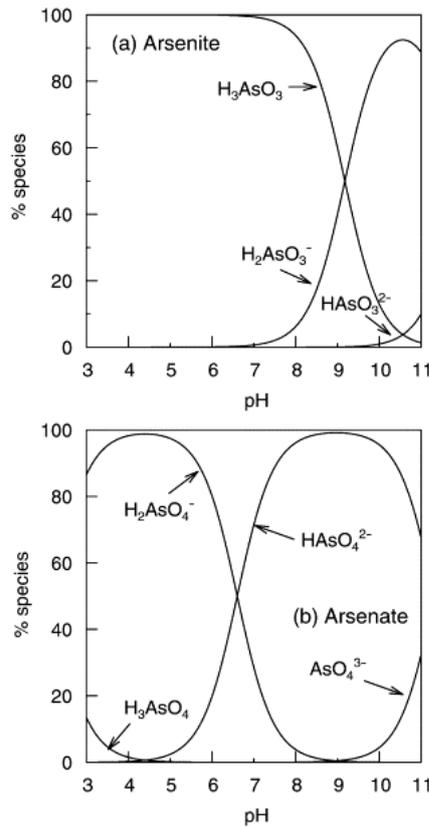


Figure 2.1. Arsenite (a) and arsenate (b) speciation as a function of pH, ionic strength of 0.01 M. Redox conditions were chosen so the indicated oxidation state dominates in each case (Smedley and Kinniburgh, 2002).

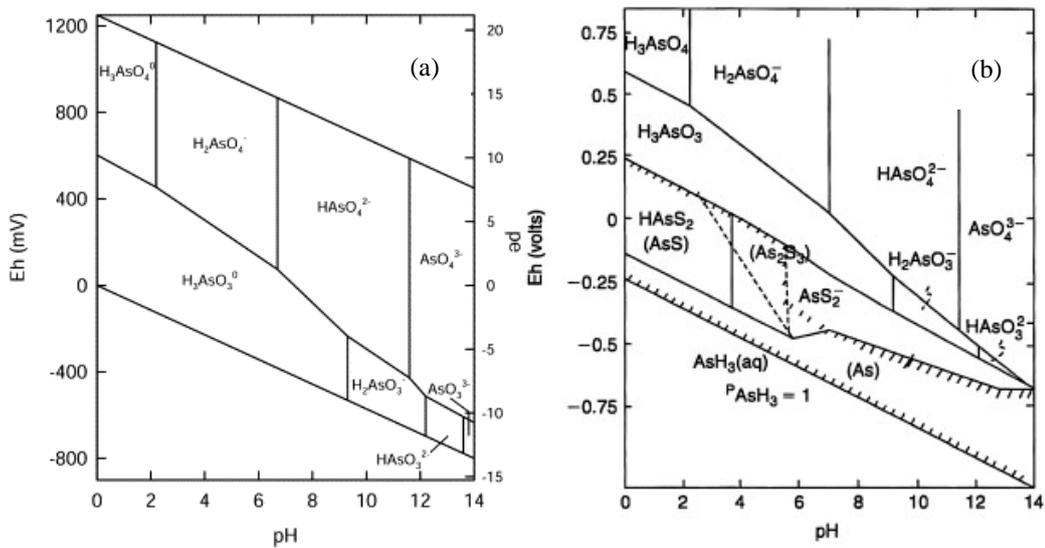


Figure 2.2. Eh-pH diagram for aqueous As species in the system As-O₂-H₂O at 25 °C and 1 bar total pressure (a) (Smedley and Kinniburgh, 2002) and in the system As-S-O₂-H₂O at 25 °C and 1 atmosphere with total arsenic 10⁻⁵ M and total sulfur 10⁻³ M (b). Solid species are enclosed in parentheses in the cross-hatched area, which indicates a solubility of less than 10^{-5.3} M (Ferguson and Gavis, 1972).

In some cases, other metals and metalloids are also present along with arsenic. In Northern Chile, the Loa River and Lluta River catchments are characterised by high levels of arsenic and boron (Romero et al., 2003, Landrum et al., 2009). In fact, As and B are typically found in geothermal waters (Ellis and Mahon, 1977) and these waters are tributaries to the Colpitas River, which flows into the Lluta River. In addition, As, B and Fe are found in the Azufre River, which is born at the foot of the Tacora Volcano and also flows into the Lluta River (Dirección General de Aguas, 2008). Arsenic, boron and iron have been considered as volcanic pollutants (Sriwana et al., 1998). The presence of these pollutants must be considered when evaluating treatment options, as they may affect the removal of arsenic.

Boron is a metalloid with high solubility. Its acid-base behaviour follows the following reaction (Snoeyink and Jenkins, 1980):



Where $pKa = -\log(Ka)$

$$Ka = \text{equilibrium constant} = \frac{\{B(OH)_4^-\}}{\{H_3BO_3\}\{OH^-\}}$$

Because of the high value of this pKa, in most natural waters boron can be found as boric acid (H_3BO_3) rather than as borate $B(OH)_4^-$, thus being harder to remove due to the lack of charge. Figure 2.3 shows that boric acid predominates at $pH < 7$. In soils, boron is often associated to aluminium (Al) and iron oxides (Kot, 2009). As shown in Figure 2.4, B ordinary shows only one valence +3 other than 0, and it is supposed to occur in nature as oxo-compounds only (Kot, 2009).

Iron (Fe) is one of the most abundant metals in the earth's crust and it is essential for plants and animals. It is highly reactive, and it can be found in oxidation states 0, +2 (ferrous) and +3 (ferric). Furthermore, the redox reactions of iron are involved in several important phenomena occurring in natural waters and water treatment systems (Snoeyink and Jenkins, 1980). Figure 2.5 presents Fe(III) species as a function of pH,

showing that the solid phase $\text{Fe}(\text{OH})_{3(s)}$ predominates at $\text{pH} > 2.7$, while Figure 2.6 indicates Fe(II) and (III) species in the presence of sulfur and carbonate, for different Fe concentrations, Eh and pH. Solids species predominating are $\text{Fe}(\text{OH})_{3(s)}$ and $\text{FeS}_{2(s)}$.

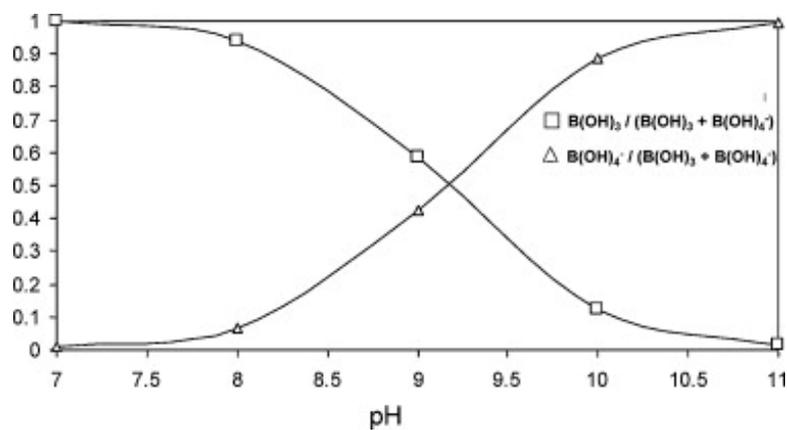


Figure 2.3. Boron speciation between pH 7 and 11 for boric acid concentration $< 0.025 \text{ M}$ (Avraham et al., 2011).

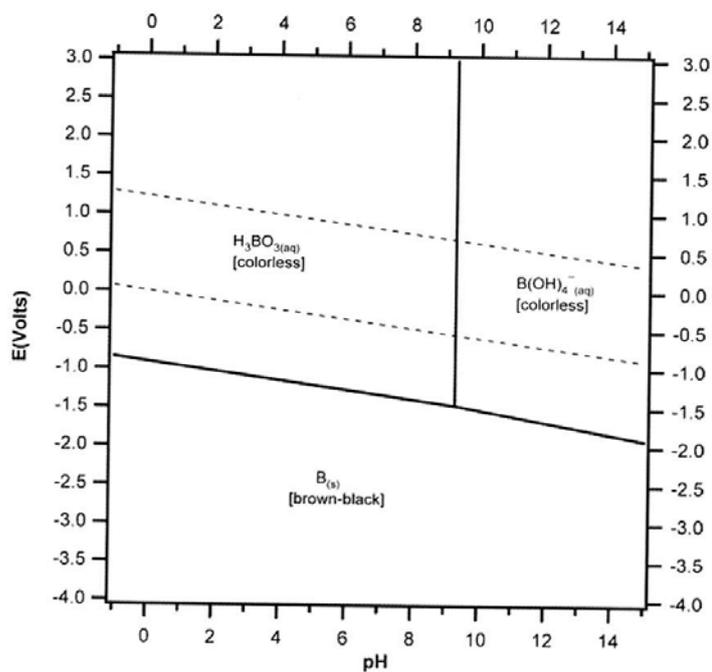


Figure 2.4. Eh-pH diagram for B species. Soluble species concentration 10^{-1} M (Schweitzer and Pesterfield, 2010).

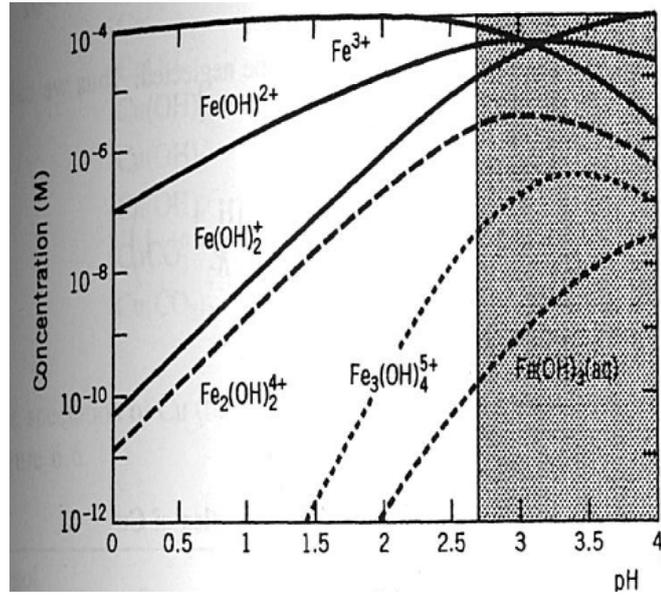


Figure 2.5. Distribution diagram of Fe(III) species in a 10^{-4} M solution. The shaded area indicates the approximate pH range of oversaturation with regard to $\text{Fe}(\text{OH})_3(\text{s})$ (Stumm and Morgan, 1996).

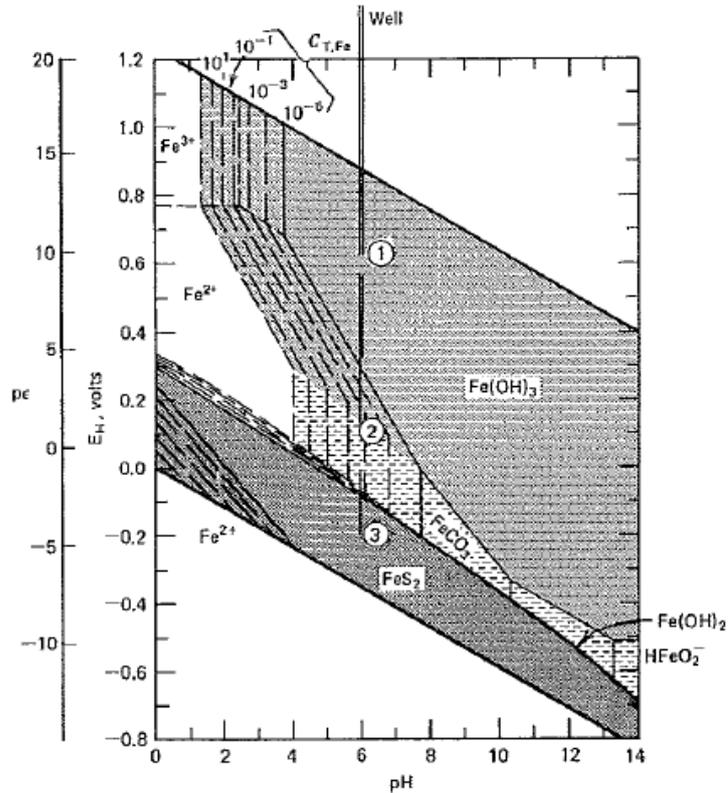


Figure 2.6. Solubility of Fe in relation to Eh and pH at 25°C and 1 atm, with total sulfur 10^{-4} M and total carbonate 10^{-3} M. The several boundaries between solid and solution phases represent different total concentration of Fe (Snoeyink and Jenkins, 1980).

2.2.2 Occurrence in the environment and impacts on human health

Serious incidents of water contamination by arsenic have been reported in different countries such as Bangladesh, India, China, USA, Chile, etc. Figure 2.7 shows the geographical distribution of arsenic sources and estimates of people at risk of chronic exposure.

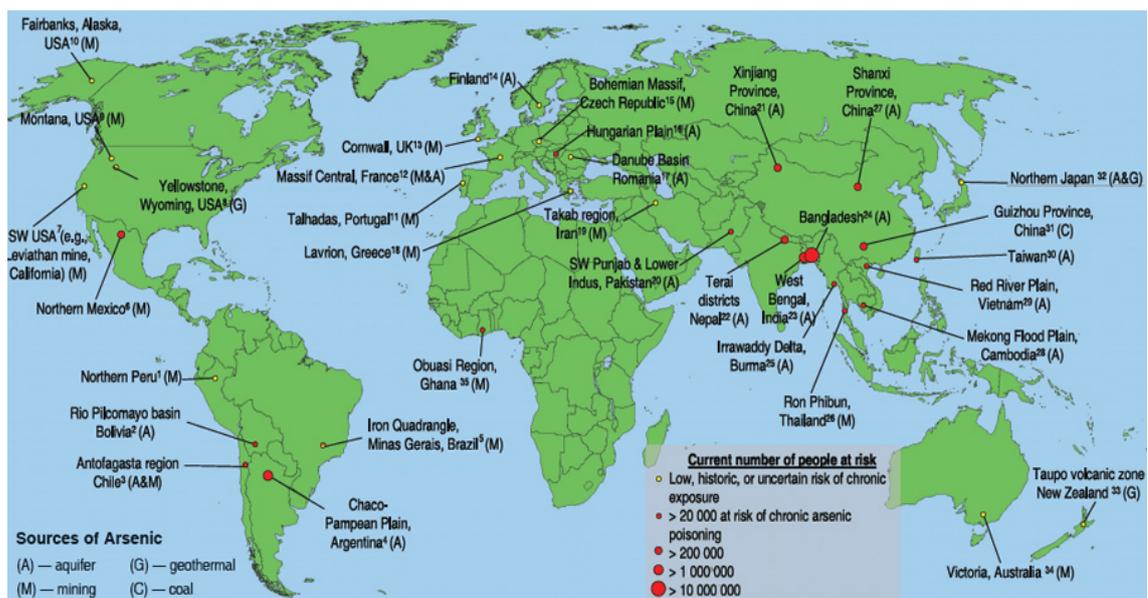


Figure 2.7. Worldwide distribution of arsenic contaminated regions, source of arsenic and numbers of people at risk of chronic exposure (Garelick and Jones, 2008).

The World Health Organisation (WHO) lowered the proposed drinking water As guideline value from 50 to 10 $\mu\text{g/L}$ in 1993. Although many regulatory agencies in industrialised nations have adopted the new guideline, many developing nations such as Bangladesh retained the higher level, since they often lack of resources to efficiently remove As from water to a level of 10 $\mu\text{g/L}$ (Smith and Smith, 2004).

In the Bengal basin, which covers most of Bangladesh and parts of the Indian states of West Bengal, Assam and Tripura, around 60 million people are thought to be at risk, since they live in areas with groundwater arsenic concentrations higher than 50 $\mu\text{g/L}$ (Mukherjee et al., 2009). The concentrations in the groundwater vary across the basin, but the highest is 4,730 $\mu\text{g/L}$, reported by Rahman et al. (2006). The aquifer is thought

to contain arsenic mobilised under anoxic conditions by bacterial activity, however the primary source of arsenic in the groundwater of the basin is still unclear (Mukherjee et al., 2009).

In Central Victoria, Australia, major creeks, rivers and lakes present As concentrations of 8 µg/L on average (Sultan and Dowling, 2006). Although the number of people at risk is low compared to those in the Bengal basin case (Garellick and Jones, 2008), it has been found that increasing soil arsenic level was associated with a significant increase in past cancer risk in socio-economically disadvantaged areas in a gold mining area in Victoria (Pearce et al., 2012).

When reduced iron minerals such as pyrite ($\text{FeS}_{2(s)}$) are oxidised, they produce acidic waters, with this phenomenon being associated with the problem of acid mine drainage. The oxidation/reduction of iron in soil and groundwaters determines the iron content of these waters (Snoeyink and Jenkins, 1980).

No guideline value for iron in drinking water has been proposed by the WHO, probably because iron at low to moderate concentrations is not generally regarded as a threat to human health or aquatic life (Kadlec and Wallace, 2009). Iron at low concentrations can be a trace nutrient but at high concentrations it can be a toxicant (US EPA, 1986). As such, the US EPA proposed a guideline value of 0.3 mg/L for human ingestion purposes, and a guideline value of 1 mg/L for protection of freshwater aquatic environments purposes (US EPA, 2009). Iron salts may not be toxic, but iron flocs or gels may be irritant or clog fish gills to effect asphyxiation (US EPA, 1986).

Despite the fact that it is generally accepted that boron is only found as H_3BO_3 and $\text{B}(\text{OH})_4^-$ (i.e. soluble and oxygenated compounds), conflicting information about boron speciation has been reported recently, since this general approach tends to oversimplify the fact that little is known about the speciation of B in natural waters, soils and sediments (Kot, 2009). As an example, it has been found that the presence of organic matter can contribute to boron adsorption, but few studies have provided convincing evidence on the adsorption of B by organic soils such as peat (Sartaj and Fernandes, 2005).

Boron is essential for plant growth (Marin and Oron, 2007), however if it is present in excess it can be toxic to the plants. Several studies have demonstrated such toxic effect, and the accumulation of boron in the water, sediments and biota of several aquatic and wetlands ecosystems (Davies et al., 2002). Boron contamination in the water environment is causing increasing concern (Xu and Jiang, 2008). The WHO drinking water guideline concentration for boron is currently set as 0.5 mg/L (WHO, 2008).

2.2.3 Remediation mechanisms

There are different mechanisms able to remove arsenic, iron and boron, being some water treatment unit processes common in them. Mostly conventional technologies have been used for this purpose, and they have been reviewed by many authors in the case of As (Mohan and Pittman Jr., 2007), by Xu and Jiang (2008) in the case of B, and by Chaturvedi and Dave (2012) in the case of Fe.

Negatively charged species are easier to remove than uncharged species, because the charges on the As(V) oxyanions allow them to be removed by sorption, anion exchange or precipitation/co-precipitation (Henken and Hutchison, 2009). This also applies to other charged species such as Fe(II), Fe(III) and $B(OH)_4^-$. As such, many remediation techniques oxidise As(III) to As(V) prior to treatment. Oxidation, co-precipitation, adsorption, ion exchange and membrane processes are the major techniques employed to remove arsenic. However, due to variations in arsenic speciation and the characteristics of the water to be treated, often more than one technology is required (Henken, 2009b). The efficiency and applicability of the above mentioned technologies vary depending on arsenic levels and the composition of the source water (Jain and Singh, 2012). For example, the effectiveness of arsenic co-precipitation with iron is relatively independent of the pH of the source water in the range 5.5-8.5. However, high levels of organic matter, orthophosphates and silicates weaken arsenic removal efficiency by competing for sorption sites on iron hydroxide precipitates (ibid). In addition, although some of these methods are quite simple, the disadvantage associated with coprecipitation/sorption methods is the large production of toxic sludge (Jain and Singh, 2012). Although membrane methods do not produce toxic sludge, they

generate toxic wastewater and require hi-tech maintenance (Mohan and Pittman Jr., 2007). Achieving arsenic treatment systems which are economically viable can also be a challenge, especially if they are small-scale or community-based in regions such as South East Asia, due to the socio economic situation of people and the high levels of As in the water (ibid).

Redox reactions are involved in the removal of iron from waters (Snoeyink and Jenkins, 1980). In an oxygenated environment, ferric iron is present as insoluble oxyhydroxides, denoted as FeOOH (Kadlec and Wallace, 2009). Although dissolved ferric iron Fe(III) is absent between pH 5 and 10, the oxyhydroxides occur in suspended form in surface and groundwaters within that pH range (Langmuir, 1997). If present in colloidal sizes, Fe can remain suspended indefinitely. Therefore, to remove these particles from water, coagulation/flocculation techniques are required (Langmuir, 1997). Colloids are removed from water either by settling if they aggregate or by filtration if they attach to the medium through which water passes (Stumm and Morgan, 1996).

Iron removal is commonly required from acid mine drainage, since it is usually rich in dissolved iron. In fact, one of the environmental problems associated with acid mine drainage is the deposition of Fe(III) hydroxides as orange precipitates downstream the acid mine drainage source, through oxidation of Fe(II) and subsequent precipitation, as the pH rises upon mixture with non-acidic water (Wang et al., 2003). Acid mine drainage is conventionally treated with finely powdered limestone (CaCO_3) or lime (Ca(OH)_2) to raise its pH, so the dissolved metals precipitate out as basic metal carbonates or oxyhydroxides. Although lime and limestone are cheap and readily available, the resulting sludge is difficult to dewater and costly to dispose of due to its metal content (Wang et al., 2003).

The role of iron in arsenic-containing water is crucial since iron may control arsenic speciation: iron oxides are especially important and effective sorbing and/or co-precipitating arsenic in both natural and artificial systems. Adsorption and co-precipitation of arsenate with iron (and aluminium) flocs are believed to be the primary arsenic removal mechanisms in water treatment plants (McNeill and Edwards, 1997).

Iron salts can be also used to remove boron (Xu and Jiang, 2008). Although there is no easy or simple method for the removal of boron from water and wastewaters (Xu and Jiang, 2008), it can be removed by different technologies, such as chemical precipitation, adsorption with activated carbon, ion exchange resins and reverse osmosis. However, they have limitations, such as the costs of chemicals, the amount of sludge and low removal capacities (Xu and Jiang, 2008). Emerging low-cost technologies have been found to have promising results, such as the use of plants (Tassi et al., 2011, Rees et al., 2011, Marin and Oron, 2007) and microalgae (Ertit Taştan et al., 2012), however the removal of boron by using efficient and economical methods is still a challenging problem (Ertit Taştan et al., 2012).

The occurrence of As, Fe and B in aquatic ecosystems may pose a risk due to possible toxic effects on biota. Chronic exposure to arsenic-contaminated water can cause cancer (Tchounwou et al., 2003). Boron and iron can also have detrimental effects if present in high concentrations (US EPA, 1986, Butterwick et al., 1989). Since arsenic and iron are highly reactive, they are easier to remove than boron. A variety of technologies have been employed to remove As, B and Fe, such as chemical precipitation, adsorption and membrane processes. Most of them are expensive and generate sludge or toxic effluents. Therefore, there is a need for cost-effective and sustainable technologies for the removal of arsenic, boron and iron from contaminated water.

2.3. Constructed wetlands for the removal of arsenic and metals

2.3.1 Constructed wetlands as a natural system for water treatment

Natural systems combine different physical, chemical and biological processes for water treatment. These processes all occur naturally, and some such as photo-oxidation and plant uptake are unique to natural systems.

The main difference between natural systems and conventional technologies is the source of energy. Despite the fact that both require the same amount of energy input for degrading the same mass of pollutant, in natural systems naturally occurring energies

that come with the land (such as solar radiation -which would allow photochemical oxidation of hydrocarbons- and the kinetic energy of the wind -which provides aeration through the surface water/air interface, allowing aerobic processes to occur-) predominate; while in conventional technologies, fossil fuel, non-renewable energies are typically employed (although in theory renewable energy sources could be used in such systems). However, due to the low power intensity in natural systems, the area required for biological processes in these systems is bigger than that required for the same processes in conventional systems. Therefore, natural systems are land intensive, whilst conventional systems are energy intensive (Kadlec and Knight, 1996).

A typical natural system is that of a wetland. Wetlands are defined as land where the water table is at or above the ground surface long enough to maintain saturated soil conditions and the growth of related vegetation (Crites et al., 2006). The capability of wetlands to treat wastewater has been demonstrated through different studies in different locations, for pre-existing natural marshes, swamps, bogs, peat lands, strands, and for systems specially constructed for water treatment (Crites et al., 2006, Kadlec and Wallace, 2009).

Wetland systems may either be natural wetlands or constructed wetlands (CW). Both can be used to treat wastewater. Constructed wetlands are engineered systems designed to simulate a natural wetland for waste treatment or other purposes (Nuttal et al., 1995). As a natural system, constructed wetlands have low investment and operating costs (Yeh, 2008).

There are two principal types of constructed wetlands (Wallace and Knight, 2006):

1. Surface-flow wetlands (SF) in which wastewater flows above the support medium. Surface-flow wetlands are also known as Free Water Surface Wetlands (FWS).
2. Subsurface-flow wetlands (SSF) in which wastewater flows through the support medium, and this flow can be vertical (VSSF) or horizontal (HSSF). Subsurface-flow constructed wetlands are also known as Vegetated Submerged Bed Wetlands (VSB).

Both types of constructed wetlands are shown in Figure 2.8.

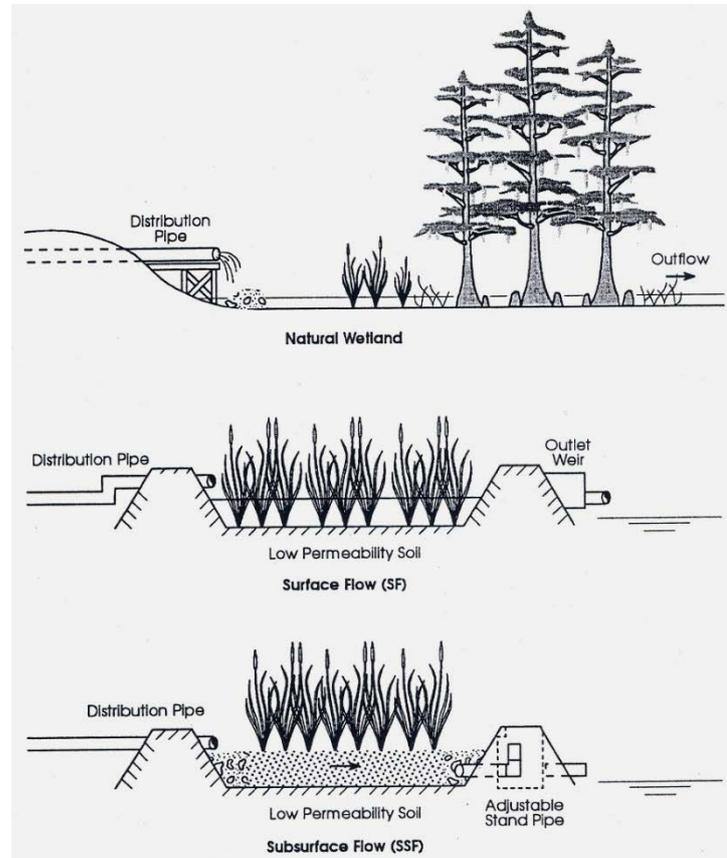


Figure 2.8. The two types of constructed wetlands: surface flow (SF) and subsurface flow (SSF) (Kadlec and Knight, 1996).

Constructed wetlands are a cost-effective way to treat industrial, agricultural and domestic wastewaters (Nuttal et al., 1995). They can reduce suspended solids, nutrients, oxygen-depleting substances, organic particulates and most other physical and chemical pollutants such as colour and bacteria. Furthermore, they are able to remove metals and metalloids, so they have been specially used for the treatment of acid mine drainage (Sobolewski, 1999, Sheoran and Sheoran, 2006, National Rivers Authority, 1992). In the US, constructed wetlands were used in more than 300 coal mine sites, to raise pH and remove iron and/or manganese (Kadlec and Wallace, 2009). Currently, constructed wetland systems are the most widely-used passive mine water treatment technology. Surface flow wetlands (also called “aerobic wetlands” or “reed beds”) are regarded as a proven technology when applied to the treatment of ferruginous, alkaline mine water

(Younger et al., 2002). For extremely acidic water, it is recommended that wetlands have an anoxic substrate of organic material. This way, a source of organic carbon is provided for sulfate-reducing bacteria (SRB). This reduction process consumes acidity and removes metals as sulfides (Younger et al., 2002).

2.3.2 Components of constructed wetlands

The main components of constructed wetlands are vegetation, media and microorganisms. These three components interact and thus constructed wetlands can be considered as complex bioreactors. Each of these components is described below.

2.3.2.1 Vegetation

The vegetation used in constructed wetlands is mostly macrophytic plants (Kadlec and Wallace, 2009), many of which occur naturally in wetland environments. The term macrophyte includes vascular plants that have tissues that are easily visible. According to their growth habitat with respect to the wetland water surface, these plants are categorised as emergent soft tissue plants, emergent woody plants, submersed aquatic plants, floating plants and floating mats (Kadlec and Wallace, 2009). All these categories are suitable for surface flow wetlands, but only the first two for subsurface flow wetlands. Emergent soft tissue plants such as *Phragmites*, *Typha* and *Schoenoplectus* (*Scirpus*) have been widely used in treatment wetlands technology (Kadlec and Wallace, 2009). Table 2.1 shows the main characteristics of emergent plants commonly used in constructed wetlands (data from Wallace and Knight, 2006).

The selection of plants should consider different factors, such as climate, latitude, maintenance and water quality (Wallace and Knight, 2006). For example, *Phragmites australis* is distributed worldwide, can be permanently inundated and is very drought resistant (Reed et al., 1995).

Table 2.1. Some plants species suitable for SF and SSF wetlands (source: Wallace and Knight, 2006).

Common name	Scientific name	Wetland type	Geographical distribution	pH range	Salinity	Comments
Cattail (broadleaf)	<i>Typha latifolia</i>	SF or SSF	Across North America	5.5-7.5	Less than 0.5 ppt*	Used as a food source by aquatic mammals
Cattail (narrowleaf)	<i>Typha angustifolia</i>	SF or SSF	Across North America	3.7-8.5	Less than 0.5 ppt	Tolerates low pH
Common reed	<i>Phragmites australis</i>	SF or SSF	Worldwide	3.7-9.0	Up to 20 ppt	Used mostly in Europe
Green bulrush	<i>Schoenoplectus (Scirpus) atrovirens</i>	SSF	North-Central North America	-	Fresh	Low maintenance SSF plant
River bulrush	<i>Schoenoplectus (Scirpus) fluviatilis</i>	SSF	North-Central North America: New Mexico, California	-	Fresh, less than 0.5 ppt	Low maintenance SSF plant

*ppt: parts per thousand

Macrophytes provide the structure that fosters many removal processes (Kadlec and Wallace, 2009), so they are essential for high-quality water treatment performance. For the removal of metals, the role of vegetation can be direct or indirect. Many studies have shown that direct uptake by the plants is often not the principal metal removal mechanism (García et al., 2010). However, wetland plants play an indirect but important role because they can:

1. Stimulate the growth of metal-oxidising bacteria by oxygen transfer into the rhizosphere (Cooper et al., 1996).
2. Provide organic matter as a carbon source for sulfate-reducing bacteria (SRB) and metal-oxidising bacteria (National Rivers Authority, 1992).
3. Provide a surface for microorganisms within the water column (National Rivers Authority, 1992) or medium (Nuttal et al., 1995).

4. Provide their roots as a surface for iron plaque, which is mostly iron (hydr)oxides precipitates. This plaque can be highly reactive with different metals and metalloids, bonding As, Mn, Zn, Cu and Pb (Hansel et al., 2002, Buddhawong et al., 2005, Kröpfelová et al., 2009).
5. Oxygenate the substrate by their roots, providing an extensive region where aerobic and anaerobic conditions are adjoining (National Rivers Authority, 1992), facilitating coupled aerobic-anaerobic processes.

2.3.2.2 Media

Gravel, grits and sand are the most common media used in subsurface flow wetlands. However, soils and other substrates have also been employed for the removal of different pollutants.

The main role of the media is to provide support to vegetation and microorganisms. In addition, the media play a role in providing different environmental conditions within the wetland, such as oxic and anoxic zones, and in providing sources for biogeochemical reactions. The selection of media that can foster particular removal processes is an important part of the wetland design. However, the use of these media may imply the need of periodic maintenance (Kadlec and Wallace, 2009).

The most commonly used media identified from the literature, together with those media which are hypothesised to be efficient in removing metals, are discussed below.

Gravel is the most common medium used, regardless of the target pollutant(s). In horizontal flow wetlands, most gravels used have been washed river gravels -usually silica quartz-, with typical sizes of 3-6 mm, 5-10 mm, and 6-12 mm recommended in the UK (Cooper et al., 1996). In vertical flow wetlands, layers of graded gravel are used usually with a top layer of washed sharp sand. The UK specification of graded gravel is given as follows: A bottom layer, 15 cm depth, having 30-60 mm round washed gravel; and a top layer, having sharp sand of 8 cm depth, 12 mm round washed gravel of 10 cm depth, and 6 mm washed pea-gravel of 15 cm depth (Cooper et al., 1996). Figure 2.9 shows this typical arrangement of the media.

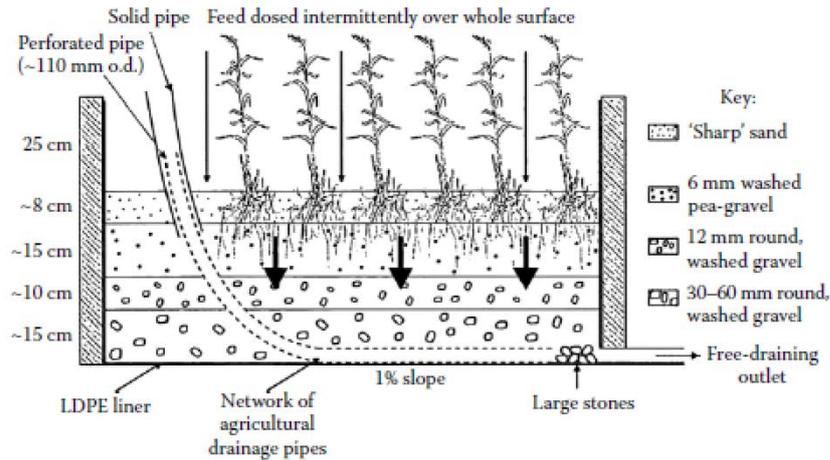


Figure 2.9. Typical arrangement of a downflow vertical flow constructed wetland (Vymazal and Kröpfelová, 2008).

Peat is partly fossilised plant organic matter, which has been found to remove different pollutants from water, including metals (Snoeyink and Jenkins, 1980, Couillard, 1994, Brown et al., 2000) and As (Cloy et al., 2009). Peat has been mainly used as a sorbent material, but a few studies have reported its use as a wetland medium (e.g. Sobolewski, 1996). Due to the organic matter content, other organic substrates could have a similar performance in the removal of metals.

Zeolite has been employed successfully as wetland medium to remove different pollutants, including metals such as Zn (Sarafraz et al., 2009). Although it can also remove As (Chutia et al., 2009, Elizalde-González et al., 2001), there is no evidence in the literature of using zeolite as a wetland medium for the removal of As.

Limestone is mostly used for the treatment of acid mine drainage, as a preliminary system for raising pH (Wallace and Knight, 2006). In constructed wetlands, limestone is occasionally used as supplementary medium but not as the main medium (Groudev et al., 2008). As such, the effectiveness of using limestone as the main wetland substrate has not been thoroughly investigated, despite the fact that its use has been recommended to enhance metal removal (Stark et al., 1996, Ye et al., 2003).

Blast furnace slags have been also used to raise pH (Kadlec and Wallace, 2009). In addition, they have shown capability to remove phosphorus (Sakadevan and Bavor, 1998). Steel slag has been used to enhance nitrogen removal (Xiong et al., 2011). Slags have high sorption capacity and thus they could be explored as alternative wetland media.

A recent study (Zurita et al., 2012) has successfully employed an iron oxide substrate to promote As retention, proving that the substrate plays an important role in As removal. Therefore, similar substrates are still to be explored in constructed wetlands to enhance As removal.

2.3.2.3 Microorganisms

Wetlands provide appropriate environmental conditions for the growth of microorganisms. Two important groups of these are bacteria and fungi, and their main characteristics are presented below.

Bacteria are prokaryotic and unicellular organisms, classified by their morphology, chemical staining characteristics, nutrition and metabolism (Kadlec and Knight, 1996). Bacteria are mostly responsible for the removal of pollutants in water treatment systems, and therefore it is important to understand their biochemical activities. Bacteria, like all organisms, must have a source of energy, carbon to synthesise new cellular material, and nutrients (inorganic elements) such as N, P, K, S, Ca and Mg (Cooper et al., 1996). Table 2.2 presents the classification of microorganisms by their source of energy and carbon.

Most bacteria are heterotrophic, so their requirements for cell synthesis (carbon source) and nutrition (energy source) are obtained from organic compounds, whereas autotrophic bacteria obtain their carbon from inorganic carbon (carbon dioxide, CO₂) (Kadlec and Knight, 1996). Inside a wetland matrix, most bacteria are found at three locations: (1) soils, (2) decaying organic matter, and (3) the solid surfaces of plants (Kadlec and Knight, 1996).

Table 2.2. General classification of microorganisms by sources of energy and carbon and some examples (Adapted from Cooper et al. (1996) and Chan (2003))

Classification	Energy source	Carbon source	Examples of organisms
Autotrophic:			
<i>Photo-autotrophic</i>	Light	CO ₂	Purple and green sulfur bacteria, algae, plants, cyanobacteria
<i>Chemo-autotrophic</i>	Inorganic oxidation-reduction reaction	CO ₂	Nitrifying, hydrogen, iron and sulfur bacteria
Heterotrophic:			
<i>Photo-heterotrophic</i>	Light	Organic carbon	Purple and green non-sulfur bacteria
<i>Chemo-heterotrophic</i>	Organic oxidation-reduction reaction	Organic carbon	Most bacteria, fungi, protozoa, and animals

Fungi are eucaryotic organisms and include yeast, molds and fleshy fungi (Kadlec and Knight, 1996). All fungi are heterotrophic and in wetlands they commonly grow associated with dead and decaying plant litter. They are important in wetlands because they mediate a significant proportion of the recycling of carbon and other nutrients. Aquatic fungi often colonise niches on decaying vegetation made available following completion of bacterial use (Kadlec and Knight, 1996).

The variety of microbial species in wetland systems functions in a wide range of physicochemical conditions. Many of these organisms are the same as those important in conventional treatment systems, therefore their growth requirements and characteristics are known (Kadlec and Knight, 1996).

The fact that the removal of pollutants in constructed wetlands is primary due to microbial activity has been a cornerstone of the technology since the beginning (Faulwetter et al., 2009). For organic carbon and nitrogen this is certainly true

(Stottmeister et al., 2003, Faulwetter et al., 2009). The metabolic processes of microorganisms play the most significant role in the removal of heavy metals (Sheoran and Sheoran, 2006), although other processes can also occur (Marchand et al., 2010).

The interaction of the constructed wetlands components: vegetation, media and microorganisms allow different physical, chemical and biological processes to occur. Vegetation provides key factors such as oxygen transfer and a source of organic matter. Vegetation also removes pollutants by direct uptake, but this may not be the most important process for metal removal. Microorganisms are often attached to plants roots, and their metabolism is important in the removal of metals. Wetland media also provides support to microorganisms and vegetation and depending on the type of media, they can also foster particular processes (for example a medium such as zeolite can enhance metal removal by sorption).

The combination of all these factors provided by the constructed wetland components allows the removal of different pollutants, including metals and metalloids.

2.4. Removal processes for metals and metalloids in constructed wetlands

2.4.1 Introduction

Subsurface flow wetlands remove various pollutants from water by different physical, chemical and biological processes. The occurrence of these processes depends on the nature of pollutants and environmental conditions. Although pollutant removal mechanisms in wetlands have been studied intensively for over two decades, for many pollutants the mechanisms are still unclear (Sheoran and Sheoran, 2006). For example, information about the mechanisms of arsenic removal in a constructed wetland is rarely found in the literature (Singhakant et al., 2009a). In addition, despite the potential removal of B by wetland systems (Davis et al., 2002), only a few studies have been performed regarding the use of constructed wetlands for this application. Since in most of them boron is not the only target pollutant (e.g. Kröpfelová et al., 2009, Arroyo et

al., 2010), removal processes have been barely identified. On the other hand, information about the removal of metals has been summarised by several authors (Dunbabin and Bowmer, 1992, Sheoran and Sheoran, 2006, Marchand et al., 2010). Given the complexity of the interactions of the different processes involved, it is difficult to identify which reactions occur in a wetland system. These reactions include processes such as settling, sedimentation, sorption, co-precipitation, cation exchange, photodegradation, phytoaccumulation, biodegradation, microbial activity and plant uptake (Sheoran and Sheoran, 2006). In general, surface wetlands are more commonly used to treat waters with high heavy metals concentrations as it has been considered that oxic/aerobic processes are more effective. However, limited information is available on the removal of heavy metals in horizontal subsurface flow wetlands, where mostly anoxic/anaerobic processes occur (Vymazal and Kröpfelová, 2008).

This section summarises up-to-date information on how three pollutants: As, Fe and B, the target pollutants of this research, can be removed in a wetland system.

2.4.2 Conceptual process maps for As, Fe and B

The main arsenic, iron and boron transformation routes in a constructed wetland are illustrated in Figure 2.10 (a), (b) and (c) respectively; and they will be discussed in the following sections. In this Figure, those processes considered to be most important in the overall removal are highlighted in bold.

2.4.3 Precipitation

Precipitation refers to dissolved species (such as H_2AsO_4^- and HAsO_4^{2-}) reacting with other dissolved species (such as Fe(III) and Ca) to form solid insoluble reaction products. This process may result from oxidation, reduction, pH changes or the mixing of chemicals into a solution (Henken and Hutchison, 2009).

Precipitation is an important removal mechanism for metals and metalloids in wetlands (García et al., 2010). Ye et al. (2003) found that in wetland microcosms, boron was

retained mostly in the sediments, concluding that boron and other pollutants (As, Se) formed insoluble precipitates (Ye et al., 2003). However, it appears that no low solubility compounds have been identified for boron (Parks and Edwards, 2005), therefore the key process which removes boron is sorption, rather than precipitation.

Key factors affecting As and Fe precipitation are: pH, the presence of other dissolved species (sulfur for example) and the redox potential. Other factors such as temperature, dissolved oxygen and a source of carbon become more important when precipitation is microbially-mediated.

Oxidation and reduction of iron occurs relatively easily depending on redox potential (Eh) and pH. Ferric iron is the dominant form under oxidised conditions (Eh>0 and pH≥6) (Faulkner and Richardson, 1989; cited by Kadlec and Wallace, 2009), and forms insoluble iron oxyhydroxide precipitates, as shown below (Wallace and Knight, 2006):

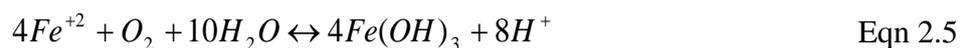


Ferric iron can exist as hydroxide (Fe(OH)₃), oxyhydroxide (FeO(OH)) or oxide such as Fe₂O₃ in neutral or slightly alkaline solution (Ehrlich, 2002). Sulfide minerals such as pyrite (FeS₂) can be formed when hydrogen sulfide (H₂S) is present. Hydrogen sulfide can reduce Fe⁺³ to Fe⁺², and precipitate it, if Fe⁺³ is present in excess (Ehrlich, 2002). Therefore, Fe can precipitate either as an oxide or as a sulfide.



In wetlands, iron precipitates form iron plaque on plant roots (Section 2.3.2.1). Therefore, iron precipitates can be either trapped in the wetland media or attached to the plants.

Some researchers consider that iron microbially-mediated oxidation, followed by precipitation of iron oxyhydroxides, is the most important iron-removal mechanism in wetlands. This process is catalysed by *Thiobacillus ferrooxidans*, the most studied acidophilic bacterium (Ehrlich, 2002) and occurs as follows (National Rivers Authority, 1992):



There are other similar bacteria which are able to oxidise iron, such as *Leptospirillum ferrooxidans* and *Sulfolobus* spp. (Ehrlich, 2002). The role of iron-oxidising bacteria is important at pH levels below 5 (Sobolewski, 1999). At higher pH values, bacterial oxidation of iron proceeds more slowly than abiotic oxidation (Kirby et al., 1999). According to McIntire et al. (1990), Fe⁺² oxidation occurs in the absence of bacteria at pH 6 or above (McIntire et al. (1990), cited in National Rivers Authority, 1992). In addition, as presented in Section 2.4.1, microbial precipitation of Fe can also cause co-precipitation of arsenic. Therefore, iron-oxidising bacteria may have a role in the removal of both Fe and As.

Despite the fact that constructed wetlands have been most widely used for acid mine drainage treatment, they generally have limited capacity to raise pH (Kadlec and Wallace, 2009). The wetland substrate may be used to adjust pH: zeolite and blast furnace slag are used to raise pH, but the effect may be transitory (Kadlec and Wallace, 2009). For coal mine waters, the incorporation of anoxic limestone drains or alkali-dosing for pH adjustment is recommended where the influent has $\text{pH} < 5.5$. The use of Reducing and Alkalinity Producing Systems (RAPS, or Successive Alkalinity Producing Systems SAPS), or compost-based wetlands are recommended for $4 < \text{pH} < 5.5$ (Mayes et al., 2009). Compost-based wetlands are constructed with a ~0.5 m thick substrate of organic waste material which promotes bacterial sulfate reduction in addition to increasing alkalinity. In addition, wetland substrates which positively influence sulfate reduction have an important role in acid mine drainage treatment, because sulfide minerals have been found in a number of wetland sediments (Sheoran and Sheoran, 2006), and therefore in wetland media.

In oxidising environments with high levels of As(V), precipitation of As(V) with Ca, Mg, Al and Fe(III) may occur (Henken, 2009a). Under reducing environments and in the presence of S and Fe, As can form insoluble sulfide compounds (Buddhawong et al., 2005; Singhakant et al., 2009a), such as orpiment As_2S_3 , in which arsenic is present as As(III), and arsenopyrite AsFeS . Orpiment may precipitate in a low Fe and S rich environment, especially under acidic conditions (Wilkin and Ford, 2006). The formation of As_2S_3 was considered to be abiotic prior to the study of Newman et al. (1997), which reported a bacterium able to precipitate As_2S_3 .

In constructed wetland environments, arsenic is retained mostly in sediments (Ye et al., 2003) or media (Buddhawong et al., 2005; Singhakant et al., 2009a, b), rather than accumulated in plants. Singhakant et al. (2009a) analysed the forms of As in a wetland using Tessier extraction and found that the residual fraction of As was the predominant As form, concluding that As was mainly retained in the pores of the wetland media (sand and gravel). In addition, the oxidisable fraction (binding to organic matter and sulfides) was the second most important fraction. Singhakant et al. (2009b) also found that the major removal mechanisms were trapping within porous media (residual fraction) and trapping with Fe and Mn on the media surface (reducible fraction).

The redox potential may indicate the presence of sulfide precipitates. Buddhawong et al. (2005) attributed the removal of As and Zn to processes that exclude precipitation as sulfides: the authors did not measure redox potential levels below 100 mV, so they assumed unfavourable redox conditions for sulfate-reducing bacteria (SRB) and concluded that sulfides could not precipitate. No further details were provided about the required redox conditions for sulfide precipitation by sulfate-reducing bacteria. Different authors have reported different redox potential values required by sulfate-reducing bacteria to thrive: less than -200 mV (Cabrera et al., 2006, Diels et al., 2006), less than -100 mV (Willow and Cohen, 2003), or between -150 and -200 mV (Tuttle, 1969). Rahman et al. (2008) noted that microbial sulfate reduction was greater under redox potential values between -160 and -190 mV, and this higher sulfate removal contributed to higher removal of As. It should be noted that different redox potentials can be found in different parts of the wetland and they cannot necessarily be represented by the measurements, since plants generate microzones of oxidising or reducing conditions in the substrate (Cohen, 2006). Therefore, measured redox potential values can be used to assess the presence of oxidising or reducing conditions, but they must be evaluated carefully.

Sulfate-reducing bacteria can mediate the precipitation of arsenosulfide minerals. These bacteria occur primarily in near-neutral anoxic environments (Sjöblom, 2003), preferring pH conditions between 5 and 8 (Cohen, 2006). However, sulfate reduction has been observed in acidic conditions (pH 3-4) (Hao, 2003). Some examples are *Desulfobacter hydrogenophilus* (autotrophic, growth on H₂ and CO₂) and *Desulfovibrio sulfodismutans* (heterotrophic) (Ehrlich, 2002).

Sulfate-reducing bacteria transform sulfate to sulfide according to the following reaction (Cohen, 2006):



where CH₂O represents a simple organic molecule, such as acetate (National Rivers Authority, 1992). The available sulfide reacts with As(III), forming insoluble As-S minerals. In addition, sulfate-reducing bacteria control their pH environment by generating alkalinity, as shown above. The optimum temperature for sulfate-reducing bacteria ranges between 28 °C and 32 °C (Hao, 2003). Sulfate reduction rates decrease as temperature decreases (Sjöblom, 2003; Sobolewski, 1999), while the solubility of oxygen increases as temperature decreases. Therefore, if As and metals are going to be removed by sulfide precipitation, high temperature is required.

Sulfate-reducing bacteria are obligate anaerobes, but they may survive a temporary exposure to oxygen and become active again under anaerobic conditions (Hao, 2003). The critical dissolved oxygen concentration below which sulfate reduction can occur is 0.1-1 mg/L (US EPA, 1985). The type of flow affects oxygen availability: vertical flow wetland systems tend to have good performance in oxygen transfer, favouring aerobic microbial populations; whilst horizontal flow wetland systems tend to be oxygen-limited and therefore will typically favour anaerobic microbial populations (Faulwetter et al., 2009; Kadlec and Knight, 1996). The method of distributing inflow also affects the availability of oxygen: batch feeding favours more aerobic processes, whilst continuous feeding favours more anaerobic processes (Faulwetter et al., 2009). Vegetation also plays a role by transferring oxygen into the rhizosphere and subsequently into the substrate.

Sulfate reduction requires a reducing environment and an electron donor. Rahman et al. (2008) reported that under oxidising conditions and with a deficiency of electron donors, sulfate reduction was limited and the removal of As was achieved via mechanisms other than precipitation of As(III) sulfides. On the other hand, under more reducing conditions and surplus carbon, sulfate reduction by sulfate-reducing bacteria contributed to an efficient As removal (Rahman et al., 2008). However, their conclusions were drawn based on their measured water quality parameters (Eh, TOC), without monitoring microbial community composition or function directly. Groudev et al. (2008) reported a numerous and diverse population of sulfate-reducing bacteria mainly in sediments of constructed wetlands treating acid mine drainage, where As and other heavy metals were precipitated as sulfides (Groudev et al., 2008); this was one of

few studies that monitored sulfate-reducing bacteria communities contributing to As removal in a constructed wetland. Another example is Duncan et al. (2004), who reported appreciable numbers of sulfate-reducing bacteria and iron-reducing bacteria. They suggested that the main As removal pathway was the biogenic precipitation of As sulfides, given that As concentrations were positively correlated with sulfur concentration, and negatively correlated with organic carbon concentration. Recently, Mattes et al. (2010) provided more details of the wetland system described in Duncan et al. (2004), highlighting that not only sulfate-reducing bacteria played a role in As removal, with iron-oxidising bacteria also making a significant contribution. However, the As removal mechanisms are not yet clearly identified (Mattes et al., 2010).

Despite the fact that some studies have reported that arsenosulfide minerals accumulate in wetland sediments when bacterial sulfate reduction was active (Langner et al., 1999, Duncan et al., 2004, Groudev et al., 2008), the importance of As_2S_3 precipitation caused by sulfate-reducing bacteria remains poorly understood (Buddhawong et al., 2005). In acid mine drainage, the initial As removal process in the presence of sulfate-reducing bacteria is not clear (adsorption or coprecipitation with other metals sulfides or ferrihydrite have been proposed). Formation of insoluble arsenic sulfide can occur after initial removal when reducing conditions have been established (Neculita et al., 2007).

Other microorganisms, such as arsenite-oxidising bacteria, arsenate-reducing bacteria and iron-oxidising bacteria, can also mediate the removal of As (directly or indirectly). Many microorganisms can oxidise or reduce arsenic, even if it is present in the mineral form (Reddy and DeLaune, 2008), and these microorganisms coexist in the soil environment (Macur et al., 2004). In constructed wetlands, some researchers have isolated different types of bacteria responsible for arsenite oxidation. Recently, in constructed wetlands treating acid mine drainage in Bulgaria, Groudev et al. (2008) isolated heterotrophic bacteria (related to the genus *Pseudomonas*) able to oxidise As(III) to As(V) at slightly acidic and neutral pH, in addition to acidophilic chemolithotrophs also able to oxidise As(III). In natural and constructed wetlands in Korea, Chang et al. (2010) isolated heterotrophic arsenic-oxidising bacteria, and despite the fact that natural and constructed wetlands presented different environments, the microorganisms presented similar bacterial groups and genes responsible for microbial

arsenite oxidation (Chang et al., 2010). Regarding arsenate reduction, Macy et al. (2000) isolated two heterotrophic bacteria able to reduce As(V) and SO₄, from an As-contaminated reed bed in Bendigo, Australia (Macy et al., 2000).

Iron-oxidising bacteria have an important role in the oxidation of Fe at pH levels below 4.5 (Hedin et al., 1994). Fe(II) oxidation occurs in the absence of bacteria at pH 6 or above (Singer and Stumm, 1970, National Rivers Authority, 1992). Iron oxidation followed by oxyhydroxide precipitation is considered by some researchers to be the most important iron-removal mechanism in wetlands (National Rivers Authority, 1992). Since Fe oxyhydroxides can sorb/coprecipitate As (Figure 2.10), iron-oxidising bacteria may cause the removal of both Fe and As. Different microorganisms (autotrophs and heterotrophs) are able to oxidise Fe (Emerson et al., 2010). Nicomrat et al. (2006) characterised the microbial communities in a constructed wetland receiving acid mine drainage and found that the acidophilic chemolithotrophs *Acidithiobacillus ferroxidans* and *Acidithiobacillus thiooxidans* were the dominant microbial species, both able to oxidise Fe and S (Nicomrat et al., 2006). Hallberg and Johnson (2005) enumerated different groups of microbes from constructed wetlands, among them iron-oxidising bacteria, which encouraged ferrous iron precipitation and concomitant arsenic removal (Hallberg and Johnson, 2005). This study was important in that it was able to demonstrate clearly that the presence of iron-oxidising bacteria caused the removal of As.

Sulfate-reducing bacteria and metal-oxidising bacteria require organic carbon for synthesis, which can be provided by vegetation, organic pollutants, or the organic portion of wetland media. A carbon source is essential to stimulate sulfate reduction, and this can be provided by organic materials such as mulch and wood chips (Lloyd et al., 2004). Supplemental carbon sources have been introduced in several wetlands to increase the rate of sulfate reduction (Sheoran and Sheoran, 2006).

Despite the fact that several studies have shown that the role of bacteria is crucial in the removal of metals and As in constructed wetlands (e.g. Sobolewski, 1999, Kosolapov et al., 2004, Hallberg and Johnson, 2005, Sheoran and Sheoran, 2006, Groudev et al., 2008), this role has largely been inferred from processes observed in other water

treatment systems and/or natural wetlands. Many publications corroborated these assumptions based on measurements in water chemistry, but they lack direct evidence of specific microbial consortia responsible for the removal of As in constructed wetlands (Faulwetter et al., 2009). These indirect studies have used a variety of approaches to the role of microorganisms in the removal. In the previously mentioned example of Rahman et al. (2008), it was assumed that under certain environmental conditions, sulfate-reducing bacteria were active and contributed to the removal. Ye et al. (2003) explained the sequestration of As in the sediments as being due to high rates of microbial activity, but no further details were provided as to how this implication was drawn. Singhakant et al. (2009a) mentioned that anoxic conditions could promote the growth of sulfate-reducing bacteria, and despite the fact that they found that As was mostly retained in bed material, they concluded that this capability could be enhanced by As sulfide precipitation. Buddhawong et al. (2005) discarded the influence of sulfate-reducing bacteria due to the high redox potential values reported (as detailed previously). These facts suggest that even though the presence of bacteria depends on environmental conditions, their actual presence in wetland systems and their role in the removal of As remains poorly understood, suggesting that further, more direct studies are required.

Faulwetter et al. (2009) reviewed more than fifty articles on microbial techniques (investigating microbial density, microbial activity and microbial diversity) in the study of wetlands. However, few studies employing microbial diversity techniques have been published (Faulwetter et al., 2009). The three most commonly applied methods for exploring microbial diversity are fluorescence in-situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE) and ribosome gene cloning (Truu et al., 2009). Studies are more often undertaken on the quantity rather than on the diversity of microbial communities (Jin and Kelley, 2007), but the communities associated with wetland systems remain poorly characterised (Lloyd et al., 2004). Various authors have recommended further studies of microbial density, diversity (Faulwetter et al., 2009) and activity (Kosolapov et al., 2004, Stein et al., 2007); suggesting a general agreement about the need for more detailed studies of the microbial communities, so as to optimise their role and improve the performance of constructed wetlands. In addition, the available reviews on microbial processes of heavy metal removal in constructed

wetlands (Kosolapov et al., 2004; Faulwetter et al., 2009) refer only briefly to the removal of arsenic driven by microorganisms.

Coprecipitation can also remove As. Coprecipitation refers to As that adsorbs onto or within the developing or fresh precipitates of other chemical species. Coprecipitation occurs at the same time as or shortly after the host solids precipitate from the solution, such as arsenic coprecipitating with iron (oxy)(hydro)oxides. On the other hand, sorption involves the incorporation of pollutants onto or within pre-existing solids (Section 2.4.4). Coprecipitation might also involve arsenic-bearing colloids or other fine-grained particles becoming trapped (absorbed) in the interiors of precipitating compounds (Henken and Hutchison, 2009). In constructed wetlands, Buddhawong et al. (2005) suggested that Fe could act as a coprecipitating agent for As, particularly in the oxic zones, whereas Singhakant et al. (2009a) concluded that organic sulfides produced by biodegradation of roots and microorganisms can co-precipitate As and Fe under reducing conditions, and also can precipitate As directly.

2.4.4 Sorption

Sorption refers to a treatment process when both adsorption and absorption are involved simultaneously, or when adsorption and absorption cannot be distinguished. Adsorption refers to the removal of ions and other dissolved species from liquids or gases by their accumulation on the surfaces of solid materials, and the adsorbed species are not a major component in the internal chemistry of the solid. Adsorption processes usually involve ion exchange. Absorption is the assimilation of chemical species into the interior of a solid substance (Henken and Hutchison, 2009).

The adsorption capacity depends on the properties of the surface, the arsenic and metals species and concentration, pH, and the presence of competing ions (Stollenwerk, 2003).

The key parameter affecting arsenic and boron sorption appears to be pH, since it affects both As and B speciation and the composition of surface functional groups. At near-neutral to acidic pH, As(V) tends to be strongly sorbed to oxide minerals. At

alkaline pH, As desorption is promoted by the negative charge of the mineral surface (Mukherjee et al., 2009). On the other hand, sorption of boron at alkaline pH is stronger (Kot, 2009).

Regarding competing anions, the sorption of arsenic oxyanions is very sensitive to the presence of phosphate (PO_4^{-3} , HPO_4^{-2} , H_2PO_4^-), sulfate (SO_4^{-2}), carbonate (CO_3^{-2}), bicarbonate (HCO_3^-) and chloride (Cl^-) (Mukherjee et al., 2009). Phosphate and silicate (SiO_4^{-4}) have the same tetrahedral configuration as arsenate, and therefore can desorb As(V) from different surfaces (Henken, 2009b). It was found that As(V) bound to a soil (which consisted of quartz, clay minerals, Fe and Al oxides) was effectively mobilised by the presence of phosphate in solution. The ability of anions in mobilising As from soil particles follows the order $\text{PO}_4^{-3} \gg \text{CO}_3^{-2} > \text{SO}_4^{-2} \approx \text{Cl}^-$ (Goh and Lim, 2005). In addition, the sorption of carbonate on ferrihydrite (hydrrous ferric oxide) decreases the sorption capacity of As(V) significantly (Appelo et al., 2002). Although it has not been reported in the literature, borate anion $\text{B}(\text{OH})_4^-$ may also compete with arsenate for sorption sites.

In constructed wetlands, sorption is considered as a main removal mechanism for metals such as Pb, Cu and Cr (Marchand et al., 2010). Sorption of arsenic, boron and iron has been documented (Buddhawong et al., 2005, Ye et al., 2003). For iron, precipitation has been reported as the main mechanism by different authors (e.g. Kadlec and Wallace, 2009, Sheoran and Sheoran, 2006, Marchand et al., 2010). However, if the environmental conditions impede Fe precipitation, for example acidic conditions, Fe could be sorbed. In addition, it has been considered that sorption is the first step of immobilisation before precipitation of metals or other processes become dominant in wetlands treating acid mine drainage (Wiessner et al., 2006).

Various surfaces are able to sorb arsenic and metals. In a constructed wetland, the main surfaces which have been found to sorb arsenic and metals include: (1) the medium (or substrate), (2) mineral particles or colloids -particularly (oxy)(hydr) oxides-, and (3) organic matter. Extracellular polymeric substances (EPS) exudated by microorganisms have been found to sorb heavy metals (Mikutta et al., 2012). However, the literature has not reported these substances as main sorption surfaces in constructed wetlands.

The most common supporting medium that has been used for arsenic and metal removal is gravel (Buddhawong et al., 2005, Kröpfelová et al., 2009, Singhakant et al., 2009a). However, Buddhawong et al. (2005) reported that the As adsorption capacity of gravel was low (in the range of up to 4.3 $\mu\text{g}/\text{kg}$), and therefore it was likely that other processes, such as binding with the Fe content of the gravel media, were responsible for the removal of As. The type and chemical composition of the supporting media should therefore be considered for improving the efficiency of metal removal in a wetland (Ye et al., 2003). However, from the available literature, it appears that the use of an adequate or specific sorbent media to enhance the removal of As in constructed wetlands has been poorly studied, despite the fact that it has been recommended by several researchers.

Metal (hydr)oxides are known for their capability of sorbing As (Hering and Kneebone, 2002) and metals (Violante et al., 2003). Arsenate sorption onto most metals (hydr)oxides and clay minerals (Inskeep et al., 2002), especially onto Fe and Mn oxyhydroxides, is well known and has been reported by a number of researchers (e.g. Kneebone et al., 2002, Pastén et al., 2006). Furthermore, it has been reported that Fe oxides have greater sorption capacity for As than do clay minerals (Gräfe and Sparks, 2006), Al oxides (Pfeifer et al., 2004) and Mn oxides (Mucci et al., 2000). On the other hand, arsenite can be sorbed mostly onto Fe hydroxides (Inskeep et al., 2002). Several authors attribute this sorption property as an important source of metal removal in constructed wetlands if Fe and/or Mn oxides are present (Sjöblom, 2003). Jensen et al. (2006) attributed the removal of B in their wetland system to the sorption capability of Fe and Al oxides.

Organic matter is able to sorb arsenite and arsenate (Redman et al., 2002), inhibiting As mobility by serving as a binding agent and/or by forming insoluble complexes (Wang and Mulligan, 2006). Organic matter can also sorb iron (Kadlec and Wallace, 2009) and boron (Parks and Edwards, 2005, Kot, 2009). In fact, sorption onto organic matter is considered as an important metal removal mechanism in wetlands (Marchand et al., 2010).

Conversely, organic matter can enhance As release from sediments and soils, mainly through competition for available adsorption sites, the formation of soluble organic matter-arsenic complexes, and the change of redox chemistry in site surfaces and As species (Wang and Mulligan, 2006). Organic matter can also compete with As for sorption sites on metallic oxides (Redman et al., 2002). In addition, during the mineralisation of organic matter, arsenate can serve as an electron acceptor, being reduced to arsenite (Ackermann et al., 2008). Hence, organic matter can remove arsenic from water, but can also release it from solid phases and therefore increase As concentration in the aqueous phase. In the case of constructed wetlands, Singhakant et al. (2009a) concluded that organic matter enhanced the adsorption capacity in the wetland bed: humic and fulvic acids from plants root decomposition could associate with As by metal-bridging mechanisms and ligand exchange-surface complexation (Singhakant et al., 2009a). Buddhawong et al. (2005) concluded that organic compounds released by root activity could be used by microorganisms as carbon sources. This decreased the redox potential, which caused the dissolution of crystalline Fe; but due to partly oxic conditions, Fe was precipitated, coprecipitating As. Despite the fact that little research has been conducted on the As binding capability of organic matter (Wang and Mulligan, 2006), the effect of organic matter on As mobility depends partly on the solubility of organic matter itself (Sharma and Sohn, 2009). Dissolved organic matter tends to mobilise As, whereas particulate organic matter tends to immobilise As.

2.4.5 Plant uptake

Direct uptake and accumulation of As and metals in plants appear to play a very minor role in the removal (Marchand et al., 2010, García et al., 2010). For example, Ye et al. (2003) reported that only 2% of the total As input was accumulated into the plant tissues, and they highlighted the minor role of plant uptake. The same conclusion was drawn by Singhakant et al. (2009a), who reported that only 0.5-1% of the total As input was accumulated in the plant tissues. Similarly, minor fractions of the total B removed have been reported in roots and shoots of cattail, *Thalia* and rabbitfoot grass in

wetlands removing B (Ye et al., 2003). Plant uptake accounted for 3% of the overall Fe removal in the study of Stefanakis and Tsihrintzis (2012).

The accumulation of As and metals depends on the type of plant (Sarma, 2011, Zhao et al., 2010). In *Lemna gibba* L. (duckweed) (Mkandawire and Dudel, 2005) and *Spirodela polyrhiza* L. (great duckweed) (Rahman et al., 2007) accumulation of As increased as the concentration of As in water increased. Duckweed has been also found to be boron (Davis et al., 2002, Marin and Oron, 2007) and iron (Sinha et al., 1994) tolerant and accumulator.

Arsenic and metals can be accumulated in different parts of the plants. Different studies have shown that roots accumulate more As than do shoots (e.g. Qian et al., 1999, Ye et al., 2003, Barley et al., 2005, Vymazal et al., 2009, Adhikari et al., 2011). In the study of Ye et al. (2003), As concentrations in roots were 2 to 10 fold greater than those of the shoots. Mean values in roots fluctuated between 5 and 30 mg/kg depending on the type of plant. The values reported by Buddhawong et al. (2005) in the roots of *Juncus effusus* were between 0.3 and 7.2 mg/kg. Similar situation has been reported for Fe, as roots contain much higher concentrations of Fe than stems or leaves (Kadlec and Wallace, 2009). On the other hand, Ye et al (2003) reported similar accumulation of B in roots and shoots.

Since most of the metal accumulation occurs in roots, harvesting has been considered unsuccessful in treatment wetlands (Kadlec and Wallace, 2009). However, Adhikari et al. (2011) suggested annual harvesting based on their reported As uptake, which fluctuated between 0.05 and 0.53 kg As ha/y. No other harvesting recommendations have been found in the available literature. If the above-ground biomass is not harvested, leaves and stems are eventually returned to the surface of the medium. Decaying plant biomass may act as a source, but it can also act as a sink for metals (García et al., 2010). It has been found that leaves tissues can release metals such as mercury, copper and zinc (Weis and Weis, 2004), but according to the literature this phenomenon has not been investigated for As.

Arsenic is taken up by plant roots (Zhao et al., 2010) and different reasons may explain why As remains there: limited translocation of As from roots to shoots (Wang et al., 2002) and the presence of Fe and S (Zhao et al., 2010). In addition, As speciation plays a relevant role in the uptake mechanism and further translocation: there is enough evidence to suggest that arsenate is taken up by the same transporters of phosphate in the roots, but it is not known what form of As is translocated from roots to shoots and how this translocation occurs. Whilst arsenate and phosphate are similar, arsenate and arsenite are not, therefore their uptake mechanisms may differ (Dhankher, 2005).

It should be noted that some studies reporting As accumulation were performed in hydroponic systems (e.g. Qian et al., 1999, Rahman et al., 2007) or in plants growing in mine waters (e.g. Mkandawire and Dudel, 2005), therefore the results may not be comparable with those in wetland systems (e.g. Ye et al., 2003, Barley et al., 2005, Buddhawong et al., 2005, Vymazal et al., 2009, Adhikari et al., 2011). Furthermore, Comino et al. (2009) found that As accumulation by *Poa annua* reached its minimum when they were planted in 100% zeolite (instead of 100% gravel, or other combinations of gravel and zeolite) due to the high As absorption capacity of zeolite. Therefore, the presence of a suitable substrate, in this case zeolite, may decrease the As uptake by plants, but may still increase the overall removal capacity.

Even though metal removal via plant uptake by macrophytes in wetlands is relatively minor compared to other processes, the indirect role of macrophytes, for example in baffling flows and contributing surface for sorption sites enhances iron removal, but experimental data has shown that plant uptake can play a more important role in Fe removal from mine waters when Fe levels are low: *Phragmites australis* is capable of removing almost 100% of Fe from water when exposed to 1 mg/L Fe (Batty and Younger, 2002). When exposed to Fe concentrations higher than 1 mg/L, plant uptake decreased in terms of percentage removal and most importantly, plant growth was inhibited. This inhibition in growth might be due to iron toxicity within the plants, and/or inhibition of nutrients uptake by the plants due to high Fe levels. Therefore, macrophytes play a critical role as a “polishing” treatment system, when the removal of the residual levels of Fe is required (Batty and Younger, 2002). In addition, Fe is found within and around the roots, mainly as iron oxides.

2.4.6 Methylation

Methylation followed by volatilisation of metalloids such as mercury (Hg), selenium (Se) and As is a well-known phenomenon that takes place in aquatic environments (Kosolapov et al., 2004). Under highly reducing and anoxic conditions, As can be converted to gaseous arsines, which are highly toxic compounds (Frankenberg Jr. and Arshad, 2002). Examples of volatile arsines are arsine (AsH_3), methylarsine ($\text{CH}_3(\text{AsH}_2)$), dimethylarsine ($(\text{CH}_3)_2\text{AsH}$) and trimethylarsine ($(\text{CH}_3)_3\text{As}$). The formation of methylarsines is a biological process that can be mediated by different organisms, such as fungi and bacteria. Sulfate-reducing bacteria are the main Hg methylating agent in soil and aquatic sediments (Bright et al., 1994). King et al. (2002) studied Hg methylation by sulfate-reducing bacteria in constructed wetlands, but no evidence of As methylation by sulfate-reducing bacteria was reported, despite the fact that it has been found that these bacteria are able to methylate As in other environments (Bright et al., 1994, Michalke et al., 2000). As such, arsenic methylation is not believed to significantly contribute to As removal in constructed wetlands. Furthermore, the relevance of this process in constructed wetlands has not been well studied (Buddhawong et al., 2005). The literature has reported that methylation occurs in metals and metalloids that include As, Hg, Se, germanium (Ge), gold (Au), platinum (Pt), etc. (Stumm and Morgan, 1996). Iron and boron are not included.

The main processes that remove arsenic, boron and iron in constructed wetlands are precipitation and sorption. Arsenic speciation plays a role in the removal, but environmental factors such as pH and the presence of other chemical species are also important. Microorganisms can enhance the removal of As by mediating redox and precipitation processes. For iron, the main removal process is precipitation, which can be chemically or microbially-mediated. Sorption may also occur if iron is present as a dissolved species. Precipitation and sorption have been reported to remove boron in wetlands (e.g. Ye et al., 2003, Jensen et al., 2006). Plant uptake (of As, B and Fe) and methylation of As might also contribute to the removal of the target pollutants in wetland systems.

2.5. Long term performance for arsenic and metals removal

The performance of wetland systems in removing metals depends on the water quality and the metal removal processes within the wetland. These processes can be aerobic or anaerobic (Sheoran and Sheoran, 2006).

Aerobic processes are governed by the volume available to collect and store chemical precipitates or the number of available removal sites. The primary removal processes in these systems include sedimentation, adsorption, ion exchange, complexation, which are finite; removal will cease unless new removal sites are generated. As subsurface flow wetlands require maintenance/reconstruction if the media becomes saturated with metals (Kadlec and Wallace, 2009), site conditions will determine whether it is more economical to simply bury the wetland system in place and construct a new one, or to excavate away the accumulated solids for proper disposal. Anaerobic systems work efficiently as long as sulfate-reducing bacteria reduce sulfate to sulfide, thus precipitating metals as metal sulfides and generating alkalinity (Sheoran and Sheoran, 2006). As presented in Section 2.4, different chemical and biological processes can remove metals in wetlands. It is generally accepted that microbially mediated processes of metal-oxidation and sulfate reduction represent the only long-term metal removal mechanism in constructed wetlands (National Rivers Authority, 1992), mainly because in natural wetlands receiving high loadings of metals for many years, sulfide and oxide deposits have been found within the substrate (Lett and Fletcher, 1980, Gammons and Fradsen, 2001).

On the other hand, chemical processes such as cation exchange, adsorption, precipitation/coprecipitation and complexation can be reversed to restore equilibrium. Therefore, metals that are adsorbed, complexed, or (co)precipitated are considered bioavailable since these processes can be reversed (Dunbabin and Bowmer, 1992). Although precipitation of metals as oxides is thought the most important metal removal mechanism in wetlands (Section 2.4.3), and is considered as a long-term removal mechanism, precipitation of metals as sulfides appears to be a more desirable way to immobilise metals, since sulfate reduction generates alkalinity (therefore helping to neutralise acidity) and sulfide precipitates are denser than oxide precipitates (National

Rivers Authority, 1992). Furthermore, sulfide precipitates are also highly insoluble (Dunbabin and Bowmer, 1992, Sheoran and Sheoran, 2006), meaning that they will not be easily re-introduced into the system. In addition, metals incorporated in mineral lattices are considered unavailable to biota (Dunbabin and Bowmer, 1992). However, only limited information exists on the removal of heavy metals by the formation of insoluble sulfides under reducing conditions, which mainly occurs in horizontal flow subsurface wetlands (Vymazal and Kröpfelová, 2008).

Given this knowledge about the metal removal processes, it has been suggested by Marchard et al. (2010) that wetlands should be designed with two compartments: the first one, reducing, to promote sulfate reduction and promote As, Hg, Se and Zn coprecipitation and a second, oxidising, to promote metal co-precipitation with iron oxides. However, other factors must be taken into consideration, such as the type and concentrations of metals/metalloids and other species. Resolubilisation/oxidation of metals from the solid/reduced phase may also occur.

Despite the fact that the lifespan of wetland systems when retaining trace metals is in the order of many decades or longer (Kadlec and Wallace, 2009), more studies on the long term performance with emphasis on the metal removal mechanisms are required (Sheoran and Sheoran, 2006). Since little is known about the temporal evolution of As removal, long term studies are also required, particularly in subsurface flow wetlands (Zurita et al., 2012). However, it has been reported by Mattes et al (2010) that after ten years of operation, the wetland system in Trail, British Columbia, Canada is still effective for removing As, Zn and Pb. This may suggest that wetlands systems for As and metal removal could be implemented and operated for long periods of time, despite of the little knowledge of the removal mechanisms.

2.6. Knowledge gaps

From the literature review, the following knowledge gaps were identified:

1. Constructed wetlands have the potential to remove metals and metalloids. However, since few studies have investigated the removal of arsenic and boron, little is known about their efficiency, or about means of optimising their performance.
2. Literature reports about the application of constructed wetlands in the removal of Fe come mainly from studies on the treatment of acid mine drainage using surface flow systems. The efficiency of subsurface flow wetlands to remove Fe, and also As and B, has not been sufficiently studied.
3. Since the focus for the treatment of acid mine drainage has been on aerobic wetlands for precipitation of iron, mainly surface flow wetlands have been employed (Kadlec and Wallace, 2009), and to a minor extent, subsurface vertical flow wetlands (Younger et al., 2002). Few studies have investigated the use of horizontal flow wetlands, i.e. those which promote anaerobic conditions.
4. To design wetlands that optimise arsenic and metal removal in constructed wetlands, the processes transforming arsenic and metals must be understood. Metal removal processes in constructed wetlands have been reviewed by several authors (e.g. Kleinmann and Girts, 1987, Sobolewski, 1999, Sheoran and Sheoran, 2006, García et al., 2010). Since arsenic and boron are metalloids, their reactivity and therefore their removal mechanisms differ to those of other metals. In addition, the removal mechanisms for As and B in constructed wetlands are not well understood, since few studies have investigated a possible application of constructed wetlands for arsenic and/or boron removal. As such, further understanding of these removal mechanisms is required to optimise As and B removal.
5. Most of the studies investigating As removal in constructed wetlands have tested highly polluted water that is not representative of realistic conditions, i.e. does not represent an actual As-containing water resource/wastewater. Using arbitrary concentrations to test systems often leads to incorrect conclusions being made about processes and influencing factors. To gain insights into the possible application of constructed wetlands, it would be preferable to undertake testing using water that is

similar in chemistry to that of a particular site where such technology might be required.

6. To date, most studies on As and metal removal have been conducted mostly in wetlands with conventional soil (surface flow systems) or gravel (subsurface flow systems) media. Little is known about how the use of alternative substrates may affect arsenic and metal removal, despite the fact that this use has been suggested in the literature so as to foster particular removal processes by providing adequate environmental conditions. For example, an organic medium can provide a source of carbon for bacterial growth, together with sorption sites. Sorption sites can also be provided by incorporating additional media, such as zeolite.

7. It has been suggested that anaerobic processes, i.e. sulfate reduction followed by sulfide precipitation, would offer more effective long-term metal removal than would aerobic processes. To promote anaerobic processes, anaerobic conditions, a source of carbon and sulfate are required. Sulfate reduction has been promoted to treat metal contaminated water (Fe, Mn, Cd and Zn) (Dvorak et al., 1992) but has been rarely studied for As contaminated waters (e.g. Duncan et al., 2004, Duncan, 2002).

8. Microbial communities may play a key role in the removal of As and metals in wetlands (Marchand et al., 2010). However, this role has been mainly inferred from environmental conditions (for example, if negative Eh values were detected and the levels of sulfate decreased, it was assumed that bacterial sulfate reduction was active) and from what is known from other water treatment systems and/or natural wetlands (for example, in anaerobic processes the reduction of sulfate to hydrogen sulfides can promote the precipitation of metal sulfides). Since the effect of microorganisms on the overall removal of metal and metalloid is still unclear (Marchand et al., 2010), specific studies looking at microbial density, activity and diversity (i.e. direct evidence of specific microbial consortia) are required (Faulwetter et al., 2009).

This research has been designed to tackle these gaps. They will be further examined in the following chapters, where specific background is presented accordingly.

The specific objectives of this research are to:

- Review current application of constructed wetlands for the removal of arsenic and metals, to identify knowledge gaps and potential promising research directions
- Study the effect of different wetland media: limestone, zeolite, cocopeat and gravel, using vertical subsurface flow wetlands, in the removal of six target pollutants, As, Fe, B, Cu, Mn and Zn, from contaminated water
- Investigate the effect of the same four media in the removal of three target pollutants, As, B and Fe, from acidic contaminated water
- Test the performance of horizontal subsurface flow wetlands using the optimal-performing media tested in vertical flow wetlands, in the treatment of acidic contaminated water
- Synthetise key findings obtained to shed light on the key pollutant removal mechanisms, and therefore make recommendations on how to improve the design of subsurface flow wetlands for the removal of As, B and Fe

2.7. Conclusions

The presence of arsenic and heavy metals in water is a worldwide problem that poses a serious pollution threat to aquatic environments. Millions of people throughout the world may develop cancer due to the intake of arsenic-containing water. Although several treatment options are available, high costs, sludge generation and isolation of contaminated areas prevent the application of conventional/centralised technologies for arsenic removal.

Constructed wetlands, particularly subsurface flow wetlands, have the potential to remove arsenic and metals. Constructed wetlands are a natural treatment technology that has been successfully applied to treat different types of wastewaters. Since they have low energy consumption and operation costs, and can be built and operated onsite, they appear as a viable alternative to treat As and metal-containing water.

Few studies have investigated the removal of arsenic using constructed wetlands. From them, it was observed that mainly precipitation, co-precipitation and sorption remove As in wetlands. However, these processes are poorly understood since they are mainly inferred from the water quality data (for example, if As and sulfate levels decrease and reducing conditions are detected, precipitation of arsenosulfides is hypothesised), so it is not possible to know which processes were involved and their contribution to the overall removal. Further studies of the speciation of arsenic in the solid phase and the role of microbial communities in the mediation of these processes can provide valuable information on these mechanisms. Different factors affect the overall performance, such as the supporting media, the pH and the presence of Fe and S, and they should be considered when designing wetland systems that enhance the removal of As.

Since there is a lack of understanding of the processes involved in the removal of arsenic and metals in subsurface flow constructed wetlands, further research is required before constructed wetlands can be confidently applied to treat water containing these pollutants.

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2.9. Journal article “Removal processes for arsenic in constructed wetlands”



Review

Removal processes for arsenic in constructed wetlands

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ABSTRACT

Arsenic pollution in aquatic environments is a worldwide concern due to its toxicity and chronic effects on human health. This concern has generated increasing interest in the use of different treatment technologies to remove arsenic from contaminated water. Constructed wetlands are a cost-effective natural system successfully used for removing various pollutants, and they have shown capability for removing arsenic. This paper reviews current understanding of the removal processes for arsenic, discusses implications for treatment wetlands, and identifies critical knowledge gaps and areas worthy of future research. The reactivity of arsenic means that different arsenic species may be found in wetlands, influenced by vegetation, supporting medium and microorganisms. Despite the fact that sorption, precipitation and coprecipitation are the principal processes responsible for the removal of arsenic, bacteria can mediate these processes and can play a significant role under favourable environmental conditions. The most important factors affecting the speciation of arsenic are pH, alkalinity, temperature, dissolved oxygen, the presence of other chemical species – iron, sulphur, phosphate –, a source of carbon, and the wetland substrate. Studies of the microbial communities and the speciation of arsenic in the solid phase using advanced techniques could provide further insights on the removal of arsenic. Limited data and understanding of the interaction of the different processes involved in the removal of arsenic explain the rudimentary guidelines available for the design of wetlands systems.

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1. Introduction

Arsenic (As) is mostly found in the earth's core and in clay- and sulphide-rich portions of the earth's crust (Henken, 2009b). Being a metalloid in group 15 on the periodic table (along with antimony, bismuth, nitrogen and phosphorus), arsenic is well known for its chronic toxicity, particularly when exposure occurs over prolonged periods. Arsenic exposure via drinking-water is related to lung, kidney, bladder and skin cancer. For example, drinking-water arsenic concentrations in excess of $50 \mu\text{g L}^{-1}$ have been associated with increased risks of cancer in the bladder and lung, whilst drinking-water arsenic levels even below $50 \mu\text{g L}^{-1}$ have been associated with precursors of skin cancer (IPCS, 2001). Therefore, the presence of arsenic in water supply poses a serious risk to human health.

Surface and ground waters in many parts of the world have been found to naturally contain As concentrations that make these waters unsuitable for human use. Significant concentrations of As have been reported in various countries such as Bangladesh, Chile, USA, China, and India. In Bangladesh, for example, about 100 million people currently drink water with As concentrations up to 100 times the World Health Organisation (WHO) drinking water guideline, which is $10 \mu\text{g L}^{-1}$ (Mohan and Pittman Jr., 2007). Two of Northern Chile's main rivers, the Loa River and the Luta River, have As concentrations of around 1400 and $240 \mu\text{g L}^{-1}$ respectively (Romero et al., 2003; Dirección General de Aguas, 2008).

To remove As from potential drinking water sources, a variety of conventional and non-conventional technologies have been studied, and these technologies have been reviewed by several authors (Mohan and Pittman Jr., 2007). However, it is known that conventional engineered treatment technologies are costly and create problems of sludge generation and disposal (Kosolapov et al., 2004; Cohen, 2006; Nelson et al., 2006). In addition, these systems often become sources of As-rich effluents and are typically located in remote isolated areas (such as mining sites), thus precluding the transportation of the effluents to large centralised treatment facilities. As such, to prevent As pollution of watercourses, it is essential to find onsite, decentralised treatment systems that are robust and have low maintenance requirements and operating costs.

Constructed wetlands are low-energy 'green' systems that have been increasingly applied in wastewater treatment since the mid-1980s (Sun and Saeed, 2009). Since the late 1990s, the application of wetland systems has accelerated, primarily due to rising costs of fossil fuel-derived energy sources and worldwide concern about the emission of greenhouse gases and climate change (Lee et al., 2009). Currently, the applications of wetland systems are mostly in the treatment of domestic sewage, especially in rural areas in developed countries in Europe and the USA (Cooper et al., 1996; Scholz and Lee, 2005; Kadlec and Wallace, 2009).

Constructed wetlands have considerable potential to remove metals and metalloids, including arsenic (Ye et al., 2003; Buddhawong et al., 2005). Some studies have been carried out to investigate the removal of metals in wetlands (Kleinmann and Girts, 1987; National Rivers Authority, 1992; Sobolewski, 1999; Sjöblom, 2003), but most have focused on acid mine drainage (AMD) treatment, primarily to remove sulphate, iron (Fe) and manganese (Mn) (Wallace and Knight, 2006). Despite their potential, few experimental studies have been specifically designed to investigate As removal in wetland systems. Kadlec and Wallace (2009) reviewed

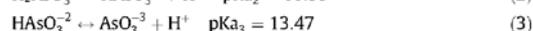
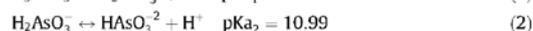
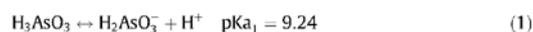
some key aspects of As behaviour in treatment wetlands, but the review was largely based on unpublished data or data found in the North American Treatment database NABD (US EPA, 1998). Other reviews are available in the literature on the removal of metals using constructed wetlands (Dunbabin and Bowmer, 1992; Sheoran and Sheoran, 2006; Yeh, 2008; Marchand et al., 2010), but they provide a general overview of metals and metalloids. Therefore, arsenic removal is only briefly covered, with little information available on the processes responsible for transformation and retention of arsenic, and the factors which control these processes.

This review aims to summarise what is currently known about the physicochemical processes for As removal in constructed wetlands, including major environmental factors that affect these processes. Microorganism-mediated mechanisms, which can also remove As by direct and indirect means, are discussed. Major knowledge gaps that currently impede wetland modelling and design for As removal are identified, together with research directions and tools that could potentially address these gaps.

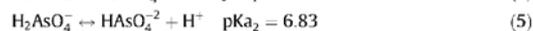
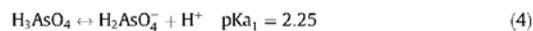
2. Arsenic chemistry

Arsenic is a highly reactive metalloid that can be found in oxidation states -3 , 0 , $+3$ and $+5$. In natural waters, arsenic occurs as arsenite (AsO_3^{3-}) and arsenate (AsO_4^{3-}), referred to as As(III) and As(V). As(III) mostly exists in reducing groundwaters and hydrothermal waters, whilst As(V) is more often present in surface waters and oxidising groundwaters (Henken and Hutchison, 2009). The main factors that control arsenic speciation are the oxidation state and pH.

As(III) commonly hydrates to arsenious acid; therefore its chemistry depends strongly on pH. The predominant As(III) species is arsenious acid, H_3AsO_3 , due to the high value of pK_{a1} (pK_{a} values reported by Wolthers et al. (2005) under the condition of 25°C and 1 bar pressure). The reactions of the dissociation of arsenious acid and its respective anions are shown below, along with the associated pK_{a} values (where $\text{pK}_{a} = -\log(K_{a})$, and K_{a} = equilibrium constant of the reaction):



As(V) commonly hydrates to arsenic acid, and its chemistry also depends on pH. The most common species are H_2AsO_4^- and HASO_4^{2-} , due to the low pK_{a1} value (pK_{a} values reported by Wolthers et al. (2005)). The reactions of dissociation of arsenic acid and its respective anions are shown below along with the associated pK_{a} values:



The toxicity of arsenic depends on its speciation; for example, arsenite is significantly more toxic than arsenate (APHA et al., 2005). It is typically more difficult to remove arsenite than arsenate from contaminated water; this is because in natural waters, under normal pH conditions (6)–(9), arsenite is mostly found as an uncharged species (H_3AsO_3), and negatively charged species

(H_2AsO_3 , HAsO_3^{2-} and AsO_3^{3-}) are found only at high pH (>9). On the other hand, arsenate is commonly found as negatively charged species (H_2AsO_4 and HAsO_4^{2-}); which are easier to remove than are uncharged species, because the charges of the As(V) oxyanions allow them to be removed by sorption, anion exchange or precipitation/coprecipitation (Henken and Hutchison, 2009).

Arsenic species in water can have different transformation pathways: (1) methylating or demethylating by interacting with biological organisms, (2) oxidising or reducing biotically or abiotically, (3) sorbing onto solids, (4) precipitating, and (5) coprecipitating. Generally, the chemistry of the water determines the chemistry of arsenic (Henken and Hutchison, 2009). Since the biogeochemical cycles of iron and arsenic are coupled in natural systems (Kneebone et al., 2002), the presence of Fe affects the speciation of As. Iron (oxy)(hydro)oxides are especially important and effective in sorbing and/or coprecipitating arsenic in both natural and artificial system (Henken, 2009a). In natural aquatic systems, arsenic speciation is often controlled by reactive surfaces with which As can associate, such as soils, clays, colloids, minerals, organic matter, and metal oxides and oxyhydroxides. Constructed wetlands can provide most of these surfaces to remove As, which suggests that in theory they should have the potential to remove arsenic, provided that the environmental conditions are conducive.

3. Arsenic removal mechanisms in constructed wetlands

Being considered complex bioreactors due to interactions between microbial communities, plants, soil and sediments, subsurface flow wetlands may remove pollutants via various physical, chemical and biological processes (Kadlec and Wallace, 2009).

The occurrence and rate of these processes depends on the nature of the pollutants and environmental conditions.

Metal removal processes in wetlands have been reviewed by different authors (Kleinmann and Girts, 1987; National Rivers Authority, 1992; Sobolewski, 1999; Sjöblom, 2003; Sheoran and Sheoran, 2006; Yeh, 2008; García et al., 2010). Some authors have focused on microbially-mediated processes (Kosolapov et al., 2004; Faulwetter et al., 2009), whilst most of them have referred more generally to a wide range of removal processes. Because the removal pathways are dependent on each other, the overall removal process is very complex, making the identification of specific removal pathways and functions more difficult (Sheoran and Sheoran, 2006). In addition, the reactivity of As differs from that of metals such as copper (Cu), zinc (Zn), Fe and Mn. Therefore, As removal processes may differ from those of other "common" metals and, As removal may occur under quite different environmental conditions. Metals such as Cu and Zn are cations and precipitate easily at basic pH, whereas arsenic species are either neutral or anions and require more specific environmental conditions (for example, the presence of other species as detailed in Section 3.1). Furthermore, the removal mechanisms for As in constructed wetlands have not been well understood (Singhakant et al., 2009a), due to the complexity of As chemistry and lack of experiments on As removal in constructed wetlands.

However, it can be observed from the studies to date that the main processes responsible for the removal of arsenic in constructed wetlands are precipitation and sorption. Arsenic speciation plays a role in the removal, but environmental factors such as pH and the presence of other chemical species are also important. Microorganisms can enhance the removal of As by mediating redox and precipitation processes. The main arsenic transforma-

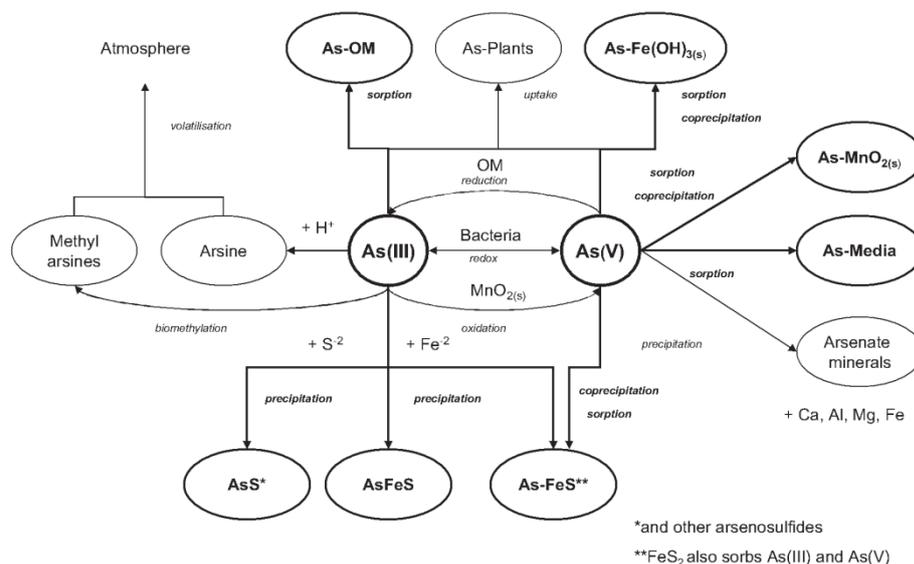


Fig. 1. The routes of arsenic transformations in a constructed wetland. The processes considered to be most important in the overall removal are highlighted in bold. Arsenite and arsenate species are located in the centre, and the black arrows show that the oxidation/reduction process can be mediated by bacteria, oxidation by manganese oxides ($\text{MnO}_2(\text{s})$), and reduction by organic matter (OM). The upper section shows that both arsenite and arsenate can be sorbed by OM, uptake by plants, and sorbed/coprecipitated by iron oxyhydroxides ($\text{Fe}(\text{OH})_3(\text{s})$). The section to the right indicates that arsenate can be sorbed/coprecipitated by $\text{MnO}_2(\text{s})$, sorbed by the media, and precipitated as minerals in the presence of calcium (Ca), magnesium (Mg), aluminium (Al) and Fe. The section to the left illustrates that arsenite can react with hydrogen (H^+) and form arsine, which can be subsequently removed through volatilisation. Arsenite also can be biomethylated, being the final methyl compounds volatile. The bottom section shows that arsenite can precipitate as sulphide/iron minerals such as realgar (AsS) and arsenopyrite (AsFeS), and can also be sorbed/coprecipitated with iron sulphide (FeS). In addition, pyrite (FeS_2) can not only sorb arsenite, but also arsenate.

tion routes in a constructed wetland, in the presence of other species (such as Fe, S and Ca), are illustrated in Fig. 1, and are discussed in more detail in the following sections. Those processes considered to be most important in the overall removal are highlighted in bold.

3.1. Precipitation

Precipitation refers to dissolved species (such as H_2AsO_4^- and HAsO_4^{2-}) reacting with other dissolved species (such as Fe(III) and Ca) to form solid insoluble reaction products. This process may result from oxidation, reduction, pH changes or the mixing of chemicals into a solution (Henken and Hutchison, 2009). In oxidising environments with high levels of As(V), precipitation of the As(V) with Ca, Mg, Al and Fe(III) may occur (Henken, 2009a). Under reducing environments and in the presence of S and Fe, As can form insoluble sulphide compounds (Buddhawong et al., 2005; Singhakant et al., 2009a), such as orpiment As_2S_3 , in which arsenic is present as As(III), and arsenopyrite AsFeS . Orpiment may precipitate in a low Fe and S rich environment, especially under acidic conditions (Wilkin and Ford, 2006). The formation of As_2S_3 was considered to be abiotic prior to the study of Newman et al. (1997), which reported a bacterium able to precipitate As_2S_3 .

In constructed wetland environments, arsenic is retained mostly in sediments (Ye et al., 2003) or media (Buddhawong et al., 2005; Singhakant et al., 2009a,b), rather than accumulated in plants. Singhakant et al. (2009a) analysed the forms of As in a wetland using Tessier extraction and found that the residual fraction of As was the predominant As form, concluding that As was mainly retained in the pores of the wetland media (sand and gravel). In addition, the oxidisable fraction (binding to organic matter and sulphides) was the second most important fraction. Singhakant et al. (2009b) also found that the major removal mechanisms were trapping within porous media (residual fraction) and trapping with Fe and Mn on the media surface (reducible fraction).

The redox potential (Eh) may indicate the presence of sulphide precipitates. Buddhawong et al. (2005) attributed the removal of As and Zn to processes that exclude precipitation as sulphides: the authors did not measure redox potential levels below 100 mV, so they assumed unfavourable redox conditions for sulphate-reducing bacteria and concluded that sulphides could not precipitate. No further details were provided about the required redox conditions for sulphide precipitation by sulphate-reducing bacteria. Different authors have reported different redox potential values required by sulphate-reducing bacteria to thrive: less than -200 mV (Cabrera et al., 2006; Diels et al., 2006), less than -100 mV (Willow and Cohen, 2003), or between -150 and -200 mV (Tuttle, 1969). Rahman et al. (2008) noted that microbial sulphate reduction was greater under redox potential values between -160 and -190 mV, and this higher sulphate removal contributed to higher removal of As. It should be noted that different redox potentials can be found in different parts of the wetland and they cannot necessarily be represented by the measurements, since plants generate microzones of oxidising or reducing conditions in the substrate (Cohen, 2006). Therefore, measured redox potential values can be used to assess the presence of oxidising or reducing conditions, but they must be evaluated carefully.

Sulphate-reducing bacteria (SRB) can mediate the precipitation of arsenosulphide minerals. These bacteria occur primarily in near-neutral anoxic environments (Sjöblom, 2003), preferring pH conditions between 5 and 8 (Cohen, 2006). Some examples are *Desulfohalobacter hydrogenophilus* (autotrophic, growth on H_2 and CO_2) and *Desulfovibrio sulfodismutans* (heterotrophic) (Ehrlich, 2002).

Sulphate-reducing bacteria transform sulphate to sulphide according to the following reaction (Cohen, 2006):



where CH_2O represents a simple organic molecule, such as acetate (National Rivers Authority, 1992). The available sulphide reacts with As(III), forming insoluble As–S minerals.

Sulphate reduction requires a reducing environment and an electron donor. Rahman et al. (2008) reported that under oxidising conditions and with a deficiency of electron donors, sulphate reduction was limited and the removal of As was achieved via mechanisms other than precipitation of As(III) sulphides. On the other hand, under more reducing conditions and surplus carbon, sulphate reduction by sulphate-reducing bacteria contributed to an efficient As removal (Rahman et al., 2008). However, their conclusions were drawn based on their measured water quality parameters (Eh, TOC), without monitoring microbial community composition or function directly. Groudev et al. (2008) reported a numerous and diverse population of sulphate-reducing bacteria mainly in sediments of constructed wetlands treating acid mine drainage, where As and other heavy metals were precipitated as sulphides (Groudev et al., 2008); this was one of few studies that monitored sulphate-reducing bacteria communities contributing to As removal in a constructed wetland. Another example is Duncan et al. (2004), who reported appreciable numbers of sulphate-reducing bacteria and iron-reducing bacteria. They suggested that the main As removal pathway was the biogenic precipitation of As sulphides, given that As concentrations were positively correlated with sulphur concentration, and negatively correlated with organic carbon concentration. Recently, Mattes et al. (2010) provided more details of the wetland system described in Duncan et al. (2004), highlighting that not only sulphate-reducing bacteria played a role in As removal, with iron-oxidising bacteria also making a significant contribution. However, the As removal mechanisms are not yet clearly identified (Mattes et al., 2010).

Despite the fact that some studies have reported that arsenosulphide minerals accumulate in wetland sediments when bacterial sulphate reduction was active (Langner et al., 1999; Duncan et al., 2004; Groudev et al., 2008), the importance of As_2S_3 precipitation caused by sulphate-reducing bacteria remains poorly understood (Buddhawong et al., 2005). In acid mine drainage, the initial As removal process in the presence of sulphate-reducing bacteria is not clear (adsorption or coprecipitation with other metals sulphides or ferrihydrite have been proposed). Formation of insoluble arsenic sulphide can occur after initial removal when reducing conditions have been established (Neculita et al., 2007).

Other microorganisms, such as arsenite-oxidising bacteria, arsenate-reducing bacteria and iron-oxidising bacteria, can also mediate the removal of As (directly or indirectly). Many microorganisms can oxidise or reduce arsenic, even if it is present in the mineral form (Reddy and DeLaune, 2008), and these microorganisms coexist in the soil environment (Macur et al., 2004). In constructed wetlands, some researchers have isolated different types of bacteria responsible for arsenite oxidation. Recently, in constructed wetlands treating acid mine drainage in Bulgaria, Groudev et al. (2008) isolated heterotrophic bacteria (related to the genus *Pseudomonas*) able to oxidise As(III) to As(V) at slightly acidic and neutral pH, in addition to acidophilic chemolithotrophs also able to oxidise As(III). In natural and constructed wetlands in Korea, Chang et al. (2010) isolated heterotrophic arsenic-oxidising bacteria, and despite the fact that natural and constructed wetlands presented different environments, the microorganisms presented similar bacterial groups and genes responsible for microbial arsenite oxidation (Chang et al., 2010). Regarding arsenate reduction, Macy et al. (2000) isolated two heterotrophic bacteria able to reduce As(V) and SO_4 from an As-contaminated reed bed in Bendigo, Australia (Macy et al., 2000).

Iron-oxidising bacteria have an important role in the oxidation of Fe at pH levels below 4.5 (Hedin et al., 1994). Fe(II) oxidation occurs in the absence of bacteria at pH 6 or above (Singer and Stumm, 1970; National Rivers Authority, 1992). Iron oxidation followed by oxyhydroxide precipitation is considered by some researchers to be the most important iron-removal mechanism in wetlands (National Rivers Authority, 1992). Since Fe oxyhydroxides can sorb/ coprecipitate As (Fig. 1), iron-oxidising bacteria may cause the removal of both Fe and As. Different microorganisms (autotrophs and heterotrophs) are able to oxidise Fe (Emerson et al., 2010). Nicomrat et al. (2006) characterised the microbial communities in a constructed wetland receiving acid mine drainage and found that the acidophilic chemolithotrophs *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* were the dominant microbial species, both able to oxidise Fe and S (Nicomrat et al., 2006). Hallberg and Johnson (2005) enumerated different groups of microbes from constructed wetlands, among them iron-oxidising bacteria, which encouraged ferrous iron precipitation and concomitant arsenic removal (Hallberg and Johnson, 2005). This study was important in that it was able to demonstrate clearly that the presence of iron-oxidising bacteria caused the removal of As.

Despite the fact that several studies have shown that the role of bacteria is crucial in the removal of metals and As in constructed wetlands (e.g., Sobolewski, 1999; Kosolapov et al., 2004; Hallberg and Johnson, 2005; Sheoran and Sheoran, 2006; Groudev et al., 2008), this role has largely been inferred from processes observed in other water treatment systems and/or natural wetlands. Many publications corroborated these assumptions based on measurements in water chemistry, but they lack direct evidence of specific microbial consortia responsible for the removal of As in constructed wetlands (Faulwetter et al., 2009). These indirect studies have used a variety of approaches to the role of microorganisms in the removal. In the previously mentioned example of Rahman et al. (2008), it was assumed that under certain environmental conditions, sulphate-reducing bacteria were active and contributed to the removal. Ye et al. (2003) explained the sequestration of As in the sediments as being due to high rates of microbial activity, but no further details were provided as to how this implication was drawn. Singhakant et al. (2009a) mentioned that anoxic conditions could promote the growth of sulphate-reducing bacteria, and despite the fact that they found that As was mostly retained in bed material, they concluded that this capability could be enhanced by As sulphide precipitation. Buddhawong et al. (2005) discarded the influence of sulphate-reducing bacteria due to the high redox potential values reported (as detailed previously). These facts suggest that even though the presence of bacteria depends on environmental conditions, their actual presence in wetland systems and their role in the removal of As remains poorly understood, suggesting that further, more direct studies are required.

Faulwetter et al. (2009) reviewed more than fifty articles on microbial techniques (investigating microbial density, microbial activity and microbial diversity) in the study of wetlands. However, few studies employing microbial diversity techniques have been published (Faulwetter et al., 2009). The three most commonly applied methods for exploring microbial diversity are fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE) and ribosome gene cloning (Truu et al., 2009). Studies are more often undertaken on the quantity rather than on the diversity of microbial communities (Jin and Kelley, 2007), but the communities associated with wetland systems remain poorly characterised (Lloyd et al., 2004). Various authors have recommended further studies of microbial density, diversity (Faulwetter et al., 2009) and activity (Kosolapov et al., 2004; Stein et al., 2007); suggesting a general agreement about the need for more detailed studies of the microbial communities, so as to optimise their role

and improve the performance of constructed wetlands. In addition, the available reviews on microbial processes of heavy metal removal in constructed wetlands (Kosolapov et al., 2004; Faulwetter et al., 2009) refer only briefly to the removal of arsenic driven by microorganisms.

3.2. Coprecipitation

Coprecipitation refers to As that adsorbs onto or within the developing or fresh precipitates of other chemical species. Coprecipitation occurs at the same time as or shortly after the host solids precipitate from the solution, such as arsenic coprecipitating with iron (oxy)(hydro)oxides. On the other hand, sorption involves the incorporation of pollutants onto or within pre-existing solids (Section 3.3). Coprecipitation might also involve arsenic-bearing colloids or other fine-grained particles becoming trapped (adsorbed) in the interiors of precipitating compounds (Henken and Hutchison, 2009). In constructed wetlands, Buddhawong et al. (2005) suggested that Fe could act as a coprecipitating agent for As, particularly in the oxic zones, whereas Singhakant et al. (2009a) concluded that organic sulphides produced by biodegradation of roots and microorganisms can co-precipitate As and Fe under reducing conditions, and also can precipitate As directly.

3.3. Sorption

Sorption refers to a treatment process when both adsorption and absorption are involved simultaneously, or when adsorption and absorption cannot be distinguished. Adsorption refers to the removal of ions and other dissolved species from liquids or gases by their accumulation on the surfaces of solid materials, and the adsorbed species are not a major component in the internal chemistry of the solid. Adsorption processes usually involve ion exchange. Absorption is the assimilation of chemical species into the interior of a solid substance (Henken and Hutchison, 2009). Various surfaces are able to sorb arsenic. The adsorption capacity depends on the properties of the surface, the arsenic species and concentration, presence of competing ions, and the pH (Stollenwerk, 2003). In a constructed wetland, the main surfaces which have been found to sorb arsenic include: (1) the medium (or substrate), (2) mineral particles or colloids – particularly (oxy)(hydr)oxides, and (3) organic matter.

Gravel is the most common supporting medium that has been used for arsenic removal (Buddhawong et al., 2005; Kröpfelová et al., 2009; Singhakant et al., 2009a). However, Buddhawong et al. (2005) reported that the As adsorption capacity of gravel was low (in the range of up to $4.3 \mu\text{g kg}^{-1}$), and therefore it was likely that other processes, such as binding with the Fe content of the gravel media, were responsible for the removal of As. The type and chemical composition of the supporting media should therefore be considered for improving the efficiency of metal removal in a wetland (Ye et al., 2003). However, from the available literature, it appears that the use of an adequate or specific sorbent media to enhance the removal of As in constructed wetlands has been poorly studied, despite the fact that it has been recommended by several researchers (refer to Section 4.6).

Arsenate sorption onto most metals (hydr)oxides and clay minerals (Inskip et al., 2002), especially onto Fe and Mn oxyhydroxides, is well known and has been reported by a number of researchers (e.g., Kneebone et al., 2002; Pastén et al., 2006). Furthermore, it has been reported that Fe oxides have greater sorption capacity for As than do clay minerals (Gräfe and Sparks, 2006), aluminium (Al) oxides (Pfeifer et al., 2004) and Mn oxides (Mucci et al., 2000). On the other hand, arsenite can be sorbed mostly onto Fe hydroxides (Inskip et al., 2002). Several authors attribute this sorption property as an important source of metal removal in con-

structed wetlands if Fe and/or Mn oxides are present (Sjöblom, 2003).

Organic matter is able to sorb arsenite and arsenate (Redman et al., 2002), inhibiting As mobility by serving as a binding agent and/or by forming insoluble complexes (Wang and Mulligan, 2006). On the other hand, it has been reported that organic matter can enhance As release from sediments and soils, mainly through competition for available adsorption sites, the formation of soluble organic matter–arsenic complexes, and the change of redox chemistry in site surfaces and As species (Wang and Mulligan, 2006). Organic matter can also compete with As for sorption sites on metallic oxides (Redman et al., 2002). In addition, during the mineralisation of organic matter, arsenate can serve as an electron acceptor, being reduced to arsenite (Ackermann et al., 2008). Hence, organic matter can remove arsenic from water, but also release it from solid phases and therefore increase As concentration in the aqueous phase. In the case of constructed wetlands, Singhakant et al. (2009a) concluded that organic matter enhanced the adsorption capacity in the wetland bed: humic and fulvic acids from plants root decomposition could associate with As by metal-bridging mechanisms and ligand exchange-surface complexation (Singhakant et al., 2009a). Buddhawong et al. (2005) concluded that organic compounds released by root activity could be used by microorganisms as carbon sources. This decreased the redox potential, which caused the dissolution of crystalline Fe; but due to partly oxic conditions, Fe was precipitated, coprecipitating As. Despite the fact that little research has been conducted on the As binding capability of organic matter (Wang and Mulligan, 2006), the effect of organic matter on As mobility depends partly on the solubility of organic matter itself (Sharma and Sohn, 2009). Dissolved organic matter tends to mobilise As, whereas particulated organic matter tends to immobilise As.

Arsenic sorption is a complex process that can occur in different surfaces in a constructed wetland and is affected by different factors, such as pH, redox potential, Fe and organic matter. Sorption onto Fe oxides is favoured by oxidising conditions and near-neutral to acidic pH (Section 4.1). As such, specific conditions must be provided to promote sorption in a wetland.

3.4. Methylation

Methylation followed by volatilisation of metalloids such as mercury (Hg), selenium (Se) and As is a well known phenomenon that takes place in aquatic environments (Kosolapov et al., 2004). Under highly reducing and anoxic conditions, As can be converted to gaseous arsines, which are highly toxic compounds (Frankenberg Jr. and Arshad, 2002). Examples of volatile arsines are arsine (AsH_3), methylarsine (CH_3AsH_2), dimethylarsine ($(\text{CH}_3)_2\text{AsH}$) and trimethylarsine ($(\text{CH}_3)_3\text{As}$). The formation of methylarsines is a biological process that can be mediated by different organisms, such as fungi and bacteria. Sulphate-reducing bacteria are the main Hg methylating agent in soil and aquatic sediments (Bright et al., 1994). King et al. (2002) studied Hg methylation by sulphate-reducing bacteria in constructed wetlands, but no evidence of As methylation by sulphate-reducing bacteria was reported, despite the fact that it has been found that these bacteria are able to methylate As in other environments (Bright et al., 1994; Michalke et al., 2000). As such, arsenic methylation is not believed to significantly contribute to As removal in constructed wetlands. Furthermore, the relevance of this process in constructed wetlands has not been well studied (Buddhawong et al., 2005).

3.5. Plant uptake

Direct uptake and accumulation of As in plants appear to play a very minor role in As removal (García et al., 2010). For example, Ye

et al. (2003) reported that only 2% of the total As input was accumulated into the plant tissues, and they highlighted the minor role of plant uptake. The same conclusion was drawn by Singhakant et al. (2009a), who reported that only 0.5–1% of the total As input was accumulated into the plant tissues.

The accumulation of As depends on the type of plant (Zhao et al., 2010). In *Lemna gibba* L. (duckweed) (Mkandawire and Dudel, 2005) and *Spirodela polyrrhiza* L. (great duckweed) (Rahman et al., 2007), accumulation of As increased as concentration of As in water increased. Harvesting could be an option for bioaccumulation, but this has been considered unsuccessful in treatment wetlands (Kadlec and Wallace, 2009). However, Adhikari et al. (2010) suggested annual harvesting based on their reported As uptake, which fluctuated between 0.05 and 0.53 kg As ha⁻¹ y⁻¹. No other harvesting recommendations have been found in the available literature. If the above-ground biomass is not harvested, leaves and stems are eventually returned to the surface of the medium. Decaying plant biomass may act as a source, but it can also act as a sink for metals (García et al., 2010). It has been found that leaves tissues can release metals such as mercury, copper and zinc (Weis and Weis, 2004), but apparently this phenomenon has not been investigated for As.

Different studies have shown that roots accumulate more As than do shoots (e.g., Qian et al., 1999; Ye et al., 2003; Barley et al., 2005; Vymazal et al., 2009; Adhikari et al., 2010). In the study of Ye et al. (2003), As concentrations in roots were 2 to 10-fold greater than those of the shoots. Mean values in roots fluctuated between 5 and 30 mg kg⁻¹ depending on the type of plant. The values reported by Buddhawong et al. (2005) in the roots of *Juncus effusus* were within that range: between 0.3 and 7.2 mg kg⁻¹.

Arsenic is taken up by plant roots (Zhao et al., 2010) and different reasons may explain why As remains there: limited translocation of As from roots to shoots (Wang et al., 2002) and the presence of Fe and S (Zhao et al., 2010). In addition, As speciation plays a relevant role in the uptake mechanism and further translocation: there is enough evidence to affirm that arsenate is taken up by the same transporters of phosphate in the roots, but it is not known what form of As is translocated from roots to shoots and how this translocation occurs. Whilst arsenate and phosphate are similar, arsenate and arsenite are not, therefore their uptake mechanisms may differ (Dhankher, 2005).

It should be noted that some studies reporting As accumulation were performed in hydroponic systems (e.g., Qian et al., 1999; Rahman et al., 2007) or in plants growing in mine waters (e.g., Mkandawire and Dudel, 2005), therefore the results may not be comparable with those in wetland systems (e.g., Ye et al., 2003; Barley et al., 2005; Buddhawong et al., 2005; Vymazal et al., 2009; Adhikari et al., 2010). Furthermore, Comino et al. (2009) found that As accumulation by *Poa annua* reached its minimum when they were planted in 100% zeolite (instead of 100% gravel, or other combinations of gravel and zeolite) due to the high As absorption capacity of zeolite. Therefore, the presence of a suitable substrate, in this case zeolite, may decrease the As uptake by plants, increasing the overall removal capacity.

Despite their minor role in As uptake, wetland plants can play an indirect but important role, because the plants: (1) stimulate the growth of metal-oxidising bacteria by oxygen transfer into the rhizosphere (Cooper et al., 1996); (2) provide organic matter as a carbon source for sulphate-reducing bacteria and metal-oxidising bacteria (National Rivers Authority, 1992), as organics released from plant roots help release sulphides to precipitate As or co-precipitate As with Fe (Singhakant et al., 2009a); (3) provide a surface for microorganisms in the wetland (National Rivers Authority, 1992; Nuttal et al., 1995); (4) use their roots as surfaces for iron plaque, which is mostly iron (hydr)oxides precipitates, to retain As (Blute et al., 2004); and (5) oxygenate the

substrate by their roots, to provide an extensive region where aerobic and anaerobic conditions are adjacent (National Rivers Authority, 1992), thereby facilitating coupled aerobic–anaerobic processes.

It would appear that the role of wetland algae in the removal of As is negligible. Buddhawong et al. (2005) reported that an algae pond (operated in parallel with constructed wetlands) did not remove As at all. Even though the use of algae as an As biosorbent has been reported (Mohan and Pittman Jr., 2007), macrophytes are likely to be or become the dominant plants in treatment wetlands (Kadlec and Wallace, 2009). It therefore appears that future research to assess the role of wetland vegetation in As removal should be focused primarily on macrophytes.

4. The effect of environmental factors on arsenic removal

A variety of environmental factors can affect the removal of As in constructed wetlands, and changes in one factor often affects another (such as pH and alkalinity; temperature and dissolved oxygen). However, many of these factors can be controlled during the design/operation of the wetlands or during any pretreatment process, such as through varying the type of wetland substrate, providing an additional carbon source, or adjusting the pH of either the influent water or of the wetland substrate.

4.1. pH

The speciation of As is significantly affected by pH. As detailed in Section 2, As(III) is mostly found as an uncharged species at neutral pH, whilst As(V) is mostly found as negative species under conditions of pH higher than 2.3. Likewise, changes in the speciation of As can affect pH, as the oxidation of As(III) to As(V) decreases the pH value, whereas the precipitation of arsenosulphides increases it.

The sorption of As to metal (oxy)(hydr)oxides depends on pH. Metal ions on the oxide surface complete their coordination shell with OH groups in the presence of water. Depending on pH, these OH groups can release or bind H^+ , developing a surface charge. As such, the sorption properties of iron and other metal (oxy)(hydr)oxides are controlled through ion exchange involving OH_2^+ , OH and O^- surface functional groups (Stollenwerk, 2003). This type of adsorption requires As to be in the form of anions (such as $H_2AsO_4^-$) to provide a proton for complexation with the OH group, forming H_2O (Hingston et al., 1972; cited by Stollenwerk (2003)). Therefore, pH is one of the most important factors controlling the adsorption of As(III) and As(V) due to its influence on As speciation and on the composition of surface functional groups. At near-neutral to acidic pH, As (V) tends to be strongly sorbed to oxide minerals. At alkaline pH, As desorption is promoted by the negative charge of the mineral surface (Mukherjee et al., 2009).

The microbial activity of sulphate-reducing bacteria is influenced by pH; these bacteria prefer neutral environments, although sulphate reduction has been observed in acidic conditions (pH 3–4) (Hao, 2003). In addition, sulphate-reducing bacteria control their pH environment by generating alkalinity (Eq. (7)).

Despite the fact that constructed wetlands have been most widely used for acid mine drainage treatment, they generally have limited capacity to raise pH (Kadlec and Wallace, 2009). The wetland substrate may be used to adjust pH: zeolite and blast furnace slag are used to raise pH, but the effect may be transitory (Kadlec and Wallace, 2009). For coal mine waters, the incorporation of anoxic limestone drains or alkali-dosing for pH adjustment is recommended where the influent has $pH < 5.5$. The use of Reducing and Alkalinity Producing Systems (RAPS, or Successive Alkalinity Producing Systems SAPS), or compost-based wetlands are recom-

mended for $4 < pH < 5.5$ (Mayes et al., 2009). Compost-based wetlands are constructed with a ~0.5 m thick substrate of organic waste material which promotes bacterial sulphate reduction in addition to increasing alkalinity.

4.2. Alkalinity

High alkalinity provides a buffer to prevent resolubilisation of non-dissolved As, because an abrupt change in pH can release sorbed or particulated As by solubilisation. Carbonate alkalinity is generally desirable, as carbonated species are likely to precipitate. Bacterial production of bicarbonate by sulphate reduction, or the presence of limestone in the medium, can lead to sufficiently high bicarbonate levels to form precipitates with metals (García et al., 2010). Changes in alkalinity can indicate changes in the speciation of As and sulphate-reducing bacteria activity, since sulphate-reducing bacteria provide alkalinity to the water (Eq. (7)) and affect its pH (Sjöblom, 2003; Cohen, 2006). As detailed in Section 4.1, the adjustment of pH in wetland systems is achieved by increasing alkalinity, generated by carbonated minerals and/or by bacterial activity.

4.3. Temperature

Biochemical processes are affected by temperature. The optimum temperature for sulphate-reducing bacteria ranges between 28 °C and 32 °C (Hao, 2003). Sulphate reduction rates decrease as temperature decreases (Sobolewski, 1999; Sjöblom, 2003), while the solubility of oxygen increases as temperature decreases. Therefore, if As is going to be removed by sulphide precipitation, high temperature is required.

Temperature cannot realistically be controlled in real-scale wetland systems. However, it may be controlled indirectly by controlling water depth (Lee et al., 2009). When water levels are reduced to their lowest mark, the water temperature is often elevated (Kadlec and Wallace, 2009), enhancing bacterial activity. The degree of temperature variation with depth will of course depend on season, with the greatest ability to control temperature during the warmer season.

4.4. Dissolved oxygen

Oxygen slowly oxidises As(III) in water (Bissen and Frimmel, 2003). In an oxygenated environment As can be removed by coprecipitation/sorption by Fe(III) oxyhydroxides, which are the dominant form of Fe (at $pH \geq 6.5$ and $Eh > 0$) (Faulkner and Richardson, 1989).

Dissolved oxygen also affects microbial activity. Sulphate-reducing bacteria are obligate anaerobes, but they may survive a temporary exposure to oxygen and become active again under anaerobic conditions (Hao, 2003). The critical dissolved oxygen concentration below which sulphate reduction can occur is $0.1\text{--}1\text{ mg L}^{-1}$ (US EPA, 1985). The type of flow affects oxygen availability: vertical flow wetland systems tend to have good performance in oxygen transfer, favouring aerobic microbial populations; whilst horizontal flow wetland systems tend to be oxygen-limited and therefore will typically favour anaerobic microbial populations (Kadlec and Knight, 1996; Faulwetter et al., 2009). The method of distributing inflow also affects the availability of oxygen: batch feeding favours more aerobic processes, whilst continuous feeding favours more anaerobic processes (Faulwetter et al., 2009). Vegetation also plays a role by transferring oxygen into the rhizosphere and subsequently into the substrate.

4.5. Competing species

The sorption of arsenic oxyanions is very sensitive to the presence of competing anions, particularly phosphate (PO_4^{3-} , HPO_4^{2-} , H_2PO_4^-), sulphate (SO_4^{2-}), carbonate (CO_3^{2-}), bicarbonate (HCO_3^-) and chloride (Cl^-) (Mukherjee et al., 2009). Phosphate and silicate (SiO_4^{4-}) have the same tetrahedral configuration as arsenate, and therefore can desorb As(V) from different surfaces (Henken, 2009c). It was found that As(V) bound to a soil (which consisted of quartz, clay minerals, Fe and Al oxides) was effectively mobilised by the presence of phosphate in solution. The ability of anions in mobilising As from soil particles follows the order $\text{PO}_4^{3-} \gg \text{CO}_3^{2-} > \text{SO}_4^{2-} \approx \text{Cl}^-$ (Goh and Lim, 2005). In addition, the sorption of carbonate on ferrihydrite (hydrated ferric oxide) decreases the sorption capacity of As(V) significantly (Appelo et al., 2002). The effect of similar anion species in the removal of As in constructed wetlands has not been reported. However, this effect may be controlled by encouraging different removal mechanisms, depending on the type of polluted water and the levels of relevant anions. For example, in groundwater, sulphate concentrations are low while phosphate and carbonate are high (Cheng et al., 2009); whereas in acid mine drainage sulphate concentrations can be several orders of magnitude higher than that of arsenate (Cheng et al., 2009), while phosphate is not present in mine-contaminated water (National Rivers Authority, 1992). One way to improve the treatment of these waters would be encouraging sulphide precipitation (sulphate reduction by sulphate-reducing bacteria) instead of sorption: the presence of sulphate will enhance the removal of As, instead of prevent it as in the case of sorption. In the case of groundwater, a source of sulphate would be required.

4.6. Supporting media

Wetland media provide support to vegetation and microorganisms, and create differing environmental conditions, such as oxic and anoxic zones. The substrates which positively influence sulphate reduction have an important role in acid mine drainage treatment, because sulphide minerals have been found in a number of wetland sediments (Sheoran and Sheoran, 2006), and therefore in wetland media. Moreover, the media offers surfaces to allow direct sorption of metal ions, and/or other particles which can sorb them. The use of alternative media to improve the removal of metals has been suggested by some researchers but not extensively studied, even though it has been recommended to improve metal removal, not only for As (e.g., Stark et al., 1996; Ye et al., 2003).

Gravel has been found to have a limited As removal capability (Lizama A. et al., in press; Singhakant et al., 2009a). Organic substrates such as peat have been found to effectively retain As in natural peat bogs and peatlands due to its affinity to organic matter (González et al., 2006; Cloy et al., 2009). However, Kalmykova et al. (2009) did not recommend peat columns to sorb As at neutral or alkaline pH due to the diminished removal capability of iron hydroxides occurring in peat (Kalmykova et al., 2009), as presented in Section 4.1. Natural and synthetic zeolites have been found to remove As (Elizalde-González et al., 2001; Payne and Abdel-Fattah, 2005; Chutia et al., 2009). However, from the literature it appears that zeolite has been used as a supporting medium in only one study of constructed wetlands aiming to remove As (Lizama A. et al., in press), even though it has been successfully employed to remove organic matter (e.g., Stefanakis et al., 2009; Stefanakis and Tshirintzis, 2009), nitrogen (Saeed and Sun, 2011), phosphorus (Sakadevan and Bavor, 1998), and zinc (Sarafraz et al.,

2009). The study of Lizama A. et al. (in press) confirmed the ability of zeolite for removing As. Limestone has been used as a supplementary medium in wetlands (Duncan, 2002; Groudev et al., 2008), but barely as the main medium (Lizama A. et al., in press), therefore its effectiveness as the main wetland substrate for the removal of As has not been thoroughly investigated, even though it appears that its capability to enhance the removal (Lizama A. et al., in press).

4.7. Sulphate and iron

The removal of As can be achieved by arsenosulphides precipitation. This process requires a source of sulphide (such as sulphide produced from sulphate reduction by sulphate-reducing bacteria). In wetlands that treat acid mine drainage, sulphate is usually available due to the exposure of sulphide-containing minerals to water and oxygen, which results in the generation of sulphate (National Rivers Authority, 1992). If sulphate is unavailable, arsenic precipitation will be limited to the formation of other minerals instead of sulphide minerals.

The removal of dissolved Fe often coincides with the removal of As, mainly via the same precipitation/coprecipitation processes, whereas the presence of Fe oxyhydroxides facilitates the removal of As by sorption, depending on the pH and the presence of competing species.

4.8. Carbon source

Sulphate-reducing bacteria and metal-oxidising bacteria require organic carbon for synthesis, which can be provided by vegetation, organic pollutants, or the organic portion of wetland media. A carbon source is essential to stimulate sulphate reduction, and this can be provided by organic materials such as mulch and wood chips (Lloyd et al., 2004). Supplemental carbon sources have been introduced in several wetlands to increase the rate of sulphate reduction (Sheoran and Sheoran, 2006).

5. Synthesis of As removal pathways

The main removal pathways of As in constructed wetlands are precipitation, coprecipitation and sorption. Even though these are chemical processes, they can be microbially-mediated. Depending on environmental conditions, arsenic can precipitate mainly as arsenosulphides (reduced species) and as arsenates (oxidised species), coprecipitate with sulphides or Fe oxides, or it can be sorbed onto the wetland substrate, metal oxides and/or organic matter.

The most important factors that affect the removal of As are pH and the presence of Fe and S. Other factors such as temperature, the presence of a carbon source and dissolved oxygen become more important in the presence of microorganisms.

It is possible to enhance particular removal processes by controlling/mediating their corresponding triggering factors. If there is sulphate, a carbon source can be added, therefore leading to the precipitation of arsenosulphides. If there is dissolved Fe, raising the pH will precipitate it, therefore As will coprecipitate. The design of wetland systems should consider the control of these key factors.

6. Design and modelling of constructed wetlands for As removal

Currently, there is no official guideline on how a wetland should be designed specifically for the removal of arsenic. Information about the design of wetlands for metals removal is also rare, but some tentative design guidelines have been proposed using simple pollutant removal models such as the zero-order model or the first-order kinetic decay models (Kadlec and Wallace, 2009). Before sufficient experiment data are collected for As removal, the design

for lab- or pilot-scale experimental wetland targeting As can be based on these relatively simple design guidelines. The zero-order model fixes the removal rate per unit area of the wetland, assuming that the concentration decays at a constant rate toward the final outlet concentration, and that this decay rate is proportional to the hydraulic loading (design flow/area) of the wetland (Eq. (8)):

$$R_A = \frac{Q}{A}(C_i - C_o) \quad (8)$$

where A is the wetland area, m^2 ; C_i the inlet concentration, $g L^{-1}$; C_o the outlet concentration, $g L^{-1}$; Q the flow rate, $L d^{-1}$; and R_A is the area-adjusted contaminant removal rate, $g m^{-2} d^{-1}$.

One study has provided a tentative areally-adjusted As removal rate of $18 g m^{-2} d^{-1}$ (PIRAMID Consortium, 2003); however this value represents a single treatment system consisting of ponds and wetlands in the Carnoulès mine, France; and the rate was obtained from aerobic ponds rather than from an anaerobic wetland. According to Kadlec and Wallace (2009), the reported values for R_A vary considerably in different studies (Fe–Mn removal); therefore, the model cannot be widely extrapolated without detailed site-specific calibration. They also suggested that the zero-order model does not accurately represent the metal removal process in wetlands.

The first-order model, which assumes plug-flow, is also widely used for a range of wetland types (Wong et al., 2006). The model assumes a first-order exponential decay of the pollutant concentration towards the final outlet concentration, again with the decay rate proportional to the hydraulic loading of the system (Eq. (9)):

$$K = \frac{Q}{A}(\ln C_i - \ln C_o) + C^* \quad (9)$$

where K is the areal constant rate, in $m d^{-1}$, and C^* is the equilibrium concentration.

Goulet et al. (2001) tested the suitability of this model to predict the retention of dissolved As (among other metals) in different seasons. The model failed to fit summer, autumn and winter data for almost every metal investigated, including As. The first-order model only considers hydraulic retention time, but other variables – such as biological and hydrological conditions – may need to be included in removal models (Goulet et al., 2001). In addition, a first-order model considering water losses or gains (Kadlec and Knight, 1996) did not represent the removal of arsenic in free surface flow constructed wetlands due to poor mass removal (Dombeck et al., 1998).

The first step in developing more useful and reliable design models for As removal is to collect reliable data on the influence of key design/operating parameter (including As loading, hydraulic loading, pH, availability of organic matter, etc.), with the ultimate aim of using the data to develop an improved set of algorithms to predict performance and to allow designers to size and design systems.

7. Key research needs

It is apparent from the literature that constructed wetlands have the potential to remove metals and metalloids. However, little is known about their efficiency, nor about means of optimising arsenic retention. Most studies describing the application of constructed wetlands in the removal of metals and metalloids come from studies on the treatment of acid mine drainage using surface flow systems. The efficiency of subsurface flow wetlands has not been sufficiently studied, since wetlands with conventional soil (surface flow systems) or gravel (subsurface flow systems) media have been most-commonly employed. Little is known about how the use of alternative substrates may affect As removal.

Microorganisms potentially play a key role in the mobility, toxicity and availability of metals in wetlands, but understanding of their removal mechanisms is still unclear (Kosolapov et al., 2004). From the available literature, it appears that under the presence of sulphate and reducing conditions (and therefore the possible presence of sulphate-reducing bacteria), As is primarily removed by sulphide precipitation; whereas under the presence of Fe and oxidising conditions (and therefore the presence of sulphate-reducing bacteria is unlikely), As is mainly removed by coprecipitation/sorption with Fe. However, Fe precipitation and As oxidation can be mediated by bacteria (Section 3.1). More evidence is required to confirm the relevance of the role of microorganisms in the removal of As. Different microbial communities can be involved in As removal, but they have not been extensively studied in constructed wetlands. In general, it does not seem necessary to inoculate wetlands with the desired microorganisms; however, they cannot establish immediately (Kadlec and Wallace, 2009). An initial seeding followed by the providing of the required environmental conditions for their growth could enhance the removal of As, but no studies have yet tested this hypothesis.

Since few studies have focused on investigating As, the removal mechanisms for As in constructed wetlands are not well understood. Nevertheless, in order to assess As speciation in the solid phase, different techniques have been used and they could be potentially applied in constructed wetlands.

To quantify the removal of As driven by the different wetlands components, a mass balance is usually carried out by quantifying the content of As in the plants, in the media/sediments and in the outlet water. However, with this conventional mass balance approach, it is difficult to distinguish whether the As retained in the wetland components is sorbed, precipitated, or both. Advanced techniques, such as scanning electron microscopy (SEM), X-ray diffraction (XRD) and synchrotron-based techniques such as X-ray spectroscopy (Landrum et al., 2009; Lombi and Susini, 2009), can be used to find out the speciation of arsenic in the solid phase and identify As species. Such information can be very useful for enhancing As removal processes in wetlands, as well as in other treatment systems. Some studies have used these techniques to examine the speciation of arsenic in aquatic environments or natural wetlands. For example, Gräfe et al. (2008) studied the co-sorption products of arsenate and copper on iron minerals with extended X-ray absorption fine structure (EXAFS), whereas Blute et al. (2004) characterised the association of arsenic and iron in the roots of *Typha latifolia* (cattail) using X-ray absorption near-edge spectroscopy (XANES). The plants, however, were obtained from a natural wetland, which contained arsenic from industrial sources, rather than from a constructed wetland (Blute et al., 2004). Regarding X-ray diffraction techniques, X-ray powder diffraction was employed by Bauer et al. (2008) to identify As association in a wetland soil in contact with groundwater rich in As, whereas synchrotron-based micro X-ray diffraction (μ -XRD) and synchrotron-based micro X-ray fluorescence (μ -SXRF) were employed by Gao and Schulze (2010) to analyse As and metal-contaminated soils from a natural wetland. Despite the fact that X-ray diffraction has been employed to study the mineralogy of solids in constructed wetlands treating acid mine drainage (e.g., Karathanasis and Thompson, 1995; Gagliano et al., 2004), it appears that it has not been employed to study As-containing minerals. To date, only Duncan et al. (2004) have reported the use of synchrotron analysis to confirm the presence of As polysulphides in a wetland system in Trail, Canada, but details of the synchrotron technique were not provided, making it difficult to apply the synchrotron technique to other As-containing samples.

Current design guidelines to predict the removal of arsenic in wetland systems are based only on zero-order model and first-order model. Neither has been shown to be effective in predicting As

removal and neither takes into account important operating conditions or substrate type. Other more elaborate models for metal removal – such as the self-organising map (Lee and Scholz, 2006), and the Wheel Jane wetlands model (Whitehead et al., 2005) – may be applied to As. However, a better understanding of the main factors that affect As removal and the key processes involved is required in order to develop more reliable and useful models, which in turn, could be used to guide wetland design.

8. Conclusions

To date, the main application of constructed wetlands in the removal of metals and metalloids has been the treatment of acid mine drainage, where arsenic was not the priority pollutant. Arsenic, as a metalloid, presents differences in reactivity and therefore in the removal processes with metals such as Cu and Zn. The literature on As removal in treatment wetlands is very limited, and studies have showed that constructed wetlands have considerable potential to remove arsenic from contaminated waters.

Major As removal mechanisms in the wetlands include precipitation, coprecipitation and sorption. While methylation and plant uptake play a minor role, microorganisms such as sulphate-reducing bacteria, iron-oxidising bacteria, arsenite-oxidising bacteria and arsenate-reducing bacteria can mediate the removal mostly by oxidation/reduction reactions, followed by precipitation, coprecipitation or sorption depending on the environmental conditions. The main environmental factors that affect As removal in wetlands include pH, alkalinity, dissolved oxygen, the presence of iron and sulphate, competing chemicals, organic carbon, and the nature of the wetland media. Before reliable modelling and design equations or guidelines can be adopted, greater understanding is required of the processes affecting As removal in wetlands, taking into account the influence of the wetland characteristics and the operating conditions.

Overall, this review is the first to focus on, and integrate available literature regarding As removal in constructed wetlands. The review has identified major removal mechanisms and environmental factors that contribute to As removal. Before constructed wetlands can be confidently applied to remove As from contaminated waters, it is necessary to understand further about the roles of two main system components: supporting media and microorganisms. The role of macrophytes, both directly and in mediating microbial processes, may also be important.

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Chapter 4: Vertical subsurface flow wetlands for the removal of As, B and Fe from acidic water



DECLARATION FOR THESIS CHAPTER 4

Declaration by candidate

In the case of Section 4.4, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Initiation, ideas, experimental design and works, data analysis, write up	75

The following co-authors contributed to the work:

Name	Nature of contribution	Extent of contribution (%)
Guangzhi Sun	Ideas and reviewing	15
Tim D. Fletcher	Ideas and reviewing	10

**Candidate's
Signature**

	Date 01/02/2013
--	---------------------------

Declaration by co-authors

The undersigned hereby certify that:

- (1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
- (2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
- (3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility
- (4) there are no other authors of the publication according to these criteria;
- (5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
- (6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

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School of Engineering & Physical Sciences, James Cook University

Signature
Guangzhi Sun



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Date
01/02/2013

4.1. Introduction

Subsurface flow wetlands have the potential to remove metals and metalloids such as arsenic. However, since their performance in this application is not completely understood, they cannot be confidently applied for this purpose. Furthermore, different authors (e.g. Ye et al., 2003) have suggested the use of alternative wetland media to optimise removal, but few studies have actually tested potential wetland media.

Results presented in Chapter 3 showed that several alternative wetland media (limestone, cocopeat and zeolite) were more effective than conventional gravel media in the removal of As, B, Fe, Cu, Mn and Zn from synthetic contaminated water. However, these results were obtained for a particular type of synthetic water, with slightly acidic pH and concentrations of heavy metals that were representative of different polluted surface waters in Chile. Furthermore, since As and Fe were mostly in a particulate form, they were predominantly removed by filtration, meaning that the effect of the different media on the removal of the dissolved fraction could not be adequately tested. As such, this chapter uses the same four media but are instead tested using a more realistic scenario in terms of pH (and hence dissolved/particulate fractioning) and heavy metal concentrations. For this purpose, the Azufre River in Northern Chile was chosen as a case study. This river has elevated levels of As, Fe and B (3, 100 and 30 mg/L, respectively) and is very acidic pH (~2) (Ríos et al., 2011). The presence of these pollutants in the river, the isolated location of the river, and the water scarcity in Northern Chile were the main reasons to choose the Azufre River as the case study to focus on in this research. These characteristics make the Azufre River an ideal candidate for a possible application of constructed wetlands as an onsite technology for the treatment of contaminated water that is needed for human use.

The main objective of this experimental stage is to test the four wetland media for the removal of As, Fe and B from synthetic water simulating the Azufre River, in vertical subsurface flow constructed wetlands. The most effective media will be selected for future experiments, where horizontal subsurface flow constructed wetlands will be tested. The same vegetation type -*Phragmites australis*- will be used as described in Chapter 1.

The key research questions and hypotheses are:

Q1: Can alternative wetland media be more effective than conventional gravel media in removing the target pollutants: As, Fe and B from acidic water?

Alternative media can be more effective than conventional gravel media in the removal of the target pollutants, mainly due to their capability to foster particular processes that gravel cannot (consistent with Chapter 3)

Q2: Which are most effective media for removing each pollutant and why?

The most effective media for removing each pollutant may differ, since the pollutant characteristics are different (e.g. As is highly reactive, while B is not)

Q3: How do the different media perform compared to the previous results (Chapter 3), i.e., under highly acidic conditions and higher pollutant concentrations?

The performance of the media will be different to that from previous results, since the water quality is considerably different: As and Fe will be mainly dissolved under the highly acidic conditions

Q4: What are the key removal mechanisms involved under these new conditions?

Since the pollutants will be mainly dissolved given the acidity of the water, the removal mechanisms cannot be limited to filtration

Q5: Are there relationships between the removal of one pollutant and that of the others?

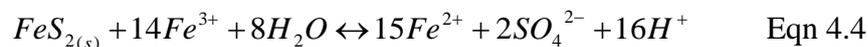
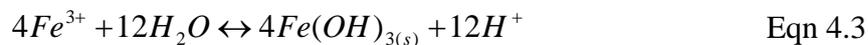
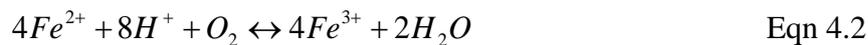
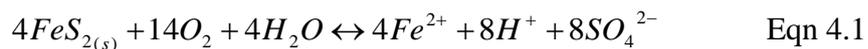
The presence of Fe will affect the removal of As, given the affinity and association between these two elements in natural aquatic systems

A laboratory-scale wetland system was constructed in order to answer these questions. The main results of these experiments are presented in a paper published in *Chemical Engineering Journal*, with additional context added on the nature of As in acidic waters (section 4.2) and the characteristics of the Azufre River (section 4.3), prior to presentation of the paper. Finally, conclusions and implications for the next experimental stage are discussed.

4.2. Acidic waters and the presence of arsenic and metals

The presence of metals and metalloids in aquatic environments is often associated with acid mine drainage. Mine drainage refers to surface waters or groundwater becoming contaminated with heavy metals, arsenic, and/or sulfuric acid as the water infiltrates mine shafts, pits, coal piles, ore processing structures and waste impoundments (Henken, 2009).

The oxidation of reduced iron minerals such as pyrite FeS_2 produces acidic waters and the problem of acid mine drainage (Snoeyink and Jenkins, 1980). In fact, the oxidation of pyrite has received attention and a general consensus on the pathway has been reached (National Rivers Authority, 1992). The corresponding equations are:



The key features of this path are (National Rivers Authority, 1992):

- Pyrite is oxidised by oxygen (as a gas dissolved in water) and ferric iron ions Fe^{3+}
- Oxidation generates acidity
- The oxidation of Fe^{2+} is the slow step (Eqn 4.2), but this oxidation can be catalysed by iron-oxidising microorganisms such as *Thiobacillus ferrooxidans*, which may increase the rate of Fe^{2+} oxidation by a million-fold and the overall rate of acid generation by up to twenty-fold

The oxidation of pyrite and other Fe^{2+} sulfides involves both iron and sulfur, as well as any arsenic impurities. Arsenic is scavenged by pyrite (arsenian pyrite) with concentrations up to 10 wt.% (Nordstrom, 2012). Furthermore, it has been suggested that arsenian pyrite (arsenic-rich pyrite) is more reactive than pyrite without arsenic (Savage et al., 2000). The major arsenic-bearing sulfides are pyrite, arsenopyrite (FeAsS), orpiment (As_2S_3) and realgar As_4S_4 (Henken, 2009). Arsenopyrite is the most common arsenic mineral (O'Neill, 1995). Arsenopyrite and pyrite dissolution contribute arsenic to ground and surface water in many parts of the USA (Welch et al., 2000). Unlike the case of pyrite, the pathway of the oxidation of arsenopyrite has been controversial (Henken, 2009) and therefore there is no general agreement on the oxidation order of iron, sulfur and arsenic. Orpiment occurs in hydrothermal deposits, volcanic vents and hot springs. It can also be formed as an oxidation product of realgar (Lengke and Tempel, 2002). Arsenic-bearing sulfide minerals occur in sediments, soils, a variety of rocks, but they are especially common in hydrothermal deposits and coals. Most sulfide minerals are very stable and insoluble if they are undisturbed under anaerobic conditions. However, they may readily decompose if natural processes or mining and other mining activities bring them into contact with aqueous solutions containing oxidising chemicals and/or oxidising bacteria (Henken, 2009).

4.3. Example of arsenic containing waters: Azufre River

The literature has reported different cases throughout the world where acidic waters contain As and metals such as Fe, Cu, Cd, Pb and Zn (e.g. Nordstrom et al., 2000, Williams, 2001). Also, a number of river systems affected by metal mine contamination has been reported, although metal mine discharges are not always acidic (Byrne et al., 2012).

However, many other cases are still being investigated. For example, in northern Chile, the Lluta River is a precious water resource in an extremely arid region (Arica and Parinacota), having As and B concentrations of 0.24 and 10 mg/L respectively (Dirección General de Aguas, 2008). The Lluta River originates from the confluence of the Azufre River and the Colpitas River. The presence of As, Fe, B and other metals is

caused by the geological characteristics of the Tacora volcano in the headwaters of the Azufre River, and also due to evaporation/concentration phenomena in the lowland portion of the catchment. The Tacora Volcano contributes salts, metals and acidity to the Azufre River, which is born at the foot of the Tacora Volcano. However, it is not clear whether its headwaters are acid drainage or hydrothermal waters, due to the existence of an old sulfur mining plant in the volcano (Dirección General de Aguas, 2008). The main pollutants in the Azufre River are As (3 mg/L), B (30 mg/L) and Fe (100 mg/L) at pH ~2 (Ríos et al., 2011, Guerra et al., 2012). On the other hand, the Colpitas River, another tributary to the Lluta River, contributes mostly B and As. These metalloids come from hydrothermal waters, tributaries to the Colpitas River.

Given the low pH and high levels of As and metals, and therefore the need for evaluating different treatment options due to the lack of water resources in the Lluta River catchment (Leiva et al., 2011), the Azufre River is a suitable case-study for a possible application of constructed wetlands for the removal of arsenic and metals. This river is located in the Chilean Altiplano -close to the Peruvian border-, around 150 km from Arica, the main city in the region. This impedes the treatment of water in Arica, therefore onsite remediation is required and conventional treatment technologies cannot be applied. Since surface waters are required mainly for irrigation purposes throughout the Lluta valley (Dirección General de Aguas, 2008), treatment must be provided in the upper part of the catchment (i.e. at of the source of the pollution, before the pollutants reach the Lluta River).

4.4. The removal of arsenic, boron and iron from an acidic wastewater in planted column reactors



The effect of substrate media on the removal of arsenic, boron and iron from an acidic wastewater in planted column reactors

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ABSTRACT

Acidic wastewaters, such as occur in acid mine drainage, typically contain heavy metals and metalloids that pose a serious threat to receiving waters, due to their high toxicity. In this study, vertical flow wetland columns, using a range of filter media, were investigated for their potential to provide an effective onsite treatment for acidic wastewaters with a pH value of 2.0 ± 0.1 . The effectiveness of four types of wetland media: gravel, cocopeat, zeolite and limestone, was studied for the removal of arsenic, boron and iron, under an average hydraulic loading of $0.073 \text{ m}^3/\text{m}^2\text{d}$. On average, limestone wetland columns gave the highest removal percentage for arsenic (99%) and iron (98%), followed by zeolite columns (92% removal for arsenic and 86% for iron). Although gravel columns were able to remove 43% of dissolved arsenic (from average input of $3.0 \pm 0.1 \text{ mg/L}$), they were not able to remove iron simultaneously. In contrast, wetland columns with cocopeat media only showed modest capacity for arsenic removal (9%), but greater capacity for removing iron (46%), and cocopeat was the only wetland media that demonstrated potential to remove boron. Overall, the results indicated that the most effective mechanism of arsenic removal in vertical flow wetlands is coprecipitation with iron, which can be enhanced by using alkaline wetland media to increase the pH of the wastewater. Combinations of media appear worthy of examination in order to optimise the efficacy and sustainability of heavy metal removal.

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1. Introduction

Acidic wastewaters, or runoff from natural arsenic-rich soil, often contain arsenic (As), boron (B) and metals that pose a serious pollution threat to waterways. Unfortunately, conventional treatment technologies for As removal such as adsorption, coagulation and membrane filtration have limitations [1], particularly related to costs, sludge generation and transportation [2,3]. Water contamination by boron poses similar problems; the presence of B in aquatic environment is causing increasing concern [4], in particular because its removal is not commonly achievable by conventional chemical treatment. Again, such conventional treatments are generally costly [5] and produce secondary waste (sludge).

During the past two decades constructed wetlands have been increasingly used, especially in rural regions, to treat domestic and agricultural effluents [6]. The growth of this technology is at least partly spurred by the rising cost of fossil fuel energies and

concern about climate change, with wetlands offering a sustainable low-energy input alternative. Wetland technology has considerable potential, as it is considered efficient and cost-effective for the treatment of metal-containing waters, including those associated with mining activities [7]. While wetlands require large areas of space, their low energy use and typically low maintenance requirements make them a potentially ideal option for treating acidic wastewaters on remote sites.

To date, the ability of constructed wetlands to remove metals and metalloids from wastewaters has been generally recognised, but not sufficiently studied [8], especially concerning the removal of arsenic [9] and boron [10]. Current knowledge about metal removal in wetlands has been obtained primarily from studies on the treatment of acid mine drainage, which usually contains sulfate, iron (Fe) and manganese (Mn) as its main pollutants [11]. Most studies to date have been conducted on surface flow wetlands, with much fewer studies conducted in subsurface flow wetlands, where the contaminated waters pass through packed media, instead of flowing above it, thus allowing more extensive contact between the contaminants and media. It may thus be hypothesised that subsurface flow wetlands could offer greater and more reliable treatment performance than surface flow wetlands, as found by Buddhawong

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et al. [12]. However, subsurface flow wetlands may need regular maintenance or even reconstruction should the media bed become saturated with metals [13].

Despite the potential advantages of subsurface flow wetlands, the lack of information about their performance hinders their ready adoption. Firstly, experiments are needed to demonstrate which wetland medium, or combination of different media, are most suited to remove these pollutants. Although various media have been suggested for such purposes [14,15], experiments are rarely carried out to verify hypothesised suitability. In a preliminary lab-scale study, cocopeat, zeolite and limestone were found to have the ability to remove As, Fe and B [16] under neutral or slightly acidic environment; however, their ability to remove these pollutants under highly acidic condition, which is usually associated with contaminated waters on mining sites, was not studied.

Secondly, experiments are required to monitor the removal of As and other pollutants, under a range of loading rates and influent concentrations, to produce data that are relevant to the treatment of specific types of wastewaters. Such information could be used to tailor the design of such wetlands to the wastewater type. Although acidic conditions are generally associated with As and metals, the concentrations of the pollutants can vary significantly, depending on site conditions. Williams [17] presented hydrochemical data for As in 34 mining sites in 7 countries of South-east Asia, Africa and Latin America, and showed that the concentrations of As fluctuated between 0.005 and 72 mg/L, whereas the pH value ranged from 0.52 to 10.0. Acidic conditions primarily result from mining, hydrothermal and volcanic activities, or sulfide oxidation [18], although groundwater movement and surface runoff can also cause low pH and high As values in waters at, or close to mining sites. For example, the Azufre River in northern Chile (Lluta River Basin) has a pH value of around 1.8, and As, B and Fe concentrations of around 3, 30 and 100 mg/L, respectively [19]. Such water quality makes the river unsuitable for human use. As such, it is necessary to run experiments designed to rigorously test the performance of wetland systems for specific pollutant types and concentrations.

Thirdly, there is a need for studies which identify and quantify the environmental factors affecting As, B and metal removal in subsurface flow wetlands, as well as the links between the removal of different pollutants that result from chemical precipitation and co-precipitation. Two key factors that affect the removal of As in wetlands are: (1) pH and (2) the presence of Fe and S [9]. A previous study [16] provided some insights into the effect of pH and Fe in the removal of As. However, the study was incomplete. For example, the study did not quantify the removal of As under highly acidic conditions (e.g. pH < 2), which is likely to be the typical environment for mine drainage and many existing polluted rivers, such as the Azufre River in Chile [20]. Regarding the second factor, iron hydrochemistry controls As aqueous mobility in acid ferruginous mine waters when pH is below 4 [17]; the interaction between Fe and As in wetland environment can have a significant effect on the removal of both pollutants. This is a phenomenon that needs to be further studied because Fe and As are so commonly found together and optimising the treatment of one will typically require consideration of the other.

The aim of this study is therefore to investigate the use of vertical flow constructed wetlands to remove three target pollutants, As, Fe and B, from a synthetic acidic wastewater that simulates highly polluted river water (for example a river polluted by acid mine drainage). The efficiencies of four wetland media are studied: cocopeat, zeolite, limestone, and river gravel, with the aim of evaluating the pollutant removal efficiency of the different wetland media. A range of environmental factors, such as temperature, pH, Eh and SO_4^{2-} levels, are monitored alongside the removal rates of the target pollutants, in order to explain the factors which must be taken into account when optimising subsurface wetlands for heavy

metal removal in acidic waters. This study is part of a broader study that will, in future phases, consider aspects such as the influence of vegetation type on pollutant removal.

2. Materials and methods

2.1. The acidic wastewater

To simulate realistic, acidic polluted water, a case-study source water was chosen: the Azufre River, Northern Chile. This river begins at the foot of the Tacora volcano, on the upper part of the Lluta River catchment (150 km from Arica, the main city of the Arica and Parinacota Region). Due to its high concentrations of As and metals and its isolated location, the Azufre River represents a typical drainage from mining sites [20]. Representative synthetic wastewater was prepared using deionised water with the following reagents added per litre of water: 3 mL 1000 mg/L arsenic standard solution (arsenic acid As_2O_5 in H_2O), 3 mL 10,000 mg/L boron standard solution (boric acid H_3BO_3 in H_2O), 0.5 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.425 mL H_2SO_4 (95–97% Merck ISO grade). As a result, the concentrations of the target pollutants in the synthetic feed were (average \pm standard deviation): 3.08 ± 0.25 mg/L As, 32 ± 2.19 mg/L B, and 107.33 ± 6.53 mg/L Fe. The resulting pH value was 2.0 ± 0.1 . Under this acidic condition, the metals were mostly dissolved, consistent with how they would be naturally found in the Azufre River.

2.2. The wetland system

Twenty subsurface vertical flow wetland columns were built using PVC pipes. Each column was 1 m tall and 100 mm in internal diameter, as illustrated in Fig. 1.

The wetland columns, placed in a greenhouse, were divided into four groups, each group employing gravel, cocopeat, zeolite and crushed limestone as the main substrate. Each group had five identical replicate columns that were operated as individual treatment units. The packed porosities of the media are 40%, 55%, 25% and 30% in gravel, cocopeat, zeolite, and crushed limestone columns, respectively. Each column had a 0.1 m deep drainage layer of 20–40 mm gravel at its base. The drainage layer was overlain with a single 0.7 m deep layer of the main substrate (gravel, cocopeat, zeolite or limestone), resulting in a total depth of 0.8 m (the main layer plus the drainage layer) (Fig. 1). In each column, a single plant of common reed (*Phragmites australis*) from the root-cuts of mature plants in a Melbourne wetland was planted. *Phragmites* was chosen primarily because of its documented tolerance to acidity (surviving well within pH range 2–8) [21]. Many other plants commonly used in wetlands have a narrow pH tolerance range, for example: pH range 4–10 for *Typha*, pH 5–7.5 for *Juncus*, and pH 4–9 for *Scirpus*. *Phragmites* is also widely used in constructed wetlands in the UK [22] and Europe, where subsurface flow wetlands are dominant, making it an ideal candidate as the 'standard' plant for use in the wetland media substrate. The *Phragmites* were given two months of establishment period to adapt to their new growth environment prior to commencement of the experiment.

During the experiments, the synthetic wastewater was stored in a 230 L continuously stirred feed tank. From the tank, two litres of synthetic wastewater were collected and dosed manually onto the top of each wetland column. The wastewater was freshly prepared each week before the first dosing, and it was kept to the next dosing day on the same week. The water passed by gravity through the wetland media during the dosing, as the outlet was non-restricted. Effluent from each column was collected underneath in an effluent collection tank. The manual dosing was carried out twice per week, giving a hydraulic loading rate of 4 L per week ($0.073 \text{ m}^3/\text{m}^2\text{d}$)

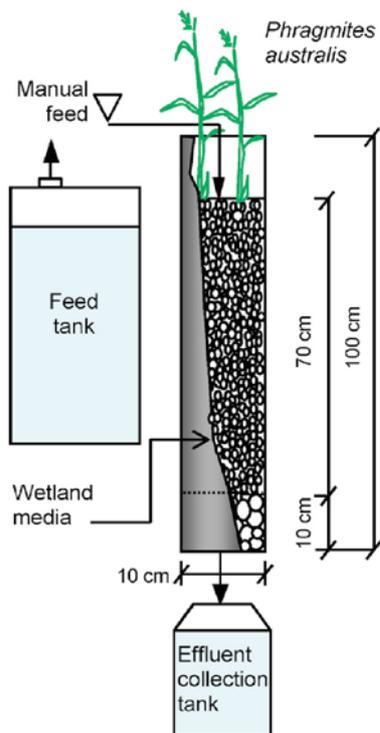


Fig. 1. A schematic diagram of the wetland column.

applied to each column. The dosing started in August 2010 and lasted for thirteen weeks.

2.3. Sampling and analysis

Water samples were collected from the inlet (feed tank) and outlet (effluent collection tank) of each column in week 1, 4, 7, 9, 11 and 13. Separate samples were taken for total and dissolved metal analysis, with analysis undertaken according to Standard Methods [23]. For the measurement of dissolved pollutants, the samples were filtered (through 0.45 μm cellulose acetate papers) and acidified (with nitric acid) immediately after sampling. The analyses of As, B and Fe concentrations were carried out for both unfiltered and filtered samples, to give total and dissolved values. The analyses of other parameters were only carried out for the unfiltered samples.

The concentrations of As, B and Fe were determined in a NATA (National Association of Testing Authorities, Australia, <http://www.nata.asn.au>) accredited laboratory (with QC procedures based on Standard Methods [23]) by ICP-OES (and ICP-MS if the parameter analysed was below the OES detection limit). ICP-OES detection limits were 0.1 mg/L for As, 0.05 mg/L for B and 0.05 mg/L for Fe, whereas ICP-MS detection limits were 0.001 mg/L for As, 0.02 mg/L for B and 0.02 mg/L for Fe. In situ parameters were measured at Monash University. Dissolved oxygen (DO), pH and conductivity values were measured using three probes that were connected to a Sension 378 m. The probes were calibrated before measurement. An ORP Testr10 probe was used to measure redox potential (Eh). Sulfate (SO_4^{2-}) was measured using DR5000 UV/Vis spectrophotometer, based on Standard Methods [23]; total

suspended solids (TSS) were measured using the same equipment, based on a photometric method for sewage and industrial wastes [24].

The mineralogical composition of gravel, zeolite and limestone was analysed by X-ray diffraction (XRD). A Scintag diffractometer with a $\text{Cu K}\alpha$ radiation source was used and the scans were performed continuously from 0° to 70° with a scan step size of 0.02° .

2.4. Calculation and statistical analysis

2.4.1. Pollutant mass removal rates

For each target pollutant, its mean mass removal rate (in $\text{mg}/\text{m}^3\text{d}$, where m^3 represents the superficial volume of a column) and removal percentage (%) in each wetland group (gravel, cocopeat, zeolite and limestone) were calculated. The mass removal rate M_R was calculated as:

$$M_R = \frac{(C_{i_{in}} - C_{i_{out}}) \times Q}{V_{\text{wetland}}} \quad (1)$$

where $C_{i_{in}}$ (mg/L) corresponds to the total concentration of As, B or Fe in the inflow; $C_{i_{out}}$ is total concentration in the outflow; Q (L/d) is daily flow rate; and V_{wetland} (m^3) is the superficial volume of a single wetland column.

2.4.2. The effect of wetland media

To analyse the performance of the different wetland media, statistical tests were performed using PASW Statistics 18 and a significance level of $\alpha = 0.05$. Prior to this testing, Kolmogorov–Smirnov test was performed to check data normality. When data were not normal, they were log-transformed; in this case, p values are indicated as $p_{\text{log-transformed}}$.

The influence of different wetland media on the wastewater treatment results was statistically assessed by one way ANOVA to compare the mean concentrations of target pollutants in gravel, cocopeat, zeolite and limestone wetland columns, as well as the environmental parameters in the columns. When significant difference caused by the media was found ($p^A < 0.05$), multiple comparison post hoc tests were performed to distinguish which wetland media were significantly different: Tukey's test was applied when the assumption of homogeneity of variances was satisfied (as determined by Levene's test), or Games–Howell's test when this assumption was not satisfied (notation p^T and p^{GW} , respectively).

If the assumption of normality was not achieved, Kruskal–Wallis analysis was performed instead of ANOVA. When significant difference was found ($p^{KW} < 0.05$), Mann–Whitney post hoc test were performed to distinguish the mean differences which were significantly different ($p^{MW} < 0.05$).

2.4.3. Correlation between the removals of different pollutants

Because As can coprecipitate with Fe, and Fe precipitation is affected by pH, Spearman correlation factor ρ was calculated for each wetland group, to discover whether any significant relation can be found between: (1) the outflow concentrations of As and Fe (dissolved and total) and (2) the outflow concentrations of Fe (dissolved and total) and pH. Spearman ρ was used instead of Pearson's correlation due to the non-normal distribution of the data.

3. Results

3.1. Overall performance of the wetland system

Table 1 presents the mean influent and effluent concentration of As, B and Fe in each group of wetland columns during the experiments (five replicates multiplied by six data sets, giving thirty

Table 1
Mean inflow and outflow concentrations – total and dissolved – of the target pollutants.

Pollutants	Mean inflow concentration (mg/L) [CV]		Mean outflow concentration (mg/L) [CV]							
	Dissolved	Total	G – gravel		C – cocopeat		Z – zeolite		L – limestone	
			Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved	Total
As	3.0 [0.10]	3.1 [0.08]	1.7 [0.18]	1.7 [0.18]	2.7 [0.13]	2.8 [0.13]	0.250 [1.42]	0.258 [1.44]	0.010 [1.79]	0.021 [0.67]
B	32 [0.08]	32 [0.07]	31 [0.08]	31 [0.07]	29 [0.07]	30 [0.07]	31 [0.10]	31 [0.10]	31 [0.09]	31 [0.09]
Fe	105 [0.07]	107 [0.06]	113 [0.07]	116 [0.06]	56 [0.08]	57 [0.09]	14.8 [0.47]	15.0 [0.47]	0.11 [0.48]	1.85 [0.47]

CV, coefficient of variation = σ/μ .**Table 2**
Mean inflow and outflow levels of monitored water quality parameters.

Parameter (unit)	Mean inflow value [CV]	Mean outflow value [CV]			
	Value	Gravel	Cocopeat	Zeolite	Limestone
pH	2.0 [1.19]	2.0 [1.17]	1.8 [1.01]	2.6 [1.04]	6.7 [1.00]
DO (mg/L)	9.93 [0.19]	10.06 [0.21]	8.94 [0.23]	10.98 [0.60]	9.37 [0.18]
T (°C)	19.4 [0.22]	18.8 [0.24]	18.8 [0.21]	19.0 [0.21]	19.0 [0.19]
Eh (Mv)	453 [0.04]	502 [0.03]	501 [0.11]	498 [0.08]	198 [0.28]
TSS (mg/L)	5 [2.06]	26 [1.54]	8 [1.32]	1 [1.99]	26 [1.77]
Electrical conductivity (mS/cm)	4.98 [0.09]	4.37 [0.13]	7.04 [0.63]	2.10 [0.35]	2.40 [0.04]
SO ₄ (mg/L)	907.23 [0.51]	938.59 [0.73]	953.66 [2.08]	1022.14 [2.12]	1142.72 [0.55]

For the pH values, CV was calculated using $-\log[\sigma]/-\log[\mu]$, where μ is the average of {H⁺} and σ is the standard deviation of {H⁺}.

values to obtain each mean value). In all wetland columns, and for the three target pollutants, the mean outflow concentrations were mostly lower than inflow concentrations (with the exception of Fe within the gravel wetlands), demonstrating that each wetland substrate type was capable of removing heavy metals from the acidic water. Pollutants were mainly in dissolved form in most effluents, with the exception of As and Fe in the effluents from the limestone wetland columns. Table 2 presents mean values of the environmental parameters being monitored, calculated similarly as the values in Table 1 (five replicates multiplied by six data sets, allowing thirty values to obtain each mean value). Table 2 shows that these values were affected differently by the types of media; while the DO values were only modestly affected, the pH was significantly affected by the media.

3.2. Removal of As

As shown in Table 1, limestone wetlands gave the lowest As outflow concentrations (dissolved and total) and thereby the highest removal efficiency. The efficiency of As removal was lower in zeolite and gravel columns. Wetland columns with cocopeat media were the least efficient for As removal. Fig. 2 gives the profile of As concentrations and removal rate over time. It can be observed that the As mass removal M_R in limestone wetlands followed the trend of the As loading throughout the experiment, whereas in zeolite wetlands the removal began to follow the trend of the As loading during the later part of the experiment. Gravel and cocopeat wetlands did not present a particular trend in removal over time. Both dissolved and total As concentration were significantly lower in the outflow than the inflow ($p^{KW} < 0.001$), confirming the

effectiveness of the wetland system as shown in Fig. 2. Furthermore, both dissolved and total As outflow concentration from each wetland substrate were significantly different to those in the inflow, and to each other (p^{MW} values shown in Table 3), indicating that each wetland substrate varied in its ability to remove As, with limestone the most effective, followed by zeolite, gravel and finally cocopeat.

3.3. Removal of Fe

Similarly to As removal, limestone wetlands gave the consistently lowest outflow concentration of total Fe. Zeolite and cocopeat wetlands, while achieving considerably lower removal than limestone, were far more effective than the gravel substrate, which generally resulted in a release of Fe (Fig. 3). The efficiency of Fe removal in cocopeat wetlands was found to improve with time, a trend that was similar to the removal of As in zeolite-based wetlands (Fig. 2). The type of substrate significantly affected the outflow concentration of dissolved and total Fe ($p^{KW} < 0.001$), with gravel being the only substrate to produce effluent concentrations which did not vary significantly from the influent concentrations (Table 4).

3.4. Removal of B

Boron removal rates appeared to be higher in all columns at the beginning of the experimental period, as indicated in Fig. 4. However, after 4 weeks of dosing, virtually no B was removed from the wastewater in gravel, limestone and zeolite columns. Cocopeat was thus the most effective substrate for boron removal. The type of substrate did affect the outflow concentration of dissolved and total

Table 3
Comparison of dissolved and total As concentration in the inflow of the wetland system and the outflow of each wetland group using Mann–Whitney post hoc tests. Bold type denotes significant difference.

	Inflow		Gravel		Cocopeat		Zeolite	
	As dissolved (mg/L)	As total (mg/L)						
Gravel	<0.001	<0.001	–	–	–	–	–	–
Cocopeat	0.029	0.023	<0.001	<0.001	–	–	–	–
Zeolite	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	–	–
Limestone	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

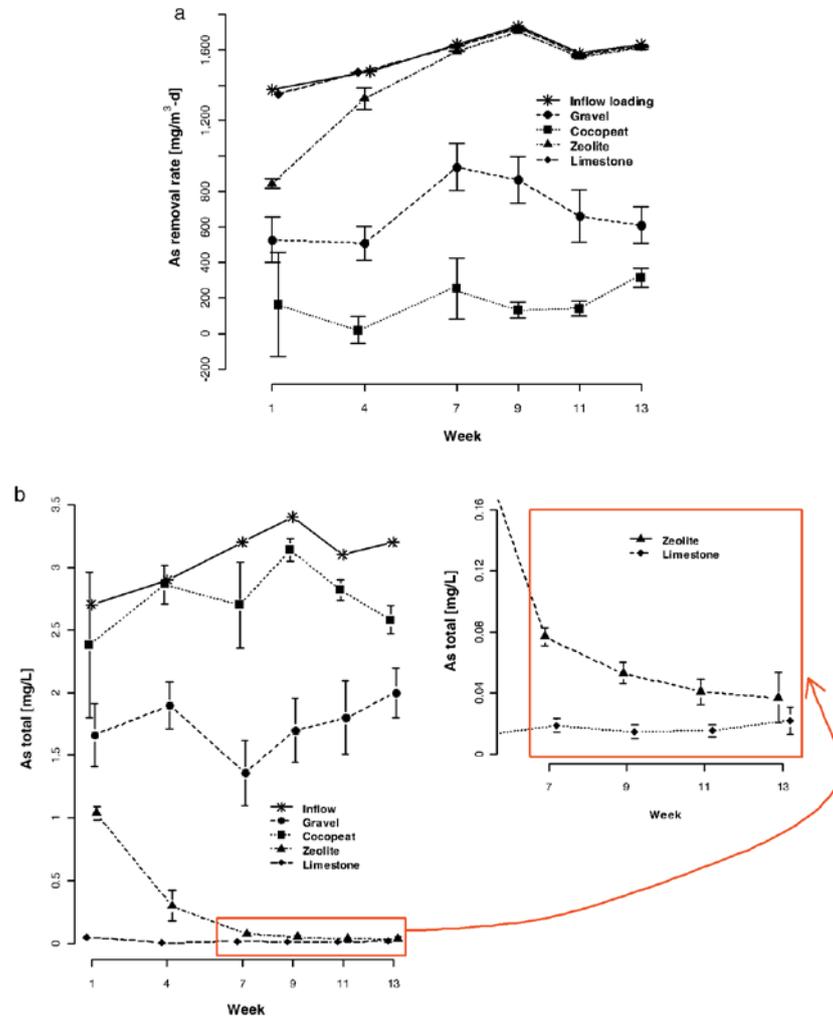


Fig. 2. Mean removal rate of total As (a), and mean inflow and outflow As total concentration in the four types of wetlands (b). Error bars indicate SD.

Table 4

Comparison of dissolved and total Fe concentration in the inflow of the wetland system and the outflow of each wetland group using Mann–Whitney post hoc tests. Bold type denotes significant difference.

	inflow		Gravel		Cocopeat		Zeolite	
	Fe dissolved (mg/L)	Fe total (mg/L)						
Gravel	0.103	0.094	–	–	–	–	–	–
Cocopeat	<0.001	<0.001	<0.001	<0.001	–	–	–	–
Zeolite	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	–	–
Limestone	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

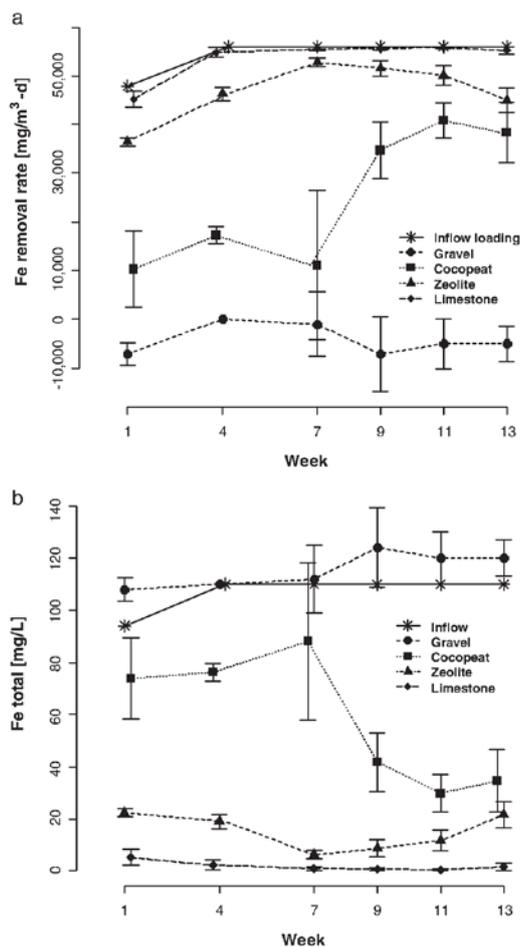


Fig. 3. Mean removal rate of total Fe (a), and mean inflow and outflow Fe total concentration in the four types of wetlands (b). Error bars indicate SD.

B ($p^{KW} = 0.04$). However, cocopeat wetlands were the only wetland type that affected significantly the concentration of B when comparing to the inflow, as shown in Table 5.

3.5. Changes in environmental parameters

3.5.1. pH and Eh

The most significant change in pH occurred, predictably, in the limestone-based wetlands, increasing from 2.0 to 6.7 (Table 2).

Table 5

Comparison of dissolved and total B concentration in the inflow of the wetland system and the outflow of each wetland group using Mann–Whitney post hoc tests. Bold type denotes significant difference.

	inflow		Gravel		Cocopeat		Zeolite	
	B dissolved (mg/L)	B total (mg/L)						
Gravel	0.307	0.394	–	–	–	–	–	–
Cocopeat	0.037	0.058	0.054	0.032	–	–	–	–
Zeolite	0.584	0.523	0.270	0.751	0.001	0.008	–	–
Limestone	0.605	0.576	0.504	0.661	0.012	0.016	0.940	0.880

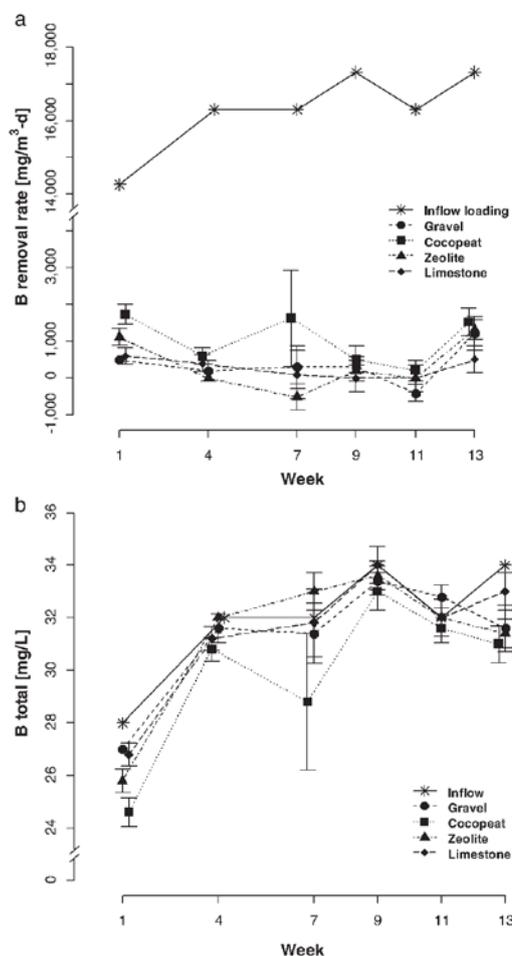


Fig. 4. Mean removal rate of total B (a), and mean inflow and outflow B total concentration in the four types of wetlands (b). Error bars indicate SD.

Zeolite wetlands also caused an increase in pH, from 2.0 to 2.6. Kruskal–Wallis analysis ($p^{KW} < 0.001$) showed that the media significantly affected the pH values of effluent from the wetland columns. Gravel and cocopeat wetlands presented an average pH slightly lower than that of the inflow, but neither of them was significantly different to the inflow ($p^{MW} = 0.82$ for gravel, $p^{MW} = 0.33$ for cocopeat). A similar trend was observed for the redox

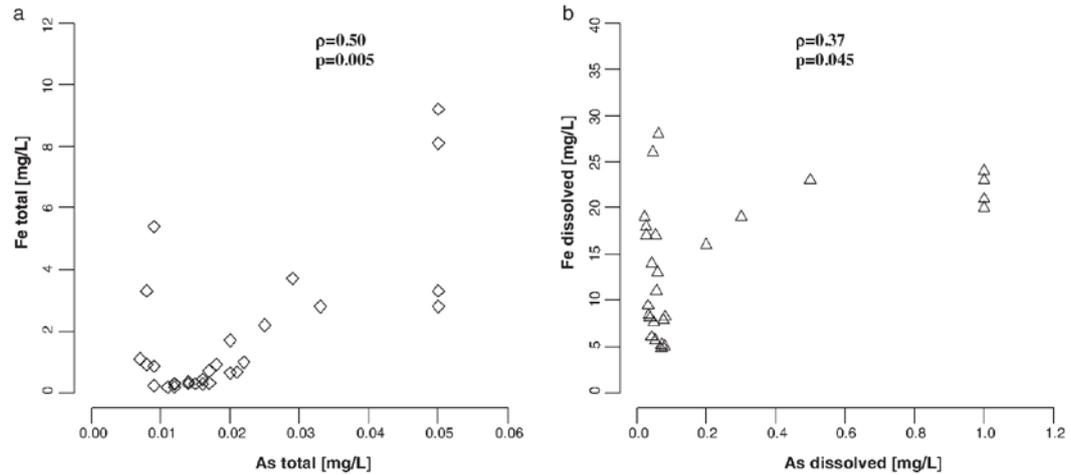


Fig. 5. Total As concentrations vs. Fe concentrations in the effluent from limestone wetlands (a), and dissolved As vs. Fe concentrations in the outflow from zeolite wetlands (b).

potential (Eh) of the effluent, as indicated by one-way ANOVA analysis result ($p^A < 0.001$); however in this case each media type (gravel, cocopeat, zeolite and limestone) was found to affect Eh significantly ($p^{GM} < 0.001$) as the wastewater passed through the columns, being gravel, cocopeat and zeolite Eh higher than the inflow, and limestone lower than the inflow (Table 2). Limestone Eh was also significantly lower to each other substrate ($p^{GM} < 0.001$). However, Eh in the gravel wetlands was not significantly different to that of cocopeat and zeolite. The zeolite and cocopeat media had similar Eh.

3.5.2. DO and sulfate

In contrast to pH and Eh, dissolved oxygen was not affected by the wetland media, with the mean DO values in the inflow and outflow of each column showing no significant difference ($p^A_{\log\text{-transformed}} = 0.176$). Sulfate concentrations were significantly influenced only by the limestone media, with SO_4^{-2} concentrations in the outflow from these columns significantly higher from SO_4^{-2} concentrations in the inflow ($p^{MW} < 0.001$). Conversely, the outflow SO_4^{-2} levels from gravel, cocopeat and zeolite wetlands presented no significant difference with those in the inflow.

3.5.3. Electrical conductivity

The wetland media were found to significantly affect electrical conductivity values of the wastewater ($p^{KW} < 0.001$). In particular, conductivity in the outflow from gravel, zeolite and limestone wetlands were significantly lower than those in the inflow ($p = 0.032$ for gravel, $p^{MW} < 0.001$ for zeolite and limestone). Conversely, electrical conductivity was greater in the cocopeat media than in the inflow, although this difference was not statistically significant.

3.5.4. Total suspended solids (TSS)

Although there were significant differences in the TSS effluent levels between the media ($p^A_{\log\text{-transformed}} = 0.001$), none of the outflow from any wetland substrate was significantly different to the inflow. Only zeolite wetlands decreased the TSS concentration (Table 2), although even in this case the decrease was not significant.

3.6. Correlation between As and Fe removal

Fig. 5 shows the plots of As outflow concentrations vs. Fe outflow concentrations in limestone and zeolite wetland columns. Removal of As and Fe were correlated as follows: (1) total As and Fe outflow concentrations from the limestone columns (Spearman $\rho = 0.5$, $p = 0.005$) and (2) dissolved As and Fe outflow concentrations from the zeolite columns (Spearman $\rho = 0.37$, $p = 0.045$).

No significant correlations were found between As and Fe concentrations (total and dissolved) in gravel and cocopeat columns.

3.7. Correlation between pH and the removal of Fe

The dissolved and total concentration of Fe from the outflow of limestone and zeolite wetlands decreased as the pH increased, as shown in Fig. 6 for limestone wetlands. Negative Spearman ρ values confirmed significant correlation between pH and Fe removal in both limestone (Spearman $\rho = -0.38$, $p = 0.037$; and Spearman $\rho = -0.59$, $p = 0.01$; for dissolved and total Fe concentration, respectively) and zeolite wetlands (Spearman $\rho = -0.47$, $p = 0.007$; and Spearman $\rho = -0.48$, $p = 0.008$; for dissolved and total Fe concentration, respectively). Conversely, the concentration of Fe was not significantly related to the pH levels in the outflow of gravel and cocopeat wetlands.

4. Discussion

4.1. The effect of wetland media on As, Fe and B removal

The results of this study show that the nature of wetland media is the main factor that determines the route and efficiency of the removal of As and Fe from acidic water. Among the four media, limestone gave the highest removal rates for As and Fe, followed by zeolite. Gravel has a moderate capacity to remove As but does not remove Fe. The ability of cocopeat to remove As and Fe is very limited, although the cocopeat columns enabled the removal of dissolved B at an average rate of 273 mg/m³d.

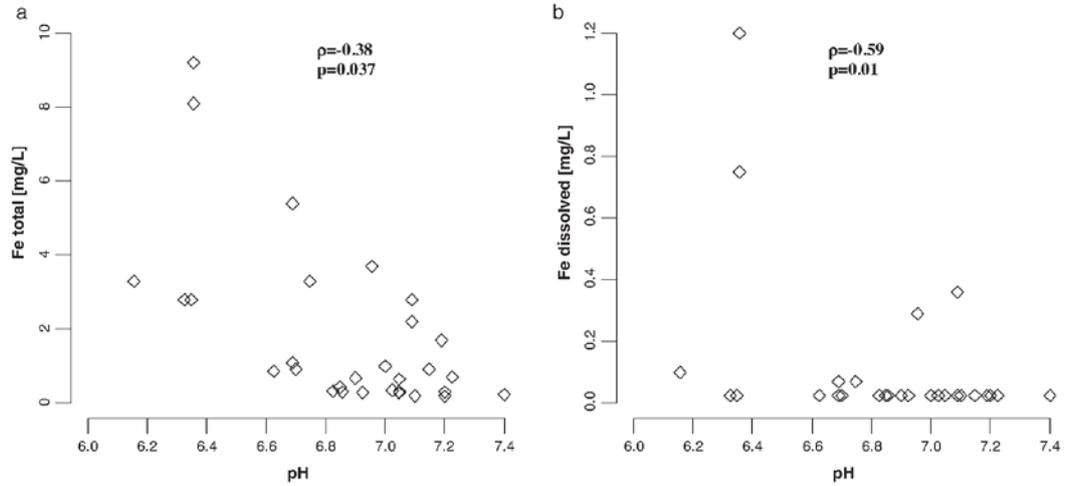


Fig. 6. Relationship between total Fe concentrations and pH values (a), and dissolved Fe vs. pH values (b), in the outflow from limestone wetlands.

4.1.1. Limestone columns

The good performance of limestone columns is attributed largely to their ability to increase pH. Iron in the effluents from the limestone wetlands is predominately in particulate form; as shown in Table 1, only 6% of the Fe is dissolved, giving a clear indication

that Fe has precipitated in the limestone wetlands. The positive correlations between outflow As and Fe concentrations (Fig. 5), and negative correlations between total Fe and pH (Fig. 6), give further indication that Fe has precipitated as a result of higher pH, and Fe precipitation has caused simultaneous As coprecipitation.

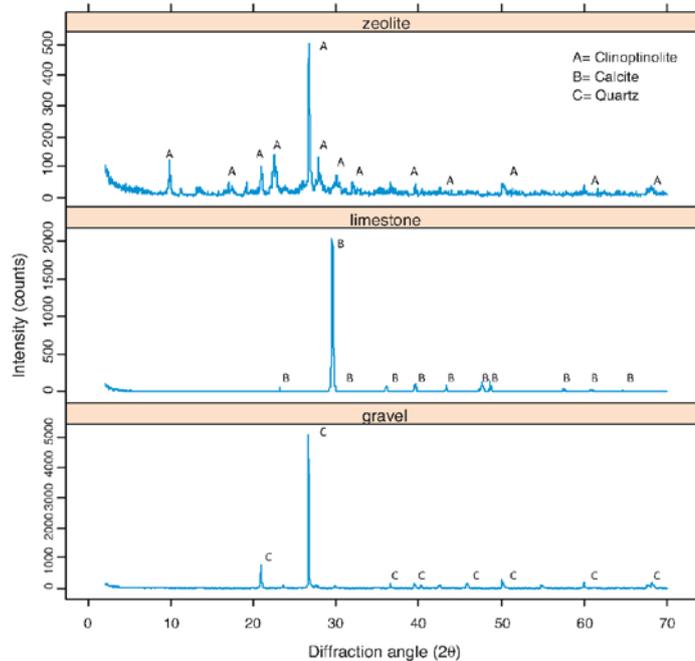


Fig. 7. X-ray diffraction (XRD) pattern of zeolite, limestone and gravel. The analysis shows that the main components of each are clinoptilolite, calcite and quartz, respectively. This information provides insights into the removal mechanisms in the corresponding wetland types.

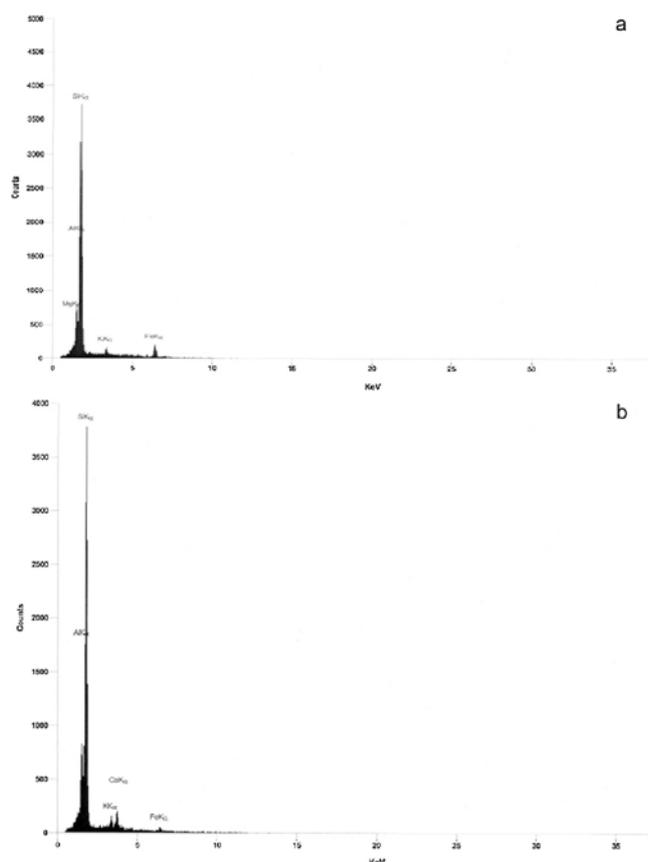


Fig. 8. Energy-dispersive spectroscopy (EDS) spectrum of gravel (a) and zeolite (b). This confirms the information provided by XRD analysis for each substrate, indicating that Si, Al, Mg, Fe and K are present in gravel, and that Si, Al, Ca, K and Fe are present in zeolite.

Other researchers have also tested the use of limestone as a supplementary substrate for As and metal removal in wetlands. Groudev et al. [25] reported the use of crushed limestone in a mixture with soil, silt, compost, cow manure and sand as the medium of four constructed wetlands designed to treat an acid mine drainage from an uranium deposit. Duncan [26] employed crushed limestone and wood pulp in three surface flow wetlands, to remove As, Cd and Zn. Ye et al. [15] suggested the use of crushed limestone to increase the removal of metals from acidic mine waste. These studies, together with the results from this study, all recommend the use of fine limestone chippings or grains in constructed wetlands, if As removal is a major objective.

In addition, limestone has previously been used as a sorbent for the removal of As (i.e. [27,28]). It should be noted that limestone is able to remove As from alkaline and no-Fe containing water, mainly due to the As-retention capacity of calcite CaCO_3 and goethite $\alpha\text{-FeOOH}$, minerals occurring in limestone [27]. Limestone used in this study was identified by XRD as mainly in the form of calcite CaCO_3 (Fig. 7). Therefore, limestone cannot only remove As by coprecipitation with Fe due to its capability to raise pH, but also by sorption onto calcite. However, as mentioned above, coprecipitation with Fe appears to be the main removal mechanism in this study.

4.1.2. Coccopeat and gravel columns

In this study, cocopeat was chosen to demonstrate how organic matter in wetland matrices may affect As, Fe and B removal. Organic substrates, such as peat, have been reported to retain As [29,30], Fe [31,32], and B [33]. As shown in Table 1, cocopeat-based columns gave low As removal (9.7%), but higher Fe removal (46.7%). Although the sorption of As(V) onto organic matter has not been studied at pH level around 2, it has been found that the ability of humic acids to bind with As(V) improves when pH value increases from 4.6 to 8.4, with the maximum binding capacity occurring at pH 7 [34]. In this study, As(V) sorption onto humic acids appears to have been negatively affected by the presence of H^+ and Fe(III), suggesting a preference of cocopeat to bind with Fe, instead of As, at low pH (around 2.0).

Cocopeat was the only wetland medium showing modest ability to remove dissolved B; the removal of B in the cocopeat columns is likely to be a result of B binding with organic matter, as suggested by Sartaj and Fernandes [35]. However, the interaction between organic matter and B is complex; it has been found that organic matter can increase B removal in soil [36], but it can also inhibit the removal of B by occluding Fe/Al oxides present in soil [37]. Low pH generally presents an unfavourable condition for B removal, due

to the boron speciation. H_3BO_3 , instead of $B(OH)_4^-$, predominates at low pH, making it more difficult for B to be removed by sorption, particularly onto organic matter, due to the lack of electrostatic attractions.

In contrast to the cocopeat columns, the gravel substrate gave higher As removal (45%) but no Fe removal. The leaching out of Fe after four weeks of operation is postulated to be a result of Fe adsorption reaching saturation, although further research will be needed to confirm this hypothesis. The mechanisms and sustainability of As removal within the gravel columns are unclear. The fact that there is no change of dissolved Fe concentration from inflow to outflow, as shown in Table 1, rules out the possibility of As coprecipitating with Fe. Binding to humic acids is also unlikely, due to the lack of organic matter in the gravel columns. X-ray diffraction analysis showed that the gravel is mainly quartz SiO_2 (Fig. 7). In addition, the energy-dispersive spectroscopy (EDS) spectrum showed that the main elements present in the gravel are Si, Al, Mg and Fe (Fig. 8a). Therefore, it is postulated that As may have been removed by sorption onto protonated alumina sites, as suggested by Clifford and Ghurye [38]. It is possible that the As may have also been exchanged by Si anions, as suggested by Arai et al., as silicate ions readily absorb onto mineral surfaces, and other oxyanions such as arsenate may replace the surface-absorbed Si anions [39].

4.1.3. Zeolite columns

Zeolite is well known for its very high cation exchange capacity. Boron is present primarily as a neutral species (H_3BO_3) at pH 2.6 (the mean pH value of the effluents from zeolite columns). As such, the ion-exchange capability of zeolite may not have been fully utilised. A higher pH, which transforms H_3BO_3 into $B(OH)_4^-$ form, would benefit ion exchange processes. In a batch experiment, it has been found that zeolite is less effective in removing B compared with another two media, fly ash and demineralised lignite, and the efficiency of zeolite improves at higher pH [40].

The capability of zeolite to remove arsenic has been reported [41], although most of the studies were on batch experiments (i.e. [42,43]). The zeolite-based wetlands showed impressive removal of As and Fe at low pH, despite the fact that low pH decreases metal removal by zeolite due to increased competition between cations and H^+ [44]. Zeolite employed in this study is mainly clinoptilolite ($NaKCa_6(SiAl)_{36}O_{72} \cdot H_2O$) as indicated by the XRD analysis (Fig. 7). This was confirmed by the EDS spectrum as the presence of Si, Al, Ca, K (and Fe) was detected (Fig. 8b). Since the dissolved concentrations of As and Fe were positively correlated (Section 3.6), and pH and Fe concentration were negatively correlated (Section 3.7), a possible removal mechanism could be: H^+ and Fe cations are exchanged by other cations – such as Ca, Na, K – present on the zeolite surface, resulting in pH increases (Table 2) and the subsequent decreases in the concentration of Fe in solution (Fig. 3b). As this process progresses, As removal increases through time (Fig. 2b), being attracted by the Fe on the zeolite sites [45]. Another possible As removal mechanism is the exchange of aluminol or silanol hydroxyl groups and anionic species, in this case arsenate. These hydroxyl groups are developed at the edges of zeolite particles in the presence of water [46].

4.2. As removal routes

The removal of arsenic from contaminated water can follow several routes: (1) methylation or demethylation by interacting with biological organisms, (2) sorption, (3) oxidation or reduction (biotic or abiotic), (4) precipitation, and (5) coprecipitation, primarily with metals such as Fe [9]. Water chemistry (particularly pH) affects arsenic speciation [47] and the main route of As removal. The presence of iron in water can significantly affect sorption, precipitation and coprecipitation processes, as demonstrated in natural

environments where biogeochemical cycles of iron and arsenic tend to be coupled [48]. This study shows that in limestone and zeolite wetland columns As is primarily removed by coprecipitation with Fe, whereas in cocopeat and gravel columns the most likely As removal route is sorption onto organic matter or media surfaces.

The nature of the (co)precipitated As–Fe particles is unknown. As described by Wang et al. [49], more effective removal of As was expected due to the simultaneous formation of iron hydroxides (due to the raise in pH) and As removal, and therefore more surface was available for arsenic. Several researchers have investigated the As retention capacity of iron hydroxides by laboratory sorption experiments. These experiments do not, however, reflect the fact of the spontaneous formation of solid phases where coprecipitation is likely to occur [50], as in the case of limestone-based wetlands. In addition, calcium arsenates can also precipitate in oxidising environments and the presence of Ca and As. Geochemical modelling and the use of advanced techniques are required to further investigate the As speciation in the solid phase.

4.3. Other factors affecting the removal of As, Fe and B

4.3.1. Vegetation

The role of microorganisms and plants can affect the transformation of metals within wetlands, because it is unlikely that the metal removal process is completely abiotic [51]. The role of vegetation in the removal of metals can either be direct (by plant uptake) or indirect (by mediating other removal processes). For As removal, however, previous studies have found that plant uptake is not a major mechanism [8,12,15]. For Fe removal, plant uptake can play a more important role when Fe levels are up to 1 mg/L [52]. In this study the Fe levels were around 100 mg/L, meaning that Fe accumulation in plants may be not considered as an important sink for Fe. The situation is similar for B removal, as plants could play a more important role in its removal depending on the concentration: for example B concentrations above 10 mg/L were toxic to the duckweed *Lemna gibba*, but at B concentration below 2 mg/L the duckweed removed B efficiently [53]. The experimental data from this study do not explicitly test nor demonstrate the role of plants in the removal of any target pollutant, but the experiment showed that *P. australis* can tolerate low pH values; as such, the plants may grow in acidic waters and be used as wetland plants for the treatment of acidic wastewaters, such as coal and metal mine drainages, as reported by Mayes et al. [54]. If the accumulation by plants is considerable, harvesting may be considered as an option for long-term As removal [55], however As [55] and metals [13] are accumulated in roots more than in shoots, therefore harvesting of aboveground parts would not contribute to the removal of these pollutants. The presence of vegetation is key as a supply of organic matter, although this is often overlooked due to the short time span of most studies [7]. Therefore, studies of long time spans are required to confirm the role of vegetation in providing more sustainable As and metal removal, through the provision of: (1) organic matter as carbon source for bacteria and adsorption medium for metals, (2) surface area (roots) for attaching bacteria and iron plaque, (3) oxygen transfer into the rhizosphere and the substrate, stimulating the growth of metal-oxidising bacteria in the first case and facilitating coupled aerobic–anaerobic processes in the second case [9].

4.3.2. Sulfate and organic carbon

Apart from pH and Fe, other factors that can significantly affect the removal of As include the presence of sulfur (S) and organic carbon (OC), which potentially allow microbial transformations to immobilise As in wetland matrices [9]. In this study, because the columns were mostly aerobic (as shown by the DO values in Table 2), sulfate could not be reduced or retained in the columns,

and the formation of As/Fe sulfide minerals was unable to occur. Accordingly, no reduction in sulfate concentration was observed. Regarding the role of organic matter, the availability of organic carbon in cocopeat wetlands did not result in significant removal of As and Fe, compared with the efficiency achieved in the limestone columns, which removed Fe and As by precipitation and coprecipitation, respectively. As no bacterial seeding was carried out prior to the experiment, it was unlikely that sufficient microbial population was present in the columns, considering that without seeding microbial population cannot establish quickly in a constructed wetland [13]. Future research will investigate the role of microorganisms in the removal of As and Fe in wetland systems.

4.4. Long-term sustainability of the wetland media

As shown in Fig. 2, the removal of As in gravel wetlands tended to decrease over time, consistent with observations in other studies (e.g. [8,16]). This observation supports the hypothesis that gravel may not be appropriate to remove As and metals, mainly due to limited sorption capacity and inevitable clogging [13]. Two studies reported Fe release from gravel wetland matrices under reducing conditions [56,57]. In this study the wetland matrices did not present reducing conditions. The Fe adsorption ability of the gravel appears to be limited at low pH conditions, as pH is a main factor favouring Fe mobility in wetlands [57]. Therefore, gravel appears not to be an appropriate substrate to remove Fe under acidic conditions. Conversely, the decrease in the removal of As over time did not occur in zeolite and limestone wetlands, despite the high inflow concentrations of pollutants over time and their higher removal capacity compared to gravel and cocopeat wetlands. As is the case with the use of organic material, zeolite and calcitic materials as wetland media will require periodic maintenance as they become saturated [13]. In addition, as reported in Section 3.5, wetlands with gravel and limestone substrate produced effluents with higher TSS than the inflow. This may indicate that these substrates degrade due to the acidity of the inflow. Furthermore, the high removal efficiency of limestone reflected in precipitation of As and Fe (Section 4.1) may also be contributing to the increase TSS levels in the outflow. Further settling/filtration may be required if gravel or limestone are used to treat highly acidic water. Given that the lifespan of the media depends on the pollutants loading, it is recommended that breakthrough experiments particularly designed to saturate the wetland media are performed to find out the effective lifespan of the wetland system. This information is key in the design of constructed wetlands aimed at the removal of As and metals using alternative media.

4.5. Implications for target pollutants removal using constructed wetlands

The results demonstrate one route to effectively remove As and Fe from the acidic wastewater in vertical flow wetlands, i.e. the increase of pH to precipitate Fe and coprecipitate As; the use of limestone and zeolite as wetland media facilitated this removal route. Enhancing this route may also decrease phytotoxicity [58,59]. However, the long-term efficiency of limestone and zeolite wetland columns is unknown. In addition, results from the gravel wetland columns showed that coprecipitation was not the only route of As removal. To further understand As removal, the role of vegetation and microorganisms needs to be investigated in carefully designed experiments. This study suggests that organic substrates can be used to enhance the removal of B, but higher pH would be required. Overall, this study has generated useful new insights for media selection and As removal routes, but there is a

long way before wetland systems can be designed and built, with confidence, to remove As, B and Fe from wastewaters.

5. Conclusions

Vertical flow wetlands with limestone medium demonstrated high efficiency for the removal of As (99%) and Fe (98%) from acidic wastewater, when the average hydraulic loading on individual wetland columns was $0.073 \text{ m}^3/\text{m}^2\text{d}$ and the As and Fe loadings were 0.23 and $7.8 \text{ g}/\text{m}^2\text{d}$, respectively.

The removal of Fe and As were correlated in wetland columns having limestone and zeolite as supporting media. The high efficiencies of the limestone columns were primarily due to their ability to raise the pH of the wastewater to stimulate Fe precipitation and As coprecipitation. Coprecipitation with Fe was shown to be the most effective route to remove As from the wastewater, but more studies are required to demonstrate the long-term efficiency and sustainability of As removal via coprecipitation.

On average, wetlands with zeolite medium removed 92% As and 86% Fe from the acidic wastewater. Conventional gravel wetland columns were able to remove 45% As, without simultaneous removal of Fe, indicating that, unlike in the limestone columns, coprecipitation with Fe was not the main route of As removal in the gravel columns.

Cocopeat was the only wetland medium that showed a reasonable ability to remove B, although on average the wetland columns with cocopeat medium only removed 9% As and 46% Fe from the wastewater.

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4.5. Conclusions and implications

Vertical flow wetlands with alternative wetland media, particularly limestone and zeolite, presented significantly higher efficiency than did gravel and cocopeat wetlands in removing As and Fe. Surprisingly, the conventional gravel media was more effective than cocopeat in removing As, but not Fe. In addition, cocopeat wetlands showed promising results in removing B. These findings support hypotheses H1, H2 and H3, since: (1) alternative media were more effective than the conventional gravel, (2) it appears that a single substrate will not give the optimum removal for all heavy metals, such that a combination of substrates is likely required, and (3) each of four wetland media presented a particular capability to remove each pollutant.

As the pollutants were mainly dissolved (as described in Hypothesis H4), different biochemical reactions may occur before physical retention in the wetland bed. It can be inferred that the key removal mechanisms involved are:

- Iron precipitation and arsenic coprecipitation in limestone wetlands, triggered by pH raise
- Iron and arsenic sorption in zeolite wetlands, due to the high ion exchange capacity
- Boron and iron sorption in cocopeat wetlands, given their affinity for organic matter

Key factors affecting the removal of As are the pH and the presence of Fe. As hypothesised in H5 (the presence of Fe will affect As speciation as these elements are associated in aquatic systems) the removal of Fe and As occurred simultaneously in limestone and zeolite wetlands, mainly due to coprecipitation of As and Fe in limestone wetlands, and sorption in zeolite wetlands.

These findings are different to those from Chapter 3. The fact that the pollutants were mainly dissolved in the inflow affected the performance of the different wetland media: filtration would not be sufficient to remove them. The removal of metals is favoured by

higher pH (Wingenfelder et al., 2005). Arsenic has high affinity for iron oxides, which is the predominant form of iron at $\text{pH} > 6.5$ (Faulkner and Richardson, 1989). Therefore, in Chapter 3, arsenic was mainly associated to iron oxides in the inflow, whereas in Chapter 4 arsenic was dissolved in the inflow. In limestone wetlands, the pH raised caused the formation of iron oxides and therefore the removal of As. This mechanism has been suggested to be more efficient than removal onto pre-existing iron oxides, due to the existence of more available surface for As retention (Wang et al., 2003).

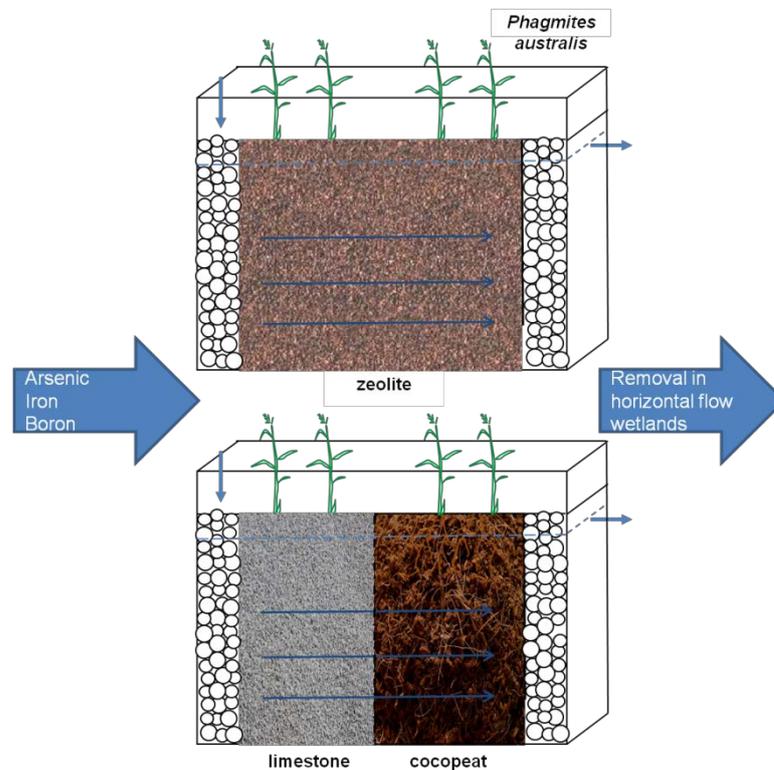
In the following experimental stage (Chapter 5), horizontal subsurface flow constructed wetlands will be employed. To optimise the removal of the target pollutants, the most effective wetland media, limestone and zeolite, will be combined to optimise the removal of As and Fe, whereas cocopeat will be selected for the removal of B.

4.6. References

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Chapter 5: Horizontal subsurface flow wetlands for the removal of As, B and Fe from acidic water



DECLARATION FOR THESIS CHAPTER 5

Declaration by candidate

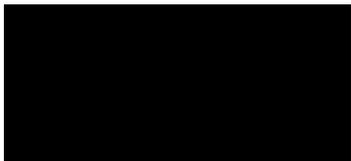
In the case of Section 5.2, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Initiation, ideas, experimental design and works, data analysis, write up	75

The following co-authors contributed to the work:

Name	Nature of contribution	Extent of contribution (%)
David T. McCarthy	Ideas and reviewing	n/a
Tim D. Fletcher	Ideas and reviewing	n/a

**Candidate's
Signature**

	Date 10/02/2013
--	---------------------------

Declaration by co-authors

The undersigned hereby certify that:

- (1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
- (2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
- (3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility
- (4) there are no other authors of the publication according to these criteria;
- (5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
- (6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location
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Signature
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5.1. Introduction

Constructed wetlands have been increasingly studied for their potential to remove arsenic and metals from water, which has been reported by different authors (e.g. Kadlec and Wallace, 2009, Marchand et al., 2010, García et al., 2010). Constructed wetlands are currently considered as a promising low cost, sustainable technology for As removal, particularly subsurface flow wetlands (Zurita et al., 2012). However, the application of horizontal subsurface flow wetlands (HSSF) for this purpose is limited (Kröpfelová et al., 2009) and as such, few studies have investigated the removal of arsenic using HSSF (e.g. Rahman et al., 2008, Rahman et al., 2011). In addition, the use of alternative media to enhance performance has been suggested (Ye et al., 2003), but not sufficiently studied.

In Chapter 4, results indicated that limestone, zeolite and cocopeat were more effective than conventional gravel media in the removal of As, Fe and B from synthetic water simulating the Azufre River, in vertical subsurface flow constructed wetlands. Limestone and zeolite were the most effective in removing As and Fe, whereas cocopeat was the most effective in removing B. As such, these three alternative media were selected as main supporting media to be used in two types of horizontal flow wetlands: one having zeolite only, and the other one having both limestone (in the inlet) and cocopeat (in the outlet), so as to enhance B removal by raising pH in the limestone section prior to sorption onto organic matter in the cocopeat section.

The main objective of this experimental stage is to verify the effectiveness of these three wetland media in the removal of As, Fe and B from synthetic water simulating the Azufre River, in horizontal subsurface flow constructed wetlands. The same vegetation type -*Phragmites australis*- will be used as described in Chapter 1.

The key research questions and hypotheses to be tested are:

Q1: Are the alternative media found effective in removing the target pollutants: As, Fe and B from acidic water in VSSF wetlands also effective in HSSF wetlands?

Alternative media in HSSF wetlands can be as effective as in VSSF wetlands, mainly due to their capability to foster specific processes, such as ion-exchange in zeolite (consistent with Chapter 4)

Q2: Which wetland type is more effective for removing each pollutant and why?

Both types of wetlands will have high As and Fe removal efficiencies, whereas limestone/cocopeat wetlands will be more effective than zeolite wetlands in removing B given the potential of cocopeat in this task (consistent with Chapter 4)

Q3: How do the different media perform compare to the previous results (Chapter 4), i.e. under continuous flow feeding in HSSF, for a longer experimental period?

Continuous flow feeding favours more anaerobic processes, which can add extra removal capacity to the system. Longer experimental period may allow observing a decrease in removal rates if the system reaches saturation

Q4: Will anaerobic conditions be found under these new conditions -since they generally predominate in HSSF wetlands- and will they affect their efficiency in removing the target pollutants?

HSSF wetlands tend to be oxygen limited, therefore favouring anaerobic processes and microbial populations, which could have a positive effect on pollutant removal

Q5: Are there relationships between the removal of one pollutant and that of the others in the different wetland media?

In limestone/cocopeat wetlands, iron and arsenic will be removed together by precipitation of iron oxyhydroxides (consistent with Chapter 4)

Q6: What is the contribution of pollutant uptake by plants to the overall removal process?

Direct uptake by plants plays a minor role, since arsenic and metals accumulate mainly in the wetland bed

A laboratory-scale wetland system was constructed in order to answer these questions. The main results of these experiments are presented in a paper submitted to *Water Research* with additional context added on the nature of microbial communities found in the wetland system (Section 5.3) after presentation of the paper. Finally, conclusions and implications are discussed.

5.2. The removal of arsenic, boron and iron from acidic wastewater using horizontal flow constructed wetlands with different wetland media

1 **The removal of arsenic, boron and iron from acidic wastewater using**
2 **horizontal flow constructed wetlands with different wetland media**

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10

11 **Abstract**

12 Arsenic (As), boron (B) and iron (Fe) are commonly found in contaminated waters, which are
13 sometimes acidic. Vertical flow subsurface constructed wetlands with alternative wetland
14 media have shown potential to remove these pollutants from acidic water. However, less is
15 known about this potential in horizontal subsurface flow wetlands. In this study, horizontal
16 flow wetlands columns were used to verify the effectiveness of two media types, one based
17 on zeolite as the main material, and the other having limestone and cocopeat. The wetlands
18 were operated under a hydraulic loading of 30 mm/d, to treat acid water with As=2.3 mg/L,
19 Fe=97.3 mg/L and B=30.8 mg/L at pH 2±0.2. Both systems were highly effective in the
20 removal of As and Fe: the zeolite wetlands removed 99.9%, 96.1% and 12% of As, Fe and B
21 respectively, whereas the removal efficiencies of the limestone/cocopeat wetlands were
22 99.8%, 87.3% and 17%. The contribution of plant uptake to As, Fe and B removal in both
23 wetland types was almost negligible (<3% in all cases). Results thus confirm the key role of
24 the wetland media in fostering specific removal processes: As co-precipitation with Fe due to
25 pH adjustment provided by limestone, and As and Fe removal by cation exchange capacity of
26 zeolite. Limestone/cocopeat wetlands may offer a more suitable treatment, given the neutral
27 pH achieved and slightly higher B removal, but zeolite wetlands are able to achieve lower
28 concentrations of Fe, despite the acidic pH in the treated effluent.

29 *Keywords:* arsenic; horizontal flow constructed wetland; supporting media; metals; reed bed;
30 water treatment

31 **1. Introduction**

32 The presence of arsenic (As) in aquatic environments is a worldwide problem (Jain and Singh
33 2012). Arsenic exposure via drinking water is known to cause bladder, lung and non-
34 melanoma skin cancer (Marshall et al. 2007). Among 21 countries with known groundwater
35 arsenic contamination (including Argentina, Bangladesh, Chile, USA, China, Mexico,
36 Poland, Canada, Hungary, New Zealand, Japan and India), the largest population at risk is in
37 Bangladesh. Here, over 100 million people drink water with arsenic levels of up to 1 mg/L,
38 which is 100 times the World Health Organisation drinking water guideline value (Mohan
39 and Pittman Jr. 2007). Therefore, the main objective when developing As treatment
40 technologies has been to remove As from groundwater for drinking water purposes. In
41 groundwater, arsenic is mainly present as arsenite, As(III). This species is harder to remove
42 than is arsenate, As(V), due to the lack of charge in the arsenious acid form H_3AsO_3 , which is
43 the As(III) predominant species at $pH < 9$ (Henken and Hutchison 2009).

44 Oxidation, co-precipitation, adsorption, ion exchange and membrane processes are the major
45 techniques employed to remove arsenic from water. However, due to variations in arsenic
46 speciation and the characteristics of the water to be treated, often more than one technology is
47 required (Henken 2009). Although some of these methods are quite simple, the disadvantage
48 associated with co-precipitation/sorption methods is the large production of toxic sludge (Jain
49 and Singh 2012). Although membrane methods do not produce toxic sludge, they generate
50 toxic wastewater and require sophisticated maintenance (Mohan and Pittman Jr. 2007).

51 Most of the hydrologic As contamination problems worldwide are the result of As
52 mobilisation under natural conditions (Mukherjee et al. 2008). However, anthropogenic
53 activities can also mobilise As. The exploitation of gold and base-metal deposits has been
54 identified as a principal cause of As contamination of surface drainage and groundwater in
55 many countries. In addition, As-contaminated waters may sometimes be acidic. Arsenic can
56 be found as dissolved species in a wide pH range (1-12) (Williams 2001). Metal mine
57 discharges have degraded many rivers around the world (Byrne et al. 2012).

58 While treatment of As-contaminated drinking waters is a worldwide challenge, surface water
59 sources for non-drinking purposes, such as irrigation or protection of aquatic ecosystems,
60 may also require treatment. In this case, conventional drinking water treatment technologies
61 may not be suitable. There is therefore a need for onsite technologies that can also remove

62 other metals/metalloids, since often, As-contaminated waters contain other pollutants. For
63 example, the presence of As and fluoride (F) in groundwater in Latin America has been
64 reported (Alarcón-Herrera et al. 2012), while surface waters in Northern Chile are also rich in
65 boron (B), antimony (Sb) and iron (Fe) (Landrum et al. 2009, Rios et al. 2011). The presence
66 of other metals alongside As must be considered when looking at treatment alternatives, as it
67 will affect the removal efficiency. For example, arsenic reactivity and speciation depends on
68 different factors, mainly pH, redox potential and the presence of Fe (Lizama A. et al. 2011).

69 Constructed wetlands have been recognised as a reliable wastewater treatment technology,
70 being also suitable for the treatment of many types of wastewater (Vymazal 2011). They have
71 the potential to remove metals and metalloids (Marchand et al. 2010), including As (Lizama
72 A. et al. 2011). However, few studies have investigated their potential to remove As, as the
73 main application of constructed wetlands has been on the treatment of acid mine drainage or
74 metal-contaminated water, where the main pollutants are iron and manganese (Wallace and
75 Knight 2006). Surface flow wetlands (SF) have been the predominant treatment of choice
76 (Kadlec and Wallace 2009).

77 Some studies have investigated the application of subsurface flow wetlands (SSF) to treat
78 arsenic and metal contaminated water, finding promising results. Buddhawong et al (2005)
79 found that subsurface flow wetlands were more effective than surface flow wetlands in
80 removing As and Zn, attributing this to the combined effect of supporting media (gravel), the
81 presence of Fe and the role of vegetation. Furthermore, to enhance As and metal removal in
82 SSF wetlands, the use of alternative media has been proposed (Ye et al. 2003), but
83 unfortunately such proposals have not yet been well tested (Lizama A. et al. 2011). Some
84 studies have been performed in vertical subsurface flow wetlands (VSSFs) with alternative
85 wetland media to enhance As and metal removal, where mainly aerobic processes occur. In
86 particular, previous work identified alternative media that enhanced As removal in VSSFs
87 under acidic conditions (Lizama Allende et al. 2011, 2012). To our knowledge, no study has
88 employed alternative media in horizontal subsurface flow wetlands (HSSFs) for this purpose.

89 Horizontal subsurface flow wetlands (HSSFs) have been most commonly used among SSF
90 systems (Lee et al. 2009), mainly treating urban and domestic wastewater from small rural
91 communities (García et al. 2004). Therefore, little is known about their efficiency to remove
92 metals and metalloids at high levels, like those found in acid mine drainage (Kröpfelová et al.

93 2009). Indeed, few studies have investigated As removal by HSSFs (e.g. Rahman et al. 2011).
94 Since HSSFs have limited oxygen transfer (Kadlec and Wallace 2009), it may be
95 hypothesised that As in these systems may be removed by sulfur or iron precipitation under
96 reducing conditions, if these elements are present. This mechanism was suggested by Mattes
97 et al. (2010), as they reported a positive correlation between As and sulfate levels, and a
98 negative correlation between As and organic carbon levels. However, this hypothesis was
99 not tested, with the As removal mechanisms not clearly identified (Mattes et al. 2010).

100 Given the multiple factors that affect As removal in SSF wetlands and the small number of
101 studies performed under specific environmental and operation conditions (e.g. As and other
102 pollutant levels, pH, hydraulic loading; type of flow, vegetation, substrate), available studies
103 may not be comparable and they may not be applicable under other conditions. Therefore,
104 specific experiments must be designed and performed for a particular application.

105 This study aims to test the effectiveness of alternative media (zeolite, limestone and
106 cocopeat) in laboratory-scale HSSF wetlands. These media were recently tested in vertical
107 flow subsurface wetlands and were found to enhance As, Fe and B removal (Lizama Allende
108 et al., 2012). In this study we employ the same vegetation type -*Phragmites australis*- and
109 water representing highly contaminated river water, similar to that found for example in the
110 Azufre River in Northern Chile (more details available in Lizama Allende et al. (2012)). We
111 present analyses of removal efficiencies and behaviour of water quality parameters, along
112 with chemical analysis of the plants and media, and use these to identify the key removal
113 mechanisms of the target pollutants and the environmental factors that affect these
114 mechanisms. The findings can be used to enhance the performance of subsurface flow
115 constructed wetlands in the removal of As, Fe and B from acidic water.

116

117 **2. Materials and methods**

118 **2.1 The wetland cells**

119 Six horizontal flow wetlands cells were built using PVC sheets (12 mm thick), with one side
120 of each wetland built with clear PVC to enable observation of water, substrate and plant roots
121 (this side was kept covered by black LDPE plastic sheeting when not being observed, to
122 prevent light-induced artefacts). Each wetland had an inlet and an outlet (65 and 100 mm

123 long, respectively), which contained gravel 20-40 mm size. Figure 1 illustrates the main
124 characteristics of the wetland columns, which were located in a greenhouse shelter (covered
125 roof but open walls) at Monash University, Clayton Campus.

126 [FIGURE1]

127 Two types of wetland columns were built, employing (i) zeolite and (ii) limestone/cocopeat
128 as the main supporting media. Each group had three replicates, operated as individual
129 treatment units. Young *Phragmites australis* were obtained from a local nursery (they were
130 harvested from a Melbourne wetland) and planted in December 2011, five months before the
131 experiments started. In each wetland, approximately 10 stems were planted, and they then
132 were submerged in tap water to allow them to establish in their new environment. *Phragmites*
133 has been selected for this and our previous studies given its tolerance to acidic conditions
134 (Cooper et al. 1996).

135

136 2.2 Synthetic water

137 Synthetic wastewater resembling the pollutant levels in the Azufre River, Northern Chile,
138 was prepared. This case-study was chosen, given the high levels of arsenic under acidic
139 environment in this river (more details available in Lizama Allende et al. (2012)).
140 Representative synthetic wastewater was prepared using tap water with the following
141 reagents added per litre of water: 3 mL 1,000 mg/L arsenic standard solution (arsenic acid
142 As_2O_5 in H_2O), 30 mL 1,000 mg/L boron standard solution (boric acid H_3BO_3 powder in
143 H_2O), 0.5 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.425 mL H_2SO_4 (95–97% Merck ISO grade). As a result, the
144 concentrations of the target pollutants in the synthetic feed were (average±standard
145 deviation): 2.6 ± 0.5 mg/L As, 30.8 ± 6.2 mg/L B, and 97.3 ± 14.0 mg/L Fe. The resulting pH
146 value was 2.0 ± 0.2 . As such, As, Fe and B were mainly in the dissolved fraction.

147

148 2.3 Operation of the wetland systems

149 The synthetic wastewater was stored in a 500 L polyethylene feed tank with a continuous
150 stirrer and pumped to each wetland column at an average rate of 150 mL/h resulting in a
151 hydraulic loading rate of 30 mm/d and a hydraulic retention time of approximately 11 days.

152 The design loading rate was chosen to reflect loading rates commonly used in HSSF wetlands
153 removing As (Kröpfelová et al. 2009, Rahman et al. 2011). The wetlands were pseudo-
154 continuously dosed for 24 hours, by having successive loadings (over a period of 18 minutes)
155 occurring once every 72 minutes. This was necessary given the very small flow rates, to
156 ensure that the volumes applied could be accurately maintained to their specification. In all
157 wetland columns, a water level of 0.6 m was maintained by having an overflow outlet hose
158 (Figure 1). The mean outflow rate was 144.9 mL/h from lime-peat (limestone/cocopeat)
159 wetlands, and 129.5 mL/h from zeolite wetlands.

160 The dosing started on May 7 and the sampling and analysis commenced on 28 May 2012 and
161 lasted for 22 weeks.

162

163 **2.4 Sampling and analysis**

164 *Water quality:* Water samples were collected from the inlet (dosing hose) and outlet (outlet
165 hose) of each wetland replicate, three times per week for the first two weeks, once a week for
166 the following six weeks, and then fortnightly for the remaining fourteen weeks. Separate
167 samples were taken for total and dissolved metal analysis, with analysis undertaken according
168 to Standard Methods (APHA, 2005). For the measurement of dissolved pollutants, the
169 samples were filtered (through 0.45 µm cellulose acetate papers) and acidified to pH<2 with
170 nitric acid immediately after sampling. The analyses of As, B and Fe concentrations were
171 carried out for both unfiltered and filtered samples, to obtain total and dissolved values. The
172 analyses of other parameters were carried out for unfiltered samples after sampling: dissolved
173 oxygen, pH and temperature were measured using HACH 51970 and HACH 51910 probes
174 that were connected to a Sension 378 meter, and they were calibrated before every
175 measurement. An ORP Testr10 probe was used to measure redox potential (Eh). Sulfate
176 concentrations were obtained using HACH DR5000 UV/VIS spectrophotometer, based on
177 Standards Methods (APHA, 2005).

178 The concentrations of As, B and Fe were determined in a NATA (National Association of
179 Testing Authorities, Australia, <http://www.nata.asn.au/>) accredited laboratory (with QC
180 procedures based on Standard Methods (APHA, 2005)) by ICP-OES and ICP-MS (if the
181 parameter analysed was below the OES detection limit). ICP-OES detection limits were 0.1
182 mg/L for As, 0.05 mg/L for B and 0.05 mg/L for Fe, whereas ICP-MS detection limits were

183 0.001 mg/L for As, 0.02 mg/L for B and 0.02 mg/L for Fe. Since in situ parameters in outlet
184 samples may not be representative of the environmental conditions inside the wetland cells,
185 in situ parameters were measured continuously at the bottom of one replicate from each of
186 the limestone/cocopeat (abbreviated to “LP”) and zeolite (“Z”) groups: Cell 2LP (lime-peat
187 cell) from limestone-cocopeat wetlands, and Cell 3Z (zeolite cell) from zeolite wetlands.
188 Dissolved oxygen (DO), pH, temperature and redox potential (Eh) were measured using four
189 probes that were connected to a Campbell CR1000 data logger. The probes used were
190 Sensorex CS511-L, CS525 ISFET, 109SS (supplied by Campbell Scientific, Australia), and
191 submersible redox sensor (supplied by TPS Pty Ltd, Australia) respectively. These probes
192 took measurements every 5 minutes for the entire experimental period. The probes were
193 calibrated before the beginning of the experiments and then at the middle of the experiments
194 according to the suppliers’ recommendations. These measurements indicate the
195 environmental conditions in the wetland cells and therefore can be used to infer how the
196 pollutants are retained in the wetland bed.

197 *Analysis of media:* Media samples were collected and analysed to find out the pollutants
198 concentration in the wetland media. At the end of the experiments, all wetland cells were
199 drained at the same rate with which they had been dosed (150 mL/h) to avoid disturbance to
200 the media. While they were being drained, media samples were collected from nine locations:
201 inlet, middle and outlet at three different horizontal levels: top, middle and bottom of each
202 wetland. Media samples were dried at 40°C until constant weight was achieved and the metal
203 content in samples from Cell 2LP, Cell 4LP, Cell 9LP (all three lime-peat wetland replicates),
204 Cell 3Z and Cell 7Z (two out of the three zeolite wetland replicates) was analysed in the same
205 NATA accredited laboratory described above.

206 *Analysis of plants:* Plants samples were obtained and analysed to discover the contribution of
207 plant uptake to the removal of the target pollutants. Plants were collected and divided into
208 two groups, depending on their physical location in each wetland: inlet or outlet. They were
209 separated into shoots and roots. Samples were dried at 55°C till constant weight was achieved
210 (Marchand 2012). Metals in these samples were determined in a NATA accredited laboratory
211 by USEPA methods 3051A and 3060A.

212

213

214 **2.5 Statistical analysis**

215 **2.5.1 Performance of the wetland system**

216 To analyse the performance of the two wetland systems, statistical tests were performed using
217 PASW Statistics 19 and a significance level of $\alpha=0.05$. Prior to this testing, Kolmogorov-
218 Smirnov test was performed to check data normality.

219 The influence of the wetland media type was statistically assessed by one way ANOVA.
220 Rather than a simple comparison between the two media types, we compare the inlet
221 concentration and the outlet concentrations of each media type, so as to detect firstly a
222 significant difference caused by any media type (i.e. we want to know if the concentrations in
223 the outlet are lower than in the inlet). The same analysis is undertaken for the environmental
224 parameters (pH, ORP, DO, sulfate). When a significant difference attributed to the media
225 types was found ($pA<0.05$), multiple comparison post-hoc tests were performed to
226 distinguish which wetland media were significantly different: Tukey's test was applied when
227 the assumption of homogeneity of variances was satisfied (as determined by Levene's test),
228 or Games-Howell's test when this assumption was not satisfied (notation pT and pGW,
229 respectively).

230 If the assumption of normality was not achieved, Kruskal-Wallis analysis was performed
231 instead of ANOVA. In these cases, when significant difference was found ($pKW<0.05$),
232 Mann-Whitney post-hoc test were performed to distinguish the mean differences which were
233 significantly different ($pMW<0.05$).

234

235 **2.5.2 Correlation between pollutant levels**

236 Spearman rank ρ correlation coefficient was obtained to determine possible relationships
237 between the removal of the target pollutants and the environmental factors, mainly As and Fe
238 concentrations in the outflow, and pH and Fe concentration in the outflow. Spearman ρ was
239 used instead of Pearson's correlation due to the non-normal distribution of most of the data.

240

241

242 **3. Results**

243 **3.1 Overall performance**

244 As presented in Table 1, both media types were highly effective in removing As and Fe, but
245 not very effective in removing B. The target pollutants remained mainly dissolved in the
246 effluent of both media types.

247 Regarding the environmental parameters, pH and Eh were the key parameters most affected
248 by the media type. Although pH was raised by both media types, it reached an almost neutral
249 value (6.95 median value) in the limestone/cocopeat wetlands, whereas it was still acidic in
250 zeolite wetlands (4.1 median value) (Table 1). Redox potential decreased to negative values
251 in the limestone/cocopeat wetlands, whereas it was still rather positive in zeolite wetlands.

252 [TABLE1]

253 **3.2 Removal trends**

254 **3.2.1 Arsenic**

255 Arsenic removal efficiencies were always greater than 99.8% for both wetland systems
256 (Table 1). As presented in Figure 2, the zeolite media produced -on average- lower As
257 concentrations than limestone/cocopeat wetlands throughout the experiment. Interestingly, As
258 levels in the outflow from both treatments were not significantly different ($p_{MW}=0.297$ for
259 total As, $p_{MW}=0.458$ for dissolved As), despite the significant difference between each
260 treatment and the inflow in the total and dissolved As concentrations ($p_{KW}<0.001$ in all
261 cases). In addition, there was no significant temporal trend in the As removal in either of the
262 media types.

263

264 **3.2.2. Iron**

265 Zeolite wetlands were more effective than limestone/cocopeat wetlands in removing Fe,
266 confirming the capability of the system to change significantly the concentration of Fe
267 ($p_{KW}<0.001$ for both total and dissolved Fe).

268 Conversely to the case of As, Fe levels in the outflow differed significantly between media
269 types ($p_{MW}<0.001$ for both total and dissolved Fe). In fact, Figure 2 shows that Fe levels

270 from zeolite wetlands were considerably lower than those from limestone/cocopeat wetlands
271 at the beginning of the experiments, but became more similar towards the end of the
272 experiments. On the other hand, Fe levels from limestone/cocopeat wetland tended to
273 decrease through time, after being quite elevated at the start of the experiment.

274

275 **3.2.3 Boron**

276 There were only moderate reductions in boron through the media: removal efficiencies were
277 17% in limestone/cocopeat wetlands, and 12% in zeolite wetlands. While B concentrations
278 were significantly lower than the influent in both media types ($pA < 0.001$), the differences
279 between media types was not significant ($pGW = 0.155$). Figure 2 shows that no discernible
280 temporal trends were observed throughout the experimental period.

281 [FIGURE2]

282 **3.3 Environmental parameters in outflow samples**

283 As shown in Table 1, pH was, not surprisingly, significantly affected by the media type. The
284 wetland system changed the pH significantly ($pA < 0.001$), with the limestone/cocopeat
285 wetlands resulting in a higher pH than that from zeolite wetlands ($pGW < 0.001$), but the latter
286 also being higher than the inflow ($pGW < 0.001$).

287 Similarly to pH, redox potential was significantly affected ($pKW < 0.001$) by media type, with
288 Eh levels in limestone/cocopeat wetlands significantly lower than those in the inflow
289 ($pMW < 0.001$) and those in zeolite wetlands ($pMW < 0.001$). Although levels in zeolite
290 wetlands did not decrease as markedly as those in limestone/cocopeat wetlands (Table 1),
291 they were still significantly different to those in the inflow ($pMW < 0.001$).

292 Although DO was also affected by the wetland system ($pA = 0.002$), DO levels in the outflow
293 from zeolite wetlands were not significantly different to those in the inflow ($pT = 1$). On the
294 other hand, DO levels in the outflow from limestone/cocopeat wetlands were significantly
295 lower than those from zeolite wetlands ($pT = 0.003$) and to those in the inflow ($pT = 0.031$).

296 Sulfate was not significantly affected by any of the wetland systems ($pA = 0.517$) and neither
297 was temperature ($pKW = 0.4$).

298 **3.4 Environmental parameters monitored in wetland cells**

299 **3.4.1 Temperature**

300 Both limestone/cocopeat (cell 2LP) and zeolite (cell 3Z) wetland cells presented a very
301 similar trend: temperature dropped as winter came just after the experiments started, and then
302 temperature rose when spring and summer came (Figure 3). However, a significant difference
303 between wetland cells was found (pMW<0.001).

304 **3.4.2 pH**

305 Neutral and stable pH values were measured in cell 2LP (Figure 3), whereas in cell 3Z pH
306 values were lower than 5 and slightly fluctuating. As expected, a significant difference
307 between cells 2LP and 3Z pH values was found (pMW<0.001).

308 **3.4.3 ORP**

309 Redox potential in Cell 2LP reached negative values quickly once the experiments started,
310 and they remained close to -400 mV (Figure 3). On the other hand, ORP values in Cell 2LP
311 decreased from positive to zero values for around half the total duration of the experiments,
312 and then they started to oscillate between 0 and 400 mV. The type of media significantly
313 affected redox potential (pMW<0.001).

314 **3.4.4 Dissolved oxygen**

315 As shown in Figure 3, DO values were low (<4 mg/L) in both wetland replicates. The lime-
316 peat cell presented lower DO values than the zeolite cell during most of the duration of the
317 experiments, except at the beginning. The type of media played a significant role in dissolved
318 oxygen levels (pMW<0.001), being higher in the zeolite media than in the
319 limestone/cocopeat wetlands.

320 [FIGURE3]

321 **3.5 Relationships between pollutant removal and environmental parameters**

322 Table 2 presents Spearman's ρ coefficient for significant correlations between the different
323 water quality parameters monitored in outflow samples, in each wetland group. A strong
324 correlation was observed between Eh and Fe levels in zeolite wetlands. A positive and
325 significant correlation between outflow concentrations of As and Fe in limestone/cocopeat

326 wetlands was observed. Conversely, a negative and significant correlation was observed
327 between outflow concentrations of As and Fe in zeolite wetlands. Iron concentration
328 decreased when pH increased in both wetland types.

329 [TABLE2]

330 **3.6 Plant uptake**

331 Plants located in the inlet accumulated higher levels of As than those in the outlet, regardless
332 of media type. However, inlet plants in the lime-peat cell accumulated higher levels of As in
333 roots than in shoots, whereas inlet plants in the zeolite cell accumulated more As in shoots
334 than in roots. Plants located in the outlet accumulated similar levels of As in both wetland
335 cells, in both roots and shoots. Total mass of As taken up by plants corresponded to 0.11%
336 and 0.09% of the total As loaded into the limestone/cocopeat and zeolite cell, respectively.

337 Iron uptake by plants was also higher in plants located in the inlet of the two wetland
338 columns. However, in both wetlands types and also in plants located in the outlet, roots
339 accumulated much higher levels of Fe than did shoots, with the root/shoot concentrations
340 ratio being higher than 100 for plants located in the outlet. Of the total loaded Fe, 2.05% and
341 0.66% was taken up by the *Phragmites* in the limestone/cocopeat and zeolite cell,
342 respectively.

343 The uptake of B was similar in plants that were in the inlet and in the outlet (Figure 4).
344 Furthermore, B was mainly present in shoots rather than roots, with B concentrations in
345 shoots four to five times those in roots, in both wetland media types. However, plant uptake
346 of B in the limestone/cocopeat wetland was considerably higher than uptake in the zeolite
347 wetlands. The proportion of B taken up by plants was 0.1% and 0.02% of the loaded B in
348 limestone/cocopeat and zeolite cell respectively.

349 [FIGURE4]

350 **3.7 Metal retention in wetland media**

351 Zeolite wetlands presented higher concentrations of As in its media at the end of the
352 experiment than did limestone/cocopeat wetlands, in which As was largely only able to be
353 detected in one of the replicates. Furthermore, in zeolite wetlands, the As concentration in the
354 media tended to decrease towards the outlet, and towards the bottom (Table 3). A similar

355 trend was observed for Fe concentrations: they were also higher than those in
356 limestone/cocopeat wetlands, tending to decrease towards the outlet and the bottom.
357 However, in limestone/cocopeat wetlands, contrary to the case of As, Fe was detected in all
358 wetland cells (from 870 to 5,900 mg/kg).

359 The cocopeat component of the limestone/cocopeat wetlands presented the highest levels of
360 boron: between 200 and 350 mg/kg. Conversely, in the limestone section, B was not detected.
361 In addition, B concentrations >10 mg/kg were only detected in the upper section of one
362 zeolite wetland (cell 7Z).

363 [TABLE3]

364 **4. Discussion**

365 **4.1 The role of wetland media**

366 Results from this study demonstrate the effectiveness of limestone, cocopeat and zeolite as
367 supporting media in HSSF wetlands for removal of As, Fe and B from acidic water. Despite
368 the fact that limestone/cocopeat wetlands appeared to be the most promising treatment system
369 for the removal of As, Fe and B; they had a similar performance to that of zeolite wetlands.
370 The higher pH, reducing and anaerobic conditions to trigger As-Fe coprecipitation and/or Fe-
371 As-S precipitation, were not sufficient to deliver a significantly better performance than
372 zeolite wetlands in the removal of As, B and Fe. This suggests that sorption capacity of
373 zeolite is more effective than precipitation by limestone and sorption capacity of cocopeat
374 under the conditions of this study.

375 *Limestone/cocopeat wetlands:* The design of these hybrid wetlands was based on the
376 capability of limestone in raising pH, therefore causing Fe precipitation and As
377 coprecipitation, as found in our previous studies. The positive correlation in the removal of
378 As and Fe (Table 3) suggests the presence of Fe precipitation and As coprecipitation. In
379 addition, given the potential of cocopeat in removing B -due to the affinity of B to organic
380 matter-, it was thus hypothesised that an initial pH adjustment (provided by the limestone
381 section of the wetland) would improve the removal of B. As such, B removal was higher than
382 in zeolite wetlands. However, a higher pH than that obtained in this study would be required
383 to have higher removal. In addition, the adsorption capacity of cocopeat for B may have
384 decreased due to the presence of As and Fe, as organic matter is also able to sorb As and Fe

385 (Redman et al. 2002) Therefore, it is likely that a number of sorption sites have been taken up
386 by As and Fe instead of B.

387 *Zeolite wetlands:* The good performance of zeolite wetlands was expected, given the capacity
388 of zeolite to remove different pollutants, including As (Chutia et al. 2009), Fe (Wingenfelder
389 et al. 2005) and B (Yüksel and Yürü 2010). The negative correlation in removal rates of As
390 and Fe suggests that As and Fe are being removed via different mechanisms: Fe cations can
391 be exchanged by Ca, Na or K cations, whereas As anions can be exchanged by aluminol or
392 silanol hydroxyl groups (Chutia et al. 2009). Furthermore, as presented in Figure 2 and 3, As
393 levels remained practically constant through time, whereas Fe levels increased, indicating a
394 likely saturation of Fe-exchange sites, which did not occur in As-exchange sites. Lizama
395 Allende et al. (2012) reported a positive correlation in removal of As and Fe and therefore it
396 was inferred that they were removed by a common mechanism. Specific sorption
397 mechanisms cannot be discarded as As can be attracted by Fe already exchanged on zeolite
398 sites (Payne and Abdel-Fattah 2005), therefore in this case the removal of Fe causes the
399 removal of As.

400 The wetland media also affected the outflow rates. Outflow rates were lower in the zeolite
401 wetlands than in the limestone/cocopeat wetlands, despite the fact that all wetlands received
402 the same inlet rate. As such, the detention time was higher in zeolite wetlands. This fact could
403 also explain the higher pollutant removal rates in zeolite wetlands, since detention time is a
404 key factor affecting metal removal efficiency in wetlands (Cohen and Staub 1992).

405

406 **4.2 The role of vegetation**

407 Our results show the important role that roots play in accumulating As, with greater retention
408 than that in the shoots. Few studies have employed *Phragmites* in constructed wetlands
409 removing As (e.g. Vymazal et al. 2009); and plant uptake values in these studies were
410 considerably lower than those reported in our study, as were the As levels in the inflow. The
411 As content in the plants is still in the range reported in the literature for other types of plants
412 (e.g. Singhakant et al. 2009b, Ye et al. 2003), but for different levels of As in the inflow. In
413 Ye et al.'s (2003) study, As in the inflow was 0.46 mg/L, and As concentration was 16.5
414 mg/kg in shoots and 110.1 mg/kg in roots; whereas in Singhakant et al.'s (2009b) study, As
415 in the inflow was 4.7 mg/L, and As concentration were in the range of 0.5-4 mg/kg in shoots

416 and 5-40 mg/kg in roots depending on the type of plant. This suggests that apart from influent
417 concentrations, other factors affect As plant uptake, for example the type of vegetation and
418 the type of substrate (Figure 4).

419 The fact that plants located in the inlet absorbed more As is consistent with what was found
420 in the zeolite and limestone/cocopeat wetlands media, since As is being removed mainly in
421 the inlet section of the wetlands. As such, less As gets through to the outlet (and lower)
422 section of the wetland and therefore less is available for plant uptake in these locations. The
423 higher uptake by plants in the zeolite cell may be explained by the higher bioavailability of
424 As, which can be still dissolved given the slight acidity in the cell (Figure 3). Iron was also
425 mainly removed in the inlet of both wetland types (Figure 4), but in this case the plant uptake
426 was higher in the limestone/cocopeat wetland cell: neutral pH possibly enhanced iron plaque
427 formation in the roots (Batty and Younger 2002). Roots in this cell developed an intense
428 orange colour, which is characteristic of iron precipitates. Since these iron precipitates were
429 attached to the roots, all Fe detected here was considered as Fe uptaken by roots. Plant roots
430 in the zeolite cell did not develop this orange colour.

431 Other studies have also reported the minor contribution of plant uptake to the overall As
432 (Singhakant et al. 2009a, Ye et al. 2003), Fe (Ye et al. 2001) and B (Ye et al. 2003) removal.
433 However, there is a need for more research regarding the indirect effect of vegetation on As
434 and metal removal (García et al. 2010), as it has been reported that vegetated systems are
435 more effective in the removal of As (Buddhawong et al. 2005), but this may not occur in the
436 removal of metals such as Cu, Cd, Pb and Zn (García et al. 2010).

437 Iron was also accumulated at a greater rate in roots than stems or leaves, consistent with
438 previous studies (Kadlec and Wallace 2009). However, Fe levels detected in plants were
439 higher than those reported in the literature: mainly between 200 and 2,000 mg/kg (ibid), but
440 they may be comparable to those reported by Ye et al. (2001), who reported 41,381 mg/kg Fe
441 in roots of cattail. Boron displayed different behaviour, being primarily accumulated in the
442 shoots. Ye et al. (2003) reported a similar finding in different plants, for similar influent B
443 concentrations. However, in another study, B was accumulated in roots more than in shoots
444 (Ye et al. 2001). Higher levels of B in shoots than in roots can be explained as B moves
445 through the plants across a concentration gradient and once in the plant, it accumulates at the
446 point where the water is lost through stomata in the leaf (Qian et al. 1999).

447 The fact that B accumulation was similar in plants located in the inlet and the outlet suggests
448 that there was not such a strong removal gradient across the system as occurred with As and
449 Fe. Higher accumulation of B in plants in the limestone/cocopeat wetland contradicts the
450 findings of several authors that high soil pH decreases boron uptake by plants (e.g. Hu and
451 Brown 1997). As such, further research is required to understand the boron uptake
452 mechanisms.

453

454 **4.3 Pollutant removal mechanisms**

455 Arsenic may be removed via different mechanisms in a constructed wetland (Lizama A. et al.
456 2011). High levels of As and Fe in the wetland media, low contribution of plant uptake and
457 very small decrease of sulfate levels in the outflow suggest that that key removal mechanisms
458 are chemical precipitation of iron and arsenic in the limestone/cocopeat wetlands, and
459 sorption in the zeolite wetlands, rather than biological precipitation of sulfides. This
460 biological process may still occur, particularly in limestone/cocopeat wetlands, given the
461 suitable conditions for sulfate reducing bacteria: neutral pH, presence of organic matter, low
462 dissolved oxygen levels and reducing conditions. However, the contribution of this process to
463 the overall removal cannot be calculated with the experimental data of this study. Since it has
464 been suggested that metal sulfide precipitation may be more desirable than metal oxides
465 precipitation due to the generation of alkalinity and the higher density of metal sulfides
466 (National Rivers Authority 1992), it may be worth enhancing this removal mechanism
467 (instead of Fe oxides precipitation) in constructed wetlands, by deliberately creating the
468 conditions for the sulfate reducing bacteria to thrive.

469 Arsenic can be also removed by sorption onto calcite (Armienta et al. 2012), the main
470 component of the limestone employed in this study. This mechanism cannot be ignored,
471 although the low levels of As detected throughout the limestone media suggest that As is
472 being removed somewhere else. Furthermore, two samples collected from the very inlet (next
473 to the gravel inlet zone, in the upper and the lower part) of Cell 4LP presented 1,600 and 250
474 mg/kg As and 45,000 and 8,000 mg/kg Fe respectively, all considerably higher than those
475 detected in the adjacent location of the wetland cell (Table 3). These results suggest that the
476 retention of both As and Fe is rather heterogeneous and occurs mainly in the inlet of the
477 limestone/cocopeat wetlands, as soon as the water comes into contact with the limestone

478 section. It is likely here that Fe precipitates are formed, capturing As as well. These particles
479 may also be trapping B, since the B concentration in the upper section of the inlet was
480 elevated.

481 Boron was primarily removed by sorption in the cocopeat substrate, not surprising given the
482 capacity of organic matter to sorb boron (de Abreu et al. 2012). On the other hand,
483 low/undetected B levels in the wetland media suggest that the sorption capacity of limestone
484 and zeolite media is low. In addition, Fe oxides are mainly trapping As and not B despite that
485 B removal by Fe oxides has been reported (Jensen et al. 2006). This suggests that borate
486 $B(OH)_4^-$ and arsenate anions AsO_4^{3-} , $HAsO_4^{2-}$ and $H_2AsO_4^-$ are competing for sorption sites
487 onto Fe oxides, and therefore borate does not affect arsenate sorption as phosphate or
488 carbonate do (Mukherjee et al. 2009). Plant uptake might also play a role, but lower B
489 concentrations would be required (Marin and Oron 2007).

490

491 **4.4 Recommendations for the removal of the target pollutant in constructed** 492 **wetlands**

493 Both media types performed effectively in removing As and Fe, with limestone/cocopeat
494 slightly less effective in removing Fe. The most appropriate system to be implemented will
495 depend on the specific requirements for the treated effluent, and also other considerations
496 such as the cost of media and maintenance requirements. If As and Fe are being trapped in
497 the inlet section of the wetlands, this could facilitate targeting maintenance or reconstruction
498 around a subset of the total wetland media, reducing cost and complexity.

499 Boron removal remains a challenge and although organic materials such as cocopeat are
500 promising, more research is needed to increase B removal in constructed wetlands. A second
501 stage of treatment could be implemented, under alkaline conditions and where B sorption by
502 organic matter and/or iron/aluminium oxides and/or clay minerals is encouraged. This second
503 stage may be effective, since As would have been removed already. A hydroponic system
504 using an accumulator species such as duckweed might also work, but this has only been
505 recommended for B concentrations below 2 mg/L (Marin and Oron 2007), therefore this
506 could be employed as a polishing system.

507 Results from this study therefore confirm the key role of media in the removal of As and
508 metals from acidic water, and the minor role of plant uptake despite the elevated levels in
509 plants tissues. Combinations of media appear as an effective way of optimising the removal
510 of different pollutants in individual wetland cells. More research is needed to confirm the
511 indirect role of vegetation in the performance of the system, and also the presence and effect
512 of microorganisms. Preliminary results have indicated the existence of different bacterial
513 communities in both zeolite and limestone/cocopeat wetlands (data not shown). Future
514 studies should focus on the role of these communities in the removal process, together with
515 the speciation of the pollutants in the solid phase using advanced techniques such as x-ray
516 diffraction (XRD) or synchrotron based techniques such as x-ray spectroscopy, or sequential
517 extraction procedures such as that proposed by Keon et al. (2001). This information is key to
518 fully understand the fate of the pollutants in the wetland matrix and therefore optimise the
519 design of constructed wetlands for the removal of As, Fe and B from contaminated water.

520

521 **5. Conclusions**

- 522 • The effectiveness of alternative wetland media was demonstrated in HSSF wetlands:
523 limestone/cocopeat and zeolite showed excellent removal efficiencies for As (99.8% and
524 99.9% respectively) and Fe (87.3% and 96.1%) for an hydraulic loading rate of 30 mm/d.
- 525 • Limestone/cocopeat wetlands could be a more suitable option depending on the
526 requirements for the final effluent, given their capacity to raise pH. However, Fe removal was
527 higher in the zeolite media.
- 528 • Key removal mechanism in limestone/cocopeat wetlands were Fe oxides
529 precipitation, which cause As coprecipitation, triggered by neutral-alkaline pH, and B
530 sorption by cocopeat. In zeolite wetlands, As and Fe were removed by ion-exchange.
- 531 • Plant uptake therefore played a minor role in the performance of the system, as the
532 accumulation of As, Fe and B were 0.11%, 2.05% and 0.1% of the total mass loading in
533 limestone/cocopeat wetlands, and 0.9%, 0.66% and 0.02% in zeolite wetlands, respectively.
- 534 • Metal accumulation in the wetland media indicated that As and Fe removal is not
535 homogeneous: in limestone/cocopeat wetlands As and Fe were mainly removed in the inlet,

536 whereas in zeolite wetland the removal decreased towards the bottom and the outlet sections
537 of the cells.

538

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546

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Table 1. Median inflow and outflow levels of the target pollutants, and median inflow and outflow levels of monitored environmental parameters. Values in brackets indicate the 5th and 95th percentiles. The data consist of a total of fifty seven measurements (three replicates multiplied by nineteen data sets).

Parameter [unit]	Inflow	Limestone/cocopeat		Zeolite	
		level	removal rate (%)	level	removal rate (%)
As [mg/L]	dissolved	2.7 [1.4,3]	0.002 [0.005,0.012]	0.001 [0.005, 0.004]	
	total	2.8 [1.4,3]	0.002 [0.005,0.015]	99.8	0.001 [0.005, 0.0042]
Fe [mg/L]	dissolved	89 [81,121]	7.8 [0.31, 25.2]	1.1 [0.005, 12.4]	
	total	91 [82,130]	8.8 [1.56, 28.2]	87.3	1.7 [0.005, 12.4]
B [mg/L]	dissolved	30 [19.8, 30.4]	25 [19.8, 30.4]	27 [19.8, 32]	
	total	31 [21,31.2]	26 [21,31.2]	15.9	28 [21.8, 32.2]
pH		2 [1.7,2.4]	6.95 [6.5, 7.4]	4.1 [2.9,6]	
T [°C]		16.1 [14.0, 22.2]	15.35 [13.0, 22.3]	15.35 [12.4,21.8]	
DO [mg/L]		12.61 [7.65, 16.2]	10.87 [7.20, 14.25]	12.25 [8.01,15.92]	
Eh [mV]		425 [395, 450]	-37 [-47.2, 59.6]	315 [99.8, 461]	
SO ₄ [mg/L]		684 [508.8, 845.3]	648.4 [474.7, 840.6]	684 [411.2, 814.8]	

Table 2. Correlation coefficients (Spearman's ρ) for the relationships between measured water quality parameters, in both limestone/cocopeat and zeolite wetlands.

	DO [mg/L]	T [°C]	pH	Eh [mV]	SO ₄ [mg/L]	As [mg/L]	Fe [mg/L]	B [mg/L]
Limestone/cocopeat								
DO [mg/L]	1							
T [°C]	-0.536**	1						
pH			1					
Eh [mV]			0.449***	1				
SO ₄ [mg/L]					1			
As [mg/L]			-0.319*			1		
Fe [mg/L]			-0.606***	-0.346**		0.410***	1	
B [mg/L]		0.408**						1
Zeolite								
DO [mg/L]	1							
T [°C]	-0.433***	1						
pH	0.513***	-0.468***	1					
Eh [mV]	-0.494***	0.344**	-0.838***	1				
SO ₄ [mg/L]		0.278*	-0.308*		1			
As [mg/L]				-0.286*		1		
Fe [mg/L]	-0.386*	0.362**	-0.764***	0.738***		-0.454***	1	
B [mg/L]		0.28*						1

Table 3. Pollutants concentration [mg/kg] in nine different locations of the wetland cells.

Limestone/cocopeat wetlands										
Wetland	Location	As			Fe			B		
		Inlet	Middle	Outlet	Inlet	Middle	Outlet	Inlet	Middle	Outlet
Cell 2LP	Top	5	<5	<5	990	1100	3200	<10	<10	350
	Middle	5	<5	<5	1100	1100	990	<10	<10	260
	Bottom	<5	<5	<5	1000	890	1800	<10	<10	320
Cell 4LP	Top	13	59	31	1100	2000	3600	<10	<10	200
	Middle	7	9	18	870	840	2700	<10	<10	230
	Bottom	10	5	<5	2200	1200	2100	<10	<10	250
Cell 9LP	Top	<5	<5	<5	910	1200	5900	<10	<10	330
	Middle	15	6	<5	1500	1100	2400	<10	<10	350
	Bottom	<5	<5	<5	870	930	2100	<10	<10	250
Zeolite wetlands										
Wetland	Location	Inlet	Middle	Outlet	Inlet	Middle	Outlet	Inlet	Middle	Outlet
Cell 3Z	Top	150	80	77	7500	6400	6500	<10	<10	<10
	Middle	31	17	12	5900	4800	4500	<10	<10	<10
	Bottom	11	9	8	5500	4700	4800	<10	<10	<10
Cell 7Z	Top	230	57	15	9300	11000	5600	21	10	<10
	Middle	58	21	13	6000	5600	5600	<10	<10	<10
	Bottom	17	11	11	6400	5000	4700	<10	<10	<10

Figure captions

Figure 1. Diagram of the experimental wetland mesocosms. In zeolite wetlands, media 1 and media 2 were zeolite, whereas in limestone/cocopeat wetlands, media 1 was limestone and media 2 was cocopeat.

Figure 2. Total concentration of As (a), Fe (b) and B (c) in the inflow and outflow from the six wetland cells. Y axis is presented in log scale on As and Fe graphs. The surge in B inflow may correspond to analytical inaccuracies, as inflow concentration should be similar to that of the previous sampling date.

Figure 3. Temperature (a), pH (b), ORP (c) and DO (d) profiles in one replicate from each group throughout the whole experimental period. The * symbol indicates where the probes were taken out for re-calibration.

Figure 4. Pollutants accumulation in plants in both wetland cells: As concentration in plants located at (a) inlet and (b) outlet; Fe concentration in plants located at (c) inlet and (d) outlet ; B concentration in plants located at (e) inlet and (f) outlet. Note the different scales on the As ((a) and (b)) and Fe ((c) and (d)) graphs.

Figure1.tif
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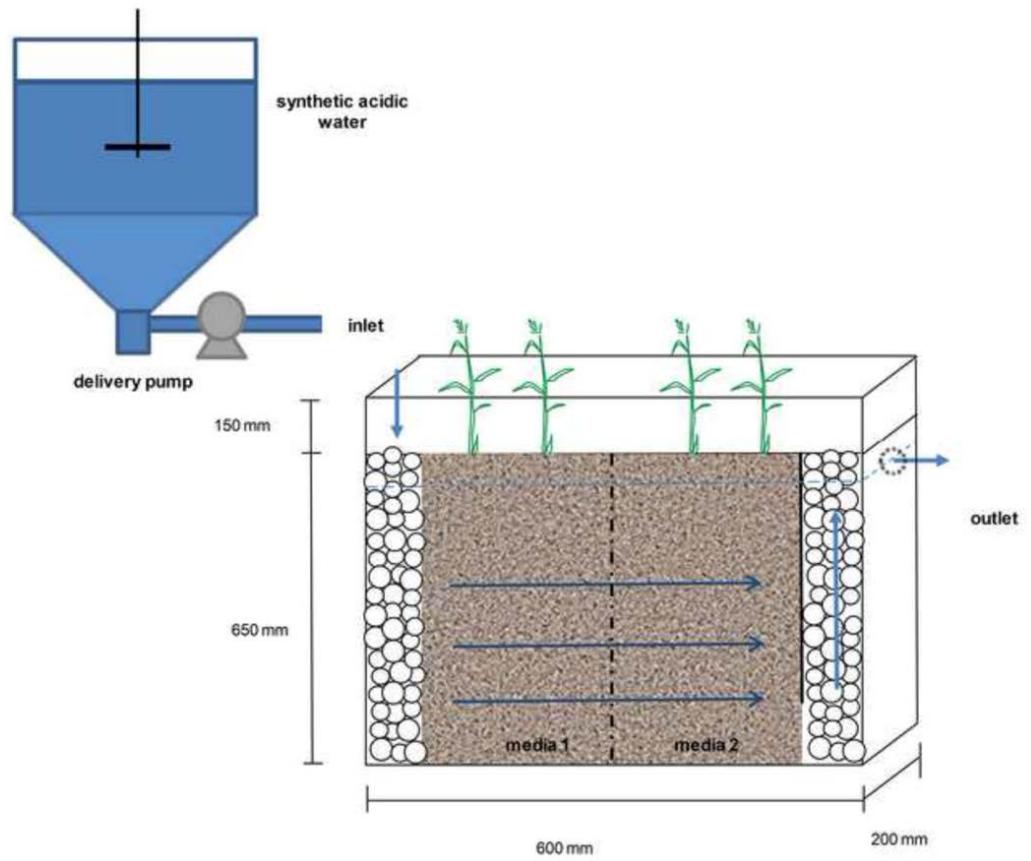


Figure2.pdf

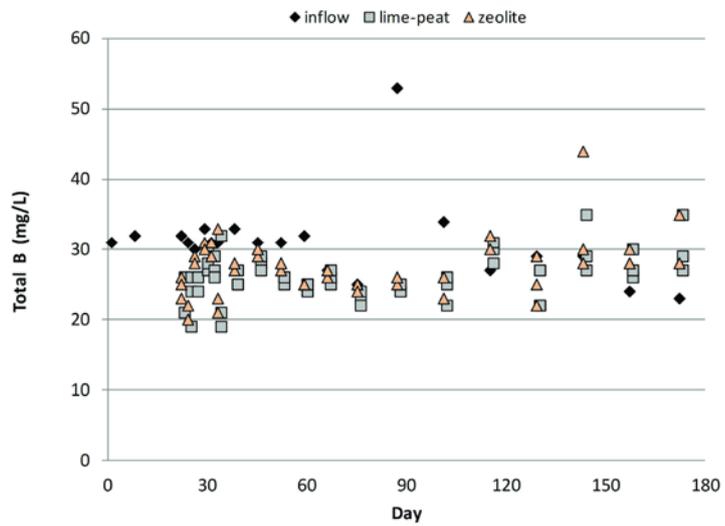
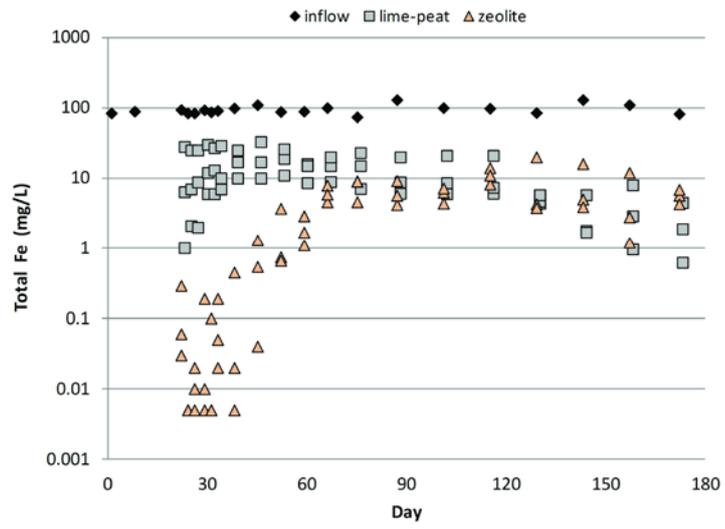
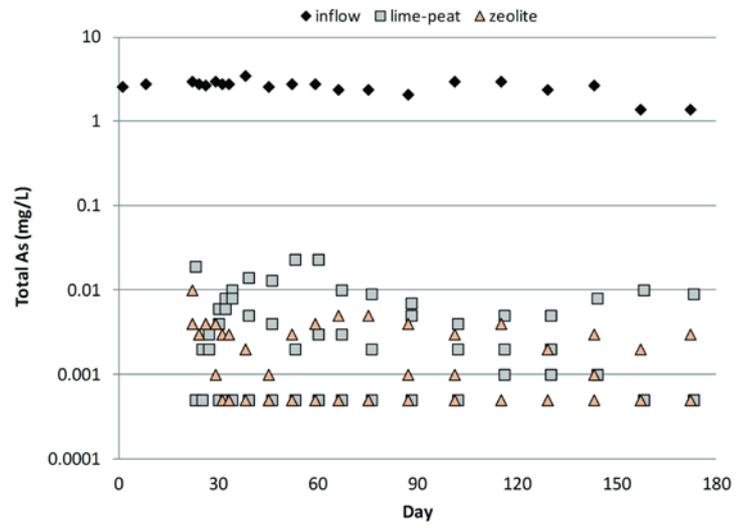


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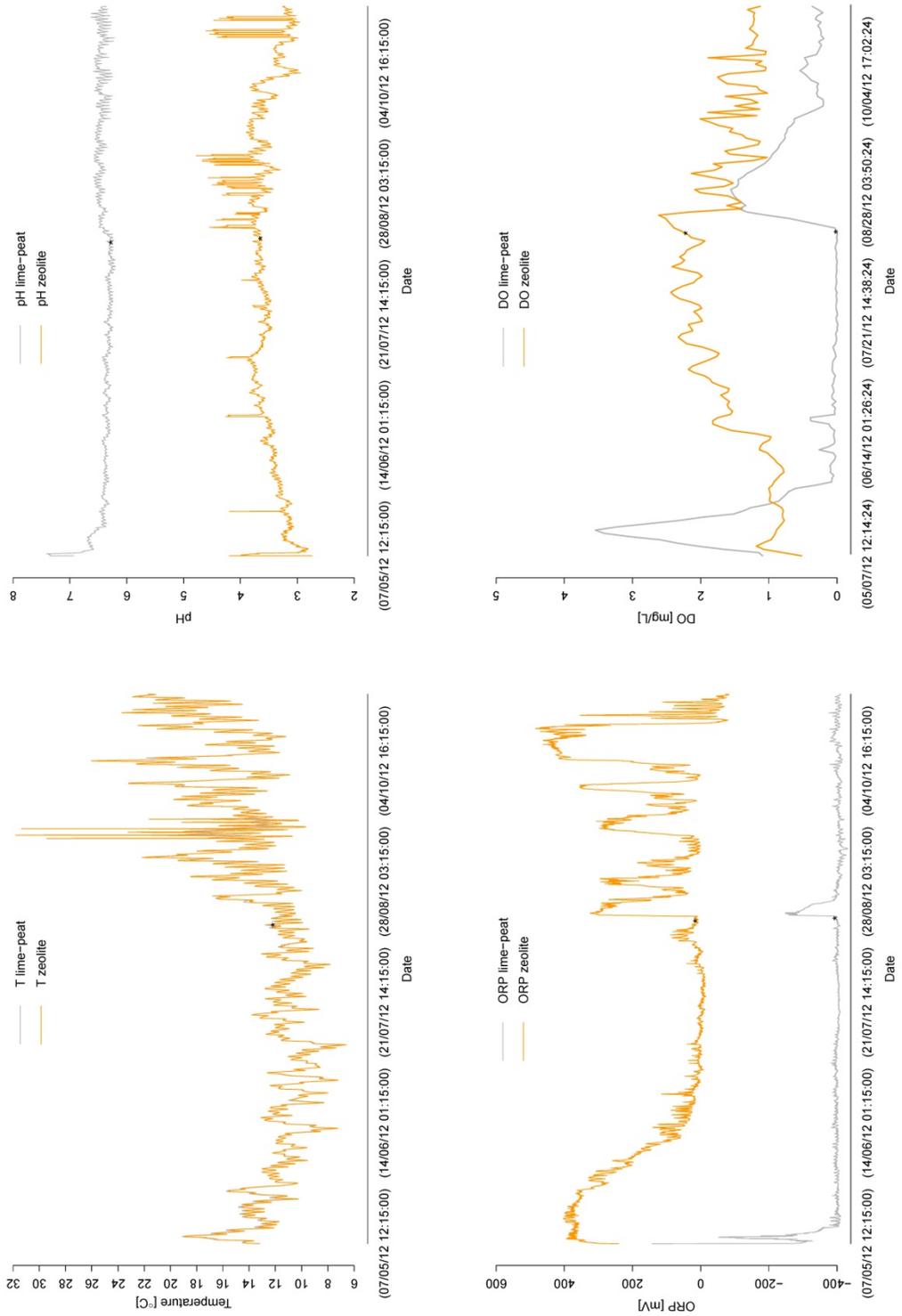
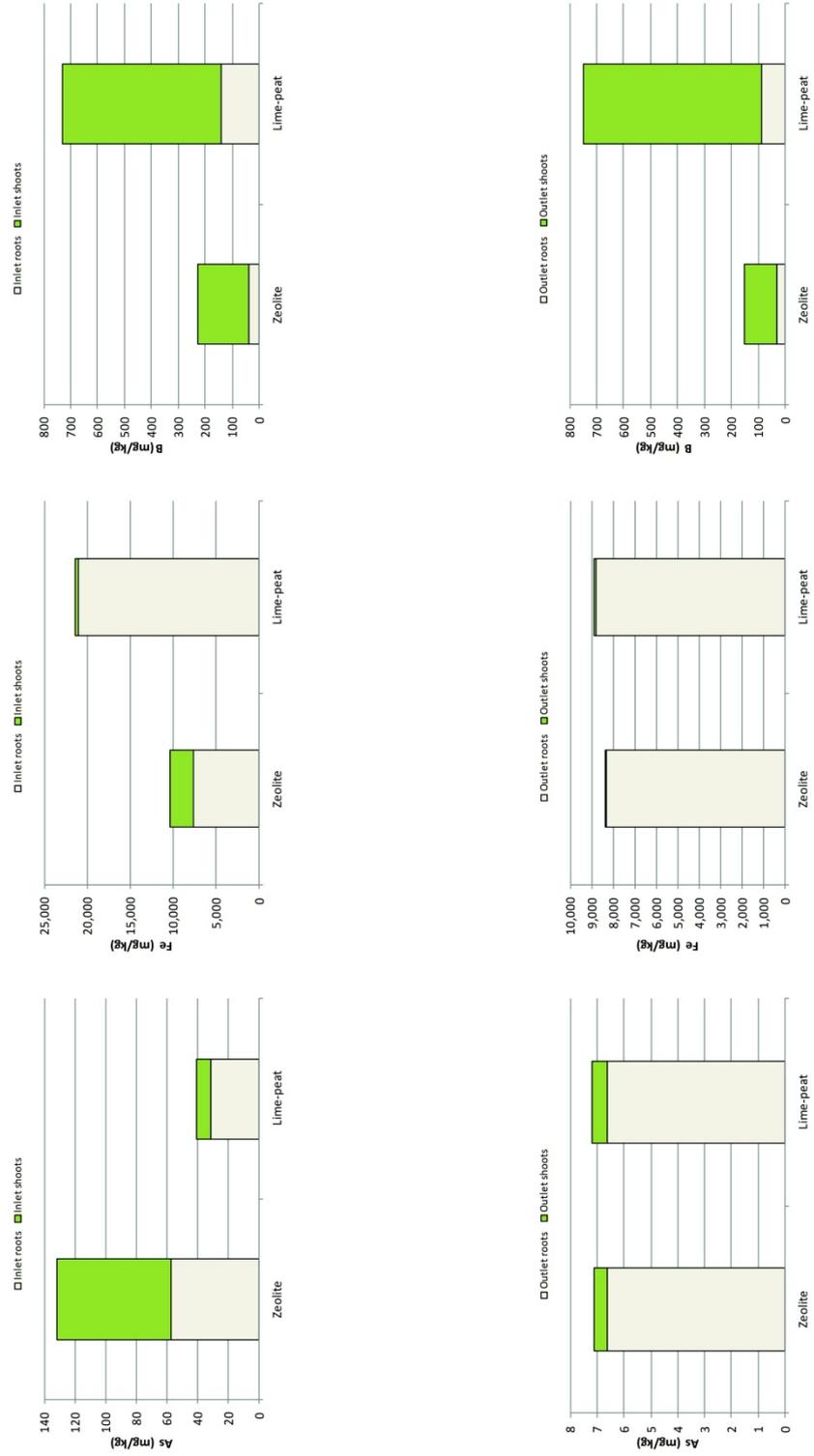


Figure4.pdf



5.3. Bacterial communities in wetland systems: preliminary findings

5.3.1 Introduction

Bacteria are essential in the removal of As and metals in constructed wetlands (Kosolapov et al., 2004, Sheoran and Sheoran, 2006). As such, research of the bacterial community composition and diversity, in this environment, is important to increase our understanding of their behaviour (Arroyo et al., 2013). Many studies assume bacterial activity based on water quality data, but they lack direct, in-depth information on the microbial consortia within specific environments (Faulwetter et al., 2009). As such, further studies of microbial density, diversity and activity have been recommended (Stein et al., 2007, Kosolapov et al., 2004).

Microbial diversity refers to the measure of the number of specific species and/or functional groups existing within an environment. To assess microbial diversity and relative abundance, molecular methods have been increasingly employed in recent times (Faulwetter et al., 2009). One such method is metagenomics which enables the study of genetic material (DNA) derived directly from environmental samples.

The objective of this study is to identify bacterial communities in both wetland types investigated using metagenomics.

5.3.2 Material and methods

Water samples were collected from the bottom of Cell 2LP (limestone/cocopeat wetland) and Cell 3Z (zeolite wetland) at the middle of the experiments (August 2012). They were filtered consecutively through 1.2, 0.45 and 0.22 μm filters immediately after collection. Filters were frozen at $-20\text{ }^{\circ}\text{C}$ prior to DNA extraction.

DNA extraction was performed using the procedure in PowerSoil® DNA Isolation Kit (Mobio Laboratories) as per the manufacturer's instructions (MO BIO, 2011), with the following modifications: the surface of each filters were carefully scrapped (using scalpel blades) to remove the sediment in 600 μL of resuspension buffer. Each filter

was treated independently. Incubation of sediment for 15 minutes at 65 °C in buffer C1 was included. Each sample was eluted in 50 µL of DNase free H₂O. The eluate from each sample was then pooled to make a single sample, representative of each wetland cell.

A single replicate sample from each cell was selected for metagenomic analysis. Metagenomics analysis was performed in the Micromon DNA Sequencing Facility (Monash University), using the Illumina Miseq Genetic Analyser. The following steps were followed for sequencing and data processing:

Sets of primers were selected from those produced by the method of Caporaso et al. (2012). These are designed to amplify regions of the 16S ribosomal RNA gene and simultaneously append both DNA adapters required to facilitate Illumina sequencing and also a DNA 'barcode' to allow resolution of individual reads to particular sequencing libraries. The primers amplify the region spanning bases 515 and 806 relative to the *E. coli* 16S rRNA gene.

Primers using index numbers 1 and 2 were used to differentiate samples on the sequencing run. Three other unrelated samples, using indexes 3-5 were also included in the same sequencing lane.

A quantitative real-time PCR (1 µL of DNA with Promega GoTaq Master Mix (A6001), according to the manufacturer's instructions) was used to validate the primers and to check for non-specific amplification.

Non specific amplification was detected in all samples, but amplification occurred late in the reaction (post-cycle 26).

Reactions were amplified using 22 cycles; this number was chosen to produce amplification before non-specific amplification was detectable by qPCR.

All five replicates were cleaned using Qiagen QIAQuick PCR Purification Kit according to the manufacturer's instructions.

The concentration of cleaned PCR products was measured using an Invitrogen Qubit dsDNA HS Assay Kit in conjunction with a Qubit 1 fluorometer.

Replicate samples were pooled and diluted to 2nM in Illumina HT1 buffer.

Samples denatured in 20 μ L volumes were denatured in 0.1N sodium hydroxide for 5 minutes at room temperature before further dilution to 6pM in Illumina HT1 buffer.

Denatured and diluted samples were mixed in an equimolar ratio to produce a final sample pool.

A Φ X174 control library was purchased from Illumina (FC-110-3001) and it was denatured and diluted using the same method as above. The control and sample library pool was mixed at 50% molar ratio to produce a final library pool for sequencing.

Six hundred μ L of the final library pool was loaded into a 300-cycle Illumina MiSeq Reagent Kit (MS-102-1001) and sequenced according to the manufacturer's instructions using paired-150b read format.

The read pairs were sub-assembled and error-corrected to single reads on a per-cluster basis using COPE (Connecting Overlapped Pair-End) (Liu et al., 2012).

After sequencing, sample data was demultiplexed and analysed using the QIIME package with the default settings (Caporaso et al., 2010).

5.3.3 Results and discussion

The taxa summary for samples from zeolite wetland (Cell 3Z, left) and limestone/cocopeat wetland (Cell 2LP, right) are shown in Figure 5.1. The analysis allowed the identification of family level data. Each coloured strip represents a different family, and the width of the strip represents the relative presence of the corresponding family within the total pool of identified microorganisms. Within this single replicate,

around 500 unique families were identified. Many of these were detected in both wetland cells, suggesting that the type of media may not be a major factor in bacterial diversity. However, further replicate data is required to confirm this.

As an example, section number 1 (Figure 5.1) indicates that 7.5% of the bacterial population in Cell 2LP and 5.7% in Cell 3Z belongs to the *Desulfobulbaceae* family, order *Desulfobacterales*. This is an order of strictly anaerobic sulfate-reducing bacteria which reduce sulfate to sulfide to obtain energy (Hao, 2003). There are two other families in this order (*Desulfobacteraceae* and *Desulfoarculaceae*), but the *Desulfobulbaceae* family was the only one identified in both wetland cells, thus the 7.5% and 5.7% mentioned above also correspond to the percentages of the order *Desulfobacterales* in each sample. It can be suggested that the environmental conditions described in Section 5.2 may be adequate for these bacteria to exist in the wetland cells.

Another relevant example is section number 2 (Figure 5.1), which indicates that 3.5% of the bacterial population in Cell 2LP and 8.8% in Cell 3Z belongs to the *Alicyclobacillaceae* family, of obligate aerobes. Among this family there are three genera: *Alicyclobacillus*, *Pasteuria* and *Sulfobacillus*. The genus *Sulfobacillus* includes acidophilic bacteria that obtain energy by oxidising ferrous iron, elemental sulfur and sulfide minerals (Bogdanova et al., 2006). Acidic conditions in Cell 3Z and its higher relative presence allow hypothesising that *Sulfobacillus sp.* may be present. Conversely, sulfate-reducing bacteria, which promote As and Fe removal by sulfide precipitation, may also promote As and Fe leaching by sulfide mineral solubilisation. However, further work is required to further define the genus and species identified within the identified *Alicyclobacillaceae* family.

The section 3 (Figure 5.1) in the limestone/cocopeat wetland, a minimal component in the zeolite wetland (0.34%), indicates that ~2.1% of the bacterial population of Cell 2LP contains bacteria belonging to the division OP11. This division was first encountered in the Yellowstone hot spring Obsidian Pool (Harris et al., 2004). OP11 bacteria have been detected in anaerobic environments and their metabolism remains

unclear, however they may play a role in the cycling of anoxic carbon, hydrogen and sulfur (Wrighton et al., 2012).

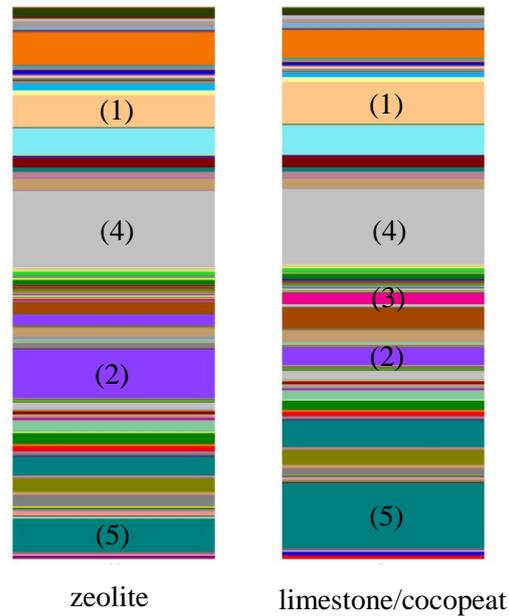


Figure 5.1. Taxonomy summary at family level for Cell 3Z (left) and Cell 2LP (right).

The largest component in both wetland cells (section 4, Figure 5.1) corresponds to the group *Proteobacteria*, which comprises many families of chemoheterotrophs and chemoautotrophs which derive nutrients from decomposition of organic material (US National Library of Medicine, 2011). In fact, *Proteobacteria* is the most numerous group (phylum) currently recognised in the domain Bacteria, being this group highly diverse in lifestyle, metabolism and ecological significance (Ettema and Andersson, 2009). Thus, it would be expected that they are commonly found in environmental samples.

Finally, section 5 corresponds to uncharacterised bacteria (6.08% in zeolite and 12% in limestone/cocopeat samples). This is not unusual as most of environmental samples have these bacteria as a major component, which shows the diversity in microbial populations (Henry, 2013).

These results suggest that the microbial community in horizontal flow wetlands having zeolite, limestone and cocopeat media is highly diverse. Sulfate-reducing bacteria that may be involved in the fate of the target pollutants within the wetland cells were identified. However, this is the first approach, using a single biological sample and metagenomic analysis, to provide direct evidence for the presence of bacteria that may function in the removal of As and metals. The inclusion of biological replicates, from multiple samples is required to further confirm and refine these data.

A recent study (Arroyo et al., 2013) has suggested that diversity and richness of bacterial communities are related to metal removal efficiency, and that plant type has a major effect on the composition, diversity and richness of the bacterial communities. More similar studies are required to fully understand the role of microbial communities in subsurface flow wetlands, and the interactions with the rhizosphere of different plants.

5.4. Conclusions

Horizontal flow wetlands with zeolite media and limestone/cocopeat media presented high efficiency in removing As, which was also similar. Zeolite wetlands were significantly more efficient than limestone/cocopeat in removing Fe, although both wetland types removed B at a similar rate. These results confirm H1 and H2 since (1) the alternative wetland media were highly effective given their particular capabilities and (2) zeolite wetlands presented even higher As, Fe and B removal rates than those in VSSF, whereas limestone/cocopeat wetlands presented higher As and B removal rates than those in limestone VSSF, although these results may not be comparable given the combined effect of limestone and cocopeat media in HSSF wetlands.

As hypothesised in H3 and H4, anaerobic/reducing conditions were detected in the wetland cells, especially in the limestone/cocopeat cell. Anaerobic microbial communities were found in both wetland types. However, zeolite wetlands still performed better than limestone/cocopeat wetlands, despite the highly reducing conditions, low dissolved oxygen levels and neutral pH measured in the

limestone/cocopeat cell. These results suggest that ion-exchange in zeolite wetlands is the most effective mechanism for both As and Fe under the conditions of this study.

Arsenic coprecipitation with iron occurred in limestone/cocopeat wetlands (as hypothesised in H5), but it was not as effective as ion-exchange in zeolite wetlands. The formation of sulfide minerals containing arsenic and/or iron cannot be discarded, although the slight decrease in sulfate levels suggest that this process was of minor importance.

The minor role of direct plant uptake in the removal of As, Fe and B was confirmed, as reported in the literature (e.g. Ye et al., 2003, Singhakant et al., 2009, Stefanakis and Tsihrintzis, 2012). Although *Phragmites australis* were able to accumulate considerable levels of the pollutants, the main sink for the target pollutants was the supporting media.

Despite the high removal rates and the duration of the experiments, the performance of the system was not particularly affected at the end of the experiments, suggesting that the alternative wetland media are able to provide efficient removal for at least six months. It is recommended that biological processes are encouraged since the sorption capacity of zeolite and cocopeat and the pH raise capability of limestone may be exhausted. Appropriate conditions must be provided for sulfate reducing bacteria to thrive: a source of carbon, sulfate, low oxygen levels, and pH between 5 and 8 (Cohen, 2006).

In summary, horizontal subsurface flow wetlands with alternative media: limestone, cocopeat and zeolite are a suitable treatment technology for the removal of As and Fe from highly acidic contaminated water. The selection of the media will depend mainly on the final use of the treated effluent: zeolite wetlands provide higher As and Fe removal rates, but under acidic pH; whereas limestone/cocopeat wetlands can provide neutral pH and a similar As removal, but a slightly lower Fe removal rate.

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Chapter 6: Conclusions and recommendations



6.1. Introduction

This chapter begins with an assessment of the strengths and weaknesses of this research. The key findings from each stage are reviewed, and design recommendations are summarised. Finally, opportunities for future work are discussed.

6.2. Strengths and weaknesses

This study has assessed the performance of subsurface flow constructed wetlands in removing arsenic and metals from highly contaminated water, testing both conventional gravel media and alternative wetland media. Two important contributions of this research project are:

1. Few studies have assessed the potential of subsurface flow wetlands in removing arsenic and metals and as such, this thesis makes an important new contribution to knowledge in the field
2. Alternative filter media for arsenic and other toxic metal removal in wetlands were tested, building substantially on earlier studies, which largely provided literature on recommended media, without explicit testing

Understanding the removal processes for arsenic and metals is crucial to enhance wetlands performance. Therefore, this research attempted to obtain knowledge of the removal routes occurring in subsurface flow wetlands. To do this, environmental parameters were measured, alongside pollutant concentrations, and then they were used to explain removal mechanisms and the main factors affecting these mechanisms. This was a powerful tool that allowed identifying the key processes that remove As and metals.

The laboratory testing of wetland systems was undertaken in an outdoor facility, so as to expose these systems to more realistic conditions such as sunlight and wind, while excluding rainfall so as to have controlled inputs, allowing a pollutant (and water) mass

balance to be achieved. However, available space and funding was limited and as such, only a limited number of wetland cells could be accommodated.

Long-term performance of subsurface flow wetlands, especially those removing As and metals, is critical. However, this cannot be fully addressed in the timeframe of a doctoral study. Attempts were made to understand longer-term behaviour in the last experimental stage, where the horizontal flow wetlands functioned for several months, but no evidence for an expected lifespan could be obtained.

This research was based on a case study, so as to ensure representativeness and reality of the testing. Synthetic water was used, since it was unfeasible to obtain real water from the Azufre River (Chile). Appropriate chemicals to achieve target As, Fe and B concentrations at low pH were employed, as these were the key parameters to focus on in this research. However, the Azufre River contains other pollutants, and since they were not of particular interest for this study, they were not added to the synthetic water. Ideally, real water should be used if feasible, as the presence of other chemical species may affect the treatment efficiency.

The effect of hydraulic and pollutant loading was not assessed in this study. The fact of having a case-study (i.e. having the same pollutant levels used in every experimental stage) was prioritised, so as to obtain further understanding of other key parameters that were considered more important: the type of wetland media and the type of flow (i.e. vertical versus horizontal), for a particular type of contaminated water. In vertical flow systems, intermittent dosing rather than continuous dosing was performed. We acknowledge that this was not representative of how wetlands are commonly operated, but since we were only interested in comparisons of media for selection, it was not relevant. Further, we refined this approach in our final stage, where water was injected almost continuously. As is always the case, there is a necessary compromise between the need for repeatability and controlled experiments, and the desire to account for 'real-world' variability. In this experiment, the primary focus has been on using well-controlled laboratory experiments, so as to be able to make inferences about likely retention processes occurring. A lack of studies using controlled laboratory experiments, where different factors can be manipulated, was detected. Therefore, it

was considered appropriate to focus on laboratory experiments, so as to carefully investigate the factors affecting the retention processes occurring.

6.3. Key findings

Subsurface flow wetlands are a cost-effective natural system, successfully used for removing various pollutants. Furthermore, they have shown capability for removing arsenic and metals. Currently, however, little guidance exists on how a wetland should be designed for the removal of arsenic, due to a lack of reliable data and understanding of the removal mechanisms. This research thus assessed the removal efficiencies of subsurface flow wetlands, both vertical and horizontal, using laboratory studies. The key findings are described below, along with recommendations for improved design and operation.

6.3.1 Vertical flow wetlands with gravel media

Vertical flow wetlands with gravel media presented lower removal efficiencies (for As, B, Fe, Cu, Mn, Zn) than those with limestone, cocopeat and zeolite media, when treating slightly acidic polluted water (in the first experimental stage of the study). However, when treating highly acidic polluted water simulating the Azufre River (second experimental stage), they presented higher As removal efficiency than cocopeat media, but much lower than limestone and zeolite wetlands. As such, gravel was discarded as a prospective wetland media, despite it is readily available and is cheap. While conventional gravel wetlands have been the most commonly used, regardless of the target pollutants, this study has verified that they are not suitable for As and metal removal (Kadlec and Wallace, 2009): As, Fe, Cu and Zn removal showed signs of declining with time in the first experimental stage, and Fe removal was non-existent in the second experimental stage. In addition, given the inability of gravel to buffer against the acidic influent, it is unlikely to be effective in treating the acidic waters that were the topic of this study.

6.3.2 Vertical flow wetlands with alternative media

Three alternative wetland media: Limestone, cocopeat and zeolite were employed. In the first experimental stage, little difference in the pollutant removal capacity of each wetland type was observed. However, in the second experimental stage, each of them presented a particular capability to remove each of the target pollutants: Limestone wetlands were the most effective in removing As and Fe, followed by the zeolite wetlands. Cocopeat wetlands were less effective in removing As, although they were more effective than gravel wetlands in removing Fe, and they were the only wetland type that showed potential to remove B. As such, the main As removal mechanism identified was coprecipitation with Fe in limestone wetlands, triggered by the rise in pH through the limestone. Sorption of As and Fe onto zeolite appeared as the second-most effective mechanism, whereas As sorption onto cocopeat was minor.

The type of media was a primary factor affecting the removal rates of the target pollutants under acidic conditions. The nature of the wetland media suggested that pH adjustment is required, since higher pH favours metal removal (Wingenfelder et al., 2005), and for both As and Fe, highest removal efficiencies were observed under neutral pH in limestone wetlands. Cocopeat media appeared as promising for B removal, due to the affinity of organic matter for B (Sartaj and Fernandes, 2005), which was still observed despite the fact that cocopeat wetlands maintained highly acidic conditions.

6.3.3 Horizontal flow wetlands with alternative media

Two types of horizontal flow wetlands were built: one with zeolite as the main media, and the other with limestone in the inlet section and cocopeat in the outlet section. Both media types showed remarkable As removal rates, with the zeolite media in this case showing higher Fe removal rates than that of limestone/cocopeat wetlands. Limestone/cocopeat wetlands showed higher B removal rate and also the capability to raise the pH from ~2 to ~7.

The minor role of As, Fe and B removal by plant uptake was confirmed, as postulated by different authors (e.g. Ye et al., 2001, Ye et al., 2003, Singhakant et al., 2009). In fact, in limestone/cocopeat wetlands, iron hydroxides were the main sink for As. Formation of these particles occurred mainly in the inlet section, made of limestone. In practice, such an arrangement would help minimise the maintenance requirements, since this section could be regularly renewed or backwashed, to remove these particles.

This study concluded that horizontal flow constructed wetlands with zeolite media are suitable for the removal As and Fe from acidic water, with 99.9% and 96% removal rates, respectively. However, if neutral pH is required, limestone/cocopeat wetlands may be preferable, although Fe removal efficiency is slightly lower (87%).

6.3.4 Design recommendations

The type of wetland media and the direction of flow are key design parameters to ensure good As and metal removal performance. Wetland media provide physical and chemical treatment, also providing support for vegetation and surface for biofilms. For the treatment of non-acidic water and thus having As and metals mainly in particulate form, provision of sorption sites and alkalinity may not be as important, as physical filtration will be the predominant mechanism. However, for the treatment of highly acidic water, provision of these factors is essential to enhance As coprecipitation with Fe, and sorption of As, Fe and B ions.

Zeolite appears to be more effective than limestone/cocopeat wetlands in removing As and Fe at low flows in a horizontal subsurface flow context. In this context, zeolite wetlands are also more effective than in a vertical flow context. The presence of an organic substrate such as cocopeat improves B removal rate, and as such a second stage where B sorption onto organic matter under alkaline conditions is recommended.

The use of *Phragmites australis* is recommended, since this species was able to tolerate acidic conditions, high levels of As, Fe and B; and live in alternative media. Although *Phragmites* is able to accumulate these pollutants, harvesting is not recommended

given the minor role of plant uptake and the higher accumulation in roots than in shoots. Other plants employed for metal removal may be used instead of *Phragmites*. If limestone is employed as wetland media, and therefore pH is neutral, *Typha latifolia* (cattail broadleaf) could be employed. If limestone is not employed, and therefore pH remains acidic, *Typha angustifolia* (cattail narrowleaf) could be employed as it tolerates low pH (Wallace and Knight, 2006).

An optimum wetland system to remove As, Fe and B from acidic water would have different sections with different media types, as combination of media has shown to be effective. The first section should have limestone. This will ensure that pH is adjusted and therefore Fe particulates are formed. In addition, this arrangement would also facilitate maintenance as a great part of the Fe and As are retained here as particulates. A second section (or even a third) could function as a polishing stage. This could be provided by zeolite and/or cocopeat. It was shown that cocopeat is effective in removing B, but zeolite may also be a good choice as As and Fe would have been mainly removed in the first section, therefore more exchange sites are available for B.

At the present time, means of dealing with maintenance of the media in subsurface flow wetlands are in experimental stage (Kadlec and Wallace, 2009). Options are to remove clogged media and replace with new media, or to remove, clean and re-install the media. In both options, there are costs associated with disposal, which are higher in the first option. To minimise the amount of As and metal-containing solids to be disposed of, the second option would be recommended.

6.4. Future work

This research has assessed broadly the performance of subsurface flow wetlands in the removal of As, B and Fe from acidic water. A better understanding of the removal mechanisms has been obtained, but a number of knowledge gaps remain. Further understanding is required to optimise design of wetlands systems that enhance As and metals removal. Long-term trials are required, to assess aspects such as clogging, lifespan of wetland media, and seasonal effects on treatment performance. Ideally,

these trials would be conducted at full scale in the field, to allow the effect of varying environmental conditions to be addressed. Design parameters should also be considered. Aspect ratio (length-to-width, L:W) higher than 10:1 ensures plug flow, however the resistance to flow increases as the length increases. It has been proposed that this value should be between 0.25:1 and 4:1 (Crites et al., 2006). For HSSF, L:W is an important decision, as longer and narrower beds will increase the pollutant loading applied to the cross sectional area of the bed; thus clogging may occur. For VF wetlands L:W is relatively unimportant (Kadlec and Wallace, 2009).

As expected, boron was the most difficult pollutant to remove. Since boron may be toxic for many plants at various concentrations (Davies et al., 2002), its removal may be required at different levels depending on the final use. Results from this study suggested that boron removal in wetlands may be achieved by encouraging sorption onto organic matter under alkaline conditions, or onto another medium with sorption capacity. Potential media that could be tested include rice husk, which has shown capability to sorb B (Man et al., 2012). Other technologies such as the use of biopolymers (Wei et al., 2011) and fly ash (Yüksel and Yürü, 2010) as sorbents have been recently explored. Phytoremediation using B hyperaccumulators also appears as a promising technology (Rámila C.D.P. et al.). Further experimental evidence is required to quantify the potential of these technologies.

The potential role of vegetation has been recognised (Marchand et al., 2010). However, studies comparing planted and unplanted systems often lead to conflicting results regarding the importance of plants (Lee and Scholz, 2007). The effect of the presence of vegetation and its type in wetlands with alternative media for the removal of As and metals is yet to be investigated. It has been reported that vegetated systems are more effective in the removal of As (Buddhawong et al., 2005, Lucas, 1982), but this may not occur in the removal of metals such as Cu, Cd, Pb and Zn (García et al., 2010). Vegetation plays an indirect but important role in metal removal because they can provide oxygen to the substrate, surface for biofilms, and organic matter. In fact, the supply of organic matter is a key point that is often overlooked, mainly to the short time span of most studies. Plant-derived organic matter promotes long-term functioning by

continuously providing carbon for bacterial metabolism and sites for metal sorption (Marchand et al., 2010).

The role of microbial communities in As and metals removal requires further attention. Biological processes ensure long term removal as long as the conditions are appropriate for the bacteria to thrive. This also involves that the system will not fail when the adsorption sites are exhausted. This study has provided evidence of the existence of several bacterial families in horizontal flow wetlands, but their genus and species could not be identified, and most importantly their role in the removal performance was not determined. To assess the effect of bacterial communities in the removal of As and metals, experiments specifically designed for this purpose are required. For example, four types of wetlands could be operated and monitored to compare their performance: type 1, seeded with bacteria relevant to As and metals removal; type 2, seeded with bacteria not relevant to As and metals removal; type 3, non-seeded; and type 4, dosed with influent which has no As or other metals of interest (to see if the bacterial communities are different, and if so, which). Particulates formed in these wetlands could be analysed using advanced techniques that can identify the speciation of As and Fe in the solid phase (e.g. x-ray spectroscopy), thus revealing the type of mechanism that caused the formation of the particulates.

This study has produced a performance data set for different wetland media in vertical and horizontal flow wetlands. When comparing these results to those from other studies, the particular conditions under which the data were obtained should also be compared. This is of special importance if a modelling tool is to be produced, as data from this study only may not be sufficient to predict treatment performance.

6.5. Conclusions

Increasing costs of fossil fuel energies are driving a push towards low-energy, sustainable water treatment systems able to remove various pollutants, including As and metals. Constructed wetlands have great potential to remove these pollutants. However, a lack of studies investigating this potential was identified.

This thesis has quantified the capability of subsurface flow constructed wetlands in removing As, B and Fe from highly contaminated water, for a range of wetland media. Laboratory experiments have provided valuable information on media selection and pollutant removal routes. Alternative wetland media presented better performance than conventional gravel media in vertical flow wetlands. In horizontal flow wetlands, these alternative media were tested, with zeolite media proving to be highly effective in removing As and Fe, followed by limestone/cocopeat wetlands. Further work is required to achieve higher B removal, with the media combining a section of limestone followed by cocopeat showing promise.

The present thesis has provided an overall and unique assessment of subsurface flow wetlands, offering useful insights for enhancing As, Fe and B removal from highly acidic water. The thesis builds a foundation on which further research can be conducted, with the aim of being able to confidently apply subsurface flow wetlands for removing As, Fe and B from contaminated waters.

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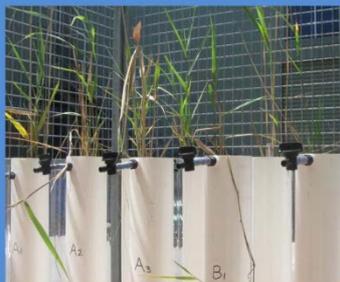
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Appendix: Conference papers



Subsurface flow constructed wetlands for the removal of arsenic and metals from acidic contaminated water



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7th International Conference on Water Sensitive Urban Design
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Enhancing the removal of arsenic and heavy metals in subsurface flow constructed wetlands using different supporting media

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Abstract

The presence of arsenic and heavy metals in drinking water sources poses a serious health risk due to chronic toxicological effects. Constructed wetlands have the potential to remove arsenic and heavy metals, but little is known about pollutant removal efficiency and reliability of wetlands for this task. This lab-scale study investigated the use of vertical subsurface flow constructed wetlands for removing arsenic, boron, copper, zinc, iron and manganese from synthetic wastewater. Gravel, crushed limestone, zeolite and cocopeat were employed as wetland media. Conventional gravel media only showed limited capability in removing arsenic, iron, copper and zinc; and it showed virtually no capability in removing manganese and boron. In contrast, alternative wetland media: cocopeat, zeolite and limestone, demonstrated significant efficiencies -in terms of percentage removal and mass rate per m³ of wetland volume- for removing arsenic, iron, manganese, copper and zinc; their ability to remove boron, in terms of mass removal rate, was also higher than that of the gravel media. The overall results clearly demonstrated the potential of using vertical flow wetlands to remove arsenic and metals from contaminated water, having cocopeat, zeolite or crushed limestone as supporting media.

Keywords

Arsenic removal; heavy metal removal; subsurface flow constructed wetlands; supporting media

INTRODUCTION

Arsenic is well known for its chronic toxicity, particularly when exposure occurs over prolonged periods. Arsenic pollution in natural waters has been reported in different countries throughout the world, such as Bangladesh, USA, China, India, and Chile. About 100 millions of people are currently drinking water with As concentrations up to 100 times 10 µg/L which is the World Health Organisation guideline (Mohan and Pittman Jr, 2007). In Chile, the Loa River has As and boron (B) concentrations around 1,400 and 21,000 µg/L, respectively (Romero et al., 2003). This river is a major waterway in Antofagasta Region and the main source of drinking and irrigation for several populated areas (Landrum et al., 2009). The presence of As and B in the Loa River is due to the El Tatio Geysir Field geothermal field, which forms the headwaters of the Salado River -a tributary to the Loa River- and these headwaters have As concentrations around 33,700 µg/L. Dissolved As is commonly present in hydrothermal waters, but El Tatio Geysir Field has the highest reported dissolved As concentration in a natural surface water (Landrum et al., 2009).

In addition to arsenic, metals/metalloids such as boron, iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) can also limit the use and reuse of water resources, either by natural or anthropogenic pollution. Boron contamination in the water environment is causing increasing concern (Xu and Jiang, 2008). In many cases, treatment of contaminated water is limited due to isolated location of the water streams and the elevated investment and operation costs of conventional technologies.

Constructed wetlands are known to be effective in removing several trace metals from contaminated water (Kadlec and Wallace, 2009). A number of studies have been carried out to investigate their metal removal efficiency (Kleinmann and Girts, 1987; Richards, 1992; Sobolewski, 1999; Sjöblom, 2003). Most of these studies have focused on acid mine drainage (AMD) treatment, primarily to remove sulfate, Fe and Mn (Wallace, 2006) by surface flow wetlands. Little is known about subsurface flow wetlands, and few literature reports are available on the performance of wetlands for the removal of As and B. Furthermore, the mechanisms of As removal have not been elaborated (Singhakant et al., 2009).

The use of alternative media in constructed wetlands has been suggested by different researchers, with the aim of improving removal performance. For example, Sarafraz et al. (2009) showed that the use of zeolite in a horizontal subsurface constructed wetland enhanced Zn sorption, and proposed zeolite as an alternative to sand and gravel. Nevertheless, little research in this area has been conducted. This study investigated the performance of four different supporting media: gravel, cocopeat, zeolite and limestone in the removal of six target pollutants: As, B, Fe, Mn, Cu and Zn using vertical subsurface flow constructed wetlands.

MATERIALS AND METHODS

The wetland system

The lab-scale wetlands consisted of twelve subsurface vertical flow wetland columns that were built using stormwater PVC pipes. Each column had 1 m height and 100 mm internal diameter and was installed in a greenhouse. The wetland columns were divided into four groups, namely group G -employing gravel as main substrate, Z -zeolite as main substrate, C -cocopeat as main substrate, and L -crushed limestone as main substrate; each group had three replicate columns. Each column had a drainage layer of 20-40 mm cream pebbles at the base which was 0.1 m deep. The drainage layer was topped with a layer of main substrates (G, Z, C, or L) that was 0.7 m deep. In each wetland column, common reed (*Phragmites australis*) was planted. The *Phragmites* were given two months to adapt to their new growth environment prior to the experiment.

Operation of the wetland system

Synthetic wastewater was prepared to simulate the concentration of the target pollutants in polluted surface waters in Chile. The synthetic wastewater was prepared using tap water, with the following reagents added per litre of water: 1 mL 1,000 mg/L arsenic standard solution (arsenic acid As_2O_5 in H_2O), 0.025 mL 10,000 mg/L boron standard solution (boric acid H_3BO_3 in H_2O), 125 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 7.2 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 3.9 mg $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$, 4.4 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.7 mg $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$. The total concentration of the metals were (average \pm standard deviation): 0.89 ± 0.05 mg/L As, 24.0 ± 0.0 mg/L B, 1.43 ± 0.40 mg/L Cu, 21.0 ± 1.4 mg/L Fe, 2.38 ± 0.82 mg/L Mn and 1.25 ± 0.44 mg/L Zn. An agitated feed tank stored the wastewater during the experiment. From it, two litres of synthetic wastewater were taken and dosed manually in each wetland, three times per week in the first month and twice per week in the second month.

Sampling and analysis

After each dosing, two types of water samples from each wetland column and from the feed tank were collected and acidified with nitric acid (HNO_3) to $\text{pH} < 2$ for total and dissolved metals analysis. For the latter, the samples were filtered through $0.45 \mu\text{m}$ cellulose acetate filters. Weekly composite samples were prepared adding an equal volume of each corresponding daily sample for every week. Metal concentrations in these composite samples for weeks 1, 3, 5 and 7 were determined by ICP-MS in a NATA accredited laboratory (4 values per column plus the inflow). In situ parameters were also monitored after each dosing (20 values per column plus the inflow). Dissolved oxygen (DO) was measured using 51970 probe, whilst for pH and conductivity 51910

and 51975 probes were used respectively. All these probes were connected to a Sension 378 meter. ORP Testr10 probe was used to measure redox potential (Eh). Sulfate (SO₄) was measured using DR5000 UV/VIS spectrophotometer based on an adapted method from Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WEF, 2005). Alkalinity was quantified using HACH alkalinity test kit, low range and high range tests.

RESULTS

Overall performance

Table 1 presents the average performance of the system during the operation period, for each group of wetland columns. As shown in Table 1, the concentration of all the target pollutants in the outflow from the gravel wetland columns was higher than that from other columns, demonstrating that the three types of alternative wetland substrate had greater removal pollutant removal efficiencies than the traditional gravel substrate. Furthermore, gravel appeared to have limited capability to remove As, Fe, Cu and Zn; and almost null capability to remove B and Mn.

Table 1. Mean inflow and outflow concentrations of the target pollutants.

	Mean inflow concentration (mg/L) [CV*]		Mean outflow concentration (mg/L) [CV*]							
			G - gravel		C - cocopeat		Z - zeolite		L - limestone	
	dissolved	total	dissolved	total	dissolved	total	dissolved	total	dissolved	total
As	0.001 [0.952]	0.890 [0.062]	0.001 [1.13]	0.253 [0.685]	0.011 [0.149]	0.013 [0.121]	0.008 [0.347]	0.011 [0.431]	0.006 [0.145]	0.009 [0.409]
B	23.5 [0.04]	24.0 [0.0]	23.5 [0.06]	24.3 [0.06]	22.0 [0.27]	22.17 [0.28]	21.33 [0.12]	21.67 [0.09]	21.67 [0.11]	21.92 [0.11]
Cu	0.047 [0.699]	1.425 [0.283]	0.34 [0.75]	0.462 [0.69]	0.012 [0.37]	0.014 [0.386]	0.007 [0.271]	0.009 [0.29]	0.013 [0.513]	0.024 [0.375]
Fe	UD**	21.0 [0.07]	0.08 [0.44]	6.7 [0.59]	0.095 [0.47]	0.238 [0.281]	0.047 [0.686]	0.246 [0.615]	0.023 [1.457]	0.205 [0.575]
Mn	2.275 [0.33]	2.30 [0.32]	2.21 [0.24]	2.233 [0.25]	0.044 [0.73]	0.046 [0.761]	0.007 [0.286]	0.016 [0.466]	0.131 [0.627]	0.143 [0.576]
Zn	0.723 [0.473]	1.245 [0.353]	0.043 [1.44]	0.664 [0.57]	0.012 [0.19]	0.013 [0.18]	0.008 [0.216]	0.01 [0.273]	0.003 [0.425]	0.008 [0.364]

*CV=coefficient of variation= σ/μ **UD: undetectable. For samples under the detection limit (0.001 mg/L for As and 0.02 mg/L for Fe), half of that value was considered for the calculations.

The changes in the monitored parameters are presented in Table 2.

Table 2. Mean inflow and outflow level of monitored water quality parameters.

Parameter	Unit	Mean value in the inflow [CV*]		Mean value in the outflow [CV]			
		Value		Gravel	Cocopeat	Zeolite	Limestone
SO ₄	mg/L	74.7 [0.083]		67.3 [0.136]	65.7 [0.07]	56.5 [0.087]	56.2 [0.098]
pH	-	5.77 [0.976]		4.95 [0.936]	5.67 [0.995]	6.13 [1.025]	7.95 [1.001]
DO	mg/L	6.3 [0.205]		6.3 [0.217]	6.1 [0.2]	6.2 [0.192]	5.9 [0.215]
T	oC	24.2 [0.121]		24.4 [0.139]	24.4 [0.133]	24.7 [0.125]	25 [0.124]
Alkalinity	mg/L CaCO ₃	14.3 [0.172]		9.3 [0.27]	11.4 [0.243]	10.8 [0.189]	155.4 [0.619]
Eh	mV	195.2 [0.082]		225.4 [0.15]	235.2 [0.137]	233.4 [0.129]	181.5 [0.143]
Conductivity	μS/cm	198.6 [0.068]		217.7 [0.112]	176.5 [0.103]	185.2 [0.459]	290.8 [0.041]

*For the pH values, the CV was calculated using $CV = -\log\{\sigma\} / -\log\{\mu\}$, where σ is the average of $\{H^+\}$ and μ is the standard deviation of $\{H^+\}$

The removal of As and Fe

Cocopeat, zeolite and limestone wetlands presented high As removal rates (average above 98%) for the entire experimental period, whereas for gravel wetlands the rates decreased over time, both as percentage and as daily mass removed per volume. In addition, the removal of As in terms of mass was almost the same for the alternative wetland media, and it only decreased when the inflow concentration decreased (Figure 1a). The removal of Fe presented a very similar trend: cocopeat, zeolite and limestone wetlands removal was around 99%, but gravel wetlands removal decreased with time (Figure 1b).

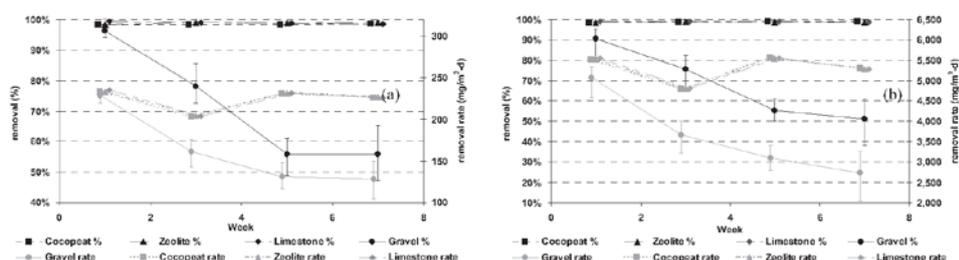


Figure 1. Mean removal rates of total As (a) and Fe (b) over time, expressed as percentage (principal axis) and as daily mass removed per unit of volume of wetland (secondary axis). Error bars indicate the corresponding minimum and maximum values.

The removal of B and Mn

Boron was removed by cocopeat, zeolite, limestone and gravel wetlands at the beginning of the experiment, but then it was leached out by cocopeat and gravel wetlands (Figure 2a). In addition, gravel wetlands barely removed Mn. As shown in Figure 2b; cocopeat, zeolite and limestone wetlands removed around 94% total Mn on average, whereas gravel wetlands even presented negative removal.

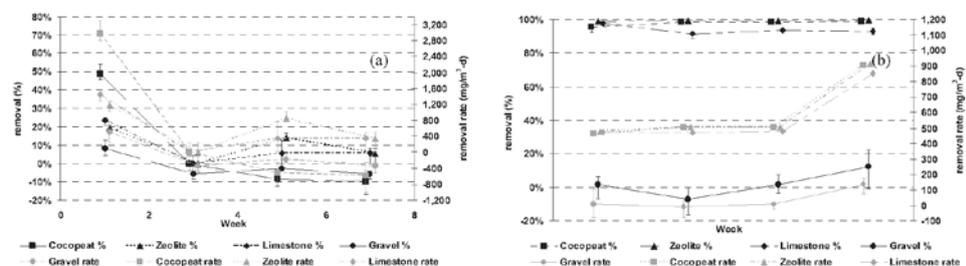


Figure 2. Mean removal rates of total B (a) and Mn (b) over time, expressed as percentage (principal axis) and as daily mass removed per unit of volume of wetland (secondary axis). Error bars indicate the corresponding minimum and maximum values.

The removal of Cu and Zn

High removal rates were observed in cocopeat, zeolite and limestone wetlands for these metals, regardless of fluctuations in inflow concentration (Figures 3a and 3b). In contrast, decreasing removal rates over time were observed in gravel wetlands, despite the fact that the daily mass removal rate increased when the inflow concentration increased.

DISCUSSION

The difference in the removal rates suggests that the removal of the target pollutants in vertical flow wetlands was primarily affected by the type of substrate. Gravel presented limited removal of As, whereas all the alternative media exhibited excess capability to remove As (average removal percentage over 98%). This supports Singhakant et al. (2009) study, who reported that the efficiency of As removal decreased over time in their sand/gravel constructed wetlands. This limitation was also observed in the performance of the gravel wetlands to remove the other five target pollutants. Furthermore, the use of sand/gravel media in subsurface flow wetlands is not recommended for the removal of metals due to limited sorption capacity and inability to form new storage sediments without clogging the wetland matrix (Kadlec and Wallace, 2009). As such, the results suggest that gravel is unsuited to be employed as wetland media for the removal of As -and metals- rich wastewater. On the other hand, the lack of similar studies prevents comparison of the performance of cocopeat, zeolite and limestone as alternative media. However, these materials have been employed in some extent to remove various of the target pollutants. The exception is cocopeat (chosen as an alternative to peat) which apparently has not been studied for metals removal.

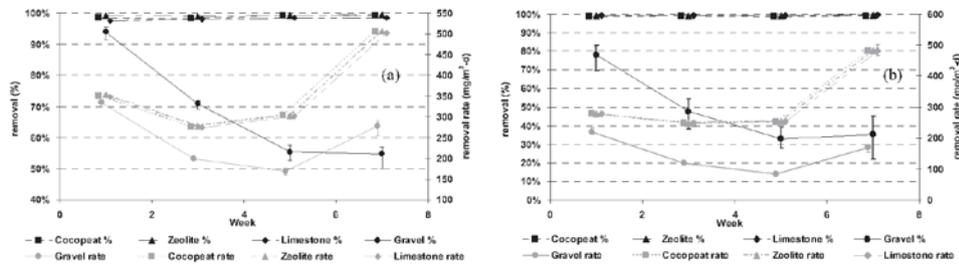


Figure 3. Mean removal rates of total Cu (a) and Zn (b) over time, expressed as percentage (principal axis) and as daily mass removed per unit of volume of wetland (secondary axis). Error bars indicate the corresponding minimum and maximum values.

The removal of heavy metals can be achieved via different processes depending on whether they are dissolved or particulated. The principle process that removes heavy metals in natural and constructed wetlands is sedimentation; however, other processes such as precipitation must occur first since sedimentation only removes particulated metals (Sheoran and Sheoran, 2006). Looking at the speciation of As, Fe and Cu in the inflow, the three of them were mostly particulated (Table 1). Therefore, physical processes such as filtration and sedimentation would be enough to remove them. According to Figure 4a and 4c, As and Fe in the outflow from gravel wetlands were also mainly particulated. Moreover, given the correlation between total As and Fe outflow concentration ($R^2=0.98$) and As and Fe removal rate ($R^2=0.97$), it can be asserted that Fe oxides trapped As by coprecipitation/sorption. Similar findings were reported by Buddhawong et al. (2005), who concluded that As binding with the Fe content of the gravel media was responsible for As removal. The sorption of metals on oxides is widely known (Stumm and Morgan, 1996). Different authors attribute this property as an important source of metal removal in constructed wetlands if Fe/Mn oxides are present (Sjöblom, 2003). Arsenate sorption onto most metals (hydr)oxides (Inskeep et al., 2002), but specially onto Fe and Mn oxyhydroxides has been reported by a number of researchers (Kneebone et al., 2002; Pastén et al., 2006). Thus, Fe oxides containing As were filtered in gravel wetlands, but given the medium gravel size (7 mm), filtration capability was limited.

In contrast, the total concentration of Cu was lower in the outflow from gravel wetlands than in the inflow from week 1 to 7, but the dissolved concentration was higher in the outflow than in the

inflow from week 3 onwards (Figure 4e). In addition, Zn was mainly particulated in the outflow from gravel wetlands (Figure 5), whereas it was mostly dissolved in the inflow (Table 1). However, the dissolved concentration was lower in the outflow than in the inflow throughout the whole period. Therefore, gravel wetlands filtered total As, Fe, Cu and Zn; released dissolved Cu, and removed dissolved Zn, but due to their limited removal capacity the total concentration of Zn increased consistently in the outflow. Both Cu and Zn can be removed by Fe oxides (Kröpfelová et al., 2009), but because conflicting information exists regarding competitive sorption of Cu and Zn on Fe oxides (Covelo et al., 2007; Violante et al., 2003), their effect on Cu and Zn removal is not clear. In cocopeat, zeolite and limestone wetlands, dissolved Cu and Zn were removed; whereas dissolved As and Fe were not (Figures 4 and 5). This shows that these wetlands were able not only to retain particulated metals, but also to remove/release dissolved metals. Different authors have proposed that the main mechanism for mobilisation of As sorbed on Fe oxides is reductive dissolution (Mukherjee et al., 2009). Since mostly aerobic conditions were found (Table 2), further experimental evidence is required to understand the As and Fe retention/mobilisation mechanisms.

Boron was mostly dissolved in the inflow (98%) and the outflow from gravel (97%), cocopeat, zeolite and limestone wetlands (99%), whereas Mn was mostly dissolved in the inflow (99%) and the outflow from gravel (99%), cocopeat (97%) and limestone wetlands (91%); but not in the outflow from zeolite wetlands (43%) (Table 1). This may indicate that the main B removal process is sorption, mainly when it is present as borate $B(OH)_4^-$. In addition, the presence of organic matter contributes to the adsorption of B in soils (Sartaj and Fernandes, 2005). The good performance of cocopeat wetlands only at the beginning of the experimental period can be explained by a limited sorption capacity of this substrate, as reported by Sartaj et al. (1999) for a peat filter. In addition, the adsorption of boron on soils depends on the pH of the solution (Kot, 2009); so lower the pH, the adsorption. Low pH could explain the low removal rates in gravel wetlands, whilst high pH could explain the highest removal rates in zeolite and limestone wetlands (Table 2).

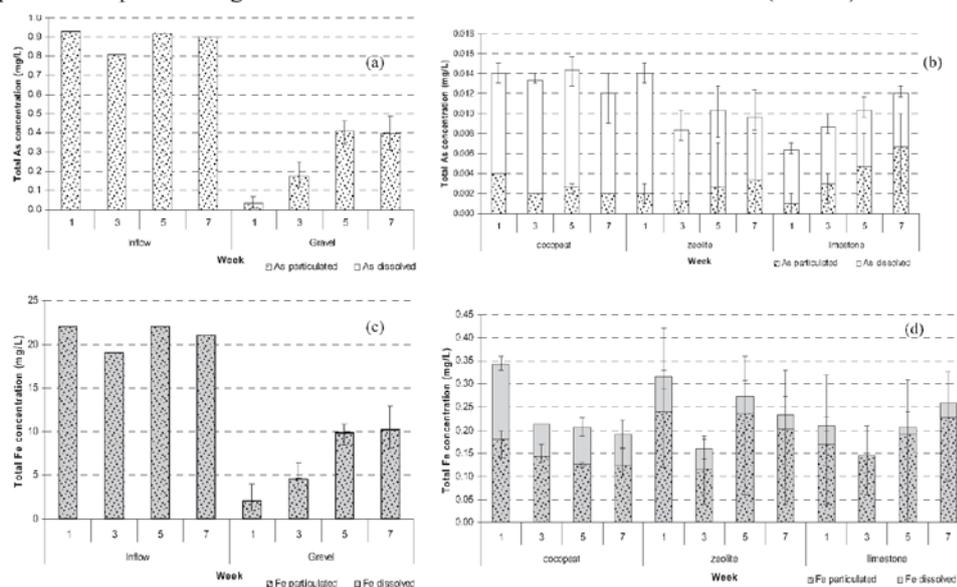


Figure 4. Total As and Fe concentration presented as dissolved and particulate fraction in the inflow and outflow of gravel wetlands (a) and (c), respectively; outflow of cocopeat, zeolite and

limestone wetlands -(b) and (d) respectively. Error bars indicate the corresponding minimum and maximum values.

The speciation of metals is the main factor that determines their bioavailability. Dissolved metals represent the most bioavailable form, especially when the metal is present as ionic or weakly complexed species (Cooper et al., 1996). Most metal removal studies only report total concentrations, however the dissolved fraction should be reported since some guidelines (such as US EPA National Water Quality Criteria) do consider it. Dissolved As(V), as a highly reactive metalloid, may have different routes, being As(III) the most toxic species. Further research is currently being undertaken to remove As efficiently in subsurface constructed wetlands.

CONCLUSIONS

This experimental study showed that cocopeat, zeolite and limestone can be used as the main media in vertical flow wetlands, to enhance the removal of As, B, Fe, Mn, Cu and Zn. In addition to providing filtration capability to remove particulated pollutants, these alternative substrates were able to provide different factors, such as organic matter (cocopeat), ion exchange sites (zeolite) and alkalinity (crushed limestone), which all contributed to the removal of the target pollutants of this study. In comparison, gravel wetlands only showed limited ability to remove As, Fe, Cu and Zn, and virtually no capability to remove B and Mn.

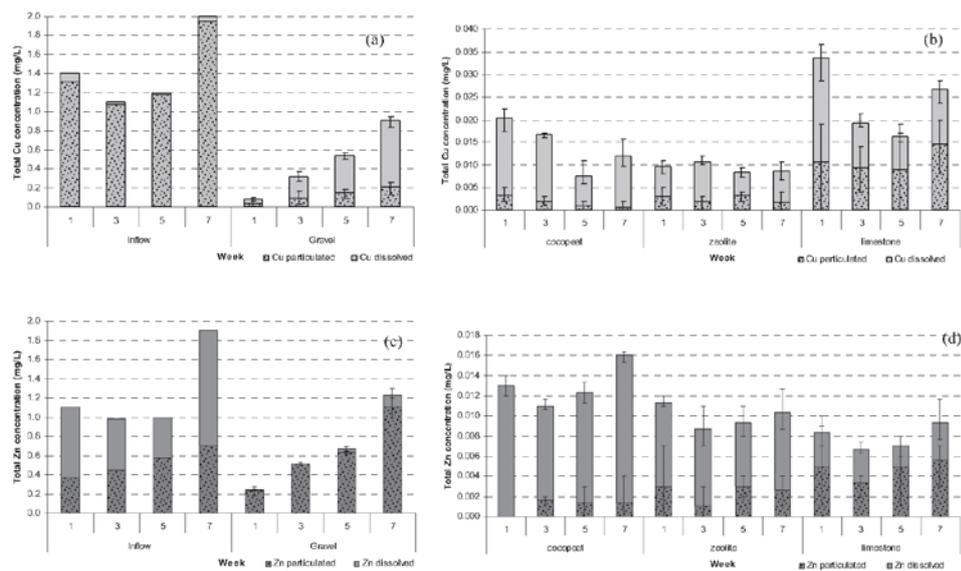


Figure 5. Total Cu and Zn concentration presented as dissolved and particulated fraction in the inflow, and outflow of gravel wetlands -(a) and (c) respectively;- outflow of cocopeat, zeolite and limestone wetlands -(b) and (d) respectively. Error bars indicate the corresponding minimum and maximum values.

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Subsurface flow constructed wetlands for the removal of arsenic and metals from acidic contaminated water

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Extended Abstract

The main aim of this study was to examine the effectiveness of alternative supporting media in subsurface flow constructed wetlands, to enhance the removal of arsenic (As), boron (B) and iron (Fe) from acidic contaminated water.

Background. Arsenic and heavy metals pollution in water is a worldwide problem. Onsite, cost-effective, low impact treatment technologies are required to remove heavy metals to avoid further degradation of aquatic ecosystems. Constructed wetlands have been successfully applied to treat several types of wastewater and their application has accelerated, primarily due to rising costs of fossil fuel-derived energy sources. They also offer low cost/easy maintenance and a wildlife habitat (Lee et al., 2009). In particular, subsurface flow constructed wetlands have the potential to remove different metals and metalloids from water, but few studies have been performed to investigate As removal (Lizama A. et al., 2011). In addition, the use of alternative media has been suggested but not sufficiently studied. Zeolite, limestone and cocopeat substrates have been found to be more effective than conventional gravel substrate under neutral to slightly acidic conditions (Lizama Allende et al., 2011), but under highly acidic conditions, their performance is unknown. These acidic conditions are generally associated with the presence of As and metals. The Azufre River, northern Chile, was selected as the case-study for its elevated levels of As and low pH (Ríos et al., 2011) to test the alternative substrates and the conventional gravel as wetland supporting media.

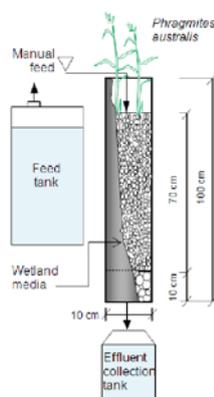


Figure 1. Wetland column design (schematic drawing).

Materials and methods. Twenty subsurface vertical flow wetlands columns were built, each column had 1 m height and 100 mm internal diameter (Figure 1). The wetland columns were divided into four groups, employing gravel, cocopeat, zeolite and limestone as the main substrate; with the aim of comparing the conventional gravel substrate with a supply of organic matter, ion exchange capacity and alkalinity respectively. Each group had five replicate columns. In each wetland column, a single plant of *Phragmites australis* from the root-cuts of mature plants was planted. Synthetic wastewater simulating the Azufre River was prepared weekly (3.08±0.25 mg/L As, 32±2.19 mg/L B, 107.33±6.53 mg/L Fe, pH 2.0±0.13). Boron and iron were also studied given their high levels naturally found in the Azufre River. Two litres of synthetic wastewater were dosed manually in each column, two times per week. Weekly composite samples from each wetland column and from the inflow were analysed for total and dissolved As, B and Fe by ICP-OES/MS.

Results and discussion. The mean As, Fe and B concentrations decreased in the outflow from every wetland column, except for Fe in gravel wetlands (Table 1). Limestone wetlands presented the highest removal rates for both As and Fe. Since in the outflow from these wetlands As and Fe were mostly particulated, and the As and Fe levels were correlated, it can be asserted that Fe precipitated causing As coprecipitation, being both removed. Although Fe tends to retain As in natural systems, gravel wetlands removed As but not Fe, indicating that other mechanisms caused As removal. Cocopeat columns were the only ones presenting promising potential for removing B, however alkaline condition would favour B removal.

Table 1. Pollutants concentration in inflow and outflow from wetland columns

Pollutant	Inflow [CV*]	Gravel outflow [CV]	Cocopeat outflow [CV]	Zeolite outflow[CV]	Limestone outflow[CV]
As _{total} [mg/L]	3.1 [0.08]	1.7 [0.18]	2.8 [0.13]	0.258 [1.44]	0.021 [0.67]
As _{dissolved} [mg/L]	3.0 [0.10]	1.7 [0.18]	2.7 [0.13]	0.250 [1.42]	0.010 [1.79]
B _{total} [mg/L]	32 [0.07]	31 [0.07]	30 [0.07]	31 [0.10]	31 [0.09]
B _{dissolved} [mg/L]	32 [0.08]	31 [0.08]	29 [0.07]	31 [0.10]	31 [0.09]
Fe _{total} [mg/L]	107 [0.06]	116 [0.06]	57 [0.09]	15.0 [0.47]	1.85 [0.47]
Fe _{dissolved} [mg/L]	105 [0.07]	113 [0.07]	56 [0.08]	14.8 [0.47]	0.11 [0.48]

*CV=coefficient of variation= σ/μ

Key findings. (1) The substrate is a critical design parameter of subsurface flow wetlands aiming to remove As, Fe and B from acidic water (2) Limestone wetlands, followed by zeolite wetlands, were the most efficient in removing As and Fe, whereas cocopeat wetlands were the most efficient in removing B (3) The main As removal mechanism was probably coprecipitation with Fe, triggered by the raise of pH in limestone wetlands.

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Arsenic removal by subsurface flow constructed wetlands

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ABSTRACT: Cost-effective, onsite, environmental-friendly treatment technologies such as constructed wetlands are required to remove arsenic (As) from water. This study investigated the performance of subsurface flow constructed wetlands in removing As from highly acidic contaminated water [arsenic 3.1 mg/L, iron (Fe) 107 mg/L, boron (B) 32 mg/L, pH 2]. Four different supporting media: gravel, cocopeat, zeolite and limestone were employed to investigate their effect on As removal. Limestone wetlands were the most effective for removal of As (99%) and Fe (98%), followed by zeolite wetlands (92% As removal and 86% Fe removal). Gravel and cocopeat wetlands removed As at lower rates (45% and 9% respectively). The most effective As removal mechanism was found to be coprecipitation with Fe, enhanced by the use of limestone and its capability to raise pH. Therefore, limestone and zeolite could be used as wetland media to enhance the removal of As from Fe-rich and acidic water.

1 INTRODUCTION

Constructed wetlands are low-cost green systems successfully applied in water treatment. They are able to remove different pollutants including arsenic (As) (Lizama Allende et al. 2011), but very little is known about their efficiency and reliability for this purpose. Surface flow wetlands have been mostly used to metals and metalloids remove, however subsurface flow wetlands could be more effective since they allow contact between wastewater and wetland substrate. The use of alternative substrates to the conventional gravel has been suggested to enhance performance (Ye et al. 2003). Furthermore, cocopeat, zeolite and limestone have potential to enhance As removal (Lizama Allende et al. 2011), although this potential has not been tested under highly acidic conditions, which is often associated to the presence of As and metals (e.g. acid mine drainage).

The main goal of this research is to investigate the performance of subsurface flow constructed wetlands with different supporting media -gravel, cocopeat, zeolite and limestone- for removal of As from acidic water simulating highly polluted water.

2 MATERIALS AND METHODS

The lab-scale wetland system consisted of twenty subsurface vertical flow wetlands. Each column

had 1 m height and 100 mm internal diameter and was installed in a greenhouse. The wetland columns were divided into four groups, employing gravel, cocopeat, zeolite and limestone as main substrate; each group had five replicate columns. In each wetland column, *Phragmites australis* was planted. Synthetic wastewater simulating the Azufre River, northern Chile—chosen as a case study given its levels of As (Ríos et al. 2011)- was prepared weekly (3.08 ± 0.25 mg/L As, 32 ± 2.19 mg/L boron (B), 107.33 ± 6.53 mg/L iron (Fe), pH 2.0 ± 0.13). Two litres of synthetic wastewater were dosed manually in each wetland, two times per week. Water samples from each wetland column and from the inflow were collected for total and dissolved metals analysis. For the latter, 0.45 µm cellulose acetate filters were used. pH was measured in situ. Weekly composite samples were analysed for As and Fe by ICP-OES or ICP-MS.

3 RESULTS

Table 1 presents the overall performance of the wetland system. Every wetland group was able to remove As. In the outflow from the wetland system, As and Fe were mainly dissolved, except in limestone wetlands where As and Fe were mostly particulate (52% and 94% respectively).

The As levels fluctuated over time, especially in zeolite wetlands where these levels mainly decreased (Fig. 1). Each type of wetland presented a different

Table 1. Mean concentrations of As and Fe in the inflow and outflow from the wetland system.

Wetland group	As (mg/L) [CV*]		Fe (mg/L) [CV]	
	Dissolved	Total	Dissolved	Total
Inflow	3.0 [0.10]	3.1 [0.08]	105 [0.07]	107 [0.06]
Gravel	1.7 [0.18]	1.7 [0.18]	113 [0.07]	116 [0.06]
Cocopeat	2.7 [0.13]	2.8 [0.13]	56 [0.08]	57 [0.09]
Zeolite	0.25 [1.42]	0.258 [1.44]	14.8 [0.47]	15 [0.48]
Limestone	0.010 [1.79]	0.021 [0.67]	0.11 [0.48]	1.85 [0.47]

*CV = coefficient of variation = σ/μ .

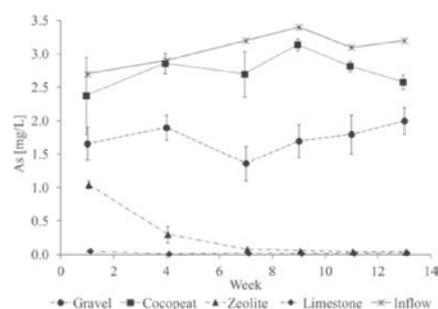


Figure 1. Mean total As concentration in the inflow and outflow from the wetland system. Error bars indicate SD.

capability to remove As and also to affect pH, being the mean values 2.0, 1.8, 2.6 and 6.7 in the outflow from gravel, cocopeat, zeolite and limestone wetlands, respectively.

4 DISCUSSION

The wetland substrate played a key role in the removal of As. The highest removal was found in limestone wetlands, followed by zeolite, gravel and cocopeat wetlands.

The fact that As and Fe were mostly particulate in the outflow from limestone wetlands, plus the positive correlation between total As and Fe (Spearman $\rho = 0.498$, $P = 0.005$), and negative correlation between total Fe and pH (Spearman $\rho = -0.585$, $P = 0.01$) indicate that As was removed by coprecipitation-sorption due to the formation of Fe (oxy) hydroxides. This appears as the main

As removal mechanism. In addition, limestone has also the capacity to remove As by sorption (Romero et al. 2011), which may increase the overall removal capacity of these wetlands. Zeolite wetlands were able to remove both As and Fe despite the low pH, given its high ion exchange capacity.

Conversely, conventional gravel wetlands removed As without removing Fe, and cocopeat wetlands removed Fe without major removal of As; indicating that there are other processes involved -apart from coprecipitation with Fe- that are worthy of examination. Even though it is known that organic matter has affinity for As, cocopeat (used as an alternative to peat as source of organic matter) did not have a particular effect on As removal, which may be explained due to the low pH which disfavours As sorption.

5 CONCLUSIONS

Limestone wetlands presented high As removal efficiency (99%) from acidic water, mainly by precipitating Fe, therefore coprecipitating As. Zeolite also presented remarkable removal, followed by gravel wetlands, whilst cocopeat wetlands presented the lowest removal. Further investigation into the lifespan of the media and role of vegetation and microorganisms is required to gain knowledge to enhance the removal of As in subsurface flow constructed wetlands.

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