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GEDRAG VAN ZWARE METALEN IN AANGELEGDE ZUIVERINGSMOERASSEN

BEHAVIOUR OF HEAVY METALS IN CONSTRUCTED TREATMENT WETLANDS

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Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences: Environmental Technology

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Els, december 2006

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LIST OF ABBREVIATIONS

AGIV	Agentschap voor Geografische Informatie Vlaanderen (Agency for Geographic Information Flanders)
AMD	Acid Mine Drainage
AVS	Acid-Volatile Sulphide
BOD	Biochemical Oxygen Demand
CEC	Cation Exchange Capacity
COD	Chemical Oxygen Demand
CW	Constructed Wetland
DL	Detection Limit
DM	Dry Matter
DO	Dissolved Oxygen
EC	Electrical Conductivity
Eh	Redox Potential
FWS	Free-Water-Surface
GF-AAS	Graphite Furnace Atomic Absorption Spectrometry
HLR	Hydraulic Loading Rate
HRT	Hydraulic Retention Time
HSSF	Horizontal SubSurface Flow
IC	Ion Chromatography
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
OC	Organic Carbon
OM	Organic Matter
PE	Population Equivalent
PT	Pre-Treatment
Q_{14}	Design peak hydraulic loading rate, supplied in 14 hours
RBC	Rotating Biological Contactor
ROL	Radial Oxygen Loss
SEM	Simultaneously Extracted Metals
SRB	Sulphate Reducing Bacteria
SS	Suspended Solids
SSF	SubSurface Flow
TF	Translocation Factor
VLAREA	Vlaams reglement inzake afvalvoorkoming en –beheer (Flemish regulations relating to waste prevention and management)
VLAREBO	Vlaams reglement betreffende de bodemsanering (Flemish regulations concerning soil remediation)
VLAREM	Vlaams reglement betreffende de milieu vergunning (Flemish regulations concerning environmental licences)
VLM	Vlaamse Land Maatschappij (Flemish Land Authority)
VMM	Vlaamse Milieu Maatschappij (Flemish Environment Agency)
VSSF	Vertical SubSurface Flow
WWTP	Wastewater Treatment Plant

GENERAL INTRODUCTION

1 Use of constructed wetlands for pollution control

An unfortunate by-product of urban, agricultural and industrial development is the production of waste streams that need to be disposed of. Rousseau (2005) reviewed the early history of wastewater collection, whereby it was eventually discharged into natural watercourses or wetlands without any treatment. However, limits in the assimilative capacity of natural receiving waters indicated a need to develop technologies that treat wastewater streams before they are being discharged (Kadlec and Knight, 1996). Conventional technologies require concrete, steel, chemicals and energy and may contribute to the depletion of non renewable resources (De Pauw and De Maeseneer, 1992). Moreover, high technological requirements and significant investment, operational and maintenance costs seriously hamper the introduction of conventional wastewater treatment systems in developing countries (Kivaisi, 2001; Sundaravadivel and Vigneswaran, 2001).

The discovery of the purifying capacity of natural wetlands has led to the development of constructed wetland (CW) technologies. The development and world-wide use of these technologies for water quality improvement since the 1950s was reviewed by Kadlec and Knight (1996) and Vymazal (2006). Wetlands, whether natural or constructed, use the nutrients present in the wastewater as resources and transform them into new biomass, soil or gases, thereby relying on natural energies from sun, wind, soil, plants and animals (Bavor et al., 1995; Kadlec and Knight, 1996). Phytoremediation, the use of plants for the treatment of contaminated soils or waters, has also gained increased attention from researchers and public opinion over the last decades (Dushenkov et al., 1995; Salt et al., 1995). Plant-based wastewater purification systems can offer a cheap and sustainable alternative for conventional approaches (Brix, 1994; EPA, 2000; Kivaisi, 2001; Scholz and Xu, 2002). Among the different systems, constructed wetlands overcome the disadvantages of natural wetlands by better hydraulic control and management (Lim et al., 2001).

Constructed wetlands are successfully used for different types of wastewater, including domestic wastewater (Vymazal, 2003; Rousseau et al., 2004), agricultural wastewater (Mantovi, 2003; Meers et al., 2005), industrial wastewater (Dunbabin and Bowmer, 1992), mine drainage (Wieder, 1989), stormwater (Carleton et al., 2001; Walker and Hurl, 2002; Van de Moortel et al., 2006) and landfill leachate (Martin et al., 1999; Bulc, 2006). Heavy metals are present in virtually all types of wastewater, ranging from very low baseline levels to elevated and toxic concentrations. In domestic wastewater, metal concentrations are generally low. On the contrary, metals can be primary pollutants in industrial, mining and landfill effluents. Industrial activities that lead to significant discharges of metals into the environment include the production of chemicals, pulp and paper, textiles, petroleum and coal, metals, machinery, etc. Heavy metals are persistent and accumulate in natural watercourses after discharge of wastewater, thereby leading to potential toxicity in receiving ecosystems and eventually humans (Kadlec and Knight, 1996). Whether designed for the treatment of domestic wastewater or metal-laden industrial effluents, CWs should prevent the discharge of heavy metals into the natural environment.

2 Removal of heavy metals in constructed wetlands

Heavy metals are effectively removed in CWs by a combination of physico-chemical and biological removal processes (Dunbabin and Bowmer, 1992; Kadlec and Knight, 1996). Despite a lot of recent research efforts, the understanding of the complex removal processes, how they interact and which are the major influencing factors, is still rather incomplete. A reason for this is that CWs depend on the interaction of many different components, including the substrate, sediment, vegetation and water column (Stottmeister et al., 2003). Natural biogeochemical processes can be involved in the removal of heavy metals from the wastewater in CWs. The way in which a wetland is constructed largely determines how wastewater treatment occurs and which mechanisms are responsible (Kosolapov et al., 2006).

Constructed wetlands in Flanders are mostly planted with common reed and used for the secondary treatment of domestic and dairy wastewater. They act as small-scale or individual treatment systems in areas where connection to the centralised sewer system is not economically feasible (VMM, 2001; Rousseau et al., 2004). There are currently no effluent standards for heavy metals in conventional and small-scale wastewater treatment plants (WWTPs) for domestic wastewater. Heavy metal concentrations in the influent and effluent of these WWTPs nevertheless must be monitored by law but they currently do not have to comply with any standards and are purely of informative nature (VLAREM II, 1995). Only few researchers have investigated the accumulation of metals in sediments and plants of CWs

(Gschlößl and Stuible, 2000; Obarska-Pempkowiak, 2001; Vymazal, 2003; Vymazal and Krása, 2003, Chagué-Goff, 2005). Before the start of this study, heavy metals in the influent and effluent of Flemish CWs have not intensively been monitored, and their accumulation in the sediments and plants had never been subject of research.

Whereas CWs are mainly used for domestic and dairy wastewater in Flanders, they have also been proposed as low cost and low maintenance treatment alternatives or as additional treatment steps for industrial effluents (Dunbabin and Bowmer, 1992). Nowadays, industries in Flanders remove heavy metals from wastewater by means of physico-chemical remediation technologies such as precipitation. However, regulatory standards are not always met and in many cases taxes are paid on the load of heavy metals. Both ecological and economical factors drive to a search for additional polishing or tertiary treatment steps to remove the marginal concentrations of heavy metals that can not be removed by classical physico-chemical methods (Brown et al., 2000; Diels et al., 2003). Qian et al. (1999) and LeDuc and Terry (2005) stated that CWs are highly efficient in removing low levels of metals from large volumes of wastewater.

When designed to remove heavy metals from industrial effluents, two approaches can be distinguished. In a first approach, CWs are designed to immobilise heavy metals in the substrate by processes of sorption and precipitation. The efficiency of these systems in metal removal has been described by Dunbabin and Bowmer (1992). The second approach is one in which metals are removed from the wastewater by accumulation in aquatic macrophytes that are regularly harvested. Floating plants that offer promising results with regard to metal removal from wastewater are *Lemna* spp. and *Eichhornia crassipes* (Zayed et al., 1998; Zhu et al., 1999). Submerged plants, such as *Potamogeton* spp. and *Myriophyllum* spp., have also been suggested as useful species for ‘polishing’ secondarily treated wastewaters as they are completely inundated and can extract metals from the water. However, work on heavy metal uptake by submerged aquatic plants is very scarce (Guilizzoni, 1991; Keskinkan, 2005).

3 General outline of the PhD thesis

Current work focuses on the behaviour of heavy metals in constructed treatment wetlands. The first chapter presents a comprehensive literature review. The experimental work of this study can be subdivided into three major parts:

- **The first part** deals with the behaviour of heavy metals in CWs for treatment of domestic wastewater in Flanders. Field studies were performed to increase insights into the removal of heavy metals in CWs and the accumulation of heavy metals in sediments and plants;
- **The second part** handles about the effects of different helophyte species on the removal of heavy metals in CWs and the differences in uptake and translocation patterns of metals in the selected helophytes, and is based on an experimental approach;
- **The third part** focuses on the potential of CWs for tertiary treatment of industrial wastewater. By means of experiments, two distinct types of CWs were evaluated: (i) subsurface flow CWs planted with the helophyte *Phragmites australis* (Cav.) Trin. ex Steud. (Common reed), aiming at removing heavy metals by immobilisation in the substrate and (ii) free-water-surface CWs planted with the hydrophyte *Myriophyllum spicatum* L. (Eurasian water milfoil), focusing on the removal of heavy metals by harvesting the plant biomass.

After presenting an overview of the different types of CWs used for wastewater treatment, **Chapter 1** reviews the most important physico-chemical and microbial removal processes of heavy metals and discusses the uptake of heavy metals by aquatic plants. The indirect effects of plants are briefly described. The effects of some important design parameters, including the selection of the substrate and vegetation type, on the removal mechanisms of heavy metals is discussed. At the end, Chapter 1 presents the major conclusions of the literature review.

In **Chapter 2**, the removal of heavy metals in different types of CWs treating domestic wastewater in Flanders is assessed. A long-term dataset of influent and effluent concentrations of heavy metals in 12 CWs was obtained from Aquafin NV and examined in order to (1) study the concentrations of heavy metals in the raw wastewater and effluents of

CWs and evaluate the removal of heavy metals, (2) assess long-term dynamics, seasonal and yearly trends and (3) make comparisons among CWs. Four different types of CWs were then selected and monitored weekly in more detail for total metal concentrations in the raw wastewater, the influents of the CWs (thus after pre-treatment) and the effluents. The regular weekly sampling campaign increased the chance of detecting temporal trends and allowed for a better study of long-term dynamics. Moreover, the monitoring of the raw wastewater and the influent of the CWs allowed to separately assess the contribution of the pre-treatment step and the CW itself in the overall metal removal. Finally, the influent and effluent of one of the studied CWs were subjected to a short-term high-frequent monitoring campaign to study the short-term dynamics.

After demonstrating in Chapter 2 that an efficient removal of heavy metals in CWs for domestic wastewater treatment was obtained, in Chapters 3 and 4 their accumulation in the sediments and plants of two distinct types of CWs was studied. In **Chapter 3**, a free-water-surface (FWS) CW was considered that had been in operation for a period of 16 years. In **Chapter 4**, a gravel-based horizontal subsurface flow (HSSF) CW that had been in operation for 3 years was subject of the study. Both CWs were planted with *Phragmites australis* and treated a mixture of domestic wastewater and stormwater. The sediment and reed biomass were sampled at different locations of the CWs in order to assess spatial variations. Total metal levels in the sediment were determined to characterise the pollution level after short- and long-term operation. The accumulation of metals in the reed biomass at the end of the growing season was investigated and the relationship with the metal levels in the sediment was evaluated. Moreover, the relative contribution of metal accumulation in the aboveground reed biomass in the overall metal removal was assessed.

Constructed wetlands in Flanders are mainly planted with common reed. Considering that quantitative information comparing growth, removal performance and differences in uptake and translocation patterns of different helophyte species is scarce, focus was given in **Chapter 5** on analysing different species of helophytes. The aim of this chapter was to (1) assess and compare the contribution of different helophyte species in relation to the removal of metals from domestic wastewater in HSSF CWs and (2) study differences in uptake and translocation patterns of metals in the selected helophyte species. To this purpose, the removal of heavy metals was analysed in gravel-based HSSF CW microcosms planted with 4 different helophyte species: *Phragmites australis* (Cav.) Trin. ex Steud. (Common reed), *Juncus effusus* L. (Soft rush), *Carex acutiformis* Ehrh. (Lesser pond sedge) and *Iris pseudacorus* L. (Yellow flag).

In Chapters 6 and 7, the potential of CWs as tertiary treatment steps for industrial effluents was approached by means of small-scale experiments. A secondary treated effluent from a non-ferro metal industry in Flanders was used in the study. **Chapter 6** assessed the potential of gravel-based subsurface flow (SSF) CWs planted with *Phragmites australis*. Next to studying the removal kinetics of heavy metals, special attention was given to elucidating the removal mechanisms. The processes of sorption onto the substrates and precipitation of metals with sulphides were studied in detail. The addition of a carbon source in order to enhance bacterial sulphate reduction and metal removal was also investigated. Finally, the effects of *Phragmites australis* were taken into consideration. The objectives of **Chapter 7** were to assess the tolerance of the submerged aquatic plant *Myriophyllum spicatum* in FWS CWs for the industrial effluent, to assess the removal kinetics and to evaluate the uptake levels in the biomass.

To conclude this work, the insights from the previous chapters are incorporated in a general discussion, after which the major conclusions from this study are drawn and future perspectives presented.

CHAPTER 1

REMOVAL OF HEAVY METALS IN CONSTRUCTED WETLANDS: A REVIEW

1 Types of constructed wetlands

Natural systems for wastewater treatment cover a wide range and are classified into three categories according to their location in the landscape and the saturation state of the soil: (1) upland or terrestrial systems, (2) wetland systems and (3) deeply flooded or aquatic systems. Aquatic systems are open-water, pond-like systems that are dominated by algae or floating, submerged or emergent plant species. In terrestrial systems, the unsaturated soil has a dual role, firstly serving as a filtration medium for pollutant removal and secondly functioning as a rooting medium for plant growth. The major terrestrial systems are on-site infiltration and low and high rate land systems (Kadlec and Knight, 1996). Wetlands, which are the transitional areas between terrestrial and aquatic ecosystems, are inundated during the whole or part of the year and are dominated by vegetation adapted to these waterlogged conditions (Price and Probert, 1997).

Constructed wetlands (CWs) are designed to provide wastewater treatment and are the engineered equivalent of their natural version. They are man-made wetlands constructed in areas where they do not naturally occur. Wetlands can provide additional benefits such as creation of controlled flooding areas, creation of habitats for fauna and flora, food production, recreational use, etc. (Sundaravadivel and Vigneswaran, 2001). Constructed wetlands that are used for primary and secondary wastewater treatment are often called constructed treatment wetlands, whereas constructed wetlands which provide advanced treatment of secondary treated wastewater and other benefits such as wildlife habitat and recreational use are often called enhancement wetlands (EPA, 2000).

Constructed wetlands are classified into the following different types according to the water flow mode and the dominant aquatic plant species (Kadlec et al., 2000) (Fig 1.1):

1) Free-Water-Surface (FWS) Constructed Wetlands: with the water flow above ground; categorised into the following subtypes according to the dominant plant species:

- Emergent aquatic macrophytes or helophytes: rooted in a substrate or floating in a mat; e.g. *Phragmites australis*, *Typha* spp., *Juncus* spp., *Carex* spp., etc.
- Floating aquatic macrophytes or pleustophytes: floating-leaved, bottom-rooted; e.g. *Nymphaea* spp., *Nelumbo* spp., etc., or free-floating; e.g. *Eichhornia crassipes*, *Lemna* spp., etc.
- Submerged aquatic macrophytes or hydrophytes: e.g. *Elodea* spp., *Myriophyllum* spp., *Potamogeton* spp., etc.

2) Subsurface Flow (SSF) Constructed Wetlands: with the water flow below ground; categorised into the following subtypes according to the water current direction:

- Horizontal SSF: planted with helophytes; e.g. *Phragmites australis*, *Juncus* spp., etc.
- Vertical SSF: planted with helophytes; e.g. *Phragmites australis*, *Juncus* spp., etc.

Free-water-surface CWs consist of shallow basins that are provided with a liner or a layer of clay to prevent contamination of the groundwater. A layer of soil or sediment supports growth of rooted macrophytes. The wastewater is directed horizontally through a dense mass of aquatic macrophytes. Horizontal subsurface flow CWs consist of shallow basins provided with a liner and filled with a granular medium such as gravel or sand, or even soil. Coarse gravel is often used in the inlet and outlet area to obtain optimum flow distribution. A drainage tube at the bottom of the outlet area allows the evacuation of effluent. Vertical subsurface flow CWs consist of one or more layers of a granular medium such as coarse sand and/or gravel, or soil. Wastewater is spread over the top surface, percolates through the filter layers and is then collected at the bottom by means of drainage tubes. The above-mentioned types of CWs can be combined with each other or with conventional wastewater treatment systems, to improve the quality of the effluent (Brix, 1994; Greenway and Simpson, 1996; Kadlec and Knight, 1996; EPA, 2000; Shutes, 2001).

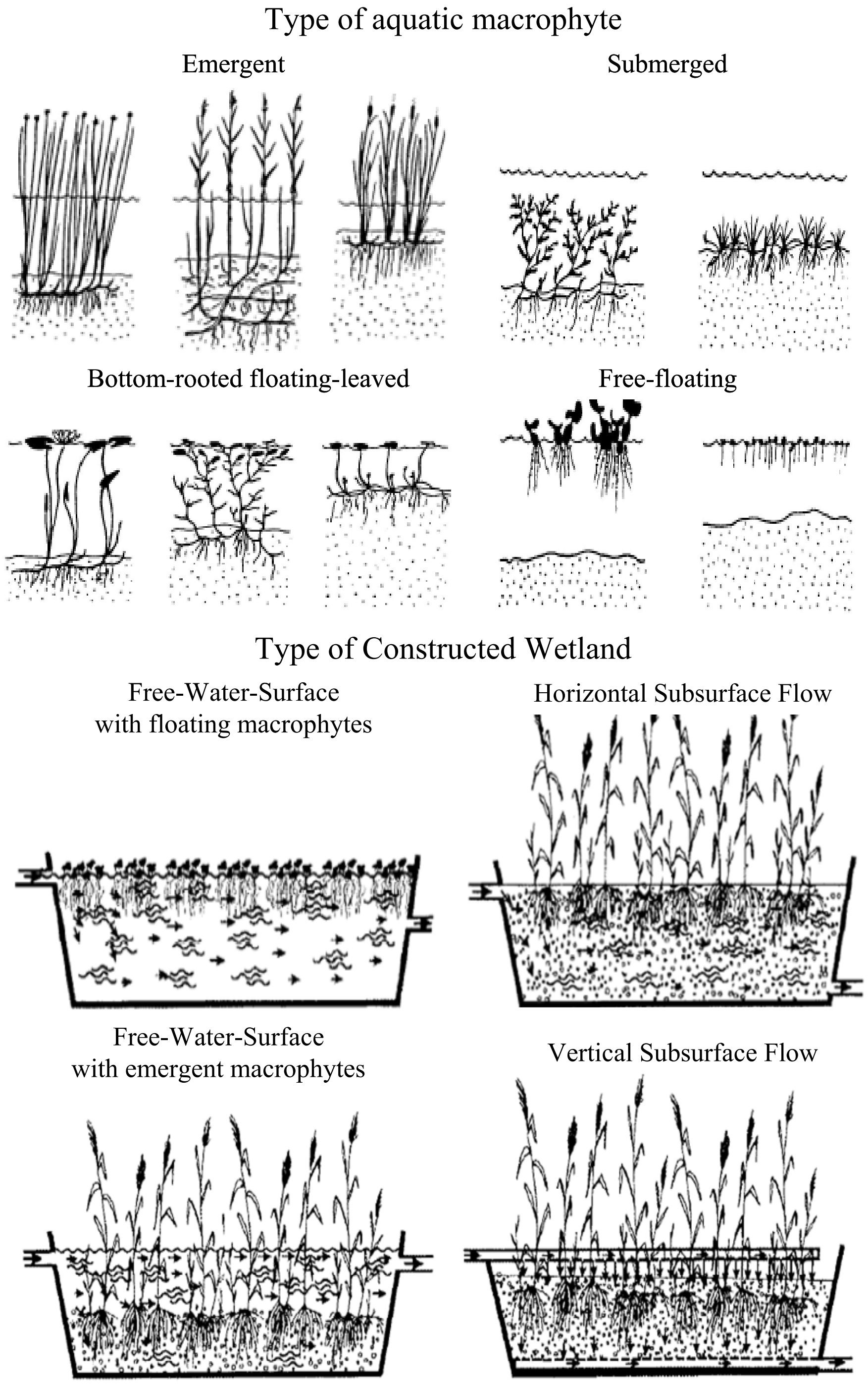

Fig 1.1 Different types of aquatic macrophytes and constructed wetlands (adapted after Vymazal, 1998; Stottmeister et al., 2003).

2 Removal of heavy metals in constructed wetlands

This section reviews the removal processes of heavy metals in constructed wetlands. Kadlec and Knight (1996) reported a wide scatter of the literature information about heavy metals in CWs. Whereas heavy metals have been subject of many research studies in CWs receiving mine drainage since the late 1980s (Wieder, 1989; Dunbabin and Bowmer, 1992), their behaviour in CWs for domestic wastewater treatment has for long received little attention (Vymazal, 2003). However, growing awareness of the consequences of long-term metal accumulation in CWs for domestic wastewater treatment has led to increased research efforts in this area over the last decade.

Research attempts have been made to increase insight in the metal removal processes, merely by two approaches: (1) monitoring of metal accumulation in the compartments of full-scale CWs and (2) studying removal processes, influencing factors and distribution of metals in the more controlled environment of laboratory and pilot-scale experiments. Experiments in controlled environments are necessary to understand metal removal processes and lead to the development of better design criteria (Song et al., 2001). The latter is especially important for systems receiving higher metal loads. Therefore, in most of the laboratory and pilot-scale experiments wastewaters with high metal concentrations such as mine drainage have been tested.

When heavy metals enter a CW, they are distributed among its compartments with the main pools being the substrate, the water column and the vegetation (Dunbabin and Bowmer, 1992; Sheoran and Sheoran, 2006). Kadlec and Knight (1996) described the following major processes responsible for the removal of heavy metals in CWs: (1) binding to the substrate, particulates and soluble organics, (2) precipitation as insoluble salts, mainly sulphides and (oxy-) hydroxides and (3) uptake by plants and micro-organisms. There are only minor gaseous removal pathways for some metals including Hg, Se and As. In their review of heavy metal removal mechanisms in CWs treating acid mine drainage (AMD), Sheoran and Sheoran (2006) have subdivided the mechanisms into physical, chemical and biological processes although the authors emphasised that the processes are dependent on each other. This section will review the most important physico-chemical and microbial removal processes of heavy metals, discuss the uptake of heavy metals by aquatic plants and conclude with the indirect effects of plants. The effects of some design parameters on the removal mechanisms of heavy metals will be discussed in the next section.

2.1 Physico-chemical removal mechanisms of heavy metals

Major physico-chemical removal processes of heavy metals occurring in CWs include (1) sedimentation and filtration, (2) sorption and (3) precipitation and co-precipitation. Most researchers investigating metal removal in CWs have focussed on the total removal without distinguishing between different removal mechanisms. However, recently there have been some attempts to study the relative importance of different removal mechanisms.

2.1.1 Sedimentation and filtration

Sedimentation and filtration are important physical processes allowing the removal of heavy metals associated with particulate matter. Sedimentation has long been recognised as a principle process in the removal of heavy metals from wastewaters (Sheoran and Sheoran, 2006).

2.1.2 Sorption

Sorption of heavy metals on a surface can occur by processes of adsorption and precipitation. Heavy metals may be adsorbed either electrostatically, resulting in the formation of relatively weak complexes (physical adsorption) or chemically, resulting in the formation of strong complexes (chemisorption). Metals are more strongly bound by chemisorption than by physical adsorption (Evangelou, 1998). Metals that are adsorbed on the surface of the substrate can be exchanged by other cations by the process of cation exchange.

2.1.3 Precipitation and co-precipitation

Next to these sorption and sedimentation reactions, metals can also precipitate with (oxy-) hydroxides, sulphides, carbonates, etc. when solubility products are exceeded (Kadlec and Knight, 1996). The stability of metals precipitated as inorganic compounds is primarily controlled by pH. At near-neutral or alkaline pH, metals are effectively immobilised (Gambrell, 1994). Bacterial production of bicarbonate by bacterial sulphate reduction, or the presence of limestone in the substrate, can lead to sufficiently high bicarbonate levels to form precipitates with metals. Metal carbonates are less stable than metal sulphides but can have a role in the initial trapping of metals (Sheoran and Sheoran, 2006).

Hydrolysis and/or oxidation of metals lead to the formation of (oxy-) hydroxides. The solubility of these oxides is very low in the range of pH normally encountered in most substrates (Evangelou, 1998). Dissolved reduced forms of Fe(II) are oxidised by abiotic reactions and bacteria and then precipitated mainly as hydroxides, a reaction which increases the acidity of the wastewater (Evangelou, 1998; Kosolapov et al., 2006):

$$Fe^{2+} + H^+ + \frac{1}{4}O_2 \rightarrow Fe^{3+} + \frac{1}{2}H_2O$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3\downarrow + 3H^+$$

After the oxidation of Fe(II), Mn(II) is oxidised to Mn(IV) which is mainly precipitated as MnO_2 but can also be precipitated as $Mn(OH)_2$. Iron and Mn precipitate sequentially because the oxidation of Mn is inhibited by the presence of Fe(II) (Kosolapov et al., 2006). Co-precipitation of metals with Fe- and/or Mn- (oxy-) hydroxides is an additional important removal pathway in oxidised substrates. Co-precipitation with Fe- and/or Mn- (oxy-) hydroxides is however not considered as a long-term removal mechanism as it is redox sensitive (Sheoran and Sheoran, 2006). These (oxy-) hydroxides are unstable in reducing conditions leading to a release of Fe, Mn and co-precipitated metals.

In anaerobic conditions and the presence of an organic carbon source, sulphate is reduced by sulphate reducing bacteria (SRB) and sulphides are formed. Sulphides then react with divalent metals to form insoluble metal sulphide precipitates (Gambrell, 1994; Evangelou, 1998; Kosolapov et al., 2006):

$$SO_4^{2-} + 2CH_2O \rightarrow H^+ + HS^- + 2HCO_3^-$$

$$H^+ + HS^- \rightarrow H_2S\ (g)$$

$$HS^- + M^{2+} \rightarrow MS\downarrow + H^+$$

With CH_2O and M^{2+} representing respectively a simple organic compound and divalent metal ion. The process of sulphate reduction buffers the pH of the solution.

2.1.4 Field evidence of physico-chemical removal processes

Walker and Hurl (2002) studied the relative importance of sedimentation in the overall removal of metals in a FWS CW for stormwater treatment. Heavy metals in stormwater tend to be associated with particulate matter, leading to their deposition within that section of the CW located close to the inlet area. The importance of sedimentation and filtration was also demonstrated in an experiment of Lim et al. (2001). They studied the removal of Cu in laboratory-scale FWS and HSSF CWs that were either planted with *Typha angustifolia* or left unplanted. Systems received primary treated sewage that was spiked with increasing Cu levels. As the dissolved Cu fractions accounted for only 3 to 6 % of the influent Cu load, sedimentation and filtration were the major removal mechanisms.

In most studies of the distribution of heavy metals in full-scale CWs for domestic wastewater, it was observed that heavy metals mainly accumulated in the substrate and sediment situated nearest to the inlet area. Gschlößl and Stuible (2000) described significant accumulation of Cu and Zn in the sand and gravel substrate of two HSSF CWs treating domestic wastewater after 10 years of operation, especially in the influent part of the beds and in the organic layer on the substrates surface. Vymazal (2003) and Vymazal and Krása (2003) studied the accumulation of metals in the sediments of gravel-based HSSF CWs treating domestic wastewater and stormwater after 3 years of operation and also observed the highest metal concentrations in the sediment of the inlet area and a general decrease along the longitudinal profile of the bed. Although the relative importance of different metal removal processes could not be quantified in these full-scale studies, it was suggested that they led to higher metal accumulation within the section situated nearest to the inlet area. This was thought to be attributed to (1) sedimentation and filtration of particulate matter and associated metals, (2) sorption of metals onto organic matter and (3) metal sulphide precipitation due to the input of organic matter and the creation of reducing conditions close to the inlet area.

O'Sullivan et al. (2004a, b) reported the efficient removal of Fe, Pb and Zn by means of bacterial sulphate reduction in 2 experimental FWS CWs planted with *Typha latifolia*, designed to treat effluents from an active Pb/Zn-mine. The process of bacterial sulphate reduction and precipitation of metals with sulphides was evidenced by (1) better removal of Pb and Zn after maturation of the CW, (2) efficient removal of SO_4^{2-} from the wastewater, (3) highly reduced conditions in the sediment, in the range of – 300 mV and (4) sequential extraction of the sediment which demonstrated that metals were mainly in the residual form, e.g. sulphide-bound. Higher metal concentrations in the substrates were observed close to the inlet area of the CWs. Sobolewski (1996) also suggested that metal sulphide precipitation is the major long-term removal process in pilot-scale peat-based CWs treating wastewater from a former open pit Cu mine. Both mineralogical analysis through scanning electron microscopy and sequential extraction of peat samples, have shown that CuS was present. However, Sobolewski (1996) also showed that other processes such as binding with the organic phase were important.

Overall, it is concluded that physico-chemical removal of metals in CWs occurs through a complex array of processes. The elucidation of these processes in full-scale CWs is a difficult task. However, quantifying these metal removal processes and assessing changes with time are important for predicting the long-term removal performance of CWs.

2.2 Biological removal mechanisms of heavy metals

2.2.1 Microbial removal processes

Micro-organisms affect the behaviour of heavy metals in constructed wetlands by (1) their role in the biogeochemical cycles which affect metal speciation, (2) biosorption, (3) reduction and (4) methylation of heavy metals (Kosolapov et al., 2006).

2.2.1.1 Bacterial sulphate reduction and oxidation of Fe and Mn

Micro-organisms govern biogeochemical cycles that immobilise heavy metals in CWs and thereby indirectly induce their removal. The main processes which lead to an immobilisation of metals are sulphate reduction and Fe- or Mn-oxidation, leading respectively to the (co-) precipitation of metals with sulphides and Fe- or Mn- (oxy-) hydroxides, important physico-chemical removal mechanisms that were described in section 2.1.3.

2.2.1.2 Biosorption of heavy metals

The microbial biomass can also sequester metals by the processes of active (energy-dependent) and passive (energy-independent) metal uptake, respectively called bioaccumulation and biosorption (Kosolapov et al., 2006). Veglio and Beolchini (1997) described the following biosorption mechanisms in their review: (i) transport across the cell membrane, (ii) precipitation, (iii) ion exchange, (iv) complexation and (v) physical adsorption. Some bacteria are also able to accumulate metals inside their cells, forming amorphous mineral inclusions. However, the storage of metals by the microbial biomass is relatively short-term due to the short life cycle of micro-organisms implying the need for additional removal pathways (Kosolapov et al., 2006).

Scholz et al. (2001), Scholz and Xu (2002) and Scholz (2003) studied Cu and Pb removal from simulated pre-treated minewater in laboratory-scale VSSF CWs packed with different media of varying adsorption capacity and cost. Different types of cheap gravel and sand were tested, next to more expensive filter media with a higher adsorption capacity including filtralite (a light expanded clay aggregate), granular activated carbon and charcoal (a carbonaceous adsorbent). The authors demonstrated that sorption onto the mature biofilm was responsible for the high performance of all systems tested. The addition of expensive adsorptive media did not enhance metal removal. Similar conclusions were derived by Lee et al. (2006) in their study of Cu and Ni removal in laboratory-scale VSSF CWs packed with different filter media.

2.2.1.3 Reduction of heavy metals

Anaerobic metal-reducing bacteria use metals as terminal electron acceptors in their anaerobic respiration. Depending on the metal species involved, this can lead to a mobilisation or immobilisation of metals. The reduction of the soluble and toxic Cr(VI) to Cr(III) results in its precipitation with hydroxides and efficient removal from wastewater. Reduction of Cr(VI) is performed by a variety of micro-organisms, including some sulphate reducing bacteria (SRB), and can occur under oxic and anoxic conditions.

Whereas the reduction of Cr(VI) leads to its effective immobilisation and retention in the substrates of CWs, reduction of other metals including Hg, Fe and Mn enhance their mobility and release from the wetland system. The reduction of Hg(II) results in the release of elemental Hg to the atmosphere. This strategy has been successfully applied to remediate Minamata Bay sediments. However, there are public concerns about atmospheric pollution. Iron- and Mn-reducing bacteria can dissolve insoluble Fe(III)- and Mn(IV)- (oxy-) hydroxides, resulting in the release of soluble Fe(II) and Mn(II), as well as the trace metals co-precipitated with these (oxy-) hydroxides. Intermediate redox conditions are detrimental to CW efficiency as they transfer metals to their most soluble state (Kosolapov et al., 2006).

2.2.1.4 Methylation of heavy metals

Some metals, including Hg, As and Se, can be biomethylated by aerobic and anaerobic bacteria resulting in the production of volatile derivatives such as dimethylmercury, dimethylselenide or trimethylarsine (Salt et al., 1995; Kosolapov et al., 2006). These volatile derivatives are generally more toxic than the inorganic metal species, because of their lipophilic character. Another toxic product of the methylation of Hg is methylmercury (CH_3Hg), a lipophilic product that is easily accumulated by aquatic organisms and transferred into the food chain. Some SRB have been identified to methylate Hg, implying the need for control when the process of sulphate reduction is aimed at to treat wastewaters that are also contaminated by Hg. However, the precipitation of HgS has been described to reduce Hg toxicity (Kosolapov et al., 2006).

2.2.2 Uptake of heavy metals by aquatic macrophytes

Salt et al. (1995) compared plants to solar driven pumps which can extract and concentrate certain elements from their environment. Aquatic macrophytes depend on the micronutrients available in the water and/or substrate, in order to meet their nutritional demands. Among the micronutrients that are required for the growth and development of all higher plants, the following heavy metals are encountered: Fe, Mn, Zn, Cu, Mo and Ni (Welch, 1995). Certain plants can also accumulate metals with no known biological function, including Cd, Cr, Pb, Co, Ag, Se and Hg (Salt et al., 1995). This section will focus on the general uptake mechanisms of heavy metals by aquatic macrophytes, whereas more information about metal accumulation in different aquatic plant species is presented in section 3.2.

Uptake of heavy metals by aquatic macrophytes is dependent on the life form of the macrophyte: rooted emergent, floating or submerged (Guilizzoni, 1991). For emergent macrophytes, root uptake is the primary source of metals. Floating and submerged species however, can also accumulate metals directly from the water by their shoots. Elucidation of a single metal uptake pathway is difficult without the use of tracers, and a comparison of several uptake patterns simultaneously is even more complex (Crowder, 1991). Radioactive tracers have demonstrated that most rooted aquatic macrophytes take up metals primarily from the sediment pore water, although some uptake may also occur from the water. For free-floating macrophytes the water is the only source of metals (Jackson, 1998).

2.2.2.1 Uptake of heavy metals by the roots

Plants take up elements in their ionic form. Aquatic macrophytes which root in the substrate, rely on the soluble metal fraction which is affected by parameters including pH, redox potential (Eh) and organic matter content of the substrate (Gambrell, 1994; Jackson, 1998). Plant roots can mobilise metals by various strategies, including (i) the excretion of metal-chelating molecules (phytosiderophores) that solubilise metals, (ii) the plasma membrane bound metal-reductases that reduce metal ions and (iii) the excretion of protons (Salt et al., 1995; Meers, 2005).

Solubilised metal ions may enter the roots by extracellular (apoplastic) or intracellular (symplastic) pathways. Most metal ions enter plant cells by specific metal ion carriers or channels. After entering the roots, metals are either stored or transported to the shoot. Transport to the shoot probably occurs in the xylem and metals can be redistributed in the shoot via the phloem. To enter the xylem, metals must cross the casparian strip that divides the endodermis from the epidermis. Metals must move symplastically to cross this strip. The symplastic transport within the endodermis is a rate limiting step. Xylem cell walls have a high cation exchange capacity which would severely retard the movement of metal cations to the shoot. Metals in the xylem and phloem may be transported chelated to organic acids, phytochelatins or metallothioneins (Raven et al., 1992; Salt et al., 1995).

Non-linear removal kinetics of heavy metals have been reported, suggesting that rooted aquatic macrophytes use several removal mechanisms. Sorption by the root is probably the fastest component of metal removal including processes such as physical adsorption and chemisorption (chelation and ion exchange). Biological processes including intracellular uptake and translocation to the shoots, and root-mediated precipitation are probably responsible for the slower components of metal removal (Salt et al., 1995; Maine et al., 2001, 2004). Keskinkan et al. (2003) referred to these processes of sorption and uptake as biosorption and bioaccumulation, as used to describe metal uptake by bacteria (section 2.2.1.).

Studies in many natural and constructed wetlands have demonstrated similar allocation patterns of metals in rooted emergent macrophyte species, with most plants having higher concentrations of metals in their belowground biomass than in their shoot tissues (Weis and Weis, 2004). Restricted translocation of metals to the shoots is at the basis of this finding.

2.2.2.2 Uptake of heavy metals by the shoots

For floating or submerged macrophytes, plants of which the shoots are in close contact with the water or are completely inundated, roots are not the sole entry points of metals. According to Guilizzoni (1991) and Keskinkan (2005), there is scarce study about heavy metal uptake by submerged and floating aquatic plants, in particular about the relative importance of shoot/root uptake. Roots of submerged aquatic macrophytes were initially thought to act as holdfasts whereas element uptake was mainly through the leaves. This was suggested by early studies which showed a good correlation between metal concentrations in the leaves and the surrounding water. For aquatic macrophytes that have roots but do not have a close association with the sediment, the water is probably the principal source of elements.

However, the situation for submerged macrophytes with a well-developed root-rhizome system and submerged foliage such as *Myriophyllum* and *Potamogeton*, is much more complex (Guilizzoni, 1991). Studies with radioactive tracers showed that elements are mainly taken up by the roots from the sediment (Jackson, 1998). Guilizzoni (1991) hypothesised that the water is the dominant source of elements when dissolved metal concentrations in the water are high or when metals are not readily available in the sediment.

This hypothesis was supported by Maine et al. (2004) who studied the causes of the increase of the Cr concentration in the shoots of the floating macrophytes *Pistia stratiotes* and *Salvinia herzogii*, when exposed to Cr-contaminated solutions. In an attempt to address whether (1) translocation from the roots to the shoots or (2) direct uptake by the shoots through contact with the solution, was the responsible mechanism, the experimental set-up included plants of which the aerial parts were exposed to the water and plants of which the aerial parts were separated from the water by means of thin sheets of polystyrene. The increase of the Cr concentration in the shoots was mainly attributed to the direct contact between the shoots and the water, and not by translocation.

Guilizzoni (1991) suggested that the uptake of ions by submerged leaves involves a binding step to the cell membrane and a transfer inside. Cations enter the abaxial epidermis by means of a multiple mechanism of passive (diffusion) or active uptake. After uptake, metals are translocated in different plant parts. It still remains unclear what factors govern root or shoot absorption and by what mechanisms the translocation occurs.

2.2.2.3 Tolerance of aquatic macrophytes

Resistance to toxic effects of metals is either performed by (i) limiting their cellular uptake (avoidance), (ii) detoxify metals once they enter the cells or (iii) develop metal resistant metabolisms. Evidence for avoidance of metal toxicity by reduced cellular uptake is very limited. Detoxification can occur by chelation, compartmentalisation or precipitation. Some metals may be chelated by organic acids or phytochelatins and accumulated within the vacuole (Salt et al., 1995).

The information about metal tolerance in wetland plants is still incomplete, although the suggestion to date is that some aquatic macrophyte species have an inherent tolerance mechanism instead of evolving tolerance when exposed to a metal-contaminated environment. This conclusion was based on the finding of several researchers that the origin of some wetland plant species, metal-contaminated sites or control sites, does not affect their tolerance when cultured in metal-contaminated solutions. This was observed for *Phragmites australis*, *Typha latifolia*, *Eriophorum angustifolium* and *Juncus effusus* (Taylor and Crowder, 1983a; Ye et al., 1997, 2003; Matthews et al., 2004).

2.2.3 Indirect effects of plants on metal removal

Aquatic macrophytes are an integral part of CWs, their interaction with the substrate and micro-organisms playing an important role (Dunbabin and Bowmer, 1992; Williams, 2002). Aquatic macrophytes can indirectly affect metal removal mechanisms in CWs by means of (1) the radial oxygen loss (ROL) in the rhizosphere, (2) the excretion of protons and exudates and (3) the input of decaying plant material. However, it has to be mentioned that more research is needed to assess the importance of the abovementioned indirect effects on metal removal in CWs. Most research involved laboratory-scale experiments, whereas field evidence of indirect effects of macrophytes on metal removal is very scarce.

2.2.3.1 Radial oxygen loss

Aquatic macrophytes are adapted to waterlogged conditions by means of transporting oxygen to the roots through their aerenchyma tissue (Armstrong et al., 1992, 1996). The flux of oxygen from the roots to the rhizosphere is called radial oxygen loss (ROL). This oxygen transport is essential for respiration and it causes the formation of an oxidative protective film, also called ‘plaque’, on the root surface. Oxidation of the rhizosphere is especially important in SSF CWs of the horizontal flow type as it creates aerobic microsites within the otherwise predominantly anaerobic substrate (Stottmeister et al., 2003). The plaques are mainly composed of Fe- (oxy-) hydroxides and other metals that are co-precipitated on the surface. There are conflicting reports as to whether the presence of plaques reduces or increases the uptake of metals by the plants (Weis and Weis, 2004). The presence of an Fe plaque on the roots of *Phragmites australis* plants was detected by Peverly et al. (1995) and Mantovi et al. (2003) in full-scale HSSF CWs, by means of scanning electron microscopy of root cross sections.

In CWs where metal sulphide precipitation is an important removal mechanism, the presence of aquatic macrophytes can negatively affect the metal removal efficiency due to the ROL by their roots. Borden et al. (2001) and Stein and Hook (2005) reported that the presence of *Scirpus acutus* or *Typha latifolia* negatively affected the removal of Zn in laboratory-scale SSF CWs in winter conditions. The authors related this seasonal effect of plants to the presence of more oxidised conditions, as evidenced by a higher redox potential, limiting sulphate reduction and precipitation of Zn with sulphides. O'Sullivan et al. (2004a, b) demonstrated less sulphate removal during the growing season of *Typha latifolia* in experimental FWS CWs treating effluents from a Pb/Zn mine and related this to the temporarily oxygenation of the substrates by plant growth. García et al. (2003) observed a decrease of the Eh with depth from the surface in gravel-based HSSF CWs treating urban wastewater. Higher redox potentials near the surface were attributed to oxygen diffusion from the atmosphere and oxygen supply from *Phragmites australis*.

2.2.3.2 Excretion of protons and exudates

Plant roots can excrete protons and exudates in the rhizosphere to acidify and mobilise metals (Salt et al., 1995; Weis and Weis, 2006). However, root exudates can also enhance metal removal in CWs by affecting the redox potential, as demonstrated by Buddhawong et al. (2005). They related the removal of As and Zn in laboratory-scale SSF CWs filled with gravel to the release of exudates by *Juncus effusus*. Systems were operated discontinuously and received synthetic mining effluents. The authors suggested that organic compounds released by the plant roots favoured a decrease of the Eh, leading to a reduction and dissolution of Fe from the gravel. In oxic zones, such as encountered in the rhizosphere, this dissolved Fe precipitated again and could co-precipitate other metals. The presence of this removal pathway was supported by patterns of the Fe concentration and redox potential in the pore water. Moreover, roots of *Juncus effusus* grown in the gravel beds had a higher As content than plants grown hydroponically in the wastewater. This was related to the Fe plaques covering the root surface.

However, it has to be noted that the abovementioned situation applies to mining and industrial effluents. In systems receiving urban wastewater with a higher organic loading, the contribution of organic compounds released by plant roots is assumed to be negligible (Stottmeister et al., 2003). Moreover, the organic loading of the wastewater would create more reducing conditions, at least in the section situated closest to the inlet area, which would favour other removal processes such as metal sulphide precipitation.

2.2.3.3 Decaying plant material

If the biomass is not harvested, large amounts of organic matter are eventually recycled in the CWs (Leuridan, 2004). Decaying plant biomass may be a source of metals through leaching and mineralisation, but it can also be a sink of metals. A number of research studies in natural wetland systems have demonstrated that metal concentrations in plant litter generally increase with time, although different mechanisms for this enrichment have been reported such as adsorption of metals from the sediment, accumulation of fine particulates in the litter and active uptake by the microbial community (Weis and Weis, 2004). The degree to which metals contained within decomposing macrophytes enter invertebrates and vertebrates remains poorly quantified. The hypothesis is that macrophytes act as vectors for metals between sediments and food webs (Jackson, 1998).

3 Design factors influencing heavy metal removal

The following section discusses some of the most important design factors affecting the removal of heavy metals in constructed wetlands, classified according to the compartment to which they apply: (1) the substrate, (2) the vegetation and (3) the water.

3.1 Substrate

Major chemical properties of the substrate that affect metal removal include the oxidation-reduction state and the organic matter content.

3.1.1 Oxidation-reduction state

The type of CW affects the prevailing oxidation-reduction conditions in the substrate and thus heavy metal removal processes. Aerobic conditions normally dominate in FWS and VSSF CWs, whereas anaerobic conditions are typical of HSSF CWs, although aerobic micro-sites are present in the rhizosphere of the helophytes because of radial oxygen loss (Stottmeister et al., 2003). Next to sedimentation, filtration and sorption processes which are common in all types of CWs, it therefore seems logical that (co-) precipitation with (oxy-) hydroxides is a removal process more typical of FWS and VSSF CWs whereas precipitation with sulphides can be an important removal process in HSSF CWs.

Changes in the oxidation-reduction state of a substrate, lead to transformations of metals between chemical forms and thereby affect their mobility and plant availability. Hydrous oxides of Fe, Al and Mn effectively adsorb or occlude most metal cations. These oxides are unstable in reducing conditions leading to a release of Fe, Al, Mn and co-precipitated metals. Metal sulphide precipitates will remain stable as long as the substrate is reduced. Upon oxidation of a sediment, sulphides will be lost and metals released. The released metals may be mostly immobilised by other processes. Immobilisation processes tend to be complimentary in that a change in redox conditions does usually not result in a large release of metals, as long as the pH does not change substantially (Gambrell, 1994).

Several studies of full-scale SSF CWs point at the importance of redox conditions in the removal of Mn and Fe. Vymazal (2005) reported higher Fe, Mn and Ni concentrations in the effluent of a HSSF CW for treatment of domestic wastewater than in the influent. Based on the significant reduction of the organics and the lack of nitrates, Vymazal (2005) suggested that anaerobic conditions prevailed in the reed beds and that Mn(IV) and then Fe(III) were reduced to Mn(II) and Fe(II) which are more soluble and were consequently partly washed out of the system. Nickel followed a similar removal pattern, suggesting that Ni which was co-precipitated with Mn- and/or Fe- (oxy-) hydroxides was released when these compounds were reduced. However, Obarska-Pempkowiak and Klimkowska (1999) reported higher Mn concentrations in the sediment towards the end of a hybrid CW. This increasing trend of Mn concentrations could be attributed to the presence of reducing conditions close to the inlet area which mobilise the Mn(II) and oxidising conditions towards the end which cause precipitation of the insoluble Mn(IV). García et al. (2003) observed an increase in the redox potential with increasing distance from the inlet in HSSF CWs for urban wastewater treatment, supporting these findings.

3.1.2 Organic matter content

Substrates with a higher organic matter content can remove more heavy metals by sorption. When the process of metal sulphide precipitation is aimed at, it is important to select a suitable carbon source. This was demonstrated by several laboratory-scale studies testing different substrate types for passive treatment of acid mine drainage, e.g. in permeable reactive barriers. Chang et al. (2000) reported that raw biomass (e.g. oak chips) was initially less efficient than biologically (e.g. compost) or chemically (e.g. sludge from a wastepaper recycling plant) treated biomass in the promotion of sulphate reduction and metal removal. Gibert et al. (2004) demonstrated that the lower the lignin content of the substrate, the higher the biodegradability and the capacity for developing bacterial activity. They demonstrated that sheep manure was a successful organic substrate favouring sulphate reduction. Results from abovementioned laboratory-scale studies could also be adopted for the enhancement of sulphidogenesis in CWs as well.

Song et al. (2001) studied the removal of Pb and Zn from synthetic mine water in unplanted laboratory-scale HSSF CWs that were filled with different mixtures of the following substrates: aged manure, hay, bark, gravel, peat moss, sand and aged sludge. The sludge and manure were added to stimulate growth of the bacterial population. Hay, bark and peat moss were added as a carbon source, and gravel and sand were used to improve hydraulic conductivity. Efficient removal of Pb and Zn was reported, independent of the substrate composition. Based on influent and effluent data, a distinction between removal pathways was not possible. Therefore, processes other than metal sulphide precipitation could also have contributed to metal removal. However, the reduction of sulphates and the detection of sulphides in the effluent suggested the important role of bacterial sulphate reduction in metal removal.

3.2 Vegetation

The type of vegetation can affect the removal of heavy metals in CWs. Different wetland plant species differ in their ability to take up and accumulate various heavy metals. The following part presents data on the accumulation of heavy metals in emergent, floating and submerged macrophytes.

3.2.1 Emergent aquatic macrophytes

Helophyte species such as *Phragmites australis*, *Typha latifolia*, *Typha domingensis*, *Schoenoplectus lacustris*, *Iris pseudacorus*, *Carex rostrata*, etc. have been tested for their removal capacities of heavy metals in a number of laboratory-scale experiments (Taylor and Crowder, 1983b; Debusk et al., 1996; Mungur et al., 1997; Stoltz and Greger, 2002; Ye et al., 2003). Full-scale treatment of a range of metal-contaminated industrial wastewaters by CWs planted with helophytes is reported by Peverly et al. (1995), Groudeva et al. (2001) and Batty and Younger (2004). In full-scale CWs, helophytes are accompanied by a gravel or soil-like substrate as a rooting medium. Dunbabin and Bowmer (1992) described the efficiency of these combined helophyte-substrate systems in metal removal and reviewed some of the full-scale CWs for the treatment of mine drainage. However, only a minor fraction of metals is removed by the helophytes themselves and can thus be removed by harvest whereas the substrate acts as the main pool for metal accumulation in CWs (Stottmeister et al., 2003).

This trend was observed in several full-scale studies of SSF CWs treating domestic and dairy wastewater (Mantovi et al., 2003; Vymazal, 2003; Vymazal and Krása, 2003). Lim et al. (2001, 2003) also reported an insignificant contribution of *Typha angustifolia* in the removal of Cu, Cd, Pb and Zn in laboratory-scale gravel-based HSSF CWs. Accumulation in the biomass of *Typha angustifolia* accounted for less than 1, 2.5, 2.6 and 0.8 % of respectively the influent Cu, Cd, Pb and Zn load. The restricted translocation of metals to the aboveground biomass of most emergent aquatic macrophytes is responsible for their low contribution in metal removal.

However, other laboratory-scale experiments demonstrated higher contributions of the helophytes, mainly of their fine roots, in the overall metal removal. Sinicrope et al. (1992) reported that about 35 and 13 % of the mass loading of respectively Cd and Zn had accumulated in the fine roots of *Scirpus lacustris* growing in SSF CW mesocosms. On the contrary, the shoots, rhizomes and coarse roots each accumulated less than 1 %. The important role of plant uptake was also reported by Cheng et al. (2002) who observed that more than 30 % of the mass loading of Cu and Mn was accumulated by *Cyperus alterniflorus* in a laboratory-scale twin-shaped vertical/reverse-vertical flow CW. Nevertheless, accumulation occurred mainly in the belowground biomass and again demonstrated the phytostabilising role rooted emergent macrophytes may have.

Table 1.1 presents a range of metal concentrations encountered in emergent aquatic macrophytes growing in natural and constructed wetlands. Table 1.2 presents a description of the reference numbers. With some exceptions, metal concentrations in helophytes growing in CWs or metal-contaminated natural wetlands were generally comparable to values reported in natural uncontaminated wetlands and do not seem to indicate that helophytes accumulate high concentrations of metals in their aboveground biomass.

Table 1.1

Concentrations of heavy metals in emergent aquatic macrophytes in constructed wetlands, uncontaminated and metal-contaminated natural wetlands or lakes (mg kg^{-1} DM).

The reference numbers are described in Table 1.2

Sampling location	**Plant species**	**Plant part**	**Cd**	**Cu**	**Pb**	**Zn**	**Cr**	**Ni**	**Co**	**Al**	**Fe**	**Mn**	**Ref n°**
Constructed wetlands													
HSSF	*Phalaris arundinacea*	Leaves	1-2	2-4	3.4-9.4	30-50		17-26		300-550	190-360	90-100	1, 2
		Stems	1.5-3.4	3-7	8-10	40-90		23-72		300-400	220-340	90-110	
		Roots	2.1-7.5	7-15	15-20	90-140		66-113		550-1200	530-970	160-300	
HSSF	*Typha domingensis*	Leaves		3.5-8.3	1.4-2.3	22-24							3
		Roots		14-17	3.4-5.6	49							
	Scirpus spp.	Leaves		2.9-7.5	1.1-1.2	14-36							
		Roots		16-19	2.2-5.9	21-33							
HSSF	*Phragmites australis*	Shoots	0.05	2.9	0.09	17				31	94	88	4
		Rhizomes	0.1	6.5	0.24	12				102	199	38	
		Roots	0.25	64	12	46				1260	3640	266	
FWS	*Phragmites communis*	Leaves	0.6-0.9	20-33	31-50	43-65					162-213	385-680	5
FWS	*Juncus* spp.	Shoots		2.5	<2	14					137		6
		Roots		4	4.5	23					718		
Uncontaminated wetlands or lakes													
Wetland	*Typha domingensis*	Leaves		4.3	3	13							3
		Roots		9.5	0.8	31							
	Scirpus spp.	Leaves		2.6	0.3	10							
		Roots		9.4	10	26							
Reed stands	*Phragmites australis*	Shoots	0.04	2.6	0.3	18				31	98	53	4
		Rhizomes	0.06	4.6	0.3	17				109	299	64	
		Roots	0.18	23	7.2	70				2583	3926	262	
Lake	*Phragmites australis*	Shoots	0.25	5.3	3.7	337							7
		Rhizomes	0.22	2.1	<1	38							
		Roots	1.2	18	9.3	245							

Table 1.1

Concentrations of heavy metals in emergent aquatic macrophytes in constructed wetlands, uncontaminated and metal-contaminated natural wetlands or lakes (mg kg^{-1} DM).

The reference numbers are described in Table 1.2 (Continued)

Sampling location	**Plant species**	**Plant part**	**Cd**	**Cu**	**Pb**	**Zn**	**Cr**	**Ni**	**Co**	**Al**	**Fe**	**Mn**	**Ref n°**
Metal-contaminated wetlands or lakes													
Submerged mine tailing impoundment	*Phragmites australis*	Shoots	1	6.4	4.1	68							8
		Rhizomes	0.1	8	8.1	80							
		Roots	4.6	80	523	1310							
	Carex rostrata	Shoots	0.4	13	13	90							
		Roots	1.4	75	305	656							
Salt-marshes	*Phragmites australis*	Leaves		3.7-11	0.1-3.0	12-79	0.2-2.3						9
		Stems		1.6-19	0.6-3.2	27-75	0.3-2.6						
		Roots		55-185	91-142	233-588	49-65						
		Rhizomes		7.3-13	6.3-8.4	25-67	4.2-11						
	Spartina alterniflora	Leaves		3.7-6.5	0.7-3.1	22-40	1.3-4.1						
		Stems		0.9-7.1	0.05-1.9	13-32	0.5-2.9						
		Roots		101-212	85-202	236-898	45-75						
		Rhizomes		6.3-53	1.9-46	30-168	1.5-17						
Sewage-contaminated lake	*Phragmites australis*	Shooths	0.05	3.3	<1	217							7
		Rhizomes	0.1	3.1	<1	31							
		Roots	0.58	15	3.5	158							
Sewage-contaminated lakes	*Phragmites communis*	Leaves	1.3-1.9	2.1-2.5	8.6-11	609-1710	1.5-1.7	1.6-2.2			76-86	228-551	10
	Typha latifolia	Leaves	1.3-1.8	2.1-3.6	6.9-19	617-1430	1.2-1.7	1.5-1.8			46-102	85-184	
	Scirpus lacustris	Leaves	1.4-3.8	3.4-4.3	11	985-2430	1.2-2.6	1.7-2.2			73-157	246-398	
Acidified lakes	*Phragmites australis*	Leaves	1.2-5.0	6.2-7.5	14-22	13-33	3.9-4.6	1.9-5.1	2.5-3.8	19-231	392-1980	82-164	11
	Iris pseudacorus	Leaves	2.6	5.8	14	21	4	4.5	2.2	216	1360	115	
	Juncus effusus	Leaves	1.3-4.8	6.3-35	0.5-17	2-39	3.8-6.4	0.4-11	3.1-8.3	17-216	1360-2420	123-1500	
	Carex remota	Leaves	2.1-2.3	6.6-20	19-27	25-95	3.5-7	8.2-13	5.1-13	36-2147	1230-2150	434-2116	
Tidal marshes	*Phragmites australis*	Leaves	0.03-0.22	2.7-8.6	0.5-7.1	11-57	0.3-1.2	0.5-5.8					12
		Stems	0.01-0.07	0.5-4.8	0.2-1.0	35-137	0.3-0.7	0.2-4.1					
		Panicles	0.02-0.12	3.1-7.0	0.4-4.5	36-132	0.1-0.4	0.5-2.3					

Table 1.2

Description of reference numbers in Table 1.1, together with location and reference

Ref n°	Description	Location	Reference
1	Gravel-based HSSF CW for domestic wastewater	Czech Republic	Vymazal and Krása (2003)
2	Gravel-based HSSF CW for domestic wastewater	Czech Republic	Vymazal (2003)
3	Gravel-based HSSF CW for secondary treated sewage, with natural wetland as control	USA	Karpiscak et al. (2001)
4	Gravel or gravel/sand-based HSSF CW for landfill leachate, with natural reed stands as control	USA	Peverly et al. (1995)
5	FWS CW for domestic wastewater	Poland	Samecka-Cymerman et al. (2004)
6	FWS CW for secondary treated domestic wastewater	New Zealand	Chagué-Goff (2005)
7	Uncontaminated and sewage-contaminated lakes	Denmark	Schierup and Larsen (1981)
8	Natural wetland vegetation developed on an old submerged mine tailings impoundment	Sweden	Stoltz and Greger (2002)
9	Metal-contaminated salt marsh	USA	Windham et al. (2003)
10	3 lakes that receive untreated municipal sewage	Poland	Szymanowska et al. (1999)
11	Acidified lakes developed in excavations after coal mining	Poland	Samecka-Cymerman and Kempers (2001)
12	Tidal marshes along the river Scheldt	Belgium	Du Laing (2006)

3.2.2 Floating and submerged aquatic macrophytes

Whereas emergent aquatic macrophytes generally accumulate low concentrations of heavy metals in their harvestable biomass (Weis and Weis, 2004) and have a small contribution to the overall removal of metals in CWs (Stottmeister et al., 2003), the situation is different for floating and submerged macrophytes. Although these macrophytes also accumulate metals by their roots, they have the additional advantage of uptake through their submerged shoots as well. Over the last decades, there has been an increasing interest in using floating and submerged plants for the removal of heavy metals from wastewater. Removal of heavy metals can be greatly enhanced by selection of the appropriate wetland plant species with a high accumulation capacity. Therefore, many research studies have screened different wetland plant species for this capacity.

Floating plants that offer promising results with regard to metal removal from wastewater and that were subject of screening studies include *Lemna minor* (Zayed et al., 1998), *Eichhornia crassipes* (Zhu et al., 1999), *Pistia stratiotes* and *Salvinia herzogii* (Maine et al., 2001, 2004). As submerged plants are completely inundated and have the ability to take up metals directly from the water, they are suggested as useful species in reducing metal concentrations in stormwater (Fritioff et al., 2005) and secondarily treated wastewaters (Keskinkan, 2005). However, according to Guilizzoni (1991) and Keskinkan (2005), there is scarce study about heavy metal uptake by submerged aquatic plants. The use of submerged macrophytes for wastewater treatment is therefore still at an experimental stage, with species like *Potamogeton epihydrus* (Goulet et al., 2005), *Ceratophyllum demersum* (Keskinkan et al., 2004) and *Myriophyllum spicatum* (Keskinkan, 2005) being tested.

Some reports of experimental work in which metal uptake in promising floating and submerged species were studied are presented in Table 1.3, together with concentrations reported in plants collected from metal-contaminated natural environments. Table 1.4 presents a description of the experimental conditions of the references listed in Table 1.3. Analysis of literature reports revealed the following general trends: (1) metal concentrations in roots are generally higher than in shoots, (2) metal concentrations in the biomass increase with increasing concentration in the water, demonstrating the potential use as bio-indicators and (3) the highest bioconcentration factors, defined as the ratio between metal concentrations in the biomass and the water, were observed at the lowest concentrations, indicating the potential for remediation of large volumes of wastewaters with low metal concentrations. Higher metal concentrations are commonly observed in floating and submerged aquatic macrophytes than in emergent wetland plants. Cardwell et al. (2002) studied the accumulation of Cd, Cu, Pb and Zn in 15 different aquatic emergent and submerged plant species growing in contaminated streams. These streams received treated sewage discharges, runoff from metal-related industry and urban stormwater runoff. *Typha* spp. and *Persicaria* spp. were the most abundant rooted emergent plant species. *Myriophyllum aquaticum* showed the highest metal levels of all plant species under study.

According to Zhu et al. (1999), a good accumulator should have the ability to (1) accumulate metals in its tissues with concentrations higher than 5000 mg kg^{-1} DM (0.5 %) and (2) bioconcentrate elements with a bioconcentration factor of more than 1000. Based on these criteria, *Eichhornia crassipes* was proposed as a good accumulator of Cd (Zhu et al., 1999) and *Lemna minor* as a good accumulator of Cd and Cu (Zayed et al., 1998). However, short-term experiments may not reveal potential toxicity symptoms of long-term exposure. Moreover, field conditions may affect the actual removal capacity, including temperature, competing ions in the water, species competition, etc. (Zhu et al., 1999). Keeping this in mind, Zayed et al. (1998) nevertheless described laboratory-scale screening experiments as an effective way of quantifying the capacity of a plant to accumulate heavy metals under standard conditions. They serve a role as a preliminary assessment of the removal capacity of the plants.

The phytoremediation efficiency may be improved by periodic harvesting of the plants in order to remove trace elements from the site and to minimise toxicity to wildlife. The trace elements that accumulated in the biomass could be recovered by ashing the biomass (Zhu et al., 1999). Moreover, Maine et al. (2001, 2004) suggested to combust the dried biomass of *Pistia stratiotes* in order to produce energy.

Table 1.3

Concentrations of heavy metals in floating and submerged aquatic macrophytes reported in laboratory and mesocosm experiments, and metal-contaminated natural environments (mg kg^{-1} DM). The reference numbers are described in Table 1.4

Plant species	**Plant part**	**Cd**	**Cu**	**Pb**	**Zn**	**Cr**	**Ni**	**Ref n°**
Laboratory and mesocosm experiments								
Floating macrophytes								
Eichhornia crassipes	Shoots	20-371	20-100			3-119	6-120	1
	Roots	220-6103	45-2800			180-3951	35-1200	
Lemna minor		13300	3360	630		2870	1790	2
Pistia stratiotes	Shoots	223-511						3
	Roots	2060-4360						
Pistia stratiotes	Shoots					79-269		4
	Roots					678-3740		
Salvinia herzogii	Shoots					93-448		4
	Roots					794-6200		
Submerged macrophytes								
Myriophyllum aquaticum	Shoots		304		549			5
Myriophyllum spicatum	Leaves	120						6
Myriophyllum triphyllum	Leaves	80						6
Metal-contaminated natural environments								
Floating macrophytes								
Eichhornia crassipes	Shoots	1-2	20-40	10-30	60-460		20-220	7
	Roots	2-10	80-1100	80-220	160-680		140-540	
Submerged macrophytes								
Potamogeton natans	Leaves	3.9	7.9	25	35	5.3	6.9	8
Potamogeton pectinatus	Shoots	1.1-1.5	806-921	151-237	246-272	2.5-2.7	57-59	9
Myriophyllum spicatum	Shoots	7.1-8.8	720-1040	469-850	313-515	1.3-1.9	18-19	9
Myriophyllum aquaticum	Leaves	1.6	156	25	1458			10
	Roots	6.5	431	64	4296			

Many papers have ranked and compared macrophytes according to their affinity with heavy metals, but the meaning of these rankings is often unclear. For example it is often not clear whether a high accumulation level is the result of a high pollution level or from an efficient uptake mechanism of the plant. Moreover, metal concentrations in macrophytes are difficult to compare because of differences in sampling times, tissues, plant species, environmental conditions, analytical procedures, etc. Therefore, Guilizzoni (1991) suggested that metal pollution studies spend less time on unrealistic accumulation experiments, but rather concentrate on simulating actual field conditions.

Table 1.4

Description of reference numbers in Table 1.3, together with location and reference

Ref n°	Description	Location	Reference
1	Single metal solutions of Cd, Cr, Cu and Ni covering a range of 0.1 - 10 mg l^{-1}, for 14 d (reduced growth at 5 and 10 mg l^{-1})	USA	Zhu et al. (1999)
2	Single metal solutions of Cd, Cr, Ni and Pb covering a range of 0.1 - 10 mg l^{-1}, for 8 d (reduced growth at 5 and 10 mg l^{-1})	USA	Zayed et al. (1998)
3	Single Cd solutions covering a range of 1 - 6 mg l^{-1}, for 21 d (phytotoxicity was observed above 2 mg l^{-1})	Argentina	Maine et al. (2001)
4	Single Cr solutions covering a range of 1 - 6 mg l^{-1}, for 31 d (phytotoxicity was observed above 2 mg l^{-1})	Argentina	Maine et al. (2004)
5	Mixed metal solution with 28, 5.6 and 104 mg l^{-1} of Zn, Cu and Fe, for 21 days	Canada	Kamal et al. (2004)
6	Single Cd solutions covering a range of 0 - 16 mg l^{-1}, for 4 d	Turkey	Sivaci et al. (2004)
7	Natural wetland polluted by discharge of industrial and domestic wastewater, and seepage from landfills	Taiwan	Liao and Wang (2004)
8	Acidified lakes developed in excavations after coal mining	Poland	Samecka-Cymerman and Kempers (2001)
9	Lakes polluted by copper mining industry	Poland	Samecka-Cymerman and Kempers (2004)
10	Metal-contaminated urban streams	Australia	Cardwell et al. (2002)

3.3 Water

Hydraulic design has been postulated as the most important factor controlling metal removal processes in CWs (Sinicrope et al., 1992; Sheoran and Sheoran, 2006). Important hydraulic parameters affecting metal removal processes include the loading application (continuous or intermittent) and the hydraulic retention time (HRT).

Metal sulphide precipitation is generally negatively affected by higher loading rates and shorter residence times. This finding was supported by laboratory-scale experiments with acid mine drainage in which bacterial sulphate reduction was governed to induce metal removal (Chang et al., 2000; Gibert et al., 2004). On the contrary, Song et al. (2001) did not observe a clear effect of the HRT, when ranged from 2 to 7 days, on the removal of Pb and Zn from synthetic mine water in laboratory-scale HSSF CWs. This could be caused by the range of HRTs tested and the prevalence of other removal mechanisms than sulphide precipitation. Stottmeister et al. (2003) suggested that the HRT affects the extent to which plants play a significant role in pollutant removal. Plants would have a greater role in HSSF CWs with sufficiently long residence times than in VSSF CWs which usually have a much shorter residence time.

The importance of the loading application strategy was demonstrated by Sinicrope et al. (1992). In their study of metal removal in mesocosms planted with *Scirpus californicus* subjected to different hydroperiods, the authors observed the highest removal efficiencies in the mesocosms that were flooded and drained two times a day. Metals were presumably removed by forming complexes with Fe- and Mn- (oxy-) hydroxides. Generally lower treatment efficiencies were observed in the treatment that was continuously inundated. The Eh of about 0 mV and the low sulphide levels in the effluent did not support significant sulphate reduction taking place in this treatment. Presumably, the experimental residence time of 1 – 1.3 days might have been too short to promote sulphate reduction.

The importance of a good hydraulic design was emphasised by Mungur et al. (1997). They studied the removal of Cu, Pb and Zn from simulated surface run-off in a laboratory-scale HSSF CW filled with gravel and planted with *Typha latifolia*, *Phragmites australis*, *Schoenoplectus lacustris* and *Iris pseudacorus*. A remarkable feature of this experiment was that the helophytes were rooted in permeable buckets filled with peat. Short-circuiting and preferential flow paths were present, as evidenced from the non-uniformly distributed patterns of metal concentrations in the peat and in the plants. Poor hydraulic design could lead to non-optimal use of the filter substrate and helophytes and thereby decrease performance with time.

4 Conclusions

Over the last decades, insight into the removal processes of heavy metals in CWs has increased considerably by the dual approach of (1) monitoring metal accumulation in the compartments of full-scale CWs and (2) studying removal processes and influencing factors by means of laboratory and pilot-scale experiments. Although in most of these experiments heavily loaded wastewaters have been tested, they have revealed useful information about the general removal mechanisms that also apply in CWs for domestic wastewater treatment.

Several studies demonstrated that heavy metals mainly accumulated in the substrate and sediment situated nearest to the inlet area of the CWs. Exceptions have been reported for Mn and Fe of which the dynamics are strongly affected by redox conditions. Anaerobic conditions prevailing in the first section of HSSF CWs can lead to mobilisation of Mn(II) and Fe(II) which can either deposit further along the CW when conditions change from reducing to oxidising, or can be partly washed out of the system.

The importance of the substrate type depends on the dominant metal removal processes aimed at. In CWs where sedimentation, filtration and sorption are dominant and aerobic conditions prevail, the substrate type is generally not of major importance and low-cost substrates such as sand or gravel are generally equally effective as expensive filter media once a mature bio-film is established. On the contrary, in systems where metal sulphide precipitation is a major removal process, the selection of a suitable substrate type governing bacterial sulphate reduction is of utmost importance. An important hydraulic parameter affecting metal removal processes is the hydraulic retention time. Metal removal is generally higher at higher residence times, especially if metal sulphide precipitation is the dominant removal mechanism.

With a few exceptions, metal accumulation in helophytes usually accounts for a negligible amount of metal removal in CWs. The major removal processes of heavy metals are of physico-chemical nature. However, helophytes can also indirectly affect metal removal by acidifying and/or oxidising the rhizosphere. Helophytes have been reported to positively, negatively or simply not affect metal removal performance in different laboratory-scale experiments. In those experiments, the dominant redox conditions of the substrate often seemed to determine the observed effect of the helophytes. Generally, the presence of helophytes did not or positively affect metal removal if the substrate was mainly aerobic, whereas helophytes have been reported to negatively affect metal removal in substrates that were strongly reduced. However, more research is needed in this area to substantiate these hypotheses.

Floating and submerged wetland plants such as *Eichhornia crassipes*, *Lemna* spp. and *Myriophyllum* spp., etc. have been reported to accumulate metals to high concentration levels and offer potential for use in CWs for metal-contaminated wastewaters. However, high accumulation levels present a problem of biomass collection and disposal in order to prevent decomposition and recycling of metals into the water phase. There are concerns about accumulation of metals in the food chain by direct consumption or decomposition, harvesting of the plants and what to do with the harvested biomass (Williams, 2002).

CHAPTER 2

REMOVAL OF HEAVY METALS IN CONSTRUCTED WETLANDS FOR TREATMENT OF DOMESTIC WASTEWATER IN FLANDERS, BELGIUM

1 Introduction

Constructed wetlands (CWs) can offer an efficient alternative for conventional wastewater treatment systems (Brix, 1994; EPA, 2000; Kivaisi, 2001). A review on their application and performance in Flanders, Belgium was presented by Rousseau et al. (2004). The number of CWs in Flanders has increased exponentially during the last decade. A total of 107 systems has been reported up till now. However, not all single-household systems, CWs on farms, etc. have been documented and were therefore not included in the database of Rousseau et al. (2004). Constructed wetlands in Flanders are used as small-scale or individual treatment systems in regions where connection to the centralised sewer system is not economically feasible (VMM, 2001). Different types of CWs are currently in operation. They range from free-water-surface (FWS) to subsurface flow (SSF) CWs and combinations of these. Subsurface flow CWs are either of the horizontal (HSSF) or vertical (VSSF) flow type. There are also a number of CWs that are preceded by conventional treatment systems and serve the role of tertiary treatment. Constructed wetlands in Flanders are mostly used for the treatment of domestic and dairy wastewater. Common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) is the dominant helophyte species which is being used in the CWs.

The effluent standards in Flanders for small-scale wastewater treatment plants (WWTPs), systems that treat wastewater of less than 2000 population equivalents (PEs), only involve COD, BOD and SS. Effluent standards for COD and BOD are respectively 125 and 25 mg l^{-1}, whereas the effluent standard for SS depends on the number of PEs that is connected to the system: 35 mg l^{-1} for 500 to 2000 PE and 60 mg l^{-1} for less than 500 PE. Moreover, a minimum reduction of the COD, BOD and SS load of respectively 75, 90 and 70 % has to be achieved. There are currently no effluent standards for heavy metals in conventional and small-scale WWTPs for domestic wastewater in Flanders. Yet, metal concentrations in the influent and effluent of these WWTPs are required to be monitored by law. However, they do not have to comply with any standards and are purely of informative nature (VLAREM II, 1995). These analyses are performed by the VMM, the Flemish Environment Agency. Minimum, maximum and mean concentrations of heavy metals in the influent and effluent for a given sampling year can be retrieved for these CWs from a database operated by the VMM (VMM, 2006). However, the database does not present information on parameters such as the number of samples, the variability and temporal trends.

A long-term dataset of influent and effluent concentrations of heavy metals in 12 CWs was obtained from Aquafin NV. The dataset comprised following types of CWs: FWS, HSSF, VSSF, combined and tertiary CWs. The influent refers to the raw wastewater that enters the pre-treatment step preceding the CWs. This pre-treatment step usually involves (1) a pre-settlement tank in the case of HSSF, VSSF and combined CWs, (2) a primary settlement ditch in the case of FWS CWs and (3) a pre-settlement tank followed by a conventional wastewater treatment system such as a rotating biological contactor (RBC) in the case of tertiary CWs. The sample collection was of low-frequent, irregular nature and varied between CWs and sampling years. The dataset was examined in order to (1) study the concentrations of heavy metals in the raw wastewater and effluents of CWs and assess the removal of heavy metals, (2) assess long-term dynamics, seasonal and yearly trends and (3) make comparisons among CWs.

Four CWs, a FWS, HSSF, VSSF and tertiary CW, were then selected and monitored weekly from the end of August 2005 until the end of May 2006 for total metal concentrations in the raw wastewater, the influents of the CWs (thus after pre-treatment) and the effluents. Although not taking into account the residence time in the CWs, the regular weekly sampling campaign increased the chance of detecting temporal trends and allowed for a better study of long-term dynamics. Moreover, the monitoring of the raw wastewater and the influent of the CWs allowed to separately assess the contribution of the pre-treatment step and the CW itself in the overall metal removal. Finally, the influent and effluent of one of the studied HSSF CWs were subjected to a short-term high-frequent monitoring campaign to study the short-term dynamics.

2 Material and Methods

2.1 Description of the constructed wetlands under study

Design characteristics and dimensions of the 12 CWs under study were collected from technical documents (Aquafin, 2003 a-l) and a geographical information system (AGIV, 2006) and are presented in Table 2.1. Constructed wetlands are operated by Aquafin NV, the Flemish company for wastewater collection and treatment. All CWs, with the exception of those located in Aalbeke and Sint-Maria-Lierde, are designed for secondary treatment of domestic wastewater but receive stormwater as well because of the combined sewer networks in Flanders, Belgium. As described by Rousseau et al. (2004), the design capacity is expressed in population equivalents (PEs) derived from the number of people that can be connected to the CW and not from organic or hydraulic loading rates.

The HSSF, VSSF and combined CWs are generally preceded by a pre-settlement tank, with the exception of the HSSF CW in Hasselt-Kiewit. That CW is preceded by a primary settlement ditch, a canal in which suspended solids are allowed to settle and which serves a similar role as the pre-settlement tanks. The pre-settlement tanks usually have 2 or 3 compartments. Sludge is periodically removed and dewatered in nearby WWTPs. The combined CWs consist of one or more parallel VSSF reed beds followed by one or more HSSF reed beds, until recently the most popular configuration of combined CWs in Flanders (Rousseau et al., 2004). These CWs are sealed with an impermeable liner. The FWS CWs are preceded by a primary settlement ditch. They have been in operation since 1989 and are the oldest of the selected CWs. The FWS CWs are not provided with a liner but have clay bottoms with a low hydraulic conductivity to prevent leaching to the groundwater. The tertiary CWs are of the HSSF type and are preceded by a pre-settlement tank and a series of rotating biological contactors (RBCs).

The HSSF reed beds are usually filled with coarse gravel (5 – 8 cm diameter) in the inlet and outlet area to provide optimum flow distribution. The remainder of the bed is usually filled with fine gravel (5 – 10 mm diameter) to a depth of 60 – 80 cm. The VSSF reed beds are generally 90 cm deep, consisting of a layer of 60 cm of filter material and a drainage layer with fine gravel. Both layers are separated by geotextile. The filter material of the VSSF reed beds is usually coarse sand (0.2 – 0.63 mm diameter) although some VSSF reed beds, e.g. Zemst-Larebeek and De Pinte-Zevergem, are filled with fine gravel (5 – 10 mm diameter).

The maximum hydraulic capacity is usually $6 \times Q_{14}$ (with Q_{14} being the design peak hydraulic loading rate, based on 150 l PE^{-1} d^{-1}, supplied in 14 hours) and the excess storm water is directed into a receiving brook by means of an overflow construction located before the pre-settlement tank or primary settlement ditch. In case of storm conditions in the tertiary CW in Aalbeke, excess stormwater bypasses the RBCs and is directed immediately towards the HSSF reed bed. In Sint-Maria-Lierde, an overflow construction located before the RBCs directs the excess stormwater towards the receiving surface water. The influent of all CWs is periodically strongly diluted with stormwater (De Moor, 2003; Story, 2004; Gardin et al., 2006). Moreover, some CWs suffer from continuous dilution because of infiltration of ground- and/or surface water into the sewer systems. This has been reported for the FWS and tertiary CWs. The influent of the tertiary CW in Sint-Maria-Lierde is also fed by runoff of agricultural land. Operational problems due to the inflow of clay and/or loam during storm conditions have been reported for the tertiary CWs and the combined CWs in Bierbeek and Ieper.

Table 2.1

Design characteristics of the 12 CWs studied. CW N° refers to the location: (1) Latem-Baarle, (2) Deurle, (3) Zemst-Kesterbeek, (4) Hasselt-Kiewit, (5) Zemst-Larebeek, (6) Sint-Niklaas-Heimolen, (7) Pervijze, (8) Ieper, (9) De Pinte-Zevergem, (10) Bierbeek, (11) Aalbeke and (12) Sint-Maria-Lierde

CW		**Lay-out**	**Year of construction**	**Design Capacity (PE)**	**Surface Area (m^2)**	**Footprint (m^2 PE^{-1})**	**Hydraulic Capacity (m^3 d^{-1})**
Type	**N°**						
FWS	1	13 FWS in series	1989	800	3000	3.8	1800 (15×Q_{14})
	2	9 FWS in series	1989	1000	3060	3.1	1800 (12×Q_{14})
HSSF	3	2 parallel HSSF	2001	350	1300	3.7	315 (6×Q_{14})
	4	8 parallel HSSF	1999	152	640	4.2	23 (1×Q_{14})
VSSF	5	2 parallel VSSF	2000	470	940	2.0	423 (6×Q_{14})
	6	2 parallel VSSF	2000	270	540	2.0	243 (6×Q_{14})
Combined	7	2 parallel VSSF + 2 parallel HSSF	2000	700	2212	3.2	630 (6×Q_{14})
	8	2 parallel VSSF + 1 HSSF	2000	400	1080	2.7	360 (6×Q_{14})
	9	2 parallel VSSF + 2 parallel HSSF	2000	750	2250	3.0	675 (6×Q_{14})
	10	2 parallel VSSF + 1 HSSF	2000	210	660	3.1	189 (6×Q_{14})
Tertiary	11	2 RBC in series + 1 HSSF	1996	500	500	1.0	450 (6×Q_{14})
	12	3 RBC in series + 1 HSSF	2000	850	425	0.5	765 (6×Q_{14})

PE: population equivalent (based on 54 g BOD PE^{-1} d^{-1}), Q_{14}: design peak hydraulic loading rate (based on 150 l PE^{-1} d^{-1})

2.2 Data collection

2.2.1 Long-term low-frequent monitoring of 12 constructed wetlands

Although there are currently no discharge criteria of heavy metals for the effluent of CWs, total concentrations of Ag, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in the influent and effluent of some CWs were measured by the VMM (VLAREM II, 1995; VMM, 2006). The influent refers to the raw wastewater before passage through the pre-treatment step. Samples are usually flow-weighed composite samples collected during 1 day. The sampling frequency varied between CWs: from 4 to 16 samples per year, usually spread over the different seasons. Total metal concentrations were analysed in different laboratories after *Aqua Regia* destruction, as described in the compendium for analysis of water (WAC/III/B/002, 2005). Depending on the laboratory, inductively coupled plasma optical emission spectrometry (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS) has been used for analysis of Ag, As, Cd, Cr, Cu, Ni, Pb and Zn. Mercury was analysed by flow injection mercury system cold vapour atomic absorption spectrometry (FIMS-CVAAS). The detection limits varied depending on the metal and the laboratory.

2.2.2 Weekly monitoring of 4 constructed wetlands

Of the 12 CWs, 4 were selected of each type for further study: (1) the FWS CW in Deurle, (2) the HSSF CW in Zemst-Kesterbeek, (3) the VSSF CW in Zemst-Larebeek and (4) the tertiary CW in Aalbeke. A weekly sampling campaign of the influents and effluents of the CWs was performed from the end of August 2005 until the end of May 2006. The CWs in Zemst-Kesterbeek, Zemst-Larebeek and Aalbeke were additionally sampled for the raw wastewater, before passage through the pre-treatment step. Operators of Aquafin NV took grab samples of the water during their weekly visits to the CWs.

Samples were collected into 150 ml plastic recipients and stored at 4 °C until transportation to the laboratory. Fifty ml of water was digested with 2 ml 65 % HNO_3 and 2 ml 20 % H_2O_2 at 150 °C for 1 h. Total metal concentrations were determined after filtration (MN 640 m filter paper, Machery-Nagel, Düren, Germany) and dilution with 1 % HNO_3 to 50 ml. Metals (Al, Cd, Cu, Cr, Pb, Ni, Zn, Fe and Mn) were analysed by means of ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) or graphite furnace atomic absorption spectrometry GF-AAS (SpectrAA-800/GTA-100, Varian, Palo Alto, CA).

2.2.3 Short-term high-frequent monitoring of the horizontal subsurface flow constructed wetland in Zemst-Kesterbeek

To study short-term dynamics, one of the 4 CWs was monitored for influent and effluent concentrations of heavy metals by means of a high-frequent monitoring campaign. The HSSF CW located in Zemst-Kesterbeek was selected for this purpose. One liter of influent and effluent were sampled every 4 h during a monitoring campaign of 8 days in September 2004 using automatic samplers (American Sigma 900, American Sigma Inc, Medina, NY). The influent refers to the influent of the reed bed, after passage through the pre-settlement tank.

Half of the water samples were filtered over a 0.45 µm filter (porafil, Machery-Nagel, Düren, Germany) and acidified with a drop of 65 % HNO_3 for analysis of dissolved metal concentrations. Twenty five ml of raw wastewater was digested with 2 ml 65 % HNO_3 and 2 ml 20 % H_2O_2 at 150 °C for 1 h. Total metal concentrations were determined after filtration (MN 640 m filter paper, Machery-Nagel, Düren, Germany) and dilution to 50 ml. Metals (Al, Cd, Cu, Cr, Pb, Ni, Zn, Fe and Mn) were analysed by means of ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) or GF-AAS (SpectrAA-800/GTA-100, Varian, Palo Alto, CA).

2.2.4 Removal efficiency

Unfortunately, none of the CWs were monitored for both influent and effluent flow rates. All CWs, with the exception of the FWS CWs, were monitored for effluent flow rates only. The FWS CWs were monitored for influent flow rates. The removal efficiency (ε) of each metal was calculated based on influent and effluent concentrations, and on the assumption of steady-state conditions and that precipitation or evapotranspiration had minimal impact on the water storage as compared to inflow and outflow:

$$\varepsilon\,(\%) = \frac{(M_i - M_e)}{M_i} \times 100\ \% = \frac{Q_iC_i - Q_eC_e}{Q_iC_i} \times 100\ \% = \frac{C_i - C_e}{C_i} \times 100\ \%$$

with $M_{i/e}$ = the metal flux in influent/effluent (mg d^{-1});
$C_{i/e}$ = the metal concentration in influent/effluent (mg l^{-1});
$Q_{i/e}$ = the mean flow rate of influent/effluent (l d^{-1});

2.3 Statistical analysis

Statistical analysis was performed using the S-plus 6.1 software package (Insightful Corp., Seattle, USA). Normality of the distribution of metal concentrations in the influents and effluents was tested by means of the Kolmogorov-Smirnov test of normality ($\alpha = 0.05$). As metal concentrations in the water were not normally distributed, significance of differences between raw wastewater, influents of the CWs and effluents were assessed by means of non-parametric Wilcoxon rank tests (α = 0.05). Seasonal effects were analysed by means of the non-parametric Kruskal-Wallis rank test (α = 0.05).

3 Results

3.1 Long-term low-frequent monitoring of 12 constructed wetlands

Table 2.2 presents the total number of influent samples that were analysed for each heavy metal of the selected CWs, together with the number of samples that had concentrations which were lower than the analytical detection limits. Only the sampling dates for which both influent and effluent data were available were taken into consideration. As detection limits of a given heavy metal varied between CWs and between sampling dates for a given CW, a range of detection limits was presented. Table 2.2 allows to derive some preliminary conclusions about which heavy metals are priority elements in the raw wastewater of the CWs. With a few exceptions, high percentages of the total number of influent samples had Ag, As, Cd, Cr, Hg, Ni and Pb concentrations below the detection limits. Copper and Zn were the most abundant trace metals in the raw wastewater of Flemish CWs.

The limited amount of samples collected per season for a given year of each of the CWs does not allow for statistical analysis of seasonal trends. When data per season were pooled over the different years for each CW, a seasonal effect was not detected in the influent or effluent of all CWs ($p > 0.05$). Moreover, there were no statistical differences in heavy metal concentrations in the influent or effluent between different sampling years in all CWs ($p > 0.05$). The low-frequent nature of sample collection does not allow to detect temporal trends. Table 2.3 therefore presents the mean influent and effluent concentrations of metals of the different CWs and the associated removal efficiencies. As detection limits of a given metal varied between CWs and between sampling dates for a given CW, concentrations lower than detection limits were considered equal to 0 mg l^{-1} in order to calculate the means. A large variability of the influent and effluent concentrations was observed for every metal.

Table 2.2

Total number of influent samples of the different CWs which were analysed for Ag, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn from the start of monitoring until the end of 2005 (N), together with the number of samples that were lower than the detection limit (N*) and the detection limits (DL) (in µg l^{-1}). CW N° refers to the location: (1) Latem-Baarle, (2) Deurle, (3) Zemst-Kesterbeek, (4) Hasselt-Kiewit, (5) Zemst-Larebeek, (6) Sint-Niklaas-Heimolen, (7) Pervijze, (8) Ieper, (9) De Pinte-Zevergem, (10) Bierbeek, (11) Aalbeke and (12) Sint-Maria-Lierde

CW		**Ag**		**As**		**Cd**		**Cr**		**Cu**		**Hg**		**Ni**		**Pb**		**Zn**	
Type	**N°**	**N** **N***	**DL**	**N** **N***	**DL**	**N** **N***	**DL**	**N** **N***	**DL**	**N** **N***	**DL**	**N** **N***	**DL**	**N** **N***	**DL**	**N** **N***	**DL**	**N** **N***	**DL**
FWS	1	27 26	25	31 29	5	31 31	5-10	49 38	5-20	31 11	15	31 29	1	41 38	10-25	31 28	20	49 0	15-20
	2	25 25	25	29 29	5	29 29	5-10	29 20	5-20	29 8	15	39 37	1	29 25	10-25	29 25	20	47 0	15-20
HSSF	3	17 17	2-4	17 15	2-8	17 17	0.4-1	17 14	5-9	17 1	5	16 12	0.1	17 13	5-10	17 12	5-6	17 0	9-10
	4	20 20	2-4	20 14	5-8	20 15	0.4-1	20 13	5-9	20 1	5	20 11	0.1	20 14	5-20	20 2	5-6	20 1	9-18
VSSF	5	8 8	2-4	8 8	2-5	8 6	0.4-1	8 7	5-9	8 1	5-10	8 7	0.1	8 2	5-10	8 4	5-6	8 0	10
	6	15 15	25	15 14	5	15 13	5	15 11	5	15 1	15	15 15	1	15 13	10	15 12	20	15 0	/
Combined	7	63 55	0.2-5	63 13	3-10	63 58	0.1-2	63 14	0.3-3	63 3	3-12	19 10	0	63 0	/	63 32	1-8	63 0	10-18
	8	21 20	0.2-5	21 19	3-10	21 21	0.1-0.4	21 10	0.3-3	21 0	3-12	11 3	0	21 0	/	21 10	1-8	21 0	18
	9	17 17	25	17 16	5	17 17	5	17 12	5	17 1	15	17 16	1	17 17	10	17 15	20	17 0	15
	10	14 14	2-4	14 13	5-8	14 14	0.4-1	14 12	5-9	14 0	5-10	14 10	0.1	14 13	5-10	14 5	5-6	14 1	9-18
Tertiary	11	22 22	25	27 26	5	27 27	5-10	27 18	5-20	27 9	15	27 26	1	27 24	10-25	27 26	5-20	27 0	15-20
	12	21 21	25	24 22	5	24 24	5-10	24 18	5-20	24 9	15	24 23	1	24 21	10-25	24 21	20	24 0	/

/: DL unknown

There were generally no significant differences between influent and effluent concentrations of Ag, Cd or Hg (Table 2.3) ($p > 0.05$). This is caused by the very low concentrations in both influent and effluent. Silver, Cd and Hg were detected in only a few of the CWs under study and the concentrations were usually lower than the detection limits (Table 2.2). A removal efficiency of 100 % based on mean influent and effluent concentrations can in some cases correspond with insignificant removal. Therefore, the removal efficiencies of these elements should be carefully considered.

Despite the low concentrations of Cr and Ni in the influents, significant positive removal efficiencies were observed in some of the CWs. The removal efficiencies of Pb were also high, between 64 and 100 %, even when influent concentrations were very low. The concentrations of As in the effluents of the HSSF, VSSF and combined CWs were significantly higher than in the influents ($p < 0.05$) corresponding with negative removal efficiencies.

A significant decrease of the Cu concentration between influent and effluent was detected in all CWs ($p < 0.05$). This was also observed for Zn, with the exception of the VSSF CW in Sint-Niklaas-Heimolen ($p = 0.23$) and the tertiary CW in Sint-Maria Lierde ($p = 0.65$). The removal efficiencies in the FWS CWs varied between 61 and 80 % for Cu and between 64 and 66 % for Zn. The highest removal efficiencies of Cu and Zn were observed in the HSSF CWs, varying between 90 and 100 %.

Table 2.3

Mean concentrations of Ag, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in the influent (Infl) and effluent (Effl) of the selected CWs (µg l^{-1}) with associated removal efficiencies (*ε*, in %). CW N° refers to the location: (1) Latem-Baarle, (2) Deurle, (3) Zemst-Kesterbeek, (4) Hasselt-Kiewit, (5) Zemst-Larebeek, (6) Sint-Niklaas-Heimolen, (7) Pervijze, (8) Ieper, (9) De Pinte-Zevergem, (10) Bierbeek, (11) Aalbeke and (12) Sint-Maria-Lierde. *: difference between influent and effluent is significant at α = 0.05; **: significant at α = 0.001

CW			**Ag**	**As**	**Cd**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
Type	**N°**										
FWS	**1**	**Infl**	3.2	0.50	0	3.1	28	0.097	0.73	4.7	113
		Effl	0	0	0	7.3	5.5 **	0.065	3.9	0	41 **
		ε (%)	*100*	*100*	*/*	*-138*	*80*	*33*	*-433*	*100*	*64*
	2	**Infl**	0	0	0	13	37	0.21	7.4	5.0	189
		Effl	0	0	0	1.9	15 *	0.49	0.86	1.8	64 **
		ε (%)	*/*	*/*	*/*	*85*	*61*	*-138*	*88*	*64*	*66*
HSSF	**3**	**Infl**	0	0.71	0	2.1	104	0.038	2.8	8.7	130
		Effl	0	5.6 *	0	0.95	0.34 **	0.006	0.54	0 *	3 **
		ε (%)	*/*	*-695*	*/*	*55*	*100*	*84*	*81*	*100*	*98*
	4	**Infl**	0	3.0	0.33	5.4	78	0.26	3.8	47	574
		Effl	0	11 **	0 *	0.32 *	6.1 **	0.035 *	0 *	3.1 **	58 **
		ε (%)	*/*	*-255*	*100*	*94*	*92*	*86*	*100*	*93*	*90*
VSSF	**5**	**Infl**	0	0	0.16	0.65	14	0.013	12	7.3	83
		Effl	0	1.7	0	2.9	0 *	0	9.1	0 *	12 *
		ε (%)	*/*	*/*	*100*	*-342*	*100*	*100*	*26*	*100*	*86*
	6	**Infl**	0	0.37	2.9	1.9	31	0	2.0	7.7	336
		Effl	0	4.0	0.45	0.85	3.3 **	0	3.3	0	588
		ε (%)	*/*	*-998*	*84*	*55*	*89*	*/*	*-63*	*100*	*-75*
Combined	**7**	**Infl**	0.47	13	0.20	5.6	55	0.084	14	11	246
		Effl	0.61	20 **	0.054	0.75 **	4.9 **	0.047	11 *	0.73 **	34 **
		ε (%)	*-31*	*-51*	*73*	*87*	*91*	*44*	*26*	*93*	*86*
	8	**Infl**	0.18	1.4	0	5.9	55	0.27	14	11	220
		Effl	0.21	1.8	0.033	0.86 *	2.0 **	0.073	7.6 **	0 **	39 **
		ε (%)	*-22*	*-28*	*/*	*85*	*96*	*73*	*47*	*100*	*82*
	9	**Infl**	0	0.49	0	2.3	39	0.14	0	3.9	169
		Effl	0	4.4 *	0	4.0	11 **	0	0	0	44 **
		ε (%)	*/*	*-807*	*/*	*-69*	*72*	*100*	*/*	*100*	*74*
	10	**Infl**	0	0.15	0	1.0	29	0.036	0.36	6.7	182
		Effl	0	8.2 **	0	0	3.3 **	0.007	0	0 **	19 **
		ε (%)	*/*	*-5395*	*/*	*100*	*88*	*80*	*100*	*100*	*90*
Tertiary	**11**	**Infl**	0	1.1	0	4.3	22	0.037	2.6	4.1	150
		Effl	0	0	0	0.81 *	3.3 **	0	0.37	0	26 **
		ε (%)	*/*	*100*	*/*	*81*	*85*	*100*	*86*	*100*	*82*
	12	**Infl**	0	0.85	0	4.4	32	0.042	3.4	9.2	209
		Effl	0	0.25	0	0.65	0.88 **	0.083	0.88	0	160
		ε (%)	*/*	*70*	*/*	*85*	*97*	*-100*	*74*	*100*	*23*

/: *ε* could not be calculated

3.2 Weekly monitoring of 4 constructed wetlands

Large variations were observed in influent concentrations of the metals throughout the study period, from the end of August 2005 until the end of May 2006. Variations of the metal concentrations in the effluents were generally smaller than in the influents indicating that peak concentrations were efficiently buffered in the CWs. Data were not normally distributed for all metals in the influents of the pre-treatment step, and the influents and effluents of the CWs ($p < 0.001$). However, for reporting purposes, the mean total metal concentrations in the water at different locations of the treatment path of the CWs during the different seasons were presented in Tables 2.4 – 2.12, together with the associated removal efficiencies. Summer '05, autumn '05, winter '05 and spring '06 are defined by following boundaries: end of August 2005 – September 21, 2005 – December 21, 2005 – March 21, 2006 – end of May 2006. Note that the pre-treatment (PT) steps of the HSSF and VSSF CWs consist of a pre-settlement tank, whereas the pre-treatment step of the tertiary CW consists of a pre-settlement tank and a series of 2 RBCs.

Although not always statistically significant, the raw wastewater generally showed the highest concentrations of Al, Cu, Zn and Pb in summer and autumn, compared to winter and spring (Tables 2.4, 2.7, 2.8 and 2.10). A similar seasonal trend was observed for these metals in the influents of most CWs as well. The Cd concentrations in the raw wastewater were lower than 1 $\mu g\ l^{-1}$ and were generally higher in winter and spring (Table 2.9). For Ni, the highest influent concentrations were observed in summer or spring (Table 2.12). On the contrary, Fe, Mn and Cr concentrations in the raw wastewater of the CWs under study were not affected by season (Tables 2.5, 2.6 and 2.11).

The pre-settlement tanks of the HSSF and VSSF CWs did generally not offer a significant reduction of the concentrations of heavy metals in the wastewater (Tables 2.4 – 2.12). This was observed throughout the monitoring period. As a result of this, the HSSF and VSSF CWs generally received the highest concentrations of heavy metals as compared to the other CWs. Concentrations of Al, Fe, Mn, Cu, Zn, Pb and Cr were generally reduced after passage through the pre-settlement tank and the RBCs preceding the tertiary CW in Aalbeke (Tables 2.4, 2.5, 2.6, 2.7, 2.8, 2.10 and 2.11). The Cd and Ni concentrations were not reduced by the pre-treatment step located before the tertiary CW, probably due to their very low influent concentrations (Tables 2.9 and 2.12).

In all CWs, the Al concentrations were reduced, with the highest removal efficiencies observed during summer or autumn (Table 2.4). Aluminium concentrations in the effluents of the CWs were not affected by season ($p > 0.05$) and were similar in the different CWs.

Table 2.4

Mean concentrations of Al in the influent of the PT (Infl PT), the CW (Infl CW) and effluent (Effl) during the different seasons (µg l^{-1}) with associated removal efficiencies of the PT, the CW and the total removal efficiency (TOT) (%). $\boldsymbol{\alpha}_{\mathbf{concentration}}$ denotes the significance of difference between the influent of the PT, CW and effluent; α_{season} denotes the significance of seasonal differences. *: difference is significant at $\alpha = 0.05$; **: significant at $\alpha = 0.001$

CW	**Season**	**Concentration (µg l^{-1})**			**$\alpha_{concentration}$**		**Removal efficiency (%)**		
		Infl PT	**Infl CW**	**Effl**	**PT**	**CW**	**PT**	**CW**	**TOT**
FWS	Summer '05	n.m.	501	83		**		83	
	Autumn '05	n.m.	198	87		**		56	
	Winter '05	n.m.	202	71		**		65	
	Spring '06	n.m.	213	69		**		68	
	α_{season}								
HSSF	Summer '05	5423	1678	81		**	69	95	99
	Autumn '05	3875	2121	84		**	45	96	98
	Winter '05	1011	825	90		**	18	89	91
	Spring '06	584	252	88		**	57	65	85
	α_{season}	*	**						
VSSF	Summer '05	644	427	81		**	34	81	87
	Autumn '05	738	805	84		**	-9	90	89
	Winter '05	298	242	67		**	19	72	77
	Spring '06	292	469	77		*	-61	84	74
	α_{season}		*						
Tertiary	Summer '05	691	137	34	**	**	80	75	95
	Autumn '05	762	196	48	**	**	74	76	94
	Winter '05	593	97	50	**	**	84	48	92
	Spring '06	388	122	94	**		68	23	76
	α_{season}		*						

n.m.: not measured

Iron concentrations in the effluents of the HSSF and VSSF CWs were higher in summer and spring than in autumn and winter, whereas the FWS CW showed the highest Fe concentration in the effluent in autumn (Table 2.5). The tertiary CW removed Fe from the wastewater ($p < 0.05$). Positive removal efficiencies of Fe were observed in the FWS CW as well, except in autumn. Iron removal in the HSSF and VSSF CWs was poor during most of the seasons. Moreover, Fe appeared to be released from the HSSF CW, although this was only statistically significant in spring ($p = 0.018$).

Table 2.5

Mean concentrations of Fe in the influent of the PT (Infl PT), the CW (Infl CW) and effluent (Effl) during the different seasons ($\mu g\ l^{-1}$) with associated removal efficiencies of the PT, the CW and the total removal efficiency (TOT) (%). $\boldsymbol{\alpha}_{\mathbf{concentration}}$ denotes the significance of difference between the influent of the PT, CW and effluent; α_{season} denotes the significance of seasonal differences. *: difference is significant at $\alpha = 0.05$; **: significant at $\alpha = 0.001$

CW	**Season**	**Concentration ($\mu g\ l^{-1}$)**			**$\alpha_{concentration}$**		**Removal efficiency (%)**		
		Infl PT	**Infl CW**	**Effl**	**PT**	**CW**	**PT**	**CW**	**TOT**
FWS	Summer '05	n.m.	345	82		**		76	
	Autumn '05	n.m.	263	485				-84	
	Winter '05	n.m.	419	190		*		55	
	Spring '06	n.m.	314	224				29	
	α_{season}			*					
HSSF	Summer '05	1614	649	1553			60	-139	4
	Autumn '05	1388	976	735			30	25	47
	Winter '05	1071	467	1054			56	-126	2
	Spring '06	1011	779	1553		*	23	-99	-54
	α_{season}			*					
VSSF	Summer '05	697	548	987			21	-80	-42
	Autumn '05	636	667	388		*	-5	42	39
	Winter '05	527	554	177		**	-5	68	66
	Spring '06	952	1240	1190			-30	4	-25
	α_{season}			*					
Tertiary	Summer '05	755	149	75	**	*	80	50	90
	Autumn '05	1088	212	64	**	*	80	70	94
	Winter '05	1086	96	52	**	**	91	46	95
	Spring '06	802	204	37	**	**	75	82	95
	α_{season}		*						

n.m.: not measured

Table 2.6 showed that Mn was generally released from the FWS, HSSF and tertiary CWs. This release of Mn was reflected in negative removal efficiencies and appeared to occur throughout the year. The VSSF CW did generally not affect the Mn concentration in the water ($p > 0.05$).

Table 2.6

Mean concentrations of Mn in the influent of the PT (Infl PT), the CW (Infl CW) and effluent (Effl) during the different seasons ($\mu g\ l^{-1}$) with associated removal efficiencies of the PT, the CW and the total removal efficiency (TOT) (%). $\boldsymbol{\alpha_{concentration}}$ denotes the significance of difference between the influent of the PT, CW and effluent; α_{season} denotes the significance of seasonal differences. *: difference is significant at $\alpha = 0.05$; **: significant at $\alpha = 0.001$

CW	**Season**	**Concentration ($\mu g\ l^{-1}$)**			**$\alpha_{concentration}$**		**Removal efficiency (%)**		
		Infl PT	**Infl CW**	**Effl**	**PT**	**CW**	**PT**	**CW**	**TOT**
FWS	Summer '05	n.m.	73	108				-48	
	Autumn '05	n.m.	73	114				-57	
	Winter '05	n.m.	68	35		*		48	
	Spring '06	n.m.	54	69				-28	
	α_{season}			*					
HSSF	Summer '05	92	88	222		*	5	-153	-141
	Autumn '05	60	54	153		*	9	-182	-156
	Winter '05	96	81	124		*	16	-53	-29
	Spring '06	125	93	160		*	26	-73	-28
	α_{season}	*							
VSSF	Summer '05	519	365	142			30	61	73
	Autumn '05	332	327	185			2	43	44
	Winter '05	373	324	300		*	13	7	20
	Spring '06	475	299	463			37	-55	3
	α_{season}			*					
Tertiary	Summer '05	216	43	93	**	*	80	-115	57
	Autumn '05	212	20	35	**		91	-77	84
	Winter '05	266	39	22	**	*	85	43	92
	Spring '06	185	56	122	**	**	70	-118	34
	α_{season}		*	**					

n.m.: not measured

Concentrations of Cu and Zn were reduced in the FWS, HSSF and VSSF CWs, whereas the tertiary CW did not reduce Cu and Zn (Tables 2.7 and 2.8). Copper removal in the FWS CW was higher in summer and autumn than in winter and spring. Effluent concentrations of Cu and Zn were generally higher in the FWS CW than in the other CWs under study.

Table 2.7

Mean concentrations of Cu in the influent of the PT (Infl PT), the CW (Infl CW) and effluent (Effl) during the different seasons (µg l^{-1}) with associated removal efficiencies of the PT, the CW and the total removal efficiency (TOT) (%). $\boldsymbol{\alpha_{concentration}}$ denotes the significance of difference between the influent of the PT, CW and effluent; α_{season} denotes the significance of seasonal differences. *: difference is significant at $\alpha = 0.05$; **: significant at $\alpha = 0.001$

CW	**Season**	**Concentration (µg l^{-1})**			**$\alpha_{concentration}$**		**Removal efficiency (%)**		
		Infl PT	**Infl CW**	**Effl**	**PT**	**CW**	**PT**	**CW**	**TOT**
FWS	Summer '05	n.m.	37	3		**		90	
	Autumn '05	n.m.	28	11		*		60	
	Winter '05	n.m.	20	10				50	
	Spring '06	n.m.	26	11				56	
	α_{season}								
HSSF	Summer '05	69	24	2		*	64	92	97
	Autumn '05	37	26	2		**	30	92	95
	Winter '05	26	24	2		**	8	91	92
	Spring '06	9	7	2		*	21	73	79
	α_{season}	*	*						
VSSF	Summer '05	4	5	2		*	-25	59	49
	Autumn '05	10	10	2		**	8	79	81
	Winter '05	5	4	2		*	18	42	52
	Spring '06	4	4	2		*	-20	54	45
	α_{season}								
Tertiary	Summer '05	15	2	2	**		87	0	87
	Autumn '05	6	2	2	*		68	0	68
	Winter '05	5	2	3	**		60	-44	42
	Spring '06	7	12	10			-73	14	-48
	α_{season}		**	*					

n.m.: not measured

Table 2.8

Mean concentrations of Zn in the influent of the PT (Infl PT), the CW (Infl CW) and effluent (Effl) during the different seasons (µg l^{-1}) with associated removal efficiencies of the PT, the CW and the total removal efficiency (TOT) (%). $\boldsymbol{\alpha}_{\mathbf{concentration}}$ denotes the significance of difference between the influent of the PT, CW and effluent; α_{season} denotes the significance of seasonal differences. *: difference is significant at $\alpha = 0.05$; **: significant at $\alpha = 0.001$

CW	**Season**	**Concentration (µg l^{-1})**			$\alpha_{concentration}$		**Removal efficiency (%)**		
		Infl PT	**Infl CW**	**Effl**	**PT**	**CW**	**PT**	**CW**	**TOT**
FWS	Summer '05	n.m.	150	37		**		75	
	Autumn '05	n.m.	102	86		*		16	
	Winter '05	n.m.	119	57		*		52	
	Spring '06	n.m.	110	55		*		50	
	α_{season}								
HSSF	Summer '05	326	165	20		*	49	88	94
	Autumn '05	240	164	19		**	32	88	92
	Winter '05	128	87	10		**	32	89	92
	Spring '06	78	46	10	*	**	41	78	87
	α_{season}	*	**	**					
VSSF	Summer '05	55	55	17		*	2	69	69
	Autumn '05	88	83	25		**	6	70	72
	Winter '05	28	24	12		*	13	49	56
	Spring '06	20	27	14		*	-34	50	33
	α_{season}	*	*	*					
Tertiary	Summer '05	103	15	13	**		86	11	87
	Autumn '05	71	15	15	**		79	0	79
	Winter '05	44	18	29	**		60	-65	33
	Spring '06	51	34	31	*		34	9	41
	α_{season}	*	*						

n.m.: not measured

The Cd concentrations were not reduced after passage through the VSSF CW (Table 2.9). The Cd concentrations in the influent and effluent of the FWS CW were lower than the analytical detection limit of 0.1 μg l^{-1} throughout the monitoring period. Positive removal efficiencies were observed in the HSSF reed beds, except during spring in the tertiary HSSF reed bed during which the Cd concentration in the effluent was significantly higher than in the influent ($p = 0.005$).

Table 2.9

Mean concentrations of Cd in the influent of the PT (Infl PT), the CW (Infl CW) and effluent (Effl) during the different seasons (μg l^{-1}) with associated removal efficiencies of the PT, the CW and the total removal efficiency (TOT) (%). **$\alpha_{concentration}$** denotes the significance of difference between the influent of the PT, CW and effluent; α_{season} denotes the significance of seasonal differences. *: difference is significant at $\alpha = 0.05$; **: significant at $\alpha = 0.001$

CW	**Season**	**Concentration (μg l^{-1})**			**$\alpha_{concentration}$**		**Removal efficiency (%)**		
		Infl PT	**Infl CW**	**Effl**	**PT**	**CW**	**PT**	**CW**	**TOT**
FWS	Summer '05	n.m.	0.10	0.10				/	
	Autumn '05	n.m.	0.10	0.10				/	
	Winter '05	n.m.	0.10	0.10				/	
	Spring '06	n.m.	0.10	0.10				/	
	α_{season}								
HSSF	Summer '05	0.70	0.59	0.10		*	15	83	86
	Autumn '05	0.39	0.42	0.10		**	-8	76	74
	Winter '05	0.96	0.62	0.22	*	*	35	65	77
	Spring '06	0.92	0.65	0.23	*	*	29	64	75
	α_{season}	*							
VSSF	Summer '05	0.10	0.39	0.38			-293	4	-278
	Autumn '05	0.10	0.30	0.28	*		-199	5	-183
	Winter '05	0.51	0.38	0.34			25	12	34
	Spring '06	0.58	0.14	0.10	*		77	26	83
	α_{season}	**		*					
Tertiary	Summer '05	0.10	0.75	0.10	*	*	-651	87	0
	Autumn '05	0.10	0.36	0.11	*	*	-264	70	-11
	Winter '05	0.20	0.26	0.22			-32	17	-10
	Spring '06	0.38	0.10	1.15	*	*	74	-1054	-202
	α_{season}	*	*	*					

n.m.: not measured, /: removal efficiency could not be calculated

With a few exceptions, the Pb concentrations were not significantly reduced after passage through the CWs (Table 2.10).

Table 2.10

Mean concentrations of Pb in the influent of the PT (Infl PT), the CW (Infl CW) and effluent (Effl) during the different seasons (µg l^{-1}) with associated removal efficiencies of the PT, the CW and the total removal efficiency (TOT) (%). $\boldsymbol{\alpha}_{\mathbf{concentration}}$ denotes the significance of difference between the influent of the PT, CW and effluent; α_{season} denotes the significance of seasonal differences. *: difference is significant at α = 0.05; **: significant at α = 0.001

CW	**Season**	**Concentration (µg l^{-1})**			$\alpha_{concentration}$		**Removal efficiency (%)**		
		Infl PT	**Infl CW**	**Effl**	**PT**	**CW**	**PT**	**CW**	**TOT**
FWS	Summer '05	n.m.	17.08	2.00		**		88	
	Autumn '05	n.m.	7.73	5.67				27	
	Winter '05	n.m.	4.22	2.53				40	
	Spring '06	n.m.	2.20	3.28				-49	
	α_{season}		*	*					
HSSF	Summer '05	26.18	10.69	2.00		*	59	81	92
	Autumn '05	15.50	9.88	2.92		*	36	70	81
	Winter '05	15.05	6.15	4.31	*		59	30	71
	Spring '06	12.89	8.31	5.78			36	30	55
	α_{season}								
VSSF	Summer '05	3.90	2.65	5.79			32	-118	-48
	Autumn '05	5.30	3.71	4.21			30	-14	21
	Winter '05	2.00	3.58	2.31			-79	35	-16
	Spring '06	2.83	7.02	4.05			-148	42	-43
	α_{season}								
Tertiary	Summer '05	5.19	2.00	2.00			61	0	61
	Autumn '05	4.43	2.13	2.05			52	4	54
	Winter '05	3.21	2.55	2.52			20	1	21
	Spring '06	3.86	2.65	4.18			31	-58	-8
	α_{season}								

n.m.: not measured

Although not statistically significant, the Cr concentrations were reduced after passage through the FWS CW during summer, autumn and winter, corresponding with positive removal efficiencies during these seasons (Table 2.11). A significant reduction of Cr was observed after passage through the HSSF CW during all seasons, with the highest removal efficiencies in summer and autumn. The VSSF and tertiary CWs showed poor removal of Cr.

Table 2.11

Mean concentrations of Cr in the influent of the PT (Infl PT), the CW (Infl CW) and effluent (Effl) during the different seasons ($\mu g\ l^{-1}$) with associated removal efficiencies of the PT, the CW and the total removal efficiency (TOT) (%). $\boldsymbol{\alpha_{concentration}}$ denotes the significance of difference between the influent of the PT, CW and effluent; α_{season} denotes the significance of seasonal differences. *: difference is significant at $\alpha = 0.05$; **: significant at $\alpha = 0.001$

CW	**Season**	**Concentration ($\mu g\ l^{-1}$)**			**$\alpha_{concentration}$**		**Removal efficiency (%)**		
		Infl PT	**Infl CW**	**Effl**	**PT**	**CW**	**PT**	**CW**	**TOT**
FWS	Summer '05	n.m.	2.38	0.50				79	
	Autumn '05	n.m.	1.53	0.60				61	
	Winter '05	n.m.	2.69	0.55				80	
	Spring '06	n.m.	0.51	0.50				3	
	α_{season}		*						
HSSF	Summer '05	6.82	2.81	0.50		*	59	82	93
	Autumn '05	3.65	2.68	0.50		**	27	81	86
	Winter '05	4.29	4.26	1.66		**	1	61	61
	Spring '06	3.40	2.85	1.52		*	16	46	55
	α_{season}		*	**					
VSSF	Summer '05	1.43	0.90	1.34			37	-49	6
	Autumn '05	2.07	0.95	0.84	*		54	12	60
	Winter '05	2.18	2.17	0.86		*	0	60	61
	Spring '06	1.88	2.83	1.52		*	-51	46	19
	α_{season}		**	*					
Tertiary	Summer '05	1.85	0.50	0.50	*		73	0	73
	Autumn '05	1.57	0.61	0.78	*		61	-29	50
	Winter '05	1.42	0.66	4.04	*		54	-516	-185
	Spring '06	0.89	1.62	2.64			-82	-64	-198
	α_{season}								

n.m.: not measured

With a few exceptions, the Ni concentration was not significantly altered after passage through the FWS, VSSF and tertiary CWs (Table 2.12). On the contrary, the HSSF CW was observed to significantly reduce the Ni concentration during all seasons ($p < 0.05$).

Table 2.12

Mean concentrations of Ni in the influent of the PT (Infl PT), the CW (Infl CW) and effluent (Effl) during the different seasons (μg l^{-1}) with associated removal efficiencies of the PT, the CW and the total removal efficiency (TOT) (%). $\alpha_{concentration}$ denotes the significance of difference between the influent of the PT, CW and effluent; α_{season} denotes the significance of seasonal differences. *: difference is significant at $\alpha = 0.05$; **: significant at $\alpha = 0.001$

CW	**Season**	**Concentration (μg l^{-1})**			**$\alpha_{concentration}$**		**Removal efficiency (%)**		
		Infl PT	**Infl CW**	**Effl**	**PT**	**CW**	**PT**	**CW**	**TOT**
FWS	Summer '05	n.m.	4.06	3.48				14	
	Autumn '05	n.m.	3.20	3.47				-8	
	Winter '05	n.m.	2.27	2.06				9	
	Spring '06	n.m.	2.86	2.00				30	
	α_{season}		*	**					
HSSF	Summer '05	9.78	5.46	2.51		*	44	54	74
	Autumn '05	5.39	4.91	2.27		**	9	54	58
	Winter '05	6.70	6.19	4.40		*	8	29	34
	Spring '06	10.64	8.41	3.06		*	21	64	71
	α_{season}	*	*	*					
VSSF	Summer '05	11.91	10.19	7.48			14	27	37
	Autumn '05	13.06	10.42	6.79		*	20	35	48
	Winter '05	18.59	15.62	9.58		**	16	39	48
	Spring '06	19.56	14.52	11.63	*		26	20	41
	α_{season}	*	*	*					
Tertiary	Summer '05	5.82	6.03	5.21			-4	14	10
	Autumn '05	3.81	3.71	2.33		*	3	37	39
	Winter '05	2.64	2.25	2.63			15	-17	0
	Spring '06	2.00	2.00	7.45		*	0	-272	-272
	α_{season}	**	**	*					

n.m.: not measured

3.3 Short-term high-frequent monitoring of the horizontal subsurface flow constructed wetland

Fig 2.1 presents the short-term dynamics of the total heavy metal concentrations in the influent and effluent of the HSSF CW Zemst-Kesterbeek. Influent and effluent concentrations were not normally distributed ($p < 0.001$ for all metals, in both fractions). However, for reporting purposes, the mean total and dissolved (< 0.45 µm) metal concentrations in the influent and effluent are presented in Fig 2.2. The variations of the metal concentrations in the effluent were generally smaller than in the influent. A significant decrease of the total concentration between influent and effluent was seen for Al, Cu, Zn and Pb ($p < 0.05$). As dissolved concentrations of most metals were low in both the influent and effluent, removal efficiencies based on this fraction were low (Fig 2.2). The dissolved concentrations of Cd, Cu, Pb, Cr and Ni in both the influent and effluent were near the detection limits of the GF-AAS (0.1 µg l^{-1} for Cd, 0.5 µg l^{-1} for Cr and 2 µg l^{-1} for Cu, Ni and Pb) or lower. Removal efficiencies of the metals were high for Al, Cu, Pb and Zn (> 81 %) and low for Cd, Cr and Ni (< 27 %). This was merely attributed to the fact that the total influent concentrations of Cd, Cr and Ni were already very low.

Total Mn and Fe concentrations were significantly higher in the effluent than in the influent with a negative removal efficiency of about 450 %. The dissolved Mn concentration in the effluent was not significantly different from the total concentration ($p = 0.18$), which means that Mn was released in a dissolved state. Moreover, the dissolved Mn concentration in the effluent was about 7 times higher than in the influent. The dissolved Fe concentration in the effluent had a mean value of 0.028 mg l^{-1} and was significantly lower than the total concentration ($p < 0.05$). Unlike Mn, Fe was released mainly in a particulate state as only 1.2 % of the total Fe was dissolved.

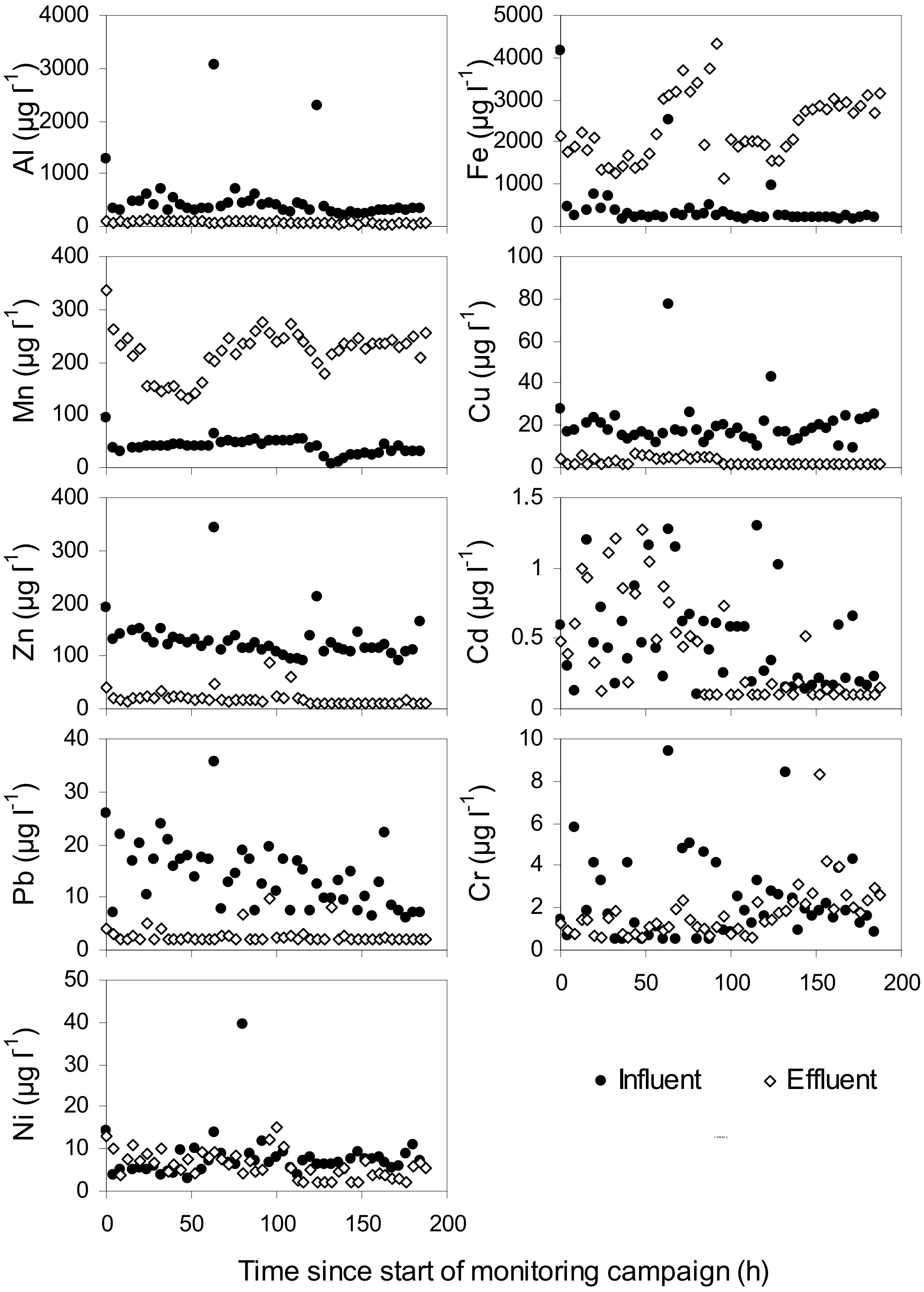

Fig 2.1 Total Al, Fe, Mn, Cu, Zn, Cd, Pb, Cr and Ni concentrations in the influent and effluent of the HSSF CW (µg l^{-1}) as a function of time since start of monitoring at 16:00, September 17, 2004 (h) (n = 48).

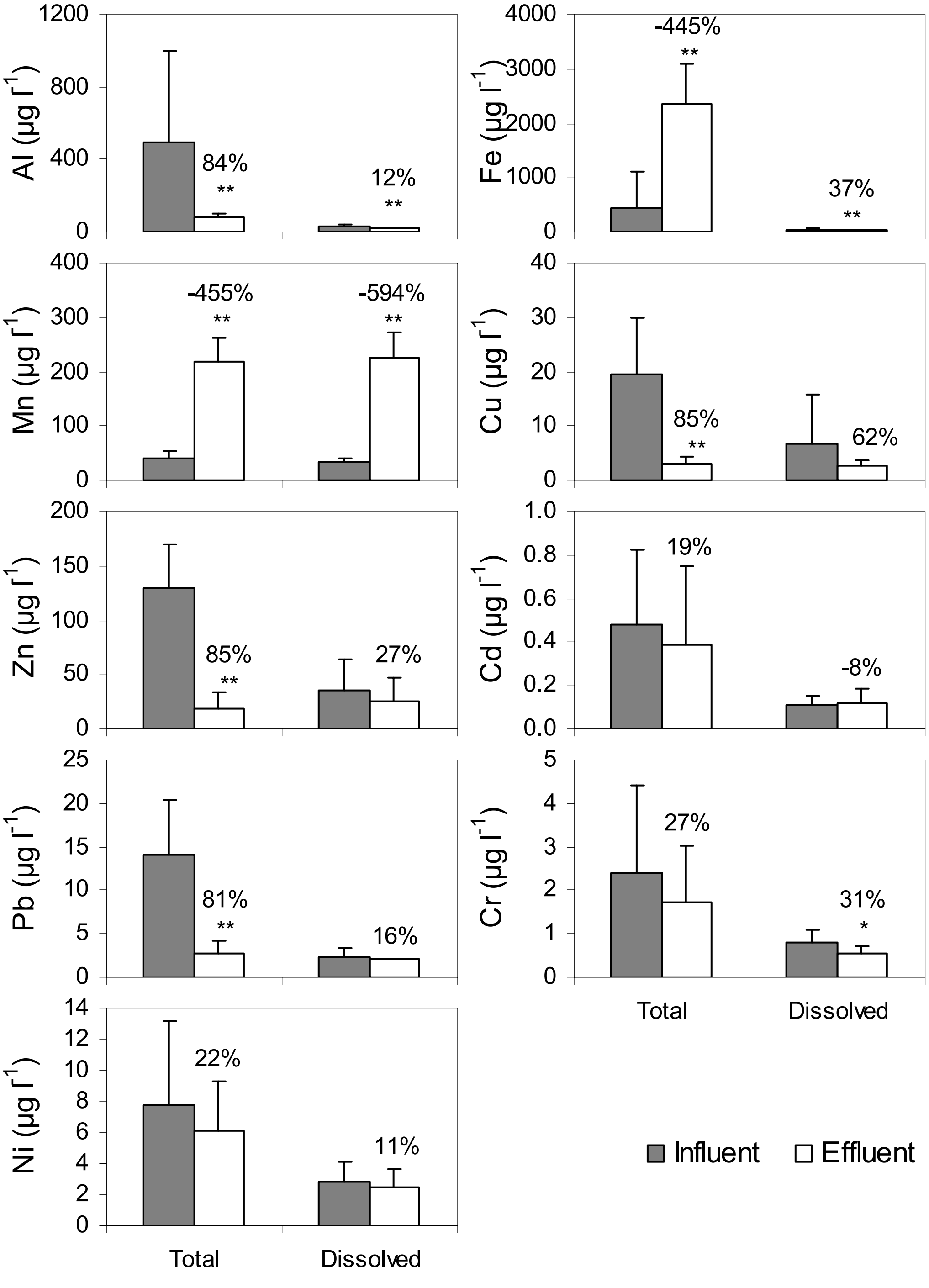

Fig 2.2 Total and dissolved Al, Fe, Mn, Cu, Zn, Cd, Pb, Cr and Ni concentrations in the influent and effluent of the HSSF CW (µg l^{-1}) and associated removal efficiencies (%) (n = 48). *: difference between influent and effluent is significant at α = 0.05; **: significant at α = 0.001.

4 Discussion

The long-term dataset obtained from Aquafin NV showed that Flemish CWs received trace concentrations of heavy metals in the raw wastewater, of which Cu and Zn were the most abundant. Important sources of Cu and Zn in the wastewater include paints, cosmetics, corrosion from pipes, and runoff from building materials (De Raeymaecker et al., 2005; VMM, 2005). The majority of influent samples had Ag, As, Cd, Cr, Hg, Ni and Pb concentrations that were lower than the detection limits. It is reminded that the long-term dataset considered the raw wastewater, thus the influent of the pre-treatment step. With a few exceptions, concentrations of heavy metals in the raw wastewater were generally similar in the CWs under study. This could be attributed to the fact that all monitored CWs received a mixture of domestic wastewater and stormwater. Mean total concentrations of Cu, Zn, Pb, Ni and Cr in the raw wastewater varied between respectively 14 – 104, 83 – 574, 3.9 – 47, 0 – 14 and 0.65 – 13 $\mu g\ l^{-1}$, levels similar to those reported by Kadlec and Knight (1996) and Vymazal (2005) for raw municipal wastewaters.

The heavy metal concentrations in the influent were generally subjected to a larger variability than in the effluent. This was observed for the long-term, weekly and short-term datasets and demonstrates the buffering effect of the CWs. Peak concentrations of heavy metals in the influent wastewater were efficiently buffered during passage through the CWs, a trend that was also observed by De Wilde (2001), De Moor (2002) and Rousseau (2005) for concentrations of COD, SS, N and P in a pilot-scale combined CW consisting of a VSSF followed by a HSSF reed bed.

There are currently no effluent standards for heavy metals in CWs in Flanders. However, it was relevant to compare the metal concentrations in the effluents with the basic environmental quality standards for surface water as the effluents of the CWs are discharged into surface water. In theory, environmental quality standards of metals are total or dissolved concentrations that do not affect the structure and function of aquatic ecosystems. However, current environmental quality standards have some shortcomings that hinder their use as good evaluators of the ecological water quality. A major shortcoming of current quality standards is that they do not incorporate metal bioavailability (Janssen et al., 2000). Therefore, comparisons between metal concentrations in the effluents and quality standards are not useful in accurately assessing the impact on the ecological quality of the receiving surface water. They are however useful in evaluating the extent of metal pollution in the effluents of the CWs. The environmental quality standards of metals in surface water are 50 µg l^{-1} for Cu, Pb, Cr and Ni, 200 µg l^{-1} for Zn, 30 µg l^{-1} for As, 1 µg l^{-1} for Cd and 0.5 µg l^{-1} for Hg (VLAREM II, 1995). Maximum total concentrations in the effluents of 12 CWs derived from the long-term dataset collected by the VMM since the start of monitoring, were 20 µg l^{-1} for As, 0.45 µg l^{-1} for Cd, 7.3 µg l^{-1} for Cr, 15 µg l^{-1} for Cu, 0.49 µg l^{-1} for Hg, 11 µg l^{-1} for Ni, 3.1 µg l^{-1} for Pb and 588 µg l^{-1} for Zn. With the exception of Zn, effluent concentrations complied with the basic environmental quality standards. The maximum value of 588 µg l^{-1} Zn was observed in the effluent of the VSSF CW in Sint-Niklaas-Heimolen, and corresponds with a negative removal efficiency of -75 %. As a positive removal efficiency was generally observed for the other metals in this CW, it remains unclear what caused this release of Zn. Effluent concentrations of Zn in the other CWs under study were lower than the standard of 200 µg l^{-1}. Total concentrations of Cd, Cr, Cu, Ni, Pb and Zn in the effluents of the 4 CWs that were subjected to a weekly monitoring campaign and in the effluent of the HSSF CW during the short-term monitoring campaign, were generally lower than basic environmental quality standards for surface water. Contrary to the other metals, standards for Fe and Mn apply to their dissolved concentrations and are 200 µg l^{-1} for both metals (VLAREM II, 1995). Dissolved concentrations of Fe and Mn in the effluent of the HSSF CW were respectively 28 ± 8 and 227 ± 46 µg l^{-1} and thus close to or lower than the basic environmental quality standards.

The low-frequent and irregular nature of sample collection did not allow the detection of temporal trends in the long-term dataset of the 12 CWs obtained from Aquafin NV. Increasing the sampling frequency of 4 selected CWs to a regular weekly strategy allowed the detection of some seasonal trends. Although not always statistically significant, the raw wastewater and influent of the CWs generally showed the highest concentrations of Al, Cu, Zn and Pb in summer and autumn ‘05, compared to winter ’05 and spring ‘06. The total precipitation volume recorded for spring ’06 (from March 21 to June 21, 2006) was considerably higher than for the other seasons, for which differences were smaller (http://www.meteoonline.be). Differences in Al, Cu, Zn and Pb concentrations in the wastewater could not clearly be related to seasonal variations in precipitation and dilution of the wastewater. Iron, Mn and Cr concentrations in the raw wastewater of the 4 CWs were not affected by season.

The long-term dataset of 12 CWs demonstrated that with a few exceptions, Cu and Zn concentrations were significantly reduced in all CWs. Comparisons of the removal efficiencies between different CWs should be carefully interpreted, as they are strongly dependent on influent concentrations and hydraulic loading rates (Kadlec and Knight, 1996). Moreover, removal efficiencies derived from the long-term dataset are based on total metal concentrations in the raw wastewater and the effluent and thus do not substantiate between the CW itself and the pre-treatment step. Monitoring of the concentrations in the influent of the CW itself, thus after passage through the pre-treatment step, provided more information and allowed better comparison of metal removal in the different CWs. However, keeping these remarks in mind, it can be roughly concluded that removal of Cu and Zn appeared to be higher in CWs with a granular filter medium than in FWS CWs. The removal efficiencies in the FWS CWs varied between 61 and 80 % for Cu and between 64 and 66 % for Zn. The highest removal efficiencies of Cu and Zn were observed in the HSSF CWs, varying between 90 and 100 %.

Weekly monitoring of the raw wastewater, the influent and effluent of 4 selected CWs provided information about the relative contribution of the CW and the pre-treatment step in the overall metal removal. The pre-settlement tanks of the HSSF and VSSF CWs did generally not offer a significant reduction of the concentrations of heavy metals in the wastewater. This was observed throughout the monitoring period. As a result of this, the HSSF and VSSF CWs generally received the highest concentrations of heavy metals as compared to the other CWs. Vymazal (2005) however reported substantial removal of most metals in the pre-treatment unit of a HSSF CW treating domestic wastewater and stormwater. The pre-treatment unit consisted of a screen and an Imhoff tank and removed 85 % of Al, 83 % of Fe, 84 % of Ni, 45 % of Cu and 93 % of Cr from the wastewater. On the contrary, the pre-treatment unit did not strongly affect the concentrations of Zn, Pb and Mn and removed 33, 21 and 2 % respectively.

Concentrations of Al, Fe, Mn, Cu, Zn, Pb and Cr were generally reduced after passage through the pre-settlement tank and the RBCs preceding the tertiary CW in Aalbeke. High mean removal efficiencies of 66, 77, 83 and 84 % were observed for Zn, Al, Mn and Fe, respectively, in the pre-treatment step during the entire monitoring period, whereas removal efficiencies of Cu, Pb and Cr varied between 35 and 39 %. The Cd and Ni concentrations were not reduced by the pre-treatment step located before the tertiary CW, probably due to their very low influent concentrations. Overall, it is concluded that the presence of a conventional wastewater treatment system such as a series of RBCs strongly reduced the inflow of most metals into the tertiary CW.

Table 2.13 presents the mean total metal concentrations in the influents of the 4 CWs and the associated removal efficiencies, together with some literature reports. Of the 4 CWs that were part of the monitoring campaign, the highest removal efficiencies of all metals except Fe and Mn, were observed in the HSSF CW in Zemst-Kesterbeek: 93, 90, 87, 70, 53, 66 and 49 % respectively for Al, Cu, Zn, Cd, Pb, Cr and Ni. This was partly attributed to the generally higher metal concentrations in the influent of this CW than in the other CWs under study. On the contrary, the tertiary CW generally received the lowest concentrations of heavy metals and was consequently the least efficient of the CWs under study. A positive relationship between influent concentrations and removal efficiencies was observed for most metals, except Fe and Mn, within the group of CWs assessed. This positive relationship has been described by Kadlec and Knight (1996). Sinicrope et al. (1992) also observed an increase of the Cu removal efficiency from 63 to 88 % in HSSF mesocosms planted with *Scirpus californicus* when Cu concentrations in the influent were increased by a factor 20. Increasing the Zn concentration in the influent from 0.3 to 2.5 mg l^{-1} on the other hand did not affect the removal efficiency in these mesocosms.

The relationship between influent metal concentrations and removal efficiencies however does not stand on its own, and the metal concentration in the influent is not the sole factor determining the removal efficiency. For example, Al concentrations in the influents of the HSSF and VSSF CWs in Flanders and their associated removal efficiencies were similar to values reported in HSSF CWs in the Czech Republic by Vymazal (2003, 2005) and Vymazal and Krása (2003). However, whereas high removal efficiencies of Al were observed by Samecka-Cymerman et al. (2004) in a FWS CW receiving domestic wastewater with low Al influent concentrations, Mays and Edwards (2001) observed negative removal efficiencies of Al in FWS CWs receiving acid mine drainage (AMD) with similar Al concentrations in the influent (Table 2.13). Factors other than the influent metal concentration clearly affect the removal efficiency as well.

Differences in influent concentrations and hydraulic loading rates (HLR) hamper comparisons between CWs (Kadlec and Knight, 1996; Vymazal, 2003). Vymazal (2003) suggested that a

HLR higher than 10 cm d^{-1} provides lower treatment effect. In their study of 2 FWS CWs receiving AMD, Mays and Edwards (2001) reported higher removal efficiencies of Mn in the CW with the lowest HLR and longest residence time. However, the authors also postulated other reasons that might explain the lower treatment efficiency of one of the FWS CWs, such as stress of the *Typha latifolia* plants and the higher alkalinity of the wastewater in the best performing CW which buffers the acidity generated by oxidation of Fe(II). Cheng et al. (2002) studied metal removal in a pilot-scale CW consisting of a vertical flow bed planted with *Cyperus alterniflorus* followed by a reverse-vertical flow bed planted with *Villarsia exaltata*, and noted that for all metals except Mn, the first chamber was responsible for metal removal. High removal efficiencies were observed, even at a fairly high HLR of 6 cm d^{-1} in this first VSSF bed. Groudeva et al. (2001) also reported very high removal efficiencies of most metals in a FWS CW treating oil-contaminated water, despite the high HLRs varying between 4.3 and 22 cm d^{-1}, which resulted in low residence times between 10 to 50 h. Overall, it is clear that many factors affect the reported removal efficiencies and that comparisons between CWs are therefore not easily drawn.

Manganese and Fe were released from the HSSF CW under study, and this release generally occurred throughout the year. Vymazal (2005) also reported a release of Fe and Mn in a HSSF CW treating domestic wastewater during the second year of operation, and related this phenomenon to the anaerobic conditions in the sediment that lead to bacterial reduction of Mn(IV) and Fe(III) and the release of the soluble Mn(II) and Fe(II) species. The release of Ni from this HSSF CW suggested that this element was co-precipitated with Fe(III) and Mn(IV) (oxy-) hydroxides. However, positive removal efficiencies of Fe and Mn have been observed in another HSSF CW after 4 years of operation by Vymazal (2003) and Vymazal and Krása (2003). The tertiary CW, also of the HSSF type, showed a release of Mn but not of Fe, indicating an oxidation reduction state that is intermediate between the reduction of Mn(IV) and Fe(III). This difference between both HSSF reed beds in this study could be attributed to the different type of wastewater that is treated. The HSSF CW in Zemst-Kesterbeek is used for secondary treatment of domestic wastewater and would receive a much higher organic loading than a similar type of CW that is used for tertiary treatment.

Although a VSSF CW is generally aerobic (Stottmeister et al., 2003) and would govern the transformation to the stable Fe(III) and then Mn(IV), the VSSF CW under study did not strongly reduce the Fe and Mn concentrations in the water. The configuration of the CW, consisting of a layer of 60 cm of fine gravel on top of a drainage layer with a depth of 30 cm, should normally enhance aerobic conditions. However, surface flooding was observed during a visit of the site at the end of May 2006. Presumably, redox conditions in the VSSF CW did not favour the removal of Fe and Mn. Cheng et al. (2002) also reported a poor removal of Mn in the first stage of a pilot-scale CW consisting of a vertical flow bed planted with *Cyperus alterniflorus*.

Whereas positive removal efficiencies of Fe and Mn have been reported in FWS CWs treating domestic sewage and AMD, poor removal was observed in the FWS CW under study (Table 2.13). Reducing conditions could have been present in that CW, leading to its low removal efficiency towards Fe and Mn. However, assumptions regarding the behaviour of Fe and Mn in CWs would need to be supported by measurements of e.g. redox potentials and concentrations of dissolved oxygen and nitrates.

Few authors have studied total and dissolved Fe and Mn concentrations in the influent and effluent and assessed transformations between the particulate and dissolved Fe and Mn fraction. Goulet and Pick (2001) reported a transformation from particulate to dissolved Fe and Mn during fall and winter in a FWS CW receiving urban and agricultural runoff. It was planted with emergent (e.g. *Typha latifolia*), floating (*Lemna minor*) and submerged (e.g. *Ceratophyllum demersum*, *Potamogeton* sp.) plants. They related the export of dissolved Fe and Mn to the reduction of Fe- and Mn-oxides in anoxic sediments. The short-term monitoring of the HSSF CW in Zemst-Kesterbeek showed export of dissolved Mn but not of Fe. This could be caused by intermediate redox conditions at the time of sampling, probably causing higher dissolved Mn concentrations in the effluent but not of Fe.

Table 2.13

Metal concentrations in the influent of the 4 CWs (Conc, µg l^{-1}) and CWs reported in literature, together with the hydraulic loading rate (HLR) (cm d^{-1}) and associated removal efficiency (*ε*, %); *: design HLR, based on the PEs that can be connected, **: HLR unknown

CW	HLR		Al	Fe	Mn	Cu	Zn	Cd	Pb	Cr	Ni	Ref n°
HSSF	4*	Conc	1238	736	75	21	113	0.6	8.4	3.2	6.2	9
		ε	*93*	*-50*	*-103*	*90*	*87*	*70*	*53*	*66*	*49*	
Tertiary	15*	Conc	137	162	38	4.5	20	0.3	2.4	0.9	3.0	9
		ε	*57*	*67*	*-50*	*1*	*-16*	*-35*	*-14*	*-172*	*-33*	
HSSF	2.7	Conc	451	1.1	0.28	11.3	198	0.43	120		9.54	1, 2
		ε	*>91*	*35*	*81*	*>82*	*>98*	*77*	*98*		*92*	
HSSF	4.1	Conc	500	300	100	4	75		6	0.5	1.5	3
		ε	*>93*	*-15*	*-269*	*>55*	*91*		*66*	*>4*	*-71*	
HSSF	4.9-6.1	Conc				60-1200	300-2500	70	300	100	300	4
		ε				*63-88*	*79-71*	*78*	*84*	*75*	*55*	
FWS	4.9*	Conc	231	327	66	26	113	<0.1	6.1	1.6	2.9	9
		ε	*67*	*9*	*-20*	*61*	*42*		*37*	*66*	*8*	
FWS	**	Conc	14-8.5	2.2-15	0.79-0.76	6.2-7.9	59-7.1	0.27-0.18	0.43-0.28		9.3-8.1	5
		ε	*97-81*	*79-86*	*99-99*	*56-43*	*68-59*	*56-17*	*81-64*		*55-33*	
FWS	1.1	Conc	20	44000	5900		<9	<6	<2			6a
		ε	*-100*	*98*	*79*							
FWS	4.1	Conc	29	205000	7400		30	20	2.2			6b
		ε	*-17*	*97*	*47*		*33*	*>70*	*26*			
FWS	4.3-22	Conc		2750-23000	640-3740	140-820		50-170	280-1070			7
		ε		*>64->96*	*>22->87*	*>93->99*		*>80->94*	*>96->99*			
VSSF	7.5*	Conc	511	748	323	6.1	49	0.3	4.3	1.8	13	9
		ε	*85*	*25*	*13*	*66*	*64*	*10*	*14*	*41*	*32*	
VSSF	6	Conc			302	1040	4250	9	10			8
		ε			*0-100*	*>98*	*100*	*>99*	*>95*			

References: (1, 2, 3): Vymazal and Krása (2003), Vymazal (2003) and Vymazal (2005): HSSF CWs for domestic wastewater; (4) Sinicrope et al. (1992): pilot-scale HSSF CW for synthetic secondary wastewater; (5) Samecka-Cymerman et al. (2004): FWS CW for domestic wastewater; (6a, b) Mays and Edwards (2001): FWS CWs for AMD; (7) Groudeva et al. (2001): FWS CW for treatment of oil contaminated water; (8) Cheng et al. (2002): pilot-scale VSSF CW (as part of a downflow/upflow CW) treating artificial wastewater; (9): This study

5 Conclusions

Copper and Zn are the most abundant heavy metals in the influent of CWs for domestic wastewater in Flanders. The heavy metal concentrations in the influent showed a larger variability than in the effluent, demonstrating the buffering effect of the CWs. Total concentrations of the heavy metals studied in the effluents were generally far below the basic environmental quality standards for surface water: 50 $\mu g\ l^{-1}$ for Cu, Pb, Cr and Ni, 200 $\mu g\ l^{-1}$ for Zn, 30 $\mu g\ l^{-1}$ for As, 1 $\mu g\ l^{-1}$ for Cd and 0.5 $\mu g\ l^{-1}$ for Hg.

Contrary to the long-term low-frequent dataset which only provided rough information concerning influent and effluent concentrations and mean removal efficiencies, the weekly monitoring campaign of 4 selected CWs provided useful additional information regarding: (1) seasonal trends, (2) the relative importance of the pre-treatment step and the CW in the overall metal removal and (3) differences between CWs. The pre-settlement tanks of the HSSF and VSSF CWs did not provide high reductions of the concentrations of heavy metals in the wastewater. On the contrary, the combination of a pre-settlement tank and a series of RBCs strongly reduced the inflow of metals into the tertiary CW.

The highest removal efficiencies of all metals except Fe and Mn, were observed in the HSSF CW, whereas the tertiary CW was the least efficient of the CWs under study. Removal efficiencies of Al, Cu, Zn, Cd, Pb, Cr and Ni in the HSSF CW were respectively 93, 90, 87, 70, 53, 66 and 49 %. Manganese was generally released from the FWS, HSSF and tertiary CWs, with the highest release observed in the HSSF CW. This release of Mn appeared to occur throughout the year. Iron was released from the HSSF CW, whereas the tertiary CW which is also of the HSSF type significantly reduced Fe. Different prevailing redox conditions in these HSSF reed beds are presumably causing the different removal patterns.

CHAPTER 3

ACCUMULATION OF METALS IN A FREE-WATER-SURFACE CONSTRUCTED WETLAND FOR DOMESTIC WASTEWATER TREATMENT

1 Introduction

Constructed wetlands (CWs) for domestic wastewater treatment have been extensively monitored when it comes to BOD, COD, suspended solids and nutrients, whereas only few researchers have investigated the accumulation of metals in sediments and plants of these systems (Kadlec and Knight, 1996). Yet, heavy metals are persistent in the environment and do accumulate in the sediments and plants of constructed wetlands. Many authors have described the sediment as the primary sink for metal accumulation and observed an increase of the total metal level in the sediment after a period of operation (Gschlößl and Stuible, 2000; Obarska-Pempkowiak, 2001; Vymazal, 2003; Vymazal and Krása, 2003, Chagué-Goff, 2005). Total metal levels give information on the pollution level but do not univocally reflect the mobility or toxicity of metals.

The metal mobility in reduced sediments of CWs is strongly limited by the presence of sulphides (Gambrell, 1994). Divalent metals such as Cd, Cu, Ni, Pb and Zn can displace Fe and Mn from their monosulphides to form highly insoluble metal sulphides (Fang et al., 2005):

$$Me^{2+} + FeS / MnS \rightarrow MeS + Fe^{2+} / Mn^{2+}$$

It is believed that heavy metals are not available for uptake by organisms in the presence of an excess of available sulphide due to the very low solubility of their sulphides (van den Hoop et al., 1997). The amount of sulphides and associated metals can be estimated by analysis of acid-volatile sulphide (AVS) and simultaneously extracted metals (SEM), operationally defined fractions that are released from the sediment after extracting the sediment with 1 M cold HCl (Allen et al., 1993).

The relationship between the SEM/AVS ratio and the toxicity of sediments was first reported by Di Toro et al. (1990). There was no toxicity of Cd for amphipods provided that the molar concentration of Cd did not exceed the molar concentration of sulphide in the sediment. When the molar concentration of Cd exceeded that of sulphide, Cd was significantly present in the pore water and toxicity was observed. This research was at the basis of the SEM/AVS concept: if the SEM/AVS ratio is < 1, no acute toxicity arising from metals is anticipated; if the ratio is > 1, the sediment might potentially exhibit toxicity due to the metals.

Since the work of Di Toro et al. (1990), a lot of research has been performed to further investigate the relationship between SEM/AVS ratios and toxicity in spiked sediments or field sediments. Good reviews are presented in Berry et al. (1996) and Hansen et al. (1996). The SEM/AVS ratio is now accepted as an accurate predictor for the absence of toxicity, whereas predictions on which sediments might be toxic are less certain. A significant number of freshwater and marine sediments collected in the US, Canada and China, with SEM/AVS ratios > 1 did not cause increased mortality (Hansen et al., 1996). This indicates that simultaneously extracted metals were also released from other phases that are controlling bioavailability in the sediments, such as organic matter and carbonates. This was supported by Yu et al. (2001) who performed a sequential extraction procedure on anoxic metal-contaminated river sediments before and after AVS extraction. The SEM/AVS ratio is very useful as a first parameter for assessment of metal toxicity of a given sediment and should be considered as a trigger for further research if the SEM/AVS ratio is > 1, e.g. analysis of the metal concentration in the pore water or toxicity testing. Toxicity predictions for sediments contaminated with a mixture of metals should use the sum of the SEM (ΣSEM) for comparison with the AVS level. If the ΣSEM/AVS is > 1, the metals could appear in the pore water in the reverse order of the solubility of their sulphides: Ni > Zn > Cd > Pb > Cu (Berry et al., 1996).

The subject of this study was a free-water-surface (FWS) CW planted with the common reed *Phragmites australis* (Cav.) Trin. ex Steud., that has been treating domestic wastewater of 1000 population equivalents (PEs) during 16 years. Different goals were at the basis of the study. Firstly, total metal levels in the sediment were determined to characterise the pollution level after 16 years of operation. Secondly, the mobility and potential toxicity of the sediment with respect to metals was assessed by means of the SEM/AVS concept. Finally, the accumulation of metals in the aboveground reed biomass at the end of the growing season was investigated and the relationship with the metal levels in the sediment was evaluated.

2 Material and Methods

2.1 Description of study site and sampling strategy

The CW under study is located in Deurle, 15 km southwest from Ghent in Flanders, Belgium. The system was designed and constructed by the Flemish Land Authority (VLM) in 1989 but was soon handed over to Aquafin NV, the Flemish company responsible for wastewater collection and treatment. The system treats domestic wastewater but receives storm water as well, because of the combined sewer networks in Flanders. The FWS CW has a theoretical design capacity of 1000 PEs (based on 54 g BOD d^{-1} PE^{-1}) and a maximum hydraulic capacity of 35.7 l s^{-1} ($12 \times Q_{14}$) (Aquafin, 2003a). An overflow construction is present to direct excess water directly into a brook, the Scheidbeek, during storm events. An aerial view and process lay-out of the FWS CW are presented in Fig 3.1 and Fig 3.2.

Influent wastewater is pumped into a primary settling ditch and then flows gravitationally through a series of 9 FWS reed beds after which it is collected in a discharge channel and eventually flows into the receiving brook. The primary settling ditch is 50 m long and has a trapezoidal cross section with a bottom width of 2 m and a width of 4 m at the water surface. Each of the 9 FWS reed beds has a length of 85 m and has a trapezoidal cross section (bottom width 2 m, width at the water surface 4 m). A water depth of about 0.5 m is observed in the reed beds. The discharge channel is 48 m long with a trapezoidal cross section. The CW has a theoretical hydraulic residence time of 9.1 days (based on 150 l d^{-1} PE^{-1})

Beds are planted with common reed (*Phragmites australis* (Cav.) Trin. ex Steud.). There is no liner present. The way the water has flown through the reed beds in the past is not registered and appears to have occurred in an uncontrolled manner, varying between flow in series and in parallel. At the time of sampling the wastewater flowed through the reed beds in series. The sediment and aboveground biomass of *Phragmites australis* were sampled in September 2004 at different locations along the treatment path in order to assess spatial variations (Fig 3.2). Sampling of the sediment was performed at the inlet and outlet of the primary settling ditch and at L/85, L/3, L, 2×L, 5×L and 9×L m from the inlet of the FWS reed beds with L being the length of one FWS reed bed, 85 m. Three replicate samples of the sediment were taken at each location. Undisturbed samples of the sediment of the FWS reed beds were taken to a depth of 30 cm by means of a liner core sampler with a diameter of 5 cm (04.15.SB, Eijkelkamp, Giesbeek, NL) and put in a polyethylene core with a plastic lid.

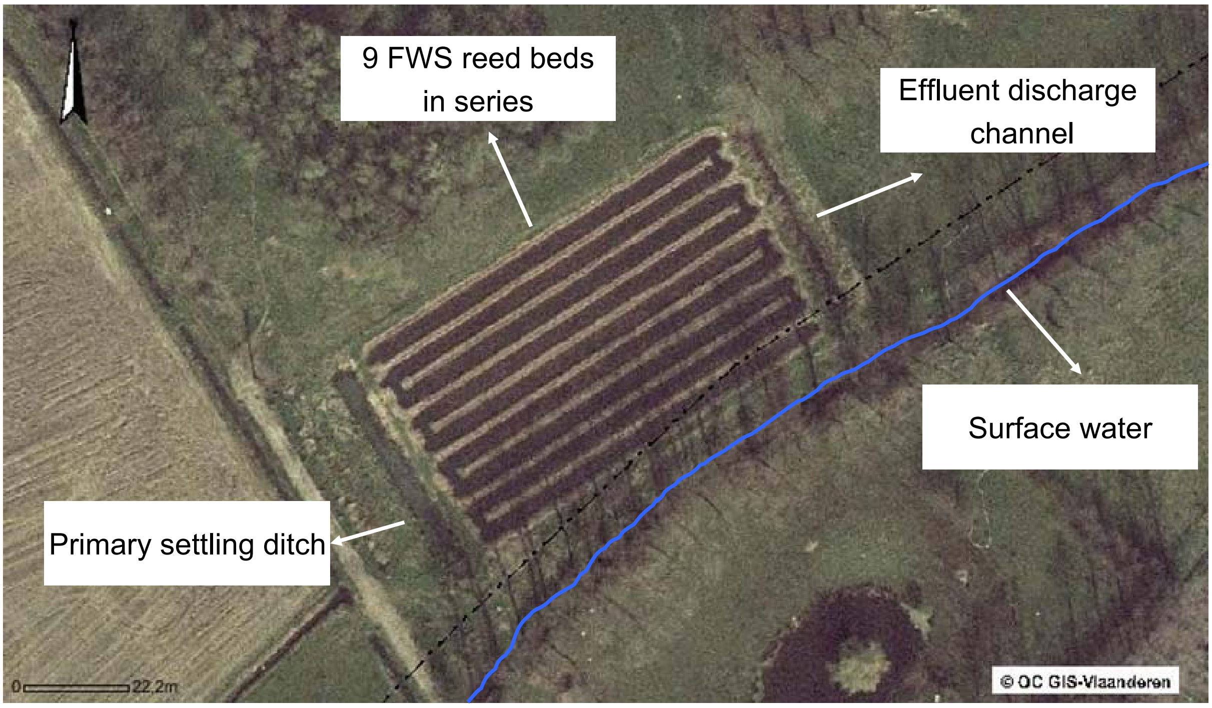

Fig 3.1 Aerial view of the FWS CW in Deurle (source: AGIV, 2006).

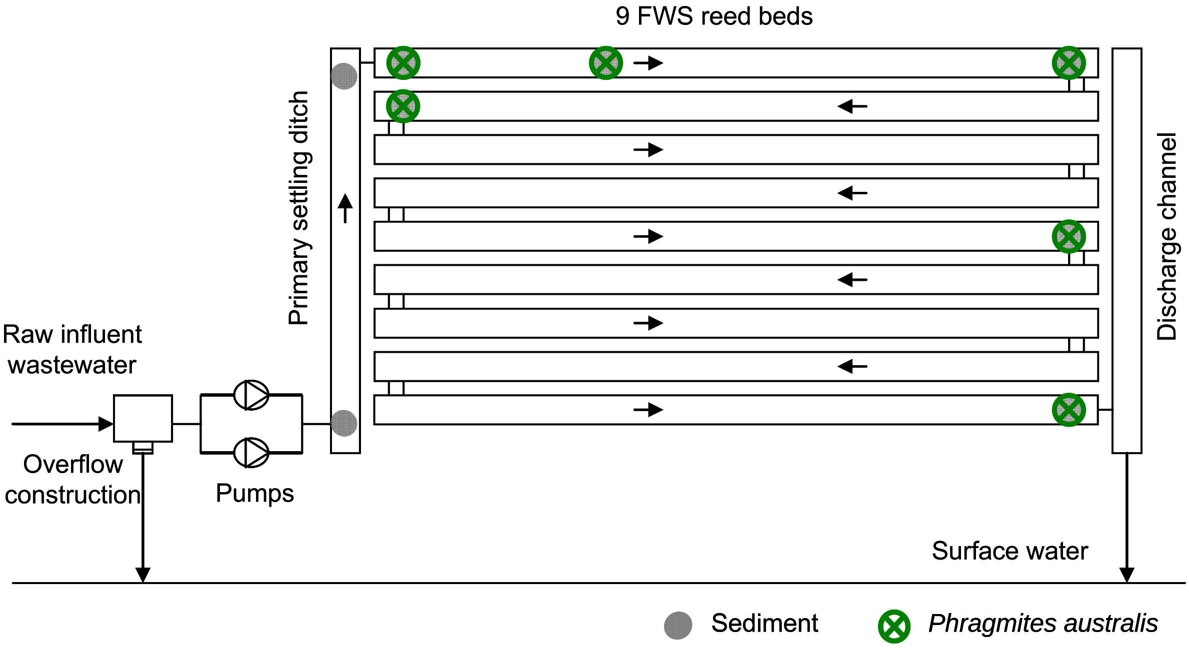

Fig 3.2 Process lay-out of the FWS CW with sampling locations of the sediment and *Phragmites australis*.

The sediment of the primary settling ditch was sampled with a polyethylene bottle as the sediment layer was too shallow and the presence of concrete at the bottom of the primary settling ditch hampered the use of the liner sampler. Sediment samples were stored into plastic bags to prevent contact with air. The sediment was analysed for total and simultaneously extracted metal concentrations, AVS, pH, electrical conductivity (EC), redox potential (Eh), cation exchange capacity (CEC), texture, $CaCO_3$, organic matter content and dry matter. Three replicate samples of the aboveground reed biomass were taken at the same locations in the FWS reed beds (Fig 3.2) and divided into leaves, leaf sheaths, stems and panicles.

2.2 Analytical procedures

Immediately after opening the polyethylene core, the sediment was divided into 2 layers of 15 cm, representing the surface and the deeper sediment. The redox potential (Eh) in the centre of both layers was measured with a redox electrode (HI 3090 B/5) and a HI 9025 meter (Hanna Instruments, Temse, Belgium). Measurements were corrected with regard to a standard hydrogen electrode, by calibrating in a solution of 0.033 M $K_3Fe(CN)_6$ and 0.033 M $K_4Fe(CN)_6$ in 0.1 M KCl.

Immediately after determination of the Eh, AVS were determined according to the method described by Tack et al. (1997a). Five g of sediment and 20 ml of 6 M HCl was added to 100 ml of deionised water in the reaction flask. The system was purged with N_2. Sulphides were then transformed to H_2S in the 1 M HCl solution in the 1st flask and collected in Zn-acetate solutions in two washing bottles connected in series. After 30 min, the suspension of the reaction flask was filtered over a white ribbon filter paper (MN 640 m filter paper, Machery-Nagel, Düren, Germany). Simultaneously extracted metals in the filtrate were determined by means of ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA). The suspensions in the washing bottles were quantitatively collected in an erlenmeyer. After addition of KIO_3 and KI, I_2 reacted with the precipitated zinc sulphides and the excess of I_2 was determined by back titration with $Na_2S_2O_3$, with a starch solution as indicator.

The surface and the deeper sediments were dried at 105 °C and ground. The dry matter content (% DM) was determined by drying the samples until constant weight at 105 °C. The organic matter content (% OM) was determined by weight loss after incineration of the oven-dried sediment samples at 550 °C during 2 h. The total metal content of the sediment was determined by *Aqua Regia* destruction of 1 g dry sediment with 7.5 ml HCl and 2.5 ml HNO_3 (Van Ranst et al., 1999). Sample suspensions were allowed to react for a minimum of 12 h and were then heated at 150°C for 2 h, before filtration over white ribbon filter paper (MN 640 m filter paper, Machery-Nagel, Düren, Germany). Metals in the filtrate were analysed by means of ICP-OES.

A 1:5 sediment:deionised water suspension was equilibrated for 18 h before measuring sediment pH using a pH-electrode (HI 1230B plastic, double-junction, combination gel, Hanna Instruments, Temse, Belgium) (Van Ranst et al., 1999). The electrical conductivity (EC) was determined with a WTW LF 537 conductivity meter (Wissenschaftlich Technischen Werkstäten, Weilheim, Germany) after stirring a 1:5 sediment:deionised water suspension for 30 minutes and filtration over white ribbon filter paper (MN 640 m filter paper, Machery-Nagel, Düren, Germany) (Van Ranst et al., 1999).

Texture analyses of composite sediment samples of each location were performed at the Research Institute for Nature and Forest (INBO 'Instituut voor Natuur- en Bosonderzoek'). Sediment samples were pre-treated with H_2O_2 to remove organic material, and with an acetate buffer solution to remove $CaCO_3$. Samples were rinsed three times by decantation after sedimentation and were then stirred for 4 hours after addition of a dispersion agent. Texture of the sediment samples was determined by means of laser diffraction (Coulter LS200, Miami, FL). The clay fraction in this method is defined by the 0-6 µm fraction, because this fraction was found to correspond with the 0-2 µm fraction determined with the classic pipette method, except for soil samples with a clay content higher than 50 % (Vandecasteele et al., 2002). The maximum clay content in the measured samples did not exceed values of 50 %. The sand fraction was defined as the > 50 µm fraction.

For a given location and depth in the FWS CW, a mixed sample of the sediment was analysed for CEC, exchangeable bases and $CaCO_3$ content. The cation exchange capacity was measured by percolation of 150 ml of 1 M NH_4OAc (pH 7) through a percolation tube filled with a mixture of 5 g of sediment and 35 g of quartz sand. The percolate was collected and exchangeable concentrations of Na, Ca, K and Mg were analysed by means of ICP-OES. The excess of ammonium was removed by rinsing with 300 ml 95 % ethanol. The exchangeable ammonium was then eluted with 500 ml of 1 M KCl and analysed in the percolate by means of steam distillation (Tecator Kjeltec System 1002 distilling unit). After distillation, the ammonia was titrated with 0.01 M HCl (Van Ranst et al., 1999).

The $CaCO_3$ content was measured by adding 25 ml of 0.25 M H_2SO_4 to 1 g of dry sediment and allowing to react for 1 h in a warm water bath at 90 °C. After cooling, 5 drops of a mixed indicator (consisting of phenolphthalein, methyl red and bromocresol green solutions) was added to the solution and the excess acid was back titrated with 0.5 M NaOH.

Plant material was rinsed with deionised water and dried until constant weight at 50 °C. The percentage of dry matter was determined by weight loss. The different plant parts were analysed for total metal levels by means of ICP-OES after digestion of 0.5 g of dry plant material with 65 % HNO_3 and 20 % H_2O_2 at 130 °C for 1 h (Du Laing et al., 2003).

2.3 Statistical analysis

Statistical analysis was performed using the S-plus 6.1 software package (Insightful Corp., Seattle, USA). ANOVA analysis of variance with 2 factors was performed to check for interactions between sampling location and depth in the FWS reed beds ($\alpha = 0.05$). The significance of differences between groups was assessed by means of a Student's t-test for comparison of 2 groups and a one-way ANOVA analysis of variance for comparison of more than 2 groups ($\alpha = 0.05$). Homogeneous subsets were determined with a Tukey post-hoc test ($\alpha = 0.05$). Pearson correlation coefficients between sediment characteristics and metal concentrations in the sediment and reed biomass were determined ($\alpha = 0.05$).

3 Results

3.1 Sediment characteristics

Significant interaction between the factors ‘location’ and ‘depth’ was seen for the EC, pH, Eh, organic matter and dry matter content (interaction location:depth $p < 0.05$). In the inlet area of the FWS reed beds, the surface sediment had a higher organic matter content (11 ± 5 %) than the deeper sediment (3.8 ± 0.5 %). Two very low values of the Eh (-637 and -662 mV) were observed in the deeper sediment of the first two sampling positions of the FWS reed beds. However, differences between characteristics of the surface and deeper sediment layer were generally small. Characteristics of both sediment layers were dependent on the sampling location ($p < 0.05$), but without a clear trend. Therefore, data of the different locations and depths within the FWS reed beds were pooled (Table 3.1). The sediment was classified as a heavy sand-silt soil. As for the FWS reed beds, differences between sediment characteristics were small within the primary settling ditch and data were pooled. A higher organic matter content and EC were encountered near the outlet, although not statistically significant ($p > 0.05$). The Eh near the outlet (-172 ± 49 mV) was lower, although not significantly, than near the inlet (-86 ± 87 mV) ($p > 0.05$).

The EC, pH, dry matter content and Eh of the sediment in the primary settling ditch and the FWS reed beds were similar (Table 3.1). The sediment in the FWS reed beds had a higher CEC (8.3 ± 3.4 cmol(+) kg^{-1} DM) and organic matter content (6.0 ± 2.7 %) than the sediment in the primary settling ditch ($p < 0.05$). This is attributed to the presence of the belowground reed biomass and the input of decaying organic matter at the end of the growing season if reeds are not harvested. Due to the input of organic matter, more sites are available for adsorption and for cation exchange. The latter is reflected in positive correlations between the organic matter content and the CEC of the sediment ($R = 0.88$, $p < 0.001$). The sum of exchangeable bases largely exceeded the CEC of the sediment because of high exchangeable levels of Ca. Presumably, the 1 M NH_4OAc reagent also extracted Ca from the $CaCO_3$ phase. A $CaCO_3$ content of 2.8 % would correspond with 56 cmol(+) kg^{-1} DM if all $CaCO_3$ would be extracted.

Table 3.1

General characteristics of the sediment of the primary settling ditch and the FWS reed beds (n = 6 for all characteristics except for CEC and exchangeable bases (n = 2) in the primary settling ditch; n = 36 for all characteristics except for CEC, exchangeable bases and $CaCO_3$ (n = 12) and for texture (n = 6) in the FWS reed beds)

Parameter	Unit	Primary settling ditch	FWS reed beds
EC	µS cm^{-1}	351 ± 64	303 ± 91
OM	%	3.7 ± 0.9	6.0 ± 2.7
DM	%	68 ± 3	66 ± 10
pH	-	7.8 ± 0.1	7.7 ± 0.2
Eh	mV	-129 ± 79	-141 ± 57
CEC	cmol(+) kg^{-1} DM	4.5 ± 1.3	8.3 ± 3.4
Na	cmol(+) kg^{-1} DM	0.40 ± 0.13	0.48 ± 0.23
K	cmol(+) kg^{-1} DM	0.18 ± 0.05	0.41 ± 0.19
Ca	cmol(+) kg^{-1} DM	38 ± 6	50 ± 10
Mg	cmol(+) kg^{-1} DM	0.71 ± 0.23	1.2 ± 0.4
$CaCO_3$	%	n.m.	2.8 ± 0.7
Clay	%	n.m.	15 ± 4
Silt	%	n.m.	31 ± 5
Sand	%	n.m.	53 ± 7

n.m.: not measured

3.2 Total metal concentrations in the sediment

Figure 3.3 presents the total metal concentrations in the sediment as a function of sampling location and depth. In the primary settling ditch, lower metal concentrations were observed in the inlet area than in the outlet area ($p < 0.05$). In the FWS reed beds, significant interaction between the factors 'location' and 'depth' was seen for all metals (interaction location:depth $p < 0.05$). At the first sampling location in the FWS reed beds, closest to the entry point of the wastewater, the highest metal concentrations were observed in the surface sediment ($p < 0.05$). Further along the treatment path, metal concentrations of the surface and deeper sediment of the FWS reed beds were similar at most sampling locations, with some exceptions. At 85 and 425 m from the inlet (L and 5×L, Fig 3.3), metal concentrations were higher in the deeper than in the surface sediment. Within both layers of the sediment in the FWS reed beds, the metal concentrations were dependent on the sampling location ($p < 0.05$), although a clear trend with distance was not observed. A higher variability was generally observed in the deeper than in the surface sediment. Total metal levels were positively correlated and strong correlations were observed between Cd-Pb, Cd-Zn, Cd-Cr, Cu-Zn, Pb-Zn, Pb-Cr, Pb-Mn and Cr-Mn ($R > 0.8$, $p < 0.001$).

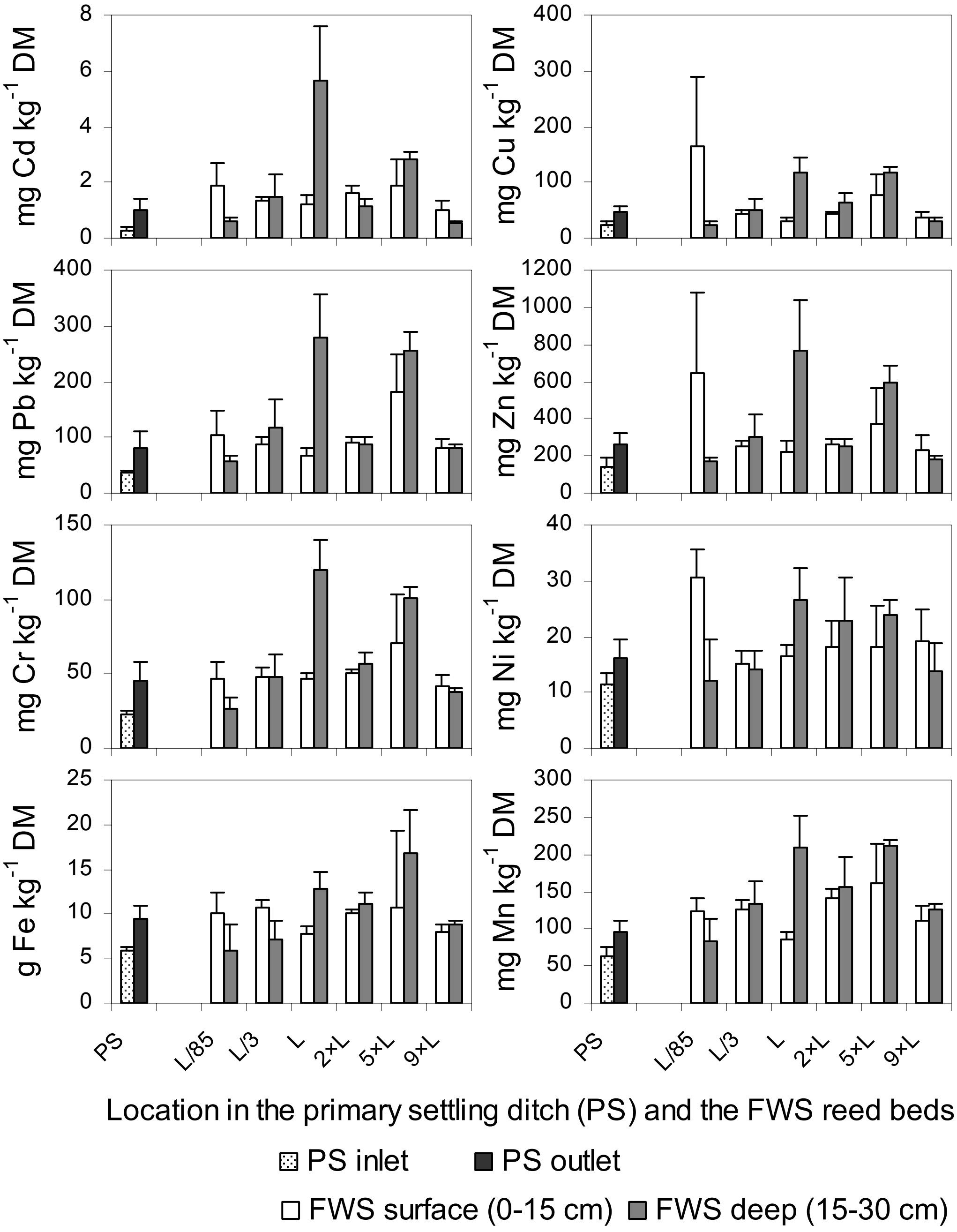

Fig 3.3 Concentrations of Cd, Cu, Zn, Pb, Cr, Ni, Mn (mg kg^{-1} DM) and Fe (g kg^{-1} DM) in the sediment of the inlet and outlet of the primary settling ditch (PS), and in the surface and deeper sediment of the FWS reed beds at different locations with respect to the inlet (L/85, L/3, L, 2×L, 5×L and 9×L; with L being 85 m) (n = 3).

3.3 Simultaneously extracted metals, acid-volatile sulphides and ΣSEM/AVS ratio

Table 3.2 presents the AVS levels, the SEM level of each metal, the sum of the SEM of Cd, Pb, Zn and Ni (ΣSEM_1), the $\Sigma SEM_1/AVS$ ratio, the sum of the SEM of Cd, Pb, Zn, Ni, Fe and Mn (ΣSEM_2) and the $\Sigma SEM_2/AVS$ ratio. As the sediment samples were not homogenised before analysis of AVS and SEM, sample heterogeneity was responsible for the high variability of the SEM and AVS levels. Within the primary settling ditch, the AVS levels of the sediment sampled near the outlet area were higher than near the inlet area. Within the FWS reed beds, AVS levels of the surface and deeper sediment were similar with some exceptions (Table 3.2). Close to the point of entry of the wastewater (at L/85 m) and at 5×L m, higher AVS levels were observed in the surface than in the deeper sediment. In both sediment layers, a decrease of the AVS level with distance was observed. This decrease mainly took place in the first reed bed of the 9 in series. Levels of AVS were positively correlated with the EC of the sediment ($R = 0.79$, $p < 0.001$) whereas AVS were not correlated with other sediment characteristics ($p > 0.05$). Levels of AVS varied between 2.5 and 146 µmol g^{-1} DM and had a mean value of 48 µmol g^{-1} DM.

Simultaneously extracted metal concentrations were affected by sampling location and depth in the FWS CW (Table 3.2). For all metals except Cu and Fe, a strong positive correlation was detected between the total and simultaneously extracted metal concentrations ($R > 0.8$, $p < 0.001$). A moderate positive correlation was also detected for Fe ($R = 0.74$, $p < 0.05$). For all metals except Cu, the distribution of the SEM levels between different locations and depths was similar to that of the total metal levels. A very high variability of the simultaneously extracted Cu levels was observed and these levels were generally very low in the surface sediment and lower than the detection limit at most locations (0.003 µmol g^{-1} DM) (Table 3.2). According to Allen et al. (1993) CuS are not extracted by 1 M cold HCl. In 18 of the 42 sediment samples (43 %), SEM-Cu were lower than detection limits. The liberated Cu presumably originated from other fractions in the sediment and therefore SEM-Cu levels were not taken into account for calculation of the SEM/AVS ratios.

Eighty three to 88 % of the ΣSEM_1 was accounted for by SEM-Zn, depending on the sampling location and depth. Lead, Ni and Cd accounted for respectively 8 to 14 %, 2 to 5 %

and 0.1 to 0.3 % of ΣSEM_1. The $\Sigma SEM_1/AVS$ ratios in the sediment of the inlet and outlet of the primary settling ditch were similar (Table 3.2). Higher AVS and ΣSEM_1 levels were observed in the outlet of the primary settling ditch, but the ratio remained the same. Within the FWS reed beds, differences between $\Sigma SEM_1/AVS$ ratios of the surface and deeper sediment were observed at some locations. Within both sediment layers, $\Sigma SEM_1/AVS$ ratios varied with distance along the treatment path, without a clear trend. The $\Sigma SEM_1/AVS$ ratios of 88 % of all sediment samples were lower than 1. The SEM/AVS ratios suggest that metals are precipitated as sulphides and are not potentially available.

When Fe and Mn were included in the SEM/AVS ratio, the ratio ($\Sigma SEM_2/AVS$) varied around 1 at most sampling locations (Table 3.2). Twenty one % of the sediment samples had a $\Sigma SEM_2/AVS$ that was higher than 3.3, whereas for 79 % the ratio varied around 1. The main part of the ΣSEM_2 is accounted for by Fe (87 to 94 %). Zinc and Mn account for respectively 3 to 9 % and 1 to 4 % of the ΣSEM_2, whereas Cd, Ni and Pb account for less than 1 %.

As for the total metal levels in the sediment, positive correlations were observed between the simultaneously extracted concentrations of some metals. Strong correlations were observed between Cd-Pb, Cd-Cr, Cd-Ni, Cr-Pb and Cr-Ni ($R > 0.8$, $p < 0.001$).

For all metals except Cu, the percentage of the total metal concentration that is simultaneously extracted in the surface and deeper sediment were similar at most sampling locations in the FWS CW. In the sediment of the primary settling ditch, the simultaneously extracted fraction was similar to values reported in the FWS CW. Table 3.3 presents the simultaneously extracted metal concentration expressed in mg kg^{-1} DM, together with the percentage of the total metal concentration that is simultaneously extracted. More than 80 % of the total Cd, Pb, Zn and Mn level is simultaneously extracted. A major part of these metals were present as metal sulphide precipitates. For Cr (30 %), Ni (46 %) and Fe (49 %), the percentage of the total metal level that is simultaneously extracted is lower. These metals are also present in other fractions in the sediment.

Table 3.2

AVS and SEM levels (µmol g^{-1} DM), ΣSEM_1 (Cd+Pb+Zn+Ni), ΣSEM_1/AVS, ΣSEM_2 (Cd+Pb+Zn+Ni+Fe+Mn) and ΣSEM_2/AVS at different locations in the sediment of the FWS CW (n = 3); < DL : < 0.003 µmol g^{-1} DM; *: one of the replicate samples had a SEM-Cu level < DL

Location in the FWS CW			AVS	SEM-Cu	SEM-Cd	SEM-Pb	SEM-Zn	SEM-Ni	ΣSEM_1	ΣSEM_1/AVS	SEM-Fe	SEM-Mn	ΣSEM_2	ΣSEM_2/AVS
Primary	Inlet	Mean	47	0.13 *	0.0034	0.19	2.5	0.077	2.8	**0.062**	51	0.95	55	**1.2**
Settling		St Dev	10	0.11	0.0015	0.05	2.0	0.007	2.1	**0.044**	4	0.43	6	**0.2**
Ditch	Outlet	Mean	90	0.14 *	0.012	0.48	4.4	0.15	5.1	**0.056**	107	1.5	113	**1.3**
		St Dev	32	0.16	0.006	0.26	1.7	0.05	2.0	**0.008**	37	0.5	39	**0.1**
FWS reed beds														
Surface sediment	L/85	Mean	104	< DL	0.013	0.59	9.3	0.22	10	**0.046**	124	2.2	137	**1.1**
		St Dev	60		0.007	0.36	9.0	0.12	9.3	**0.009**	51	0.6	58	**0.0**
	L/3	Mean	91	< DL	0.010	0.49	3.9	0.13	4.5	**0.049**	100	2.1	107	**1.2**
		St Dev	20		0.005	0.26	1.3	0.07	1.6	**0.009**	34	0.6	36	**0.2**
	L	Mean	38	0.023	0.008	0.27	2.6	0.11	3.0	**0.09**	42	1.1	46	**1.3**
		St Dev	12	0.008	0.003	0.12	1.0	0.01	1.2	**0.04**	9	0.2	9	**0.2**
	2×L	Mean	15	0.24	0.007	0.35	3.2	0.14	3.7	**0.62**	81	1.9	86	**13**
		St Dev	11	0.12	0.001	0.07	0.8	0.01	0.9	**0.81**	8	0.4	9	**15**
	5×L	Mean	58	< DL	0.008	0.61	3.3	0.12	4.0	**0.071**	64	1.8	70	**1.2**
		St Dev	27		0.005	0.40	1.4	0.07	1.9	**0.006**	23	0.3	25	**0.1**
	9×L	Mean	23	< DL	0.0045	0.29	2.5	0.12	2.9	**0.16**	37	1.4	41	**2.4**
		St Dev	13		0.0030	0.09	1.1	0.05	1.2	**0.09**	9	0.5	9	**1.5**
Deeper sediment	L/85	Mean	59	< DL	0.0042	0.25	2.0	0.078	2.4	**0.041**	61	1.2	65	**1.1**
		St Dev	8		0.0005	0.10	0.3	0.024	0.4	**0.012**	7	0.5	8	**0.0**
	L/3	Mean	71	0.020	0.010	0.42	3.6	0.12	4.1	**0.075**	74	1.9	80	**1.5**
		St Dev	36	0.016	0.013	0.44	3.6	0.11	4.1	**0.023**	57	1.4	63	**0.1**
	L	Mean	38	0.29	0.031	0.89	8.9	0.30	10	**0.41**	80	2.3	92	**3.6**
		St Dev	19	0.31	0.027	0.78	7.1	0.30	8.2	**0.50**	54	1.6	63	**4.1**
	2×L	Mean	10	0.40 *	0.011	0.39	4.1	0.22	4.7	**0.73**	108	2.9	115	**18**
		St Dev	10	0.40	0.004	0.05	1.3	0.05	1.4	**0.52**	34	1.8	37	**12**
	5×L	Mean	4.4	0.93	0.018	1.2	8.6	0.21	10	**2.9**	151	3.6	165	**47**
		St Dev	1.7	0.48	0.004	0.5	2.4	0.05	2.9	**2.2**	46	0.3	49	**36**
	9×L	Mean	34	0.024	0.005	0.35	2.5	0.067	2.9	**0.08**	47	2.0	52	**1.5**
		St Dev	2	0.030	0.001	0.03	0.1	0.012	0.1	**0.00**	3	0.1	3	**0.0**

Table 3.3

Simultaneously extracted metal levels (mg kg^{-1} DM) and respective percentages of the total metal levels with standard deviation (st dev) and range (minimum and maximum values) (n = 42)

Metal	**SEM (mg kg^{-1} DM)**							**% of total metal concentration**						
	mean	**±**	**st dev**	**range**				**mean**	**±**	**st dev**	**range**			
Cu	10	±	19	0	-	90		15	±	23	0	-	90	
Cd	1.2	±	1.1	0.2	-	6.8		82	±	46	18	-	269	
Pb	102	±	79	16	-	364		92	±	35	20	-	166	
Zn	290	±	250	53	-	1269		85	±	29	24	-	169	
Ni	8.7	±	6.2	1.8	-	38		46	±	20	14	-	116	
Cr	16	±	12	2.7	-	61		30	±	11	7	-	51	
Fe	4527	±	2369	1491	-	11143		49	±	22	12	-	107	
Mn	106	±	54	34	-	267		80	±	22	20	-	134	

3.4 Accumulation of metals in aboveground biomass of *Phragmites australis*

Metal concentrations in the leaves, stems, leaf sheaths and panicles were significantly affected by sampling location, except for Cd in all plant parts ($p > 0.35$). However, a clear trend along the treatment profile of the FWS CW could not be observed. The mean metal concentrations in the leaves, leaf sheaths, stems and panicles are presented in Table 3.4. For all metals except Cd, significant differences between metal concentrations in the different plant parts were detected. The highest Cr concentration was observed in the stems and the highest Mn concentration was observed in the leaves. For Cu and Pb, the highest concentration was observed in the panicles. The panicles also contained high concentrations of Fe, Ni and Zn although these were not always different from concentrations in other plant parts (Table 3.4).

Within the different plants parts of *Phragmites australis*, significant correlations were observed between some metals. Within the leaves, Cr and Ni ($R = 0.86$) and Mn and Zn ($R = 0.77$) concentrations were positively correlated ($p < 0.001$). Within the leaf sheaths, positive correlations were observed between Cr and Fe ($R = 0.81$) and between Mn and Ni ($R = 0.80$) ($p < 0.001$). The Cr, Fe and Zn concentrations in the stems of *Phragmites australis* were positively correlated ($R > 0.7$, $p < 0.05$). Within the panicles, positive correlations were detected between Cr, Cu, Fe and Pb ($R > 0.76$, $p < 0.001$).

For some metals, correlations were observed between concentrations in different plant parts of *Phragmites australis*. For all metals, the concentration in the leaves was poorly correlated with the concentration in the other plant parts. The Cu, Fe and Mn concentration in the leaf sheaths and stems were positively correlated ($R > 0.74$, $p < 0.001$). For Cr and Fe, a positive correlation was observed between the concentrations in the leaf sheaths and the panicles ($R > 0.82$, $p < 0.001$). The Zn and Fe concentrations in the stems and panicles were positively correlated ($R > 0.72$, $p < 0.001$).

Table 3.4

Metal concentrations in the leaves, leaf sheaths, stems and panicles of *Phragmites australis* (mg kg^{-1} DM) (n = 18) in the FWS CW. Small letters denote homogeneous subsets (Tukey post-hoc test)

	Leaves		Leaf sheaths		Stems		Panicles	
Cd	0.054 ± 0.017	a	0.049 ± 0.020	a	0.037 ± 0.014	a	0.051 ± 0.026	a
Cu	3.7 ± 0.9	b	1.9 ± 0.4	a	1.3 ± 0.4	a	5.3 ± 1.9	c
Pb	1.1 ± 0.3	b	0.75 ± 0.28	ab	0.51 ± 0.21	a	1.73 ± 1.06	c
Zn	51 ± 10	b	38 ± 6	a	72 ± 29	c	82 ± 15	c
Cr	0.81 ± 0.14	a	0.82 ± 0.23	a	1.3 ± 0.4	b	0.96 ± 0.28	a
Ni	0.68 ± 0.17	b	0.77 ± 0.16	b	0.47 ± 0.21	a	0.81 ± 0.24	b
Fe	152 ± 17	c	56 ± 9	b	34 ± 7	a	144 ± 73	c
Mn	239 ± 102	c	88 ± 27	b	30 ± 12	a	81 ± 16	b

Based on the observed metal concentrations in the different plant parts of *Phragmites australis*, an estimation of the metal mass accumulated in the aboveground reed biomass was made. A biomass standing stock of 1.5 kg DM m^{-2} was assumed, based on reports from constructed and natural wetlands in temperate regions (Gries and Garbe, 1989; Adcock and Ganf, 1994; Asaeda and Karunaratne, 2000; Karunaratne et al., 2003; Soetaert et al., 2004; Vymazal and Kröpfelová, 2005; Lesage et al., accepted). Considering that the total planted surface area of the 9 reed beds was 3060 m², a total aboveground biomass production of 4590 kg DM was estimated. To estimate the biomass of the different plant parts, the following percent division of the aboveground biomass standing stock was used: stems: 56 %, leaf sheaths: 19 %, leaves: 24 % and panicles: 1% (Gries and Garbe, 1989; Gessner et al., 1996; Karunaratne et al., 2003). The mass of metals in the different plant parts was then estimated by multiplying the metal concentrations with the dry mass of each plant part. Table 3.5 presents the estimated metal masses in the aboveground plant parts of *Phragmites australis* and the relative contribution of each plant part in the aboveground metal accumulation. Metals were mostly stored within the leaves and stems, accounting for 74 to 87 % of the total mass within the aboveground reed biomass. Leaf sheaths accounted for 12 to 25 % of the accumulated metal mass, whereas the relative contribution of the panicles was very low and never exceeded 3 %. If the aboveground reed biomass would be annually harvested, the following amounts of metals could be removed in decreasing order, expressed on a mg m^{-2} $year^{-1}$ basis: 137 Mn, 101 Fe, 91 Zn, 3.1 Cu, 1.7 Cr, 1.1 Pb, 0.87 Ni and 0.065 Cd.

Table 3.5

Estimated metal masses (in g) accumulated in the leaves, leaf sheaths, stems and panicles of *Phragmites australis* in the FWS CW and their relative contribution (%) in the total metal accumulation in the aboveground reed biomass

	Leaves		**Leaf sheaths**		**Stems**		**Panicles**	
	mass	**%**	**mass**	**%**	**Mass**	**%**	**mass**	**%**
Cd	0.059 ± 0.019	30	0.043 ± 0.018	21	0.096 ± 0.037	48	0.0023 ± 0.0012	1
Cu	4.1 ± 1.0	43	1.7 ± 0.3	18	3.4 ± 1.0	36	0.24 ± 0.09	3
Pb	1.2 ± 0.4	36	0.65 ± 0.24	20	1.3 ± 0.5	41	0.079 ± 0.049	2
Zn	56 ± 11	20	33 ± 5	12	184 ± 74	66	3.8 ± 0.7	1
Cr	0.90 ± 0.15	18	0.71 ± 0.20	14	3.4 ± 0.9	67	0.044 ± 0.013	1
Ni	0.75 ± 0.18	28	0.67 ± 0.14	25	1.2 ± 0.5	46	0.037 ± 0.011	1
Fe	168 ± 18	54	49 ± 8	16	86 ± 19	28	6.6 ± 3.4	2
Mn	264 ± 113	63	77 ± 24	18	76 ± 31	18	3.7 ± 0.7	1

3.5 Correlations between metal concentrations in the sediment, aboveground biomass of *Phragmites australis* and sediment characteristics

Table 3.6 presents the Pearson correlation coefficients between the total and simultaneously extracted metal concentrations and the organic matter content and CEC of the sediment. The correlations between the simultaneously extracted and total metal concentrations have already been reported in section 3.3 to describe the distribution of both metal fractions over locations and depths in the FWS CW. A good correlation was generally observed between the simultaneously extracted and the total metal levels, except for Cu (Table 3.6).

A strong positive correlation was detected between the total Cu, Ni and Zn concentrations in the sediment and the organic matter content ($R > 0.8$, $p < 0.001$). A positive correlation with the organic matter content was also observed for most simultaneously extracted metals. Total Cu, Pb, Zn, Cr, Fe and Mn concentrations in the sediment were positively correlated with the CEC ($R \geq 0.78$, $p < 0.001$). Simultaneously extracted levels of Pb, Zn, Cr and Mn were also strongly correlated with the CEC ($R \geq 0.78$, $p < 0.001$) (Table 3.6). A correlation with other sediment characteristics such as pH, Eh, AVS, EC, dry matter, clay, silt, or sand content was not observed.

Metal concentrations in the different plant parts of *Phragmites australis* and the simultaneously extracted or total metal concentrations in the sediment were not significantly correlated ($p > 0.05$). It has to be noted that correlations were performed on a limited dataset of 6 points, comprising the means of each parameter at the 6 sampling locations in the FWS reed beds.

Table 3.6

Pearson correlation coefficients between the total and simultaneously extracted metal concentrations, the organic matter content (% OM) and the CEC in the sediment of the FWS CW (n = 14). *: significant at α = 0.05; **: significant at α = 0.001

		SEM		**% OM**		**CEC**	
Cd	**Total**	0.94	*	0.56	*	0.7	*
	SEM			0.6	*	0.73	*
Cu	**Total**	0.39		0.95	**	0.79	**
	SEM			0.39		0.65	*
Pb	**Total**	0.91	**	0.65	*	0.83	**
	SEM			0.77	*	0.92	**
Zn	**Total**	0.95	**	0.87	**	0.85	**
	SEM			0.9	**	0.86	**
Cr	**Total**	0.92	**	0.59	*	0.78	**
	SEM			0.62	*	0.79	**
Ni	**Total**	0.89	**	0.83	**	0.68	*
	SEM			0.69	*	0.69	*
Fe	**Total**	0.74	*	0.68	*	0.82	**
	SEM			0.7	*	0.71	*
Mn	**Total**	0.84	**	0.63	*	0.81	**
	SEM			0.68	*	0.8	**

4 Discussion

4.1 Metal contamination of the sediment

Within the primary settling ditch, lower total metal concentrations were encountered in the inlet than in the outlet area. This could be caused by the gradual settling of suspended solids during passage through the ditch. The presence of an overflow construction before the primary settling ditch allows to discharge wastewater directly into the surface water in case of storm conditions. However, with the regular occurrence of storm weather, sediment could be flushed out of the inlet area and settle again at the outlet area, evidenced by a higher organic matter content near the outlet. Higher metal concentrations observed at this point demonstrate the positive relationship between metal concentrations and organic matter content. Higher AVS levels and a lower Eh of the sediment sampled near the outlet of the primary settling ditch correspond with the higher organic matter content observed at this point. Lower AVS levels near the inlet are caused by the input of oxygen with the wastewater.

Within the FWS reed beds, differences between metal concentrations in the surface and deeper sediment were generally small and a clear trend with distance was not observed. The origin of metals in the sediment can not be related to the discharge of domestic sewage. In their study of a natural wetland receiving effluent of a municipal WWTP for more than 35 years, Chagué-Goff and Rosen (2001) clearly observed the highest concentrations of Zn, Pb and Cu near the point of discharge and a decrease of metal concentrations with depth. Yu et al. (2001) also observed a decrease of metal concentrations with depth in marine sediments, resulting from pollution of effluents of waste metal recycling industries. However, these authors assessed the metal distribution by considering thin sections of 1 – 2 cm of sediment cores, whereas this study considered two layers of 15 cm. The lack of a spatial trend with distance or depth in the FWS CW could also originate from the low mass loading of metals in the influent wastewater as reported in Chapter 2.

Table 3.7 presents the total metal concentrations in the sediment of the FWS reed beds and a comparison with (1) literature reports for sediments of constructed and natural wetlands, rivers and lakes, (2) the most stringent soil remediation standards in Flanders that apply to soils of land-use class I and II, including nature, forestry and agriculture and (3) background values in uncontaminated soils. The latter standards were corrected for the organic matter and clay content as required by VLAREBO (1996). Due to a higher organic matter (6.0 ± 2.7 %) and clay content (15 ± 4 %) of the sediment in the FWS reed beds than in a standard reference soil (respectively 2 and 10 %), the background values and standards for remediation were higher. From Table 3.7, one can conclude that metal levels were higher than background values, but lower than the most stringent soil remediation standards. A comparison of total metal levels in the sediment with baseline concentrations in uncontaminated soils is valuable in evaluating the extent of metal pollution but does not allow the assessment of potential risks (Tack et al., 1997b). Total concentrations of Cd, Cu, Pb and Zn in the sediment of the FWS CW were similar to values reported by Samecka-Cymerman (2004) and Chagué-Goff (2005) for FWS CWs treating domestic wastewater, but were significantly higher than concentrations in an uncontaminated lake (Schierup and Larsen, 1981).

The range of Fe concentrations in the sediment of the FWS CW was similar to values reported by Chagué-Goff (2005) and Chagué-Goff and Rosen (2001) in respectively a constructed and natural wetland receiving domestic wastewater (Table 3.7). Iron concentrations were considerably higher than values reported by Szymanowska et al. (1999), Vymazal (2003), Vymazal and Krása (2003) and Samecka-Cymerman et al. (2004). The sediment of the FWS CW studied by Samecka-Cymerman et al. (2004), and the 3 sewage-contaminated lakes studied by Szymanowska et al. (1999) consist mainly of sand, which presumably explains the low Fe concentrations monitored in the sediment. Vymazal (2003) and Vymazal and Krása (2003) studied metal concentrations in the sediment of a gravel-based HSSF CW treating domestic wastewater after 4 years of operation and observed low Fe concentrations. However, the Fe originated solely from the wastewater as the filter material does not contribute to the sediment composition.

4.2 SEM/AVS ratio and metal mobility

Total metal levels give an idea about the pollution level of the sediment, but say little or nothing about the mobility of metals (Gambrell, 1994). Larsen and Schierup (1981) studied the distribution of Cd, Cu, Pb and Zn in the sediment and *Phragmites australis* biomass of 2 lakes in Denmark. One lake was contaminated by discharge of mechanically treated sewage from a nearby town for more than 10 years, whereas the other lake served as an uncontaminated control. Total metal concentrations were about 80 times higher in the sewage-contaminated lake than in the uncontaminated lake. However, based on metal concentrations in the interstitial water, bioavailability of metals was 4 to 116 times greater in the uncontaminated than in the sewage-contaminated lake. The more alkaline pH, low redox values and higher organic carbon content of the sewage-contaminated lake favoured non-available forms of the heavy metals. The authors suggested that heavy metals were immobilised as sulphides or chelated to organic matter. Chagué-Goff (2005) assessed the mobility of metals in a FWS CW by means of the sequential extraction procedure of Tessier et al. (1979) and showed that metals were mainly associated with organics/sulphides and the residual phase.

Table 3.7

Total Cd, Cu, Pb, Zn, Cr, Ni, Mn (mg kg^{-1} DM), Fe and Al (g kg^{-1} DM) concentrations in the sediment of the FWS CW compared with literature data, soil remediation standards (SRS) and background values (BV); m and r denote mean and range respectively

		Cd	Cu	Pb	Zn	Cr	Ni	Al	Fe	Mn	Ref n°
Constructed wetlands											
FWS CW	m	1.8	67	124	355	58	19		10	139	12
	r	0.5-7.7	19-308	50-364	157-1139	20-140	7-35		3.9-21	54-254	
FWS CW	r	1.0-1.9	16-32	114-147	71-131				0.02-0.03	287-319	1
FWS CW	r		6-19	11-75	39-72				12-13	39-77	2
HSSF CW	m	27.5	110	155	273		45.4	12	0.44	434	3,4
HSSF CW	r	0.2-2.5	15-288	14-162	65-934	26-41	32-42	7.5-20	23-35	261-947	5
Natural wetlands, rivers and lakes											
Uncontaminated lake	m	0.03	1.8	<1	3.8						6a
Natural wetland receiving treated sewage	r		0-110	0-100	0-400	0-90	0-30	20-80	50-250	500-1500	7
Sewage-contaminated lake	r	0.2-1.6	4-20	2-44	20-300						6b
Sewage-contaminated lakes	r	1.2-1.8	2.1-3.0	9.7-13	475-1100	1.2-1.9	1.6-3.0		0.03-0.1	39-178	8
River estuary	r		5.1-7	36-59	114-197		17-22				9
Metal-contaminated river	r		15-1239	12-460	44-1623		8.6-216				10
Criteria											
BV		1.2	19	51	77	40	11				11a
SRS		2.9	219	253	747	142	125				11b

References: (1) Samecka-Cymerman et al. (2004): FWS CW for domestic wastewater; Poland; (2) Chagué-Goff (2005): FWS CW for secondary treated domestic wastewater, after 4 years of operation; New Zealand; (3,4): Vymazal and Krása (2003) and Vymazal (2003): gravel-based HSSF CW for domestic wastewater, after 3 years of operation; Czech Republic; (5) Lesage et al. (accepted): gravel-based HSSF CW for domestic wastewater, after 3 years of operation; Belgium; (6) Schierup and Larsen (1981): uncontaminated lake (a) and sewage-contaminated lake (b); Denmark; (7) Chagué-Goff and Rosen (2001): natural wetland receiving effluent from a municipal WWTP for more than 35 years; New Zealand; (8) Szymanowska et al. (1999): 3 lakes receiving untreated municipal sewage; Poland; (9) Fang et al. (2005): Pearl river estuary; China; (10) Yu et al. (2001): metal-contaminated river; Taiwan; (11) VLAREBO (1996): background values (a) and soil remediation standards (b), corrected for OM and clay content; (12) This study

The SEM/AVS ratio gives an idea about potential toxicity and mobility of metals in anoxic sediments. A high variability of the AVS and SEM levels was observed at most sampling locations and depths. As the surface and deeper sediment samples were not homogenised before analysis of AVS and SEM, sample heterogeneity was responsible for the high variability between the 3 replicate samples. Sample heterogeneity can be high as AVS and SEM levels have been reported to change with depth, even on a small scale of a few centimetres (Fang et al., 2005). According to Van den Berg et al. (1998) SEM/AVS ratios of mixed homogenised sediments are less meaningful because of the high variability in depth. However, a subdivision in more sub-samples in relation to depth was not performed in this study. The SEM/AVS ratio was determined as a parameter complementary to the assessment of the pollution level of the sediment of the CW after 16 years of operation. The SEM/AVS ratio was not a subject of study on itself, but taken into account for reasons of preliminary mobility and toxicity predictions. The mean AVS and SEM levels are therefore meaningful.

A decrease of the AVS level with distance was observed in both sediment layers. The surface sediment in the inlet area of the first FWS reed bed had a higher AVS level than the deeper sediment. However, most literature reports describe an increase of the AVS level with depth (Van de Berg et al., 1998; Fang et al., 2005) although some authors have demonstrated higher AVS levels in the surface than in the deeper sediment as well. Yu et al. (2001) observed a decrease of the AVS levels with depth at one site of a metal-contaminated river and related this pattern to differences in sulphur inputs between upper and lower sediments. The AVS content in sediments varies with factors affecting the supply of organic matter and sulphates, and the redox state of the sediment. Fang et al. (2005) observed no correlation between the AVS and TOC level in sediments from a river estuary and attributed lower AVS levels in the surface sediments to oxygen diffusion from the overlying water. In the FWS CW under study, the AVS pattern is presumably related to organic matter. The decrease of AVS levels with distance, mainly in the first reed bed of the 9 in series, could be caused by the higher organic loading in the wastewater near the inlet area. Sulphides can only be formed in very reduced sediments and in the presence of organic matter. A Eh of -141 mV as observed in the FWS reed beds can cause sulphate reduction. The surface sediment in the inlet area of the first FWS reed bed had a higher organic matter content than the deeper sediment, which can therefore explain the higher AVS level in the surface sediment at this location.

Levels of AVS in the sediment of the FWS CW varied between 2.5 and 146 µmol g^{-1} DM and had a mean value of 48 µmol g^{-1} DM. The reported AVS levels are similar to values reported by Yu et al. (2001) in marine sediments (2 – 45 µmol g^{-1} DM) and van den Hoop et al. (1997) in freshwater and marine sediments (< 0.1 – 50 µmol g^{-1} DM). Van den Hoop et al. (1997) showed that freshwater sediments did not contain less AVS than marine sediments, although lower sulphate concentrations are generally observed in freshwater environments. Fang et al. (2005) reported lower AVS levels in sediments of a river estuary, varying between < 0.01 and 6 µmol g^{-1} DM. These lower values could be related with the more dynamic nature of the study site and oxygen diffusion from overlying water.

The $\Sigma SEM_1/AVS$ ratios of 88 % of all sediment samples of the FWS CW were lower than 1. The SEM/AVS ratios indicate that metals are precipitated as sulphides and are not potentially available. The major part of the ΣSEM_1(Cd+Pb+Zn+Ni) was accounted for by Zn (83 – 88 %). Van den Hoop et al. (1997) also observed the highest contribution of Zn in the SEM of freshwater and marine sediments (for about 80 %), whereas Cd accounted for less than 1 %. When Fe and Mn were included in the SEM/AVS ratio, the ratio ($\Sigma SEM_2/AVS$) varied around 1 at most sampling locations. This could indicate that metal sulphide complexes were extracted and that other sulphide minerals were not extracted by 1 M cold HCl. Allen et al. (1993) reported that sulphide was not released from pyrite (FeS_2) under these operational conditions. The main part of the ΣSEM_2 is accounted for by Fe (87 to 94 %).

A good correlation was generally observed between the simultaneously extracted and total metal concentrations, except for Cu. Fang et al. (2005) also observed a close relationship between total and SEM concentrations in the sediments of a river estuary. A large percentage of the total metal concentration was simultaneously extracted, the highest for Pb (50 – 95 %) and the lowest for Ni (0 – 40 %), whereas the ratios for Cd, Cu and Zn varied between 20 – 60 % (Fang et al., 2005). In the sediment of the FWS CW under study, more than 80 % of the total Cd, Pb, Zn and Mn level is simultaneously extracted, suggesting that sulphide precipitation was an important binding mechanism for these metals.

Copper was not extracted together with the AVS in 43 % of the samples, which corresponds with the finding of Allen et al. (1993) that CuS is not solubilised by means of 1 M cold HCl. However, for some samples Cu was detected in the simultaneously extracted phase. The extracted Cu was probably released from another phase responsible for Cu immobilisation in the sediment. The total Cu concentration in the sediment is strongly correlated with the organic matter content, a phase which was thought to be important in the immobilisation of Cu. As for Cu, binding with other phases was thought to be important for Ni, Cr and Fe as well. Yu et al. (2001) performed a sequential extraction procedure according to the method of Tessier et al. (1979) before and after AVS extraction of metal-contaminated anoxic river sediments, and demonstrated that other phases such as organic carbon, carbonates and Fe-oxides contributed to the SEM. This coincided with low metal concentrations in the pore water despite a SEM/AVS ratio exceeding 1, and showed that metals were bound by other phases in the sediment.

4.3 Metal accumulation in the biomass of *Phragmites australis*

Metal concentrations in the aboveground plant parts of *Phragmites australis* were not correlated with the simultaneously extracted or total metal concentrations in the sediment, a finding corresponding with many studies in natural and constructed wetlands which showed that *Phragmites australis* restricts the translocation of metals to the aboveground plant parts (Stoltz and Greger, 2002; Windham et al., 2003; Weis and Weis, 2004). These studies showed similar allocation patterns of metals in emergent macrophyte species. Most wetland plants have higher concentrations of metals in their belowground biomass than in their shoot tissues. Leaf tissues generally have the next highest concentrations, followed by stems and rhizomes. Metal concentrations in aboveground plant parts of *Phragmites australis* in the FWS CW under study were generally comparable to values reported in natural uncontaminated wetlands and do not seem to indicate that helophytes accumulate high concentrations of metals in their aboveground biomass (Table 1.1, Chapter 1). Within the aboveground reed biomass, metals were mainly stored in the leaves and the stems (74 to 87 %), whereas the leaf sheaths (12 to 25 %) and the panicles (< 3 %) accounted for a smaller proportion of the accumulated metal mass. By harvesting the aboveground reed biomass, small amounts of Cu, Cr, Pb, Ni and Cd are removed (< 3 mg m^{-2} $year^{-1}$), whereas about 90, 100 and 140 mg m^{-2} $year^{-1}$ of respectively Zn, Fe and Mn can be removed.

Chapter 2 presented information on the metal loading in the influent and effluent of the FWS CW, determined by a weekly monitoring campaign performed from the end of August ’05 until the end of May ’06. A mean flow of 308 m^3 day^{-1} was reported in 2004 (VMM, 2006).

The estimated mass loadings of Cu, Pb, Zn, Cr, Ni, Fe and Mn in the influent of the FWS reed beds during the year 2004 were 2.9, 0.69, 12, 0.19, 0.33, 37 and 7.5 kg, respectively. The percentages of the total Cu, Pb, Zn, Cr, Ni, Fe and Mn mass loadings that were accumulated in the aboveground reed biomass were 0.32, 0.47, 2.2, 2.7, 0.81, 0.84 and 5.6 %, respectively. As Cd concentrations in the influent wastewater were lower than the detection limit of 0.1 µg l^{-1}, a maximum mass loading of 0.011 kg was estimated and a minimum contribution of accumulation in the aboveground biomass of 1.8 % was derived. Overall, low percentages of the mass loadings of heavy metals are accumulated in the aboveground reed biomass and could be removed by harvest. Metal accumulation in plants usually accounts for a negligible amount of the metal removal in CWs (Mantovi et al., 2003; Stottmeister et al., 2003; Vymazal, 2003; Vymazal and Krása, 2003; Lesage et al., accepted). Metals sequestered in the belowground plant parts are eventually buried within the sediment.

4.4 Suggestions regarding end-use of the CW and long-term immobilisation of metals

The sediment acts as the primary sink for metals in the FWS CW studied. However, this immobilisation of metals in the sediment may eventually affect the end-use of the CW when its life expectancy is exceeded. Two major end-use strategies are dredging or leaving the sediment in the CW. In a first strategy, about 500 m^3 sediment would need to be disposed of if a layer of 30 cm would be dredged. However, the reed bed is situated in a nature area, implying the need for fulfilment of the most stringent criteria for reuse of the sediment as soil, as demanded by the Flemish regulations relating to waste prevention and management. The mean metal levels in the sediment of the CW are higher than the criteria of 1.2 mg Cd kg^{-1} DM, 19 mg Cu kg^{-1} DM, 51 mg Pb kg^{-1} DM, 77 mg Zn kg^{-1} DM, 40 mg Cr kg^{-1} DM and 11 mg Ni kg^{-1} DM, corrected for organic matter and clay content (VLAREA, 1998). Therefore, dredging the sediment and reusing it as soil is not suggested as a suitable strategy.

A second end-use strategy would be to leave the sediment in the FWS CW. Then, the question arises how to manage the site. A first management option could be to maintain the sediment in flooded conditions. The wetland could then be transformed from a constructed treatment wetland into a more natural one, providing other benefits such as wildlife habitat or recreational use. In a second, much easier ‘management’ option, the site would just be abandoned. The sediment is then left susceptible to natural cycles of drying or flooding, depending on the climatic conditions. However, as precipitation with sulphides was suggested as an important mechanism of metal immobilisation, the selected management option will affect the behaviour of metals.

If no management is applied, oxidation of the sediment will eventually lead to sulphide oxidation and acidification. This may lead to a temporary release of metals. Tack et al. (1996) observed an increase of the solubility of some elements upon oxidation of a calcareous dredged sediment. Acidity generated through the process of sulphide oxidation was responsible for the dissolution of a small amount of carbonates and the release of associated elements such as Co, Ni and Mn. In the case of the sediment of the FWS CW, a mean $CaCO_3$ content of 2.8 % was observed, corresponding with a buffering capacity of 0.56 mol H^+ kg^{-1} DM. The sediment had a mean AVS level of 48 µmol g^{-1} DM. If all metal sulphides would be oxidised, a maximum acidity of 0.2 mol H^+ kg^{-1} DM could be generated. It was concluded that the buffering capacity of the sediment is sufficiently high to prevent a large increase in acidity upon drainage and oxidation. Moreover, other immobilisation processes including adsorption or precipitation with Fe- and Mn- (oxy-) hydroxides, carbonates and complexation with organic matter will attenuate the intensity of metal release during oxidation.

Gambrell (1994) suggested that a change from reducing to oxidising conditions does generally not result in a large release of metals due to the complimentarity of immobilisation processes, under the condition that the pH does not change substantially. Upon oxidation of the calcareous dredged sediment, Tack et al. (1996) however reported an increase of the water soluble concentrations of Zn and Cd whereas Cu and Pb concentrations were unaffected. The latter elements were more strongly adsorbed by Fe- and Mn- (oxy-) hydroxides and organic matter than Cd and Zn. Tack et al. (1999) also observed a much lower leachability for Cu and Pb in an oxidised dredged sediment derived soil, compared to Zn and Cd.

Overall, irrespective of the metal considered, both short- and long-term mobility of metals are expected to be the lowest when the sediment remains in reduced conditions. This, in combination with the complexity of the sediment system and the uncertainty of predictions regarding long-term immobilisation upon changes in oxidation state, leads to the advise that the sediment of the FWS CW is best kept in reducing conditions.

5 Conclusions

Although the FWS CW had been treating domestic wastewater for a period of 16 years at the time of sampling, the metal pollution level of the sediment was low to moderate. Total concentrations of Cd, Cu, Pb, Zn, Cr and Ni in the sediment were 1.8, 67, 124, 355, 58 and 19 mg kg^{-1} DM, respectively, values higher than background reference levels, but much lower than the most stringent soil remediation standards. Moreover, SEM/AVS ratios indicate a low mobility of metals in the sediment and no potential toxicity. The SEM/AVS ratios were lower than 1 in 88 % of all sediment samples.

Within the aboveground reed biomass, metals are mainly accumulated within the leaves and stems. Leaves, leaf sheaths and stems accounted for 97 – 99 % of the total metal mass in the aboveground reed biomass whereas the contribution of the panicles was negligible. Overall, low percentages of the mass loadings of heavy metals are accumulated in the aboveground reed biomass and could be removed by harvest.

It can be concluded that the sediment was the main pool for metal accumulation within the FWS CW. Precipitation with sulphides was suggested as an important immobilisation process. No problems are expected after closure of the site with regard to metals if the sediment is maintained in reduced conditions. Predictions about long-term immobilisation after oxidation of the sediment are less certain.

CHAPTER 4

ACCUMULATION OF METALS IN A HORIZONTAL SUBSURFACE FLOW CONSTRUCTED WETLAND FOR DOMESTIC WASTEWATER TREATMENT

1 Introduction

Constructed wetlands (CWs) can offer an efficient alternative for conventional wastewater treatment systems (Brix, 1994; EPA, 2000; Kivaisi, 2001). A review on their application and performance in Flanders, Belgium was presented by Rousseau et al. (2004). Chapter 2 showed that concentrations of heavy metals in the influent wastewater of Flemish treatment wetlands were generally low and that most metals were efficiently removed from the wastewater after passage through the CWs. Constructed wetlands have been extensively monitored when it comes to BOD, COD, SS and nutrients whereas knowledge on metals remains somewhat limited (Kadlec and Knight, 1996). Yet, heavy metals are persistent in the environment and do accumulate in the sediments and plants of constructed wetlands. Only few researchers have investigated the accumulation of metals in sediments and plants of CWs for domestic wastewater treatment (Gschlößl and Stuible, 2000; Obarska-Pempkowiak, 2001; Vymazal, 2003; Vymazal and Krása, 2003; Samecka-Cymerman et al., 2004). Three major processes are responsible for metal removal in CWs: (i) binding to sediments and soils, (ii) precipitation as insoluble salts (mainly sulphides and (oxy-) hydroxides) and (iii) uptake by plants and bacteria (Kadlec and Knight, 1996).

The purpose of this study was to investigate the accumulation of metals in a horizontal subsurface flow (HSSF) CW treating domestic wastewater after three years of operation. The HSSF CW is filled with gravel and planted with the common reed *Phragmites australis* (Cav.) Trin. ex Steud. The CW has a design capacity of 350 population equivalents (PEs). Chapter 2 showed that heavy metals, except Fe and Mn, were efficiently removed in the CW under study. Goal of this chapter was to study whether significant metal accumulation has occurred after 3 years of operation. The sediment and reed biomass were sampled in the beginning of September 2004 at different locations along the treatment path of the CW in order to assess spatial variations. Metal concentrations were determined in the sediment and in the leaves, stems and belowground biomass of *Phragmites australis*. Moreover, the relative contribution of metal accumulation in the aboveground reed biomass in the overall metal removal was assessed. Gambrell (1994) described that fine-textured sediments containing an appreciable amount of organic matter tend to accumulate metals whereas coarse-textured materials are generally not contaminated due to their low affinity for metals. Therefore, the contribution of the gravel itself was not assessed and the sediment present in the pores of the gravel was considered to be responsible for metal accumulation in the filter medium of the reed bed.

2 Material and Methods

2.1 Description of the study site and sampling strategy

The CW under study is located in Zemst in Flanders, Belgium and is operated by Aquafin NV. The system was designed for secondary domestic wastewater treatment but receives stormwater as well, because of the combined sewer networks in Flanders. Description of the study site was based on Aquafin (1996, 1997, 2003h). The system has been in operation since 2001 and has a theoretical design capacity of 350 population equivalents (PEs) (based on 54 g BOD d^{-1} PE^{-1}) and a theoretical hydraulic residence time of 6.7 days (based on 150 l d^{-1} PE^{-1}). The process lay-out of the CW is presented in Fig 4.1. Effluent is discharged into a brook, the Kesterbeek. An overflow construction was built to direct excess water directly into this brook during storm events. The maximum hydraulic capacity of the CW is 22.5 m^3 h^{-1} ($6\times Q_{14}$). Influent wastewater is collected in a pre-settlement tank consisting of 3 compartments with a total volume of 33 m^3 and a minimum residence time of 1 h. Sludge is regularly removed from the pre-settlement tank and dewatered at the wastewater treatment plant Mechelen-Noord.

Wastewater is pumped to the inlet area of the two parallel HSSF reed beds that are alternately fed. Wastewater is distributed uniformly across the width of the reed beds by means of equidistant vertical riser pipes. Each reed bed has a length of 50 m, a width of 13 m and a depth of 0.5 m at the inlet area with a bottom slope of 0.7 %. Beds are provided with a liner and are planted with common reed (*Phragmites australis* (Cav.) Trin. ex Steud.). Beds are filled with fine gravel (5 – 10 mm diameter) and the inlet and outlet area is filled with coarse gravel (5 – 8 cm diameter). Effluent is collected by drainage tubes and regulation of the hydraulic gradient can be performed by adjusting height of an elbow effluent construction.

The sediment and *Phragmites australis* biomass were sampled in the beginning of September 2004. Sampling of the sediment and reed biomass was performed at different locations in one of the two parallel reed beds in order to assess spatial effects (Reed bed 1, Fig 4.1). Sampling was performed at increasing distance from the inlet at 1, 5, 25 and 50 m, with three locations across the width of the reed bed at each distance, further referred to as left, middle and right (with regard to the water flow direction). To avoid confusion about the term ‘sediment’, a description is presented herewith. The sediment was defined as the soil-like material that was present in the pores of the gravel filter medium. In other words, anything that was not root or gravel was referred to as sediment. The sediment originates mainly from suspended solids in the inflowing wastewater and from decaying reed biomass.

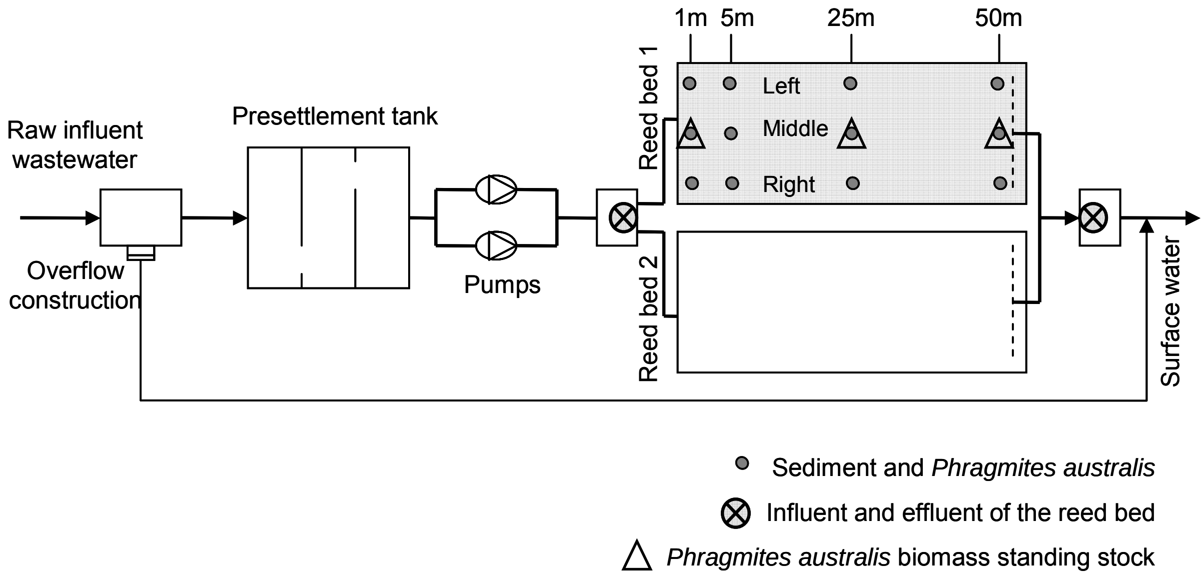

Fig 4.1 Process lay-out of the horizontal subsurface flow constructed wetland and sampling locations.

The sediment was sampled by means of a stainless steel cylindrical corer (8 cm diameter, length of 31 cm) that was driven into the gravel substrate to a depth of 30 cm. The reed bed would be heavily disturbed by sampling of deeper profiles of the substrate. Vymazal and Krása (2003) did not find statistical differences in metal concentrations in the sediment from various depth profiles in a HSSF CW treating domestic wastewater. Thus, it could be assumed that the core of 30 cm of the substrate was generally representative of the entire depth profile. Three replicate samples were taken at each location. Each core of the substrate contained three fractions: the gravel, sediment and belowground biomass of *Phragmites australis*. The aboveground parts of 3 *Phragmites australis* plants were sampled at each location and divided into leaves and stems (leaf sheaths were not differentiated from stems). An estimation of the aboveground biomass standing stock was made by harvesting all aboveground biomass in a square of 1 m² at some locations in the CW (Fig 4.1). The assessment of the biomass standing stock was performed just after maximal growth of *Phragmites australis*, in early September 2004.

2.2 Analytical procedures

Each core of the substrate was separated into three fractions, gravel, sediment and belowground biomass, by means of a rinsing procedure. Gravel was rinsed with deionised water over a sieve (1 mm diameter). Remaining sediment was removed from the gravel and the belowground biomass by ultrasonication in deionised water at 37 kHz for 10 min (Bandolin Sonorex Super RK103H, Bandolin, Berlin, Germany). The belowground biomass of *Phragmites australis* was removed from the rinsed gravel. The rinsing suspension was homogenised by stirring and 4 sub-samples of 100 ml were taken. Two sub-samples were analysed for metals by digesting 25 ml of the rinsing suspension with 2 ml 65 % HNO_3 and 2 ml 20 % H_2O_2 at 150 °C for 1 h. After cooling, 2 ml of 20 % H_2O_2 was added and samples were evaporated almost to dryness at 150 °C. This last step was repeated 4 times after which the residue was filtered (MN 640 m filter paper, Machery-Nagel, Düren, Germany) and the filtrate was diluted to 50 ml with 1 % HNO_3 for metal analysis. The other 2 sub-samples were filtered and the sediment mass was determined by difference in weight of the MN 640 m filter paper. Plant material was dried at 50 °C until constant dry weight and digested with HNO_3 and H_2O_2 for metal analysis (Du Laing et al., 2003). Metals (Al, Cd, Cu, Cr, Pb, Ni, Zn, Fe and Mn) were analysed by means of ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) or GF-AAS (SpectrAA-800/GTA-100, Varian, Palo Alto, CA).

2.3 Statistical analysis

Statistical analysis was performed using the S-plus 6.1 software package (Insightful Corp., Seattle, USA). The significance of differences in metal concentrations in the sediment, substrate, belowground biomass, leaves and stems of *Phragmites australis* between sampling locations was assessed using ANOVA analysis of variance with 2 factors, ‘distance’ and ‘position’ ($\alpha = 0.05$). Factor 1, ‘distance’, indicates the distance from the inlet of the CW and was differentiated into the following subsets: 1, 5, 25 and 50 m. Factor 2, ‘position’, indicates the sampling position at each distance and was differentiated into the following subsets: left, middle and right (with regard to the water flow direction). Homogeneous subsets were determined with a Tukey post-hoc test ($\alpha = 0.05$). Pearson correlation coefficients ($\alpha = 0.05$) were determined between mean metal concentrations in leaves, stems, belowground biomass and sediments of the different sampling locations.

3 Results

3.1 Metal concentrations in the sediment

The metal concentrations in the sediment of each sampling location are presented in Fig 4.2. Significant interaction between the factors ‘distance’ and ‘position’ was seen for all metals (interaction distance:position $p < 0.05$) except for Fe and Ni ($p = 0.093$ and 0.16 for respectively Fe and Ni). It can thus be postulated that the pattern of the metal concentration in the sediment as a function of distance from the inlet depends on the position in the reed bed (left, middle, or right). Moreover, differences between positions depend on the distance from the inlet.

The Cd, Cu, Pb, Zn and Cr concentration in the sediment sampled at the left side was higher than at the other positions within the first 5 m of the reed bed ($p < 0.05$) (Fig 4.2). For all metals except Mn, the concentration in the sediment decreased with distance. A strong decrease of the Zn concentration in the sediment was seen along the different profiles ($p < 0.05$). A similar decrease was also seen for Cd and Cu ($p < 0.05$), although the pattern was slightly different between positions in the reed bed. The decrease of the Al concentration in the sediment occurred more gradually along the different profiles. The Al concentration did not decrease within the first 5 m but was significantly lower from a distance of 25 m onward ($p < 0.05$). A similar pattern was seen for Pb along the profiles in the left and the middle. The decrease of the Fe, Cr and Ni concentration in the sediment along the different profiles was less pronounced than for the other metals. The Mn concentration in the sediment increased along each longitudinal profile ($p < 0.05$), a pattern that was unique within the group of metals studied (Fig 4.2).

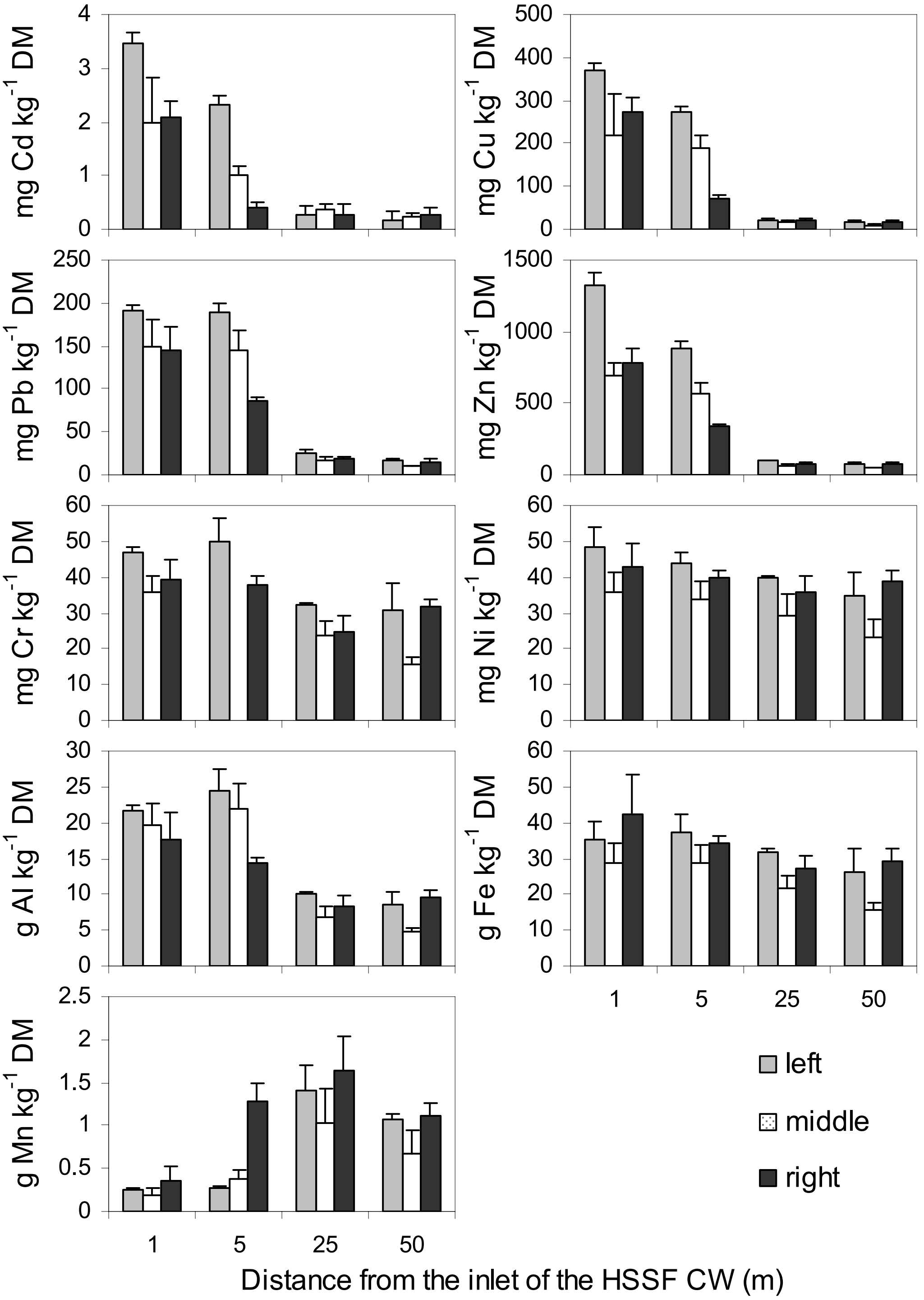

Fig 4.2 Total Cd, Cu, Pb, Zn, Cr, Ni, Al, Fe and Mn concentration in the sediment (mg kg^{-1} DM) as a function of distance from the inlet and position (left, middle and right) across the width of the HSSF CW (n = 6).

3.2 Metal concentrations in the *Phragmites australis* biomass

For most metals, there was a significant interaction between the factors ‘distance’ and ‘position’ on the concentration in the belowground biomass (interaction distance:position $p < 0.05$ for all metals except Cr and Zn, with $p = 0.89$ for Cr and $p = 0.92$ for Zn). For all metals except Cr and Zn, an effect of position was detected within the first 5 m of the reed bed with generally higher concentrations at the left side of the reed bed. Contrary to the sediments and belowground biomass, there was no significant interaction between the factors ‘distance’ and ‘position’ on the concentration in the leaves and stems for most metals (interaction distance:position $p > 0.1$ for all metals except for Cd in the leaves ($p = 0.045$) and for Cu in both leaves and stems ($p < 0.005$)). For most metals, concentrations in leaves or stems were the same at the left, middle, or right side of the reed bed. Higher concentrations of Mn and Cu in both leaves and stems were encountered at the right side of the bed, whereas the Cd concentration in leaves was higher at the left side of the reed bed. As for the belowground biomass, these differences where mainly seen within the first 5 m of the reed bed.

It can be postulated that the factor ‘position’ had less impact on the metal concentration in the different plant parts than on that in the sediment. Moreover, the limited amount of replicate samples at each sampling location ($n = 3$), makes it difficult to draw straightforward conclusions about spatial effects at a certain distance. Therefore, data of the 3 positions at each distance were pooled ($n = 9$) and the metal concentration in the belowground biomass, leaves and stems as a function of distance from the inlet is presented in Table 4.1.

For all metals except Mn, the concentration in the belowground biomass decreased with distance ($p < 0.05$ for Cd, Cu, Pb, Zn, Al and Fe; $p = 0.66$ and 0.10 for Cr and Ni, respectively). The decrease of the Zn, Al and Fe concentration in the belowground biomass was already significant at 5 m from the inlet. A significant decrease of the Cd, Cu and Pb concentration in the belowground biomass was seen at 25 m from the inlet. An exceptional trend was seen for Mn, with increasing concentrations in the belowground biomass along the longitudinal profile. For all metals except Mn, an exponential fit was found between the metal concentration in the belowground biomass and the distance from the inlet ($R^2 > 0.6$).

Table 4.1

Metal concentrations in the belowground biomass (BG), leaves and stems of *Phragmites australis* (mg kg^{-1} DM) as a function of distance from the inlet (m) (n = 9) in the HSSF CW. Small letters denote homogeneous subsets (Tukey post-hoc test)

Metal	Distance	BG		Leaves		Stems	
Cd	**1**	0.47 ± 0.17	b	0.071 ± 0.031	b	0.083 ± 0.045	b
	5	0.37 ± 0.24	ab	0.062 ± 0.049	ab	0.031 ± 0.017	a
	25	0.20 ± 0.13	a	0.031 ± 0.014	a	0.036 ± 0.019	a
	50	0.19 ± 0.08	a	0.028 ± 0.009	a	0.050 ± 0.042	ab
Cu	**1**	44 ± 12	b	2.2 ± 1.5	a	0.91 ± 0.41	a
	5	29 ± 20	b	4.1 ± 2.4	b	3.3 ± 2.1	c
	25	8.0 ± 1.4	a	4.4 ± 0.9	b	2.3 ± 1.2	bc
	50	6.9 ± 1.7	a	3.6 ± 0.9	ab	1.5 ± 0.3	ab
Pb	**1**	20 ± 5	b	1.1 ± 0.4	b	0.43 ± 0.34	a
	5	14 ± 9	b	0.53 ± 0.26	a	0.42 ± 0.25	a
	25	2.0 ± 1.0	a	0.74 ± 0.34	ab	0.34 ± 0.10	a
	50	1.3 ± 0.5	a	0.97 ± 0.61	ab	0.39 ± 0.14	a
Zn	**1**	184 ± 49	c	23 ± 11	a	15 ± 7	a
	5	129 ± 44	b	49 ± 11	b	39 ± 18	b
	25	46 ± 11	a	29 ± 8	a	25 ± 9	a
	50	32 ± 9	a	20 ± 6	a	11 ± 3	a
Cr	**1**	22 ± 13	a	0.95 ± 0.37	a	0.90 ± 0.38	a
	5	19 ± 19	a	0.72 ± 0.29	a	1.1 ± 0.4	a
	25	15 ± 12	a	0.86 ± 0.53	a	1.3 ± 0.6	a
	50	15 ± 13	a	0.82 ± 0.37	a	1.0 ± 0.7	a
Ni	**1**	9.3 ± 3.7	a	0.29 ± 0.13	a	0.29 ± 0.17	a
	5	13 ± 8	a	0.45 ± 0.18	a	0.36 ± 0.17	a
	25	7.0 ± 3.2	a	0.41 ± 0.16	a	0.52 ± 0.36	a
	50	6.4 ± 3.8	a	0.30 ± 0.08	a	0.20 ± 0.13	a
Al	**1**	2534 ± 737	b	21 ± 8	a	17 ± 8	a
	5	1430 ± 1119	a	16 ± 7	a	11 ± 6	a
	25	676 ± 398	a	14 ± 7	a	10 ± 6	a
	50	522 ± 268	a	14 ± 3	a	10 ± 5	a
Fe	**1**	6433 ± 1365	b	114 ± 26	a	28 ± 10	a
	5	2670 ± 1427	a	90 ± 21	a	27 ± 7	a
	25	1655 ± 661	a	86 ± 24	a	36 ± 17	a
	50	1653 ± 1178	a	98 ± 29	a	36 ± 19	a
Mn	**1**	58 ± 30	a	60 ± 22	a	19 ± 8	a
	5	107 ± 73	a	108 ± 62	a	30 ± 16	a
	25	285 ± 112	b	226 ± 99	b	68 ± 26	b
	50	243 ± 131	b	236 ± 117	b	57 ± 24	b

The Cr, Ni, Al and Fe concentrations in leaves and stems were independent of the factor 'distance' ($p > 0.05$). A significant effect of distance on the concentration in leaves and stems was only observed for Mn, Zn, Cd and Cu ($p < 0.05$). Moreover, similar trends were seen in leaves and stems for these elements. The Mn concentration in leaves and stems increased along the longitudinal profile and was higher from a distance of 25 m onward. Variations in metal concentrations in the aboveground biomass were generally much smaller than in belowground biomass and sediments, except for Mn.

Metal concentrations in the belowground biomass were generally higher than in leaves and stems, with the exception of Mn. The Mn concentrations in the leaves and belowground biomass did not differ and were higher than that in the stems ($p < 0.05$). The Al, Cr, Cd and Ni concentrations in leaves and stems were not significantly different and were lower than these in the belowground biomass ($p < 0.05$). For Fe, the following order of increasing concentration was seen: stems < leaves < belowground biomass. For Cu, Zn and Pb, the order was dependent on the factor ‘distance’. The Cu, Zn and Pb concentration in leaves and stems did not differ and was lower than that in the belowground biomass within the first 25 m of the reed bed for Zn and Pb, and within the first 5 m for Cu. At further distances along the treatment path the order changed slightly for these metals. The concentration in the stems was lower than in the leaves from 25 m onward for Cu and at 50 m from the inlet for Zn ($p < 0.05$). At a distance of 50 m from the inlet, the Pb concentration in the belowground biomass did not differ from that in the leaves but was still significantly higher than that in the stems ($p < 0.05$).

3.3 Correlations between metal concentrations in leaves, stems, belowground biomass and sediment

Correlations between metal concentrations in the different compartments of the 12 sampling locations in the reed bed are presented in Table 4.2. Metal concentrations in the belowground biomass and sediments were positively correlated. For Cd, Cu, Pb, Zn, Al and Mn this correlation was significant ($p < 0.05$), whereas for Cr, Ni and Fe it was not ($p = 0.054$, 0.26 and 0.071 for Cr, Ni and Fe, respectively).

There was a strong positive correlation between the Mn concentration in leaves, stems, belowground biomass and sediments. A change of the Mn level in the sediment was reflected in the Mn level in the belowground biomass, stems and leaves as well. Unlike for Mn, changes in the Zn, Pb, Ni and Fe level in the sediment or belowground biomass were generally not reflected in the aboveground biomass (Table 4.2). Higher concentrations of Cd and Al in the rooting medium of the plants led to higher concentrations of these elements in the belowground biomass, leaves and stems as well, although this was not statistically significant for stems (Table 4.2).

An opposite pattern was seen for the Cu concentration in the leaves, which was strongly negatively correlated with the Cu concentration in the sediment ($p < 0.05$). A negative correlation was also detected between the Cr concentration in the stems and the sediment ($p < 0.05$). In other words, higher concentrations of Cu and Cr in the sediment were accompanied by lower levels of these metals in the leaves or stems. Although significant correlations were found for some metals between the concentration in the aboveground biomass and the sediment, variations in the concentration in the aboveground reed biomass between sampling locations were much lower than in the sediment.

Table 4.2

Pearson correlation coefficients between metal concentrations in the sediment and belowground biomass (BG), leaves and stems of *Phragmites australis* in the HSSF CW (*: significant correlation at $\alpha = 0.05$; **: significant at $\alpha = 0.001$)

Metal		**Stems**	**BG**	**Sediment**
Cd	**Leaves**	0.19	0.91 **	0.90 **
	Stems		0.33	0.55
	BG			0.88 **
Cu	**Leaves**	0.84 **	-0.70 *	-0.71 *
	Stems		-0.46	-0.39
	BG			0.91 **
Pb	**Leaves**	0.22	0.08	0.01
	Stems		0.32	0.36
	BG			0.99 **
Zn	**Leaves**	0.92 **	0.19	0.05
	Stems		0.17	0.01
	BG			0.92 **
Cr	**Leaves**	0.39	-0.34	-0.23
	Stems		-0.30	-0.65 *
	BG			0.59
Ni	**Leaves**	0.36	0.08	-0.32
	Stems		0.14	-0.08
	BG			0.46
Al	**Leaves**	0.67 *	0.64 *	0.59 *
	Stems		0.51	0.34
	BG			0.76 **
Fe	**Leaves**	-0.15	0.44	0.10
	Stems		-0.13	-0.40
	BG			0.54
Mn	**Leaves**	0.96 **	0.79 *	0.89 **
	Stems		0.85 **	0.88 **
	BG			0.68 *

3.4 Mass balance and relative importance of accumulation in the aboveground *Phragmites australis* biomass in the overall metal removal

During the year 2004, a mean effluent flow rate of 104 $m^3 d^{-1}$ was reported in the HSSF CW (VMM, 2006). The influent flow rate was not monitored. In order to estimate the mass loading of heavy metals into the reed bed under study, the mean influent and effluent flow rates were considered equal to 52 $m^3 d^{-1}$. This approach ignores the effects of precipitation and evapotranspiration on the water balance in the reed bed and the temporal variations in flow rates. However, as influent flow data were not given, mass loadings of heavy metals were estimated as following. Mean influent and effluent concentrations of the CW collected during the weekly monitoring programme from the end of August '05 until the end of May '06, presented in Chapter 2, were multiplied with the flow rates to calculate the mass loadings in the influent and effluent. Table 4.3 presents the estimated mass loadings in the influent and effluent of the studied reed bed of the HSSF CW during the year 2004, together with the estimated metal masses in the different compartments of the reed bed. The last column of the table presents the metal masses that can by removed by harvest on a mg m^{-2} $year^{-1}$ basis. First, a description on how the masses in the different compartments of the reed bed were derived is presented.

The sediment mass in the substrate cores was higher close to the inlet area and varied between 33 ± 14 and 46 ± 9 kg DM m^{-3} substrate at 1 m, and between 21 ± 4 and 50 ± 12 kg DM m^{-3} substrate at 5 m. The sediment mass was higher at the left side of the reed bed within the first 5 m of the reed bed ($p = 0.064$ at 1 m, $p < 0.001$ at 5 m). The sediment mass was not affected by the sampling location from a distance of 25 m onward (19 ± 3 kg DM m^{-3} substrate). The metal concentration per m^3 substrate at each sampling location was calculated by multiplying the metal concentration in the sediment with the sediment mass in each core, and was plotted as a function of distance. A good exponential fit was found for all metals ($R^2 > 0.74$) except Mn. Therefore, the Mn concentration per m^3 substrate was linearly interpolated whereas the exponential fit was used for the other metals to determine the metal concentration per m^3 substrate at increasing distances between inlet and outlet. The metal mass accumulated in the sediment was then derived by multiplying with the substrate volume at each distance and integrating over the length of the reed bed.

The aboveground biomass standing stock was 1.2, 1.4 and 0.73 kg DM m^{-2} at 1, 25 and 50 m from the inlet, respectively. Linear interpolation led to a total aboveground biomass of 760 kg DM in the reed bed under study. Leaves were assumed to account for 25 % of the aboveground biomass, whereas stems with associated leaf sheaths accounted for the remaining 75 %, based on Gries and Garbe (1989), Gessner et al. (1996) and Karunaratne et al. (2003). The metal mass accumulated in the leaves and stems was estimated by multiplying the mean metal concentration in the leaves and stems with the leaf and stem biomass. The belowground biomass production was not determined, but a ratio of the aboveground and belowground biomass (AG/BG) of 1 was assumed. Adcock and Ganf (1994) reported AG/BG ratios of ~ 0.42 for *Phragmites australis* growing in a gravel-based HSSF CW for tertiary effluent treatment and for reeds growing in a natural lagoon. However, other studies showed higher AG/BG ratios for *Phragmites australis* growing in CWs than for natural reed stands. Peverly et al. (1995) reported an AG/BG ratio of ~ 1 for reeds growing in a gravel-based HSSF CW for landfill leachate treatment. Tanner (1996) observed an AG/BG ratio of ~ 0.8 for *Phragmites australis* growing in gravel mesocosms. An AG/BG ratio of more than 2 was observed by Gries and Garbe (1989) for reeds growing in a soil-based HSSF CW. Lower AG/BG ratios varying between 0.5 and 0.7 for natural reed stands have been reported by Asaeda and Karunaratne (2000), Karunaratne et al. (2003) and Soetaert et al. (2004).

The metal concentration in the belowground biomass was an exponential function of distance for all metals ($R^2 > 0.6$) except Mn. Linear interpolation of the Mn concentration in the belowground biomass was performed. The metal mass accumulated in the belowground biomass was calculated by multiplying the metal concentration in the belowground biomass with the biomass and by integrating over the entire length of the reed bed.

For all metals, less than 2 % of the mass removed from the wastewater after passage through the reed bed was accumulated in the aboveground reed biomass and could therefore be removed by harvest (Table 4.3). The remainder is stored belowground, either in the sediment or in the belowground reed biomass. The masses of Cd, Cu, Pb, Cr and Ni removed by harvest of the aboveground biomass were very low. About 30 mg m^{-2} of Zn could be removed by yearly harvest. The highest masses that could be removed by harvest were observed for Mn and Fe.

Table 4.3

Estimated metal masses in the influent, effluent, sediment, belowground (BG) and aboveground (AG) reed biomass, together with the mass removed from the water (Δ) in 2004 (in g), the percentage of the metal mass removed from the wastewater that is accumulated by the aboveground reed biomass (% AG) and the mass that can be removed by harvest on a mg m^{-2} y^{-1} basis

	Water			Sediment	Reed biomass		% AG	Harvest
	Influent	**Effluent**	**Δ**		**BG**	**AG**		
Cd	11	3.2	7.3	8.4	0.2	0.0	0.5	0.1
Cu	395	39	357	847	13	1.8	0.5	2.8
Pb	159	74	85	637	4.4	0.4	0.4	0.6
Zn	2137	269	1869	2991	59	19	1.0	29
Cr	61	21	40	355	13	0.8	2.0	1.2
Ni	117	60	57	404	6.3	0.3	0.5	0.4
Al	23504	1640	21864	139739	802	10	0.04	15
Fe	13964	20912	-6948	317438	1949	37		56
Mn	1417	2881	-1464	9398	164	55		85

4 Discussion

4.1 Metal contamination of the sediment

Metal concentrations in the sediment were significantly affected by the position across the width of the reed bed (left, middle or right). Firstly, this points at the heterogeneity of metal concentrations in the sediment and at the importance of sufficient sampling locations. Secondly, this gives evidence of unequal distribution of the influent wastewater across the width of the reed bed in the inlet area. This is supported by the fact that differences between positions were generally higher within the first 5 m of the reed bed, closest to the inlet area. The Cd, Cu, Pb, Zn, Cr and Al concentrations in the sediment sampled at the left side were higher than at the other positions. Moreover, a higher sediment mass at the left side of the reed bed supports the hypothesis of unequal influent distribution. Cooper et al. (2005) also reported unequal influent distribution in some HSSF tertiary reed beds evidenced by differences in sludge levels.

A strong decrease of the Cd, Cu, Pb and Zn concentrations in the sediment was seen within the first 25 m of the reed bed. Concentrations of these metals were elevated close to the inlet area and decreased towards background values rapidly. Vymazal (2003), Vymazal and Krása (2003) and Obarska-Pempkowiak and Klimkowska (1999) also reported a decrease of these metals in the sediment of CWs treating municipal wastewater. Table 4.4 presents the total metal concentrations in the sediment of the HSSF CW and a comparison with (1) literature reports for sediments of constructed and natural wetlands, and lakes, (2) the most stringent soil remediation standards in Flanders that apply to soils of land-use class I and II, including nature, forestry and agriculture and (3) background values in uncontaminated soils.

Metal levels in the sediment of the HSSF CW were similar to values reported in literature for CWs for domestic wastewater treatment. Gschlößl and Stuible (2000), Vymazal and Krása (2003) and Vymazal (2003) did not report excessive metal levels in the sediment of CWs treating domestic wastewater compared to natural wetland sediments and state that there was no risk at the time of study. Metal concentrations in the sediment of the HSSF CW studied and these reported in literature were however much higher than those reported by Schierup and Larsen (1981) in the sediment of an uncontaminated lake. Elevated total concentrations of Cd, Cu and Zn in the inlet area of the HSSF CW were encountered after 3 years of operation. The highest Cd, Cu and Zn concentrations in the sediment were found at 1 m from the inlet. The Cd, Cu and Zn concentrations were respectively 2.5 ± 0.8, 288 ± 84 and 934 ± 299 mg kg^{-1} DM, and were higher than the most stringent soil remediation standards in Flanders (Table 4.4). From a distance of 25 m onward, the levels were similar or lower than background values for a standard soil. The Cr and Ni levels in the sediment were low. A concluding remark on the abovementioned comparisons of total metal levels in the sediment with background values and soil remediation standards is that they are only useful in evaluating the extent of metal pollution. However, they do not allow the assessment of the potential risks associated with metal accumulation in the sediment as other physico-chemical and biological factors will affect the bioavailability and toxicity of metals (Gambrell, 1994; Tack et al., 1997b).

Table 4.4

Total Cd, Cu, Pb, Zn, Cr, Ni, Mn (mg kg^{-1} DM), Fe and Al (g kg^{-1} DM) concentrations in the sediment of the HSSF CW, compared with literature data, soil remediation standards (SRS) and background values (BV); m and r denote mean and range respectively

		Cd	Cu	Pb	Zn	Cr	Ni	Al	Fe	Mn	Ref n°
Constructed wetlands											
HSSF CW	r	0.2-2.5	15-288	14-162	65-934	26-41	32-42	7.5-20	23-35	261-947	11
HSSF	m	27.5	110	155	273		45.4	12	0.4	434	1,2
HSSF	r	0.5-3.4	4-127	4-25	13-402	10-35	7-30				3a
HSSF	r	0.3-4	17-143	7.3-90	52-490	38-259	28-71				3b
Hybrid	r	1-5.2	18-65	10-45	23-130	11-41				21-218	4
FWS	r	1.0-1.9	16-32	114-147	71-131				0.02-0.03	287-319	5
FWS	r	0.5-7.7	19-308	50-364	157-1139	20-140	7-35		3.9-21	54-254	6
Natural wetlands, rivers and lakes											
Uncontaminated lake	m	0.03	1.8	<1	3.8						7a
Natural wetland receiving treated sewage	r		0-110	0-100	0-400	0-90	0-30	20-80	50-250	500-1500	8
Sewage-contaminated lake	r	0.2-1.6	4-20	2-44	20-300						7b
Sewage-contaminated lakes	r	1.2-1.8	2.1-3.0	9.7-13	475-1100	1.2-1.9	1.6-3.0		0.03-0.1	39-178	9
Criteria											
BV		0.8	17	40	62	37	9				10a
SRS		2	200	200	600	130	100				10b

References: (1, 2) Vymazal and Krása (2003) and Vymazal (2003): gravel-based HSSF CW for domestic wastewater, after 3 years of operation; Czech Republic; (3) Gschlößl and Stuible (2000): sand/gravel-based HSSF CW (a) and sand-based HSSF CW (b) for domestic wastewater treatment, after 10 years of operation; Germany; (4) Obarska-Pempkowiak and Klimkowska (1999): Hybrid CW consisting of a HSSF reed bed and a series of ditches and dams for domestic wastewater, after 5 years of operation; Poland; (5) Samecka-Cymerman et al. (2004): FWS CW for domestic wastewater; Poland; (6) This work, Chapter 3: FWS CW for domestic wastewater, after 16 years of operation; Belgium; (7) Schierup and Larsen (1981): uncontaminated lake (a) and sewage-contaminated lake (b); Denmark; (8) Chagué-Goff and Rosen (2001): natural wetland receiving effluent from a municipal WWTP for more than 35 years; New Zealand; (9) Szymanowska et al. (1999): 3 lakes receiving untreated municipal sewage; Poland; (10) VLAREBO (1996): background values (a) and soil remediation standards (b) for a standard reference soil (2% OM, 10% clay); (11): This study

After 3 years of operation, the sediment in the inlet area was significantly contaminated with Zn, Cu and Cd. A contamination problem with Pb could occur within the first 5 m of the reed bed in the near future. A very simple calculation was performed to estimate the lifetime before saturation of the sediment with metals would inhibit the efficiency of the reed bed. It is assumed that saturation levels of Zn, Cu and Cd were reached within the first 5 m of the reed bed after 3 years of operation and that the ‘saturation front’ in the sediment proceeds along the treatment path in a linear way. Taking into account that the reed bed has a total length of 50 m, a rough estimation would be that the system will be able to retain metals efficiently for at least an additional 27 years. However, it appears unlikely that saturation levels of metals were reached within the first 5 m of the reed bed at this stage of operation. Metals enter the system mainly in a particulate state and will continue to be efficiently filtered out of the water within the first section of the reed bed, leading to gradually increasing metal levels close to the inlet area and a higher life expectancy. Kadlec and Knight (1996) state that the system’s life is in the order of hundreds of years or longer, based on calculated metal loading rates. However, life expectancy is strongly affected by the maintenance of the reed bed. Surface flooding of the inlet area was noted in September 2004 in this study (personal observation) and in March 2004 by Pauwels (2004). Surface flooding did not reach a distance of 5 m so far although further flooding and thus bypassing of the first section of the reed bed could accelerate the migration of metals in the reed bed. Cooper et al. (2005) and Rousseau et al. (2005) underline the importance of maintenance on the operational lifetime of the reed bed.

Elevated total concentrations of Cd, Zn, Cu and Pb were present within the first 5 m of the reed bed. However, it are mainly the chemical forms of the metals and the processes affecting transformations between these forms that are determining the risks rather than the total metal concentrations (Gambrell, 1994). Sulphides were observed within the first 5 m of the reed bed (qualitative assessment). Precipitation as metal sulphide complexes was thought to be an important removal process for metals within the first 5 m of the reed bed, next to filtration and sedimentation of suspended solids (with associated metals), sorption processes and complexation with organic matter. However, when the reed bed reaches its operational lifetime, it is important to realise that metal immobilisation is dependent on redox conditions and thus management of the site. If the reed bed would be abandoned and drained, sulphide oxidation and mineralisation of organic matter can lead to the release of metals, possibly causing a flush of metals out of the reed bed during rain events. According to Gambrell (1994), a change from reducing to oxidising conditions usually does not result in a large release of metals except for highly organic sediments such as in this study, where significant acidification and consequent metal release are likely to occur upon oxidation. The uncontrolled flush of heavily-contaminated water could have devastating impacts on receiving watercourses. If managers decide not to (immediately) remediate the substrate upon its closure, it is suggested to maintain the site in a waterlogged condition to guarantee the immobilisation of heavy metals.

4.2 Trends in Fe and Mn concentrations in the sediment

Iron levels in the sediment decreased from 35000 ± 9300 to 23000 ± 7200 mg kg^{-1} DM and were very high compared to a mean level of 440 mg kg^{-1} DM reported by Vymazal (2003) in the sediment of a HSSF CW (Table 4.4). Iron levels were comparable to levels reported in sediments of a FWS CW for domestic wastewater treatment (Chapter 3) and a natural wetland receiving domestic sewage (Chagué-Goff, 2005). Kadlec and Knight (1996) reported similar Fe levels in average upland soils. In theory, the only inputs of Fe into the reed bed would be the wastewater and the decaying reed biomass. The decrease of the Fe concentration in the sediment with distance, suggests that there was an input of Fe with the influent wastewater during the operational lifetime of the reed bed. Iron levels in the raw wastewater varied between 1 and 1.6 mg l^{-1}, as reported in Chapter 2. These levels fell within the range of 0.1 and 1.9 mg l^{-1} reported by Kadlec and Knight (1996) for raw municipal wastewater and do not indicate an excessive input of Fe. Normal levels in the wastewater and comparable levels in the sediment to upland soils, led to the assumption that Fe was already present in the gravel filter from the start of operation. It seems likely that a fine grain fraction was present in the pores of the gravel filter at the time of construction.

The Mn concentration in the sediment increased along the treatment path and reached a maximum value of 1360 ± 430 mg kg^{-1} DM. The pattern of the Mn concentration in the sediment as a function of distance underlines the importance of redox conditions in the sediment. Reduction of Mn-oxides would occur mostly in the beginning of the reed bed, where the organic loading is high and DO is rapidly removed from the wastewater and is used as an electron acceptor in the microbial degradation of organic matter. After Mn(IV) is reduced to Mn(II), it can migrate through the reed bed leading to a higher dissolved Mn concentration in the effluent as described previously. Three reasons are presented for the higher Mn concentration in the sediment further along the treatment path. Firstly, Mn can migrate through the reed bed in the form of colloidal complexes. However, part of these colloids can be retained in the reed bed by filtration processes further along the treatment path. Secondly, part of the Mn could deposit as Mn-oxides when redox conditions change again from reducing to oxidising. Obarska-Pempkowiak and Klimkowska (1999) also related the increase of the Mn concentration in the sediment of a hybrid CW, to changing redox conditions. *Phragmites australis* plants release oxygen into the rhizosphere (Armstrong et al., 1994). In the inlet area of the reed bed where the organic loading is high, this oxygen would be consumed rapidly for aerobic degradation processes. However, further along the treatment path oxygen leaking from the belowground biomass could form Mn-oxides. Brix (1997) reported oxygen release rates varying widely from 0.02 to 12 g m^{-2} d^{-1} for *Phragmites*. García et al. (2003) reported an increase of the Eh with distance from the inlet area of HSSF reed beds, corresponding with the progressive oxidation of organic matter. Moreover, the Eh decreased with depth as mechanisms responsible for water oxygenation such as diffusion from the atmosphere and radial oxygen loss (ROL) from the belowground biomass of the reed plants mainly occur at the top of the gravel beds (García et al., 2003). The importance of root oxygenation was underlined by Stein and Hook (2005), who stated that seasonal variation in root oxygenation could cause a shift in microbial metabolic pathways. The authors reported a shift from anaerobic to aerobic conditions in *Scirpus* planted laboratory-scale wetlands in winter, leading to elevated redox potentials, inhibition of sulphate reduction and less Zn removal. As a last possible reason for higher Mn concentrations in the sediment at increasing distance from the inlet, the higher sequestration of Mn into the reed biomass further along the treatment path is mentioned. When the reed biomass is not frequently harvested, as was the case in the reed bed under study, the Mn accumulated in the reed biomass eventually becomes buried within the sediment.

4.3 Metal accumulation in the biomass of *Phragmites australis*

Metal concentrations in belowground biomass and sediments were positively correlated. An exponential decrease of the metal mass in the sediment and belowground biomass was seen for all metals except Mn. This could give evidence of an exponential removal pattern of metals in CWs as mentioned by Kadlec and Knight (1996). Metal concentrations in the pore water have not been investigated in this study to substantiate this hypothesis. The Mn concentration in leaves, stems, belowground biomass and sediment were strongly positively correlated reflecting the high mobility of Mn and uptake into the different compartments. For the other metals, changes in the metal level in the sediment or belowground biomass were generally not reflected in the aboveground biomass although significant correlations were found for some metals. Vymazal (2003) and Vymazal and Krása (2003) also reported aboveground metal concentrations in *Phalaris arundinacea* that were unaffected by sampling position.

Studies in many natural and constructed wetlands have showed that *Phragmites australis* restricts the translocation of metals to the aboveground plant parts (Stolz and Greger, 2002; Windham et al., 2003; Weis and Weis, 2004). These studies showed similar allocation patterns of metals in emergent macrophyte species. Most wetland plants had higher concentrations of metals in their belowground biomass than in their shoot tissues. The metal concentrations in the leaves and stems of *Phragmites australis* in the HSSF CW under study were similar to values reported in literature for *Phragmites australis* growing in uncontaminated reed stands, constructed and natural wetlands (Table 1.1, Chapter 1) and do not indicate excessive accumulation in the reed biomass. As the belowground biomass was only rinsed with deionised water before analysis, the metal concentrations in the belowground biomass presumably include metals that were adsorbed and/or co-precipitated on the Fe-(oxy-) hydroxide plaques covering the root surface. Gries and Garbe (1989) and Mays and Edwards (2001) suggested that a major part of the metals were bound as co-precipitates in such plaques.

Less than 2 % of the annually removed mass from the wastewater is accumulated in the aboveground reed biomass, whereas the remainder is retained in the sediment or belowground reed biomass. Obarska-Pempkowiak (2001) reported a relative contribution of the reed biomass of 14 – 22 % for Cd, Cu and Pb in the annual metal removal. The highest accumulation was found in the rhizomes. According to Sinicrope et al. (1992) 35 % of Cd, 6 % of Cu and 13 % of Zn added in the wastewater was accumulated in the roots of *Scirpus californicus* in mesocosms exposed to artificial domestic wastewater. Metals are mainly accumulating in the belowground organs of *Phragmites australis* and are eventually recycled to the sediment with the decomposition of the reed biomass that starts at the end of the growing season. The relative contribution of the aboveground reed biomass in the overall metal accumulation was generally very low. If the aboveground biomass of the reed bed would be harvested, about 2 g of Cu, 10 g of Al, 20 g of Zn, 40 g of Fe, 55 g of Mn and trace amounts of Cd, Pb, Cr, or Ni (< 1 g) would be removed annually.

5 Conclusions

After 3 years of operation, the sediment in the inlet area was significantly contaminated with Zn, Cu and Cd (respectively 934 ± 299, 288 ± 299 and 2.5 ± 0.8 mg kg^{-1} DM) whereas Pb (162 ± 32 mg kg^{-1} DM) could form a contamination problem within the near future. The Cr and Ni levels in the sediment were low. At this stage of operation, the contamination problem is situated within the inlet area and metal concentrations in the sediment decreased towards background values further along the treatment path. Metal concentrations in belowground biomass and sediments were positively correlated whereas changes in the metal level in the sediment or belowground biomass were generally not reflected in the aboveground biomass. Metals sequestered in the reed biomass are mainly stored in the belowground biomass and eventually recycled to the sediment.

Less than 2 % of the annually removed mass from the wastewater is accumulated in the aboveground reed biomass. The sediment is the most important sink for metal accumulation in the reed bed. According to a rough estimation, the system will be able to retain metals efficiently for at least an additional 27 years. Whether the shift of metals from domestic wastewater to sediments of CWs is a sustainable affair should be an important topic of discussion. It is however convincingly shown that metals are accumulating in sediments of CWs even when daily metal loads in the influent are very low. When the reed bed exceeds its operational lifetime, careful management of the site will be needed to prevent unwanted flushing of metals into the receiving watercourses.

CHAPTER 5

EFFECT OF HELOPHYTE SPECIES ON THE REMOVAL OF HEAVY METALS IN HORIZONTAL SUBSURFACE FLOW CONSTRUCTED WETLANDS

1 Introduction

Constructed wetlands (CWs) are more and more being used as small-scale treatment systems for domestic wastewater in Flanders, Belgium. Different types of CWs are currently in operation, ranging from free-water-surface (FWS) over horizontal subsurface flow (HSSF) to vertical subsurface flow (VSSF) CWs and combinations of these types. Horizontal subsurface flow CWs are increasingly gaining popularity as a secondary and tertiary treatment step for domestic wastewater, mainly because they have a higher performance and better odour control than FWS CWs and because they are less subjected to clogging problems as compared to VSSF CWs (EPA, 1993; Rousseau et al., 2004). Constructed wetlands in Flanders are mainly planted with *Phragmites australis* because of its fast growth rate and its tolerance to a wide range of environmental conditions such as pH, temperature, salinity, toxic components and reducing conditions (Massacci et al., 2001; Stottmeister et al., 2003).

Heavy metals are effectively removed in CWs by a complex combination of physico-chemical and biological removal processes, including sedimentation, binding to substrates, precipitation as insoluble salts (mainly sulphides and (oxy-) hydroxides) and uptake by plants (Kadlec and Knight, 1996). Research towards the distribution of heavy metals in helophyte-based CWs for domestic wastewater treatment has increased considerably over the last decade. Nowadays, it is commonly believed that the substrate acts as the primary sink for heavy metal accumulation and that only a negligible fraction of the influent loading can be removed by accumulation in the aboveground biomass of helophytes (Gschlößl and Stuible, 2000; Vymazal, 2003; Vymazal and Krása, 2003; Lesage et al., 2005). Helophytes generally store heavy metals in their belowground biomass (Weis and Weis, 2004). Therefore, the overall process of metal removal in helophyte-based CWs can be approached as one of phytostabilisation.

Salt et al. (1995) described a good phytostabilising plant as (1) showing tolerance to elevated levels of heavy metals, (2) being capable of immobilising the heavy metals belowground, either by root uptake, precipitation or reduction and (3) having low translocation of heavy metals to the shoots. Differences in uptake and translocation patterns between emergent macrophytes are important as they affect the behaviour of heavy metals in wetlands (Weis and Weis, 2004). For example, Windham et al. (2003) showed that *Spartina alterniflora* allocates more metals in the aboveground tissues than *Phragmites australis* in salt marshes. Upon senescence and decay, a larger pool of metals stored in the aboveground biomass of *Spartina alterniflora* becomes available for deposit feeders and for entering the aquatic environment. From a phytostabilisation perspective, *Phragmites australis* is the preferred plant species as it sequesters more metals belowground. When making the extrapolation from natural to constructed wetland conditions, the same considerations apply and differences in uptake patterns should affect the choice of a suitable helophyte species for use in constructed treatment wetlands. However, quantitative information comparing the removal performance of different emergent macrophytes in CWs is scarce (Tanner, 1996; Brisson et al., 2006).

Goals of the presented study were (1) to assess and compare the contribution of different helophyte species towards the removal of metals from domestic wastewater in HSSF CWs and (2) to study differences in uptake and translocation patterns of metals in the selected helophyte species. For this purpose, the removal of Cd, Cu, Pb and Zn was studied in gravel-based HSSF CW microcosms planted with 4 different commonly used helophyte species: *Phragmites australis* (Cav.) Trin. ex Steud. (Common reed), *Juncus effusus* L. (Soft rush), *Carex acutiformis* Ehrh. (Lesser pond sedge) and *Iris pseudacorus* L. (Yellow flag). All plants are perennial rhizomatous plants (Mennema, 1994). *Phragmites australis* is a plant of the Poaceae family with a wide geographical distribution, extending from cold temperate regions to the tropics (Mennema, 1994; Massacci et al., 2001; Karunaratne et al., 2003). In some regions of America and Australasia it is a non-native plant species, posing weed risk (Chambers and McComb, 1994; Tanner, 1996). *Iris pseudacorus* belongs to the Iridaceae family and is a native plant in Europe and North Africa, but is non-native in America and Australasia. Its characteristic yellow flowers were responsible for the introduction of *Iris pseudacorus* as an ornamental plant in Northern America and has created weed problems ever since (APIRS, 2006). *Carex acutiformis*, a plant of the Cyperacea family, is a native plant in Europe and Asia, but non-native in America. *Juncus effusus* is a plant of the Juncaceae family with a worldwide geographical distribution (Flora, 2006).

The effects of the different helophyte species on the removal of metals was assessed by including an unplanted system in the experimental setup. Constructed wetland microcosms were fed with synthetic wastewater that simulated domestic wastewater with elevated levels of Cd, Cu, Pb and Zn. These metals were selected because they are most abundant in domestic wastewater (Chapter 2) and most frequently exceed basic environment quality standards in surface water (VMM, 2005). Metal levels were analysed in the influent, effluent and pore water at intermediate positions along the treatment profile. Metal concentrations in the plants were determined after 4 months in order to characterise the distribution of metals over the above and belowground biomass, to assess the relative contribution of metal accumulation in the harvestable plant parts with respect to the loading into the HSSF CW microcosms and to compare different helophyte species.

2 Material and Methods

2.1 Experimental set-up of the horizontal subsurface flow constructed wetland microcosms

Constructed wetland microcosms were 1 m long, 0.135 m wide and filled with gravel (3 – 8 mm diameter, porosity of 0.38) to a depth of 0.10 m (Fig 5.1). Rhizon pore water samplers (0.1 µm diameter) were fixed at 2 cm above the bottom, at 0.25, 0.5 and 0.75 m from the inlet (Rhizon soil moisture samplers for metals and organic matter, Eijkelkamp BV, Giesbeek). A bottom slope of 1 % (0.01 m m^{-1}) was provided. An inlet and outlet area of 0.10 m long, filled with coarse gravel (8 – 16 mm diameter, porosity of 0.43), was created in order to obtain optimum influent distribution and effluent collection. The water level was maintained just below the gravel surface at a constant height of 0.095 m, by means of an elbow construction at the outlet. The inlet construction consisted of a small pipe fixed into the gravel at the inlet area. Three baffles made of plastic were placed to a depth of 2 cm above the bottom of the system, at regular intervals between inlet and outlet to enhance plug flow conditions.

Young vegetative propagules of the helophyte species were collected from a local plant nursery. The belowground biomass of the helophytes was rinsed with tap water and plants were grown in 25 % Hoagland nutrient solution (Qian et al., 1999) for 8 weeks before transplantation into the microcosms. Five replicates of each plant species were analysed for dry weight and heavy metal concentrations at the start of the experiment. Each microcosm was planted with 5 plants of one of the 4 selected species. Two replicates of each planted wetland and two unplanted wetlands were constructed in a greenhouse. A light regime of 16 h was maintained throughout the experiment. Temperature varied between 10 and 22 °C and relative humidity varied between 28 and 53 %.

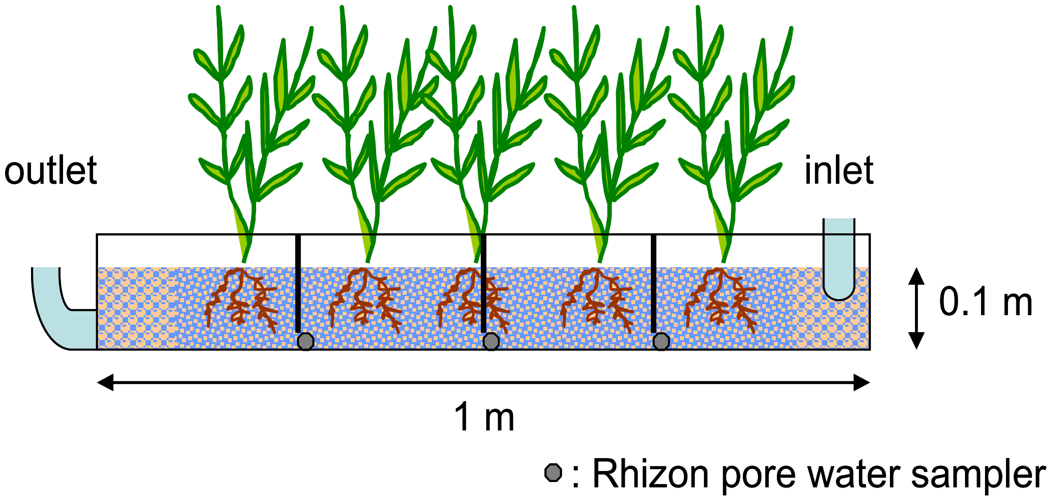

Fig 5.1 Schematic presentation of the horizontal subsurface flow constructed wetland microcosms.

2.2 Removal of heavy metals in the constructed wetland microcosms

The HSSF CW microcosms were fed with synthetic wastewater, starting November 2003. Ten times concentrated synthetic wastewater was diluted with tap water in a central distribution tank and intermittently fed to the microcosms by means of an immersed pump at a flow rate (Q_i) of 0.8 l day^{-1} (200 ml every 6 h), corresponding with a nominal residence time of 6.3 days. Concentrated synthetic wastewater was made once a week and stored in a closed tank. Evapotranspiration was compensated in order to ensure steady state conditions by daily addition of deionised water by means of sprinklers fixed in 4 positions along the length of the systems. Correction for evapotranspiration was based on regular observations of the water level in the systems. The influent, effluent and pore water were regularly sampled and analysed for Cd, Cu, Pb and Zn by means of ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) or GF-AAS (SpectrAA-800/GTA-100, Varian, Palo Alto, CA) after acidifying the samples with a drop of 65 % HNO_3. Metal concentrations that were lower than the analytical detection limits of the ICP-OES or GF-AAS, were replaced by the detection limits for further data treatment: 0.1 µg l^{-1} for Cd, 2 µg l^{-1} for Cu, Pb and 30 µg l^{-1} for Zn.

Synthetic domestic wastewater contaminated with Cd, Cu, Pb and Zn with nominal concentrations of 0.1, 0.5, 0.5 and 10 mg l^{-1} respectively, was initially used. The composition of the synthetic wastewater is presented in Table 5.1. Carbon and nutrient concentrations were representative of concentrations in domestic wastewater (160 mg l^{-1} C, 100 mg l^{-1} N_{tot} and

15.5 mg l^{-1} P_{tot}) (Kadlec and Knight, 1996; VMM, 2001). During the first 71 days of the experiment (1st loading period), the Cd, Cu, Pb and Zn concentrations in the synthetic wastewater stored in the distribution tank were 0.12 ± 0.00, 0.46 ± 0.06, 0.35 ± 0.05 and 10 ± 0 mg l^{-1}, respectively, corresponding to the nominal concentrations. However, the distribution system appeared to withhold heavy metals as evidenced by lower actual Cd, Cu, Pb and Zn concentrations in the influent wastewater of the microcosms of 0.095 ± 0.001, 0.24 ± 0.01, 0.15 ± 0.02 and 4.1 ± 0.1 in mg l^{-1}, respectively.

Table 5.1

Composition of the synthetic wastewater during the 1st loading period (mg l^{-1})

Chemical compound	Concentration	Chemical compound	Concentration
Nutrients		***Trace elements***	
$NH_4H_2PO_4$	57.5	$FeSO_4.7H_2O$	1.49
$(NH_4)_2SO_4$	94.3	$MnSO_4$	0.28
KNO_3	517	$CuSO_4.5H_2O$	1.9
$MgSO_4.7H_2O$	202	$Pb(NO_3)_2$	0.8
$CaCl_2.2H_2O$	110	$ZnSO_4.7H_2O$	44
NaCl	127	$CdSO_4.8H_2O$	0.31
$Na_2SO_4.10H_2O$	350	***Carbon source***	
		Glucose	400

From day 71 to day 114 (2nd loading period), higher Cu, Pb and Zn concentrations in the influent wastewater of 0.67 ± 0.54, 1.3 ± 0.3 and 11 ± 2 mg l^{-1}, respectively, were fed to the microcosms by increasing the Cu, Pb and Zn levels in the distribution tank to 1, 1.5 and 25 mg l^{-1}, respectively. The Cd concentration in the influent wastewater was not changed and was 0.093 ± 0.02 mg l^{-1} during that period. Nutrient and organic carbon levels were not altered during the 2nd loading period. After 114 days, helophytes from one of the replicates were harvested. Above and belowground biomass were divided, rinsed with deionised water and dried at 50 °C until constant weight. Aboveground plant parts of *Phragmites* were divided in leaves and stems. Plant material was analysed for heavy metals by means of ICP-OES after digestion with 65 % HNO_3 and 20 % H_2O_2 (Du Laing et al., 2003).

2.3 Mass balance

A mass balance was calculated at the end of the experiment to assess the relative contribution of accumulation in the biomass of the helophytes in the overall metal removal. A description on how this mass balance was derived is presented hereafter.

For each metal and each microcosm, the total mass removed from the water (M_{water}) was calculated as follows:

$$M_{water} = M_{water,1st} + M_{water,2nd}$$

With $M_{water,\ 1st/2nd}$ the metal masses removed during the 1st or 2nd loading period.

The masses removed during the 1st or 2nd loading period were calculated as follows:

$$M_{water,1st/2nd} = \left[\left(Ci_{1st/2nd} \times Qi_{1st/2nd}\right) - \left(Ce_{1st/2nd} \times Qe_{1st/2nd}\right)\right] \times t_{1st/2nd}$$

With $Ci_{1st/2nd}$ and $Ce_{1st/2nd}$ the mean metal concentration in the influent and effluent (mg l^{-1}), $Qi_{1st/2nd}$ and $Qe_{1st/2nd}$ the influent and effluent flow rates (l d^{-1}) and $t_{1st/2nd}$ the duration (d) of the 1st or 2nd loading period.

The masses of each metal that were accumulated in the different plant parts of the helophytes were calculated as follows:

$$M_{plant\ part} = \left[\Sigma_{i=1-5}\left(Ce_{plant\ part\ i} \times DMe_{plant\ part\ i}\right)\right] - 5 \times \left(Ci_{plant\ part\ i} \times DMi_{plant\ part\ i}\right)$$

With $Ce_{plant\ part,i}$ and $DMe_{plant\ part,i}$ the metal concentration (mg kg^{-1}) and dry matter (kg) of a specific part (above or belowground biomass) of plant i (1 – 5, indicating the location between inlet and outlet) at the end of the experiment, and $Ci_{plant\ part}$ and $DMi_{plant\ part}$ the mean initial metal concentration (mg kg^{-1}) and dry matter (kg) of that plant part. For *Phragmites australis*, a differentiation between leaves and stems was included.

2.4 Statistical analysis

Statistical analysis was performed using the S-plus 6.1 software package (Insightful Corp., Seattle, USA). Non-parametric Kruskal-Wallis rank tests ($\alpha = 0.05$) were performed to assess the significance of effects of (1) distance, (2) type of HSSF CW microcosm and (3) influent loading on the metal levels in the water. The significance of differences in initial metal concentrations in the above and belowground biomass of the helophytes was analysed by means of Student's t-tests ($\alpha = 0.05$). Pearson correlation coefficients were determined between metal concentrations, and above and belowground biomasses of the helophytes ($\alpha = 0.05$).

3 Results

3.1 Removal of heavy metals from the wastewater

Heavy metal concentrations in the pore water decreased very rapidly from inlet to outlet in all microcosms. Fig 5.2 presents the mean metal concentrations in the influent, pore water and effluent of the systems during the two loading periods. Standard deviations were not presented to enhance readability of the figure. During the 1st loading period, a sharp decrease of the metal concentrations was observed within the first 0.25 m of the microcosms after which the metal concentrations did not decrease significantly anymore ($p > 0.05$). No significant differences were observed between the different microcosms ($p > 0.05$).

After a period of 71 days, the influent Cu, Pb and Zn concentrations were increased respectively 3, 3 and 8 times. As for the 1st loading period, the Cu, Cd and Pb concentrations decreased sharply within the first 0.25 m of all microcosms and did not decrease from that point on ($p > 0.05$) (Fig 5.2). Moreover, increasing the Cu and Pb concentration in the influent did not affect their removal in none of the microcosms. The removal of Cd was also not affected by increasing the levels of the other heavy metals in the influent. During the 2nd loading period, the unplanted microcosms showed a sharp decrease of the Zn concentration to 30 µg l^{-1} within the first 0.25 m of the microcosms, a trend similar to that observed within the 1st loading period. Higher Zn levels were observed in the pore water at 0.25 m from the inlet in the planted microcosms: 271 µg l^{-1} for *C. acutiformis*, 528 µg l^{-1} for *I. pseudacorus*, 523 µg l^{-1} for *J. effusus* and 305 µg l^{-1} for *P. australis*. However, further along the treatment path the Zn concentrations in the pore water varied around or were lower than 30 µg l^{-1} in all microcosms (Fig 5.2).

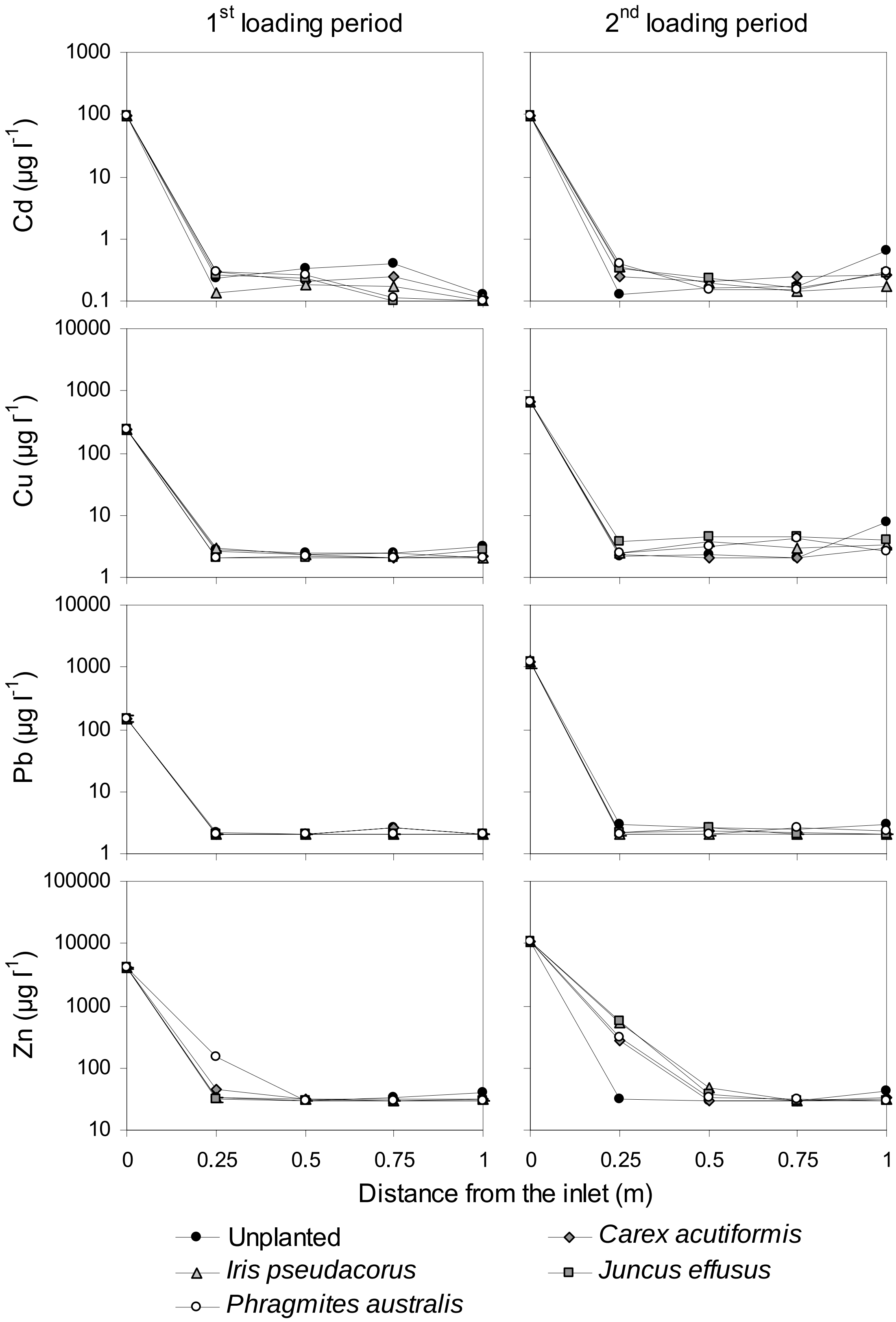

Fig 5.2 Metal concentrations in the water (µg l^{-1}) as a function of the distance from the inlet of the different HSSF CW microcosms (m), during the 1st (n = 14) and 2nd (n = 8) loading period.

3.2 Growth of the helophytes in the constructed wetland microcosms

Above and belowground plant parts of all helophytes had developed new biomass during the 114 days of the experiment, without symptoms of toxicity. A decrease of the biomass of *P. australis* was observed with increasing distance from the inlet (Fig 5.3). A different trend was observed for the other helophyte species for which the biomass first showed an increase and later a decrease as a function of distance from the inlet. A positive correlation between the above and belowground biomasses was observed for *I. pseudacorus* and *P. australis* ($R \geq 0.98$, $p < 0.05$). The biomasses of the leaves and stems of *P. australis* were also significantly positively correlated ($R = 1.00$, $p < 0.01$).

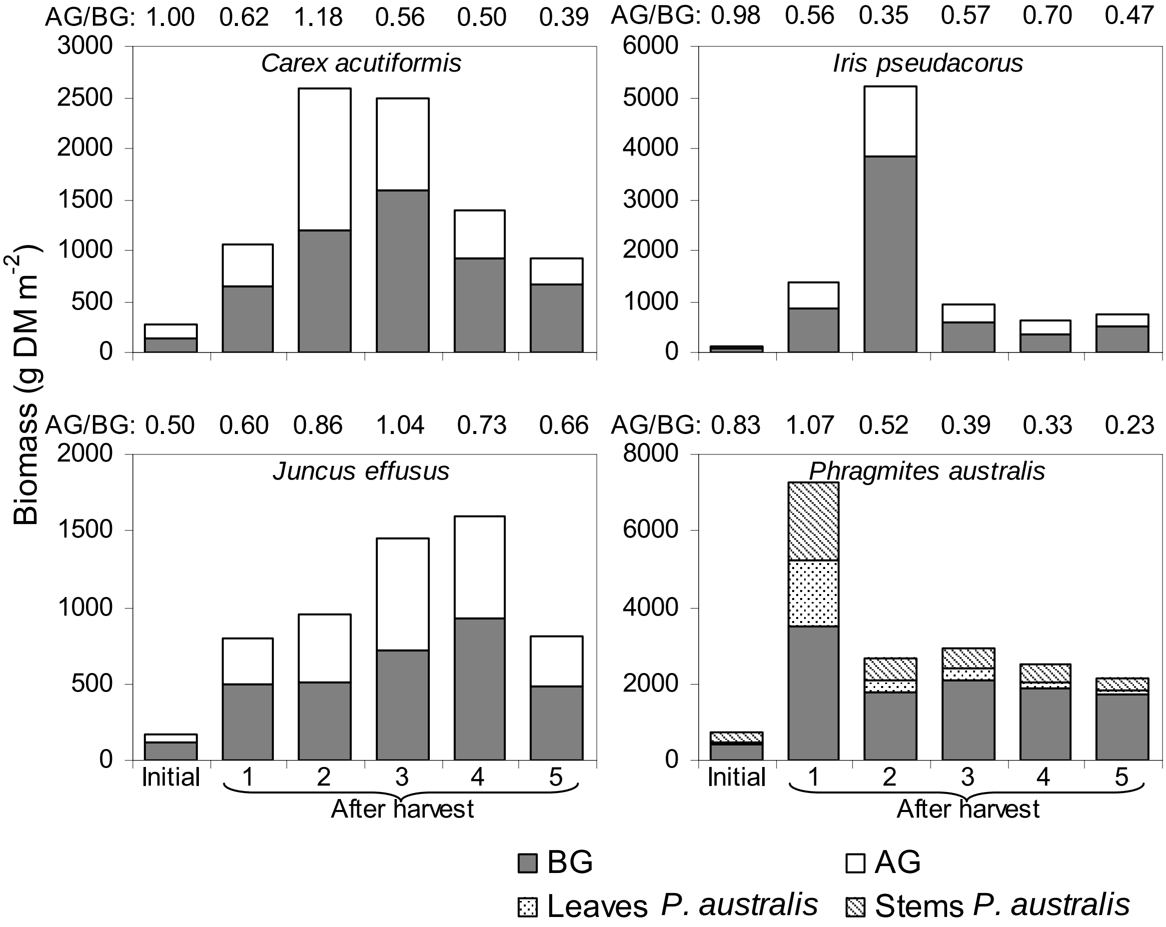

Fig 5.3 Below (BG) and aboveground (AG) biomasses (g DM m^{-2}), together with the ratios (AG/BG), of the helophytes at the start and end of the experiment, with 1 – 5 denoting the location with respect to the inlet of the HSSF CW microcosms.

Phragmites australis showed the highest aboveground biomasses of the helophytes under study, varying between 3750 g DM m^{-2} at the inlet and 403 g DM m^{-2} at the outlet of the microcosm. The lowest aboveground biomasses were observed for *J. effusus*, with a range of 301 to 741 g DM m^{-2}. *Carex acutiformis* and *I. pseudacorus* showed a similar range of the aboveground biomass of respectively 259 – 1398 and 236 – 1138 g DM m^{-2}. Ratios of the above and belowground biomass were generally lower than 1 (Fig 5.3).

3.3 Metal accumulation in the helophytes

3.3.1 Metal concentrations in the aboveground biomass of the helophytes

Fig 5.4 presents the metal concentrations in the above and belowground biomass of the different helophyte species at the start and end of the experiment. In the aboveground biomass of *C. autiformis* and *I. pseudacorus*, and in the leaves of *P. australis*, the highest Cd and Zn concentrations were observed in these plants located the closest to the inlet area, with a sharp decrease between the first and second plant. Cadmium and Zn concentrations in the stems of *P. australis* were less affected by spatial variations. *Juncus effusus* showed higher Cd and Zn concentrations in the plants located further along the treatment path. Cadmium concentrations in *J. effusus* varied between 0.24 and 1.1 mg kg^{-1} DM, being higher than concentrations observed in the other helophytes (Fig 5.4). The range of Zn concentrations observed in the aboveground biomass of *I. pseudacorus* was the lowest of all helophyte species and varied between 14 – 58 mg kg^{-1} DM (Fig 5.4).

The Cu concentrations in the aboveground biomass of *I. pseudacorus* and *J. effusus* did not display a clear trend with distance from the inlet (Fig 5.4). With the exception of the very high Cu concentration of 147 mg kg^{-1} DM in the aboveground biomass of the *C. acutiformis* plant at the first location, concentrations in *C. acutiformis*, *I. pseudacorus* and *J. effusus* were similar to the initial Cu concentrations. For *P. australis*, a decrease of the Cu concentration in the leaves and stems was observed with increasing distance from the inlet. The range of Cu concentrations observed in the different helophyte species was similar.

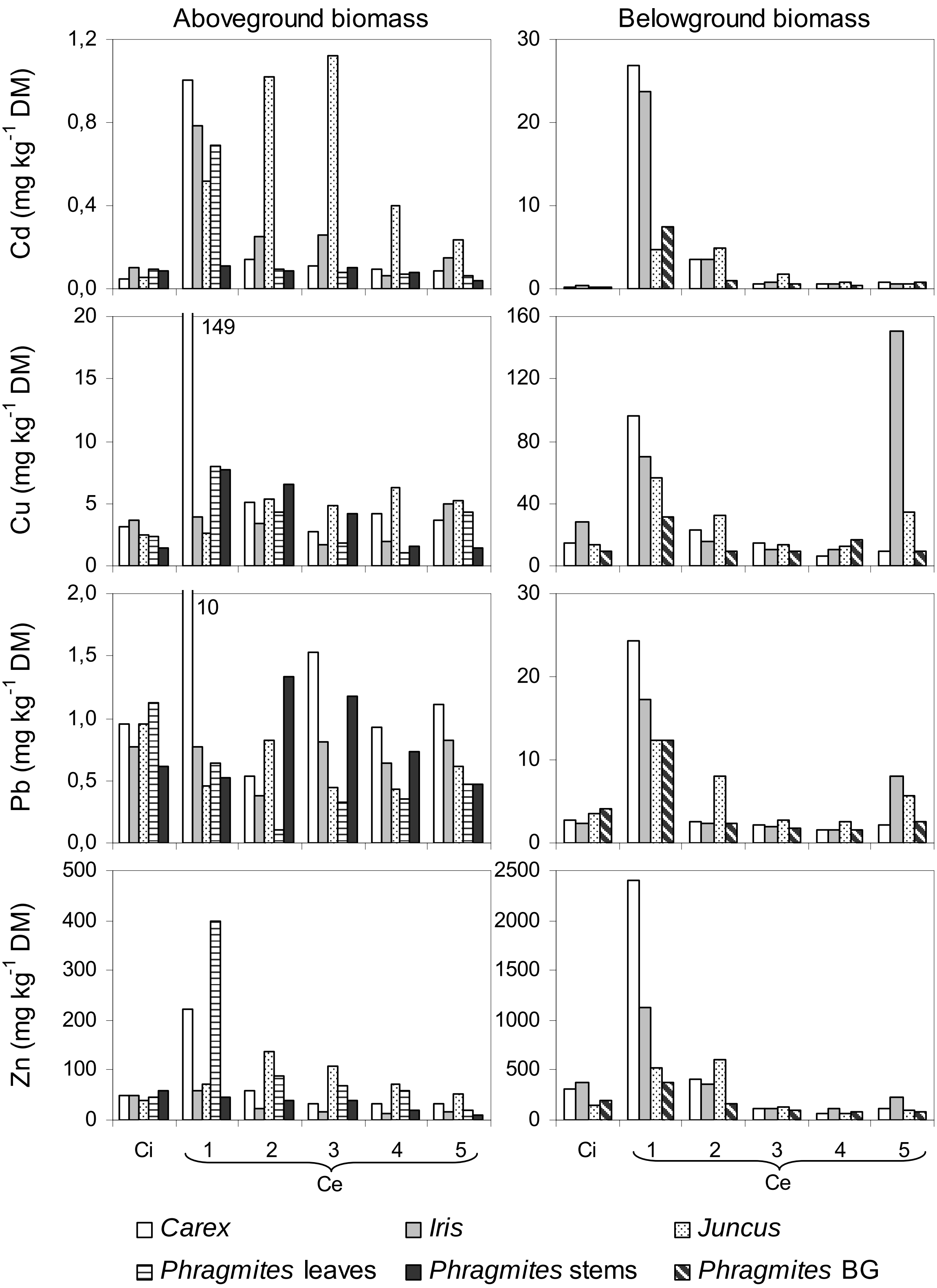

Fig 5.4 Metal concentrations in the above and belowground biomass of the helophytes at the start (C_i) and end (C_e) of the experiment (mg kg^{-1} DM) with 1 – 5 denoting the location with respect to the inlet of the HSSF CW microcosms.

With the exception of the high Pb concentration of 10 mg kg^{-1} DM in the aboveground biomass of the *C. acutiformis* plant located closest to the inlet area, the Pb concentrations in the aboveground biomass of all helophyte species were similar to or lower than the initial Pb concentrations. Moreover, Pb concentrations in the aboveground biomass of the different helophyte species were similar and varied between 0.1 – 1.5 mg kg^{-1} DM (Fig 5.4).

3.3.2 Metal concentrations in the belowground biomass of the helophytes

Metal concentrations in the belowground biomass of the helophytes displayed a general decreasing trend with distance from the inlet. Closest to the inlet area, the highest metal concentrations in the belowground biomass were generally observed in *C. acutiformis*. In the belowground parts of the *C. acutiformis* plant in this first position, the Cu, Pb and Zn concentrations at the end of the experiment were 7 to 9 times higher than their initial levels whereas Cd concentrations were about 100 times higher than initially. Further along the treatment path, metal concentrations in the belowground biomass of the different helophyte species were generally similar (Fig 5.4).

Cadmium, Cu, Pb and Zn concentrations in the above and belowground biomass of *C. acutiformis* were significantly positively correlated ($R \geq 0.98$, $p < 0.01$), indicating a similar spatial trend for all metals in both plant parts. Significant correlations were generally not observed between metal concentrations in the above and belowground biomass of *J. effusus*. For *P. australis*, the Cd and Zn concentration in the leaves were positively correlated with their concentrations in the belowground biomass ($R \geq 0.98$, $p < 0.01$). The same was observed for Cd, Cu and Zn in *I. pseudacorus* ($R \geq 0.98$, $p < 0.01$ for Cd and Zn; $R = 0.89$, $p < 0.05$ for Cu).

3.3.3 Translocation factor

Metal concentrations in the belowground biomass were generally higher than in the aboveground biomass. This was observed for all helophyte species, at the start of the experiment ($p < 0.01$) and after harvesting the plants. The translocation factor (TF) is defined as the ratio of the metal concentration in the above and belowground biomass (Deng et al., 2004). With only a few exceptions, the TF in plants located closest to the inlet area was lower than the initial TF, caused by a higher increase of the metal concentration in the below than in the aboveground biomass. The translocation factors in helophytes growing closest to the inlet area of the microcosms were generally lower than in helophytes that were located further along the treatment path. The TFs at the start and end of the experiment are presented in Table 5.2. With a few exceptions, the TFs were lower than 1, indicating restricted translocation of heavy metals to the aboveground biomass of the helophytes.

Table 5.2

Translocation factor (TF) of the metals in the helophytes at the start and end of the experiment, with a differentiation of the shoots of *Phragmites australis* in leaves and stems. Mean ± st dev are presented for the initial TF (n = 5), a range is presented of the TFs in the plants after harvest

Metal	**Time**	***Carex acutiformis***	***Iris pseudacorus***	***Juncus effusus***	***Phragmites australis***	
					Leaves	**Stems**
Cd	**Start**	0.17 ± 0.02	0.32 ± 0.01	0.27 ± 0.02	0.48 ± 0.01	0.44 ± 0.01
	End	0.04 - 0.16	0.03 - 0.30	0.11 - 0.66	0.09 - 0.17	0.02 - 0.19
Cu	**Start**	0.22 ± 0.01	0.13 ± 0.04	0.18 ± 0.02	0.24 ± 0.01	0.14 ± 0.02
	End	0.18 - 1.54	0.03 - 0.21	0.05 - 0.51	0.06 - 0.47	0.10 - 0.70
Pb	**Start**	0.34 ± 0.01	0.33 ± 0.01	0.27 ± 0.02	0.27 ± 0.02	0.15 ± 0.03
	End	0.22 - 0.72	0.04 - 0.41	0.04 - 0.17	0.05 - 0.21	0.04 - 0.65
Zn	**Start**	0.15 ± 0.01	0.12 ± 0.02	0.25 ± 0.01	0.23 ± 0.02	0.29 ± 0.01
	End	0.09 - 0.48	0.05 - 0.13	0.14 - 0.99	0.23 - 1.07	0.12 - 0.43

3.3.4 Correlations between metal concentrations and above and belowground biomass of the helophytes

The Cd and Zn concentrations in the leaves of *P. australis* were positively correlated with the biomass of the leaves ($R \geq 0.99$, $p < 0.01$). The Cd and Pb concentrations in the belowground biomass of *P. australis* were also positively correlated with the biomass ($R \geq 0.97$, $p < 0.05$). For the stems of *P. australis*, a correlation between the metal concentrations and the biomass was not observed. Correlations were not detected between the biomass and the metal concentrations in the above or belowground plant parts of *C. acutiformis*, *I. pseudacorus* and *J. effusus* ($p > 0.05$).

3.4 Relative contribution of metal accumulation in the helophytes in the overall metal removal from the wastewater

Table 5.3 presents the masses of each metal that were removed from the water in the different microcosms together with the masses that were accumulated in the above and belowground biomass of the helophytes. The difference between the masses removed from the water and the masses accumulated in the helophytes, is the rest fraction that is retained within the gravel substrate of the CWs. The relative contribution of each compartment in the overall metal removal is presented as well. Interestingly, the mass of metals removed in the unplanted microcosms was similar to that removed in the planted ones.

With the exception of the contribution of the aboveground biomass of *C. acutiformis* in the Cu removal from the water (6 %), accumulation of metals in the aboveground biomass of *C. acutiformis*, *I. pseudacorus* and *J. effusus* generally accounted for less than 1 % of the overall metal removal. It has to be noted that 86 % of the total mass of Cu that was accumulated in the aboveground biomass of *C. acutiformis* was recovered within the plant that was located closest to the inlet of the microcosm, due to its very high Cu concentration of 147 mg kg^{-1} DM. A higher contribution of the aboveground biomass in the overall metal removal, was observed for *P. australis*: 2.1, 4.6, 30 and 43 % for respectively Pb, Cd, Cu and Zn. The *P. australis* plant located closest to the inlet of the CW showed the highest biomass and metal concentrations and accounted for 72 to 95 % of the total metal mass recovered in the aboveground biomass of the microcosm.

Accumulation of metals in the belowground biomass of *C. acutiformis*, *I. pseudacorus* and *J. effusus* accounted for a higher proportion of the metal removal than the aboveground biomass of these plant species. Differences were observed, depending on the plant species and the metal. Accumulation of Pb in the belowground biomass of *C. acutiformis*, *I. pseudacorus* and *J. effusus* varied between 1 and 2 % of the mass of Pb removed from the water. A higher contribution of the belowground biomass of these helophyte species was observed for Cd, Cu and Zn. The lowest contribution of accumulation of Cd, Cu and Zn in the belowground biomass with respect to the mass of metals removed from the water, was observed for *J. effusus*, being respectively 2, 3 and 6 %. The belowground biomass of *P. australis* accounted for a very high percentage of the overall metal removal, varying between 34 % for Pb and 114 % for Cu. As was observed for the aboveground biomass of *P. australis*, the highest proportion of the total metal mass accumulated in the belowground biomass of the CW was recovered in the plant located closest to the inlet, varying between 80 and 92 %.

Significant amounts of metals were retained within the gravel substrate in the microcosms planted with *C. acutiformis*, *I. pseudacorus* and *J. effusus*, varying between 83 to 99 % of the mass of metals removed from the water. The gravel substrate of the microcosm planted with *P. australis* accumulated 9 and 64 % of respectively the mass of Cd and Pb removed from the water. Masses of Cu and Zn recovered within the biomass of *P. australis* exceeded the masses removed from the wastewater, resulting in negative mass balances.

Table 5.3

Metal masses removed from the water (M_{water}), accumulated in the above ($M_{aboveground}$) and belowground ($M_{belowground}$) biomass of the helophytes and the rest fraction (M_{rest}) (mg), together with the relative contribution of each compartment in the metal removal (%) for the different HSSF CW microcosms

Metal	**HSSF CW**	**WATER**	**HELOPHYTES**					**REST**	
	microcosm	M_{water}	$M_{aboveground}$		**%**	$M_{belowground}$	**%**	M_{rest}	%
Cd	**Unplanted**	7						7	100
	Carex	7	0.02		0.2	0.51	7	6	93
	Iris	7	0.02		0.2	0.77	11	6	89
	Juncus	7	0.04		0.5	0.15	2	7	97
	Phragmites	7	0.32	0.26 (leaves) 0.06 (stems)	4.6	6	87	1	9
Cu	**Unplanted**	25						25	100
	Carex	25	1.51		6.0	2.40	9	21	85
	Iris	25	0.13		0.5	4.19	17	21	83
	Juncus	25	0.24		0.9	1.57	6	23	93
	Phragmites	25	7.48	3.25 (leaves) 4.23 (stems)	30	29	114	-11	/
Pb	**Unplanted**	30						30	100
	Carex	30	0.13		0.4	0.50	2	30	98
	Iris	30	0.02		0.1	0.61	2	30	98
	Juncus	30	0.01		0.0	0.32	1	30	99
	Phragmites	30	0.65	0.24 (leaves) 0.42 (stems)	2.1	10	34	19	64
Zn	**Unplanted**	413						413	100
	Carex	414	4.06		1.0	46	11	364	88
	Iris	414	0.81		0.2	50	12	363	88
	Juncus	414	4.28		1.0	14	3	395	95
	Phragmites	414	179	155 (leaves) 25 (stems)	43	352	85	-117	/

/: Not applicable (negative mass balance)

4 Discussion

4.1 Performance of the constructed wetland microcosms

All HSSF CW microcosms proved to be highly efficient in removing metals from the wastewater. Metal concentrations in the pore water decreased very rapidly from inlet to outlet. Increasing the Cu and Pb levels in the influent 3 times did not affect their removal in all microcosms. Zinc removal in the planted microcosms occurred less rapidly than in the unplanted ones after increasing the influent Zn concentration 8 times. However, further along the treatment path the Zn concentrations in the pore water were similar in all microcosms. The removal of Cd was not affected by increasing the levels of the other metals in the influent. Removal efficiencies of ≥ 99 % were observed throughout the experiment with mean Cd, Cu, Pb and Zn concentrations in the effluents varying between < 0.1 – 0.65 µg l^{-1}, < 2 – 7.8 µg l^{-1}, < 2 – 3 µg l^{-1} and < 30 – 43 µg l^{-1}, respectively, during both loading periods.

Whereas metal removal in the unplanted microcosm was solely attributed to adsorption and precipitation processes in the filter substrate, a fraction of the metal removal in the planted microcosms can be attributed to uptake by the helophytes. Yet, accumulation of metals in the aboveground biomass of *C. acutiformis*, *I. pseudacorus* and *J. effusus* generally accounted for less than 1 % of the metal removal. However, accumulation of metals in the biomass of *P. australis* appeared to be a more important removal process. This is presumably caused by the short duration of the experiment in which the initial establishment of the *P. australis* biomass was taking place. Lower percentages of metals are expected to be removed by accumulation in the biomass in full-scale CWs, as described elsewhere (Mantovi et al., 2003; Stottmeister et al., 2003; Vymazal, 2003; Vymazal and Krása, 2003; Lesage et al., accepted). However, some laboratory-scale experiments reported higher contributions of the helophytes, mainly of their fine roots, in the overall metal removal (Sinicrope et al., 1992; Cheng et al., 2002). Metals sequestered in the belowground plant parts are eventually buried within the sediment.

4.2 Uptake and translocation patterns of heavy metals in the helophytes

Tables 5.4, 5.5 and 5.6 present the metal concentrations observed in the above and belowground biomass of the different helophyte species, together with values reported in literature and a description of the referred wetlands. However, it should be kept in mind that comparisons between metal concentrations in aquatic macrophytes are complicated by different factors, including differences in (1) metal concentrations in the surrounding water or soil, (2) physico-chemical characteristics affecting metal mobility (pH, Eh, organic matter content, etc.), (3) plant species, plant part, growth stage and (4) analytical procedure of metal analysis (Guilizzoni, 1991; Du Laing et al., 2003). Although comparisons should be considered as indicative, following general conclusions could be drawn:

(1) Metal concentrations in the belowground biomass were generally higher than in the aboveground biomass.

Studies in many natural and constructed wetlands have demonstrated similar allocation patterns of metals in emergent macrophyte species, with most wetland plants having higher concentrations of metals in their belowground biomass than in their shoot tissues (Tables 5.5 and 5.6). Helophytes are able to balance uptake and translocation to the shoots, even under heavily polluted conditions (Deng et al., 2004). With a few exceptions, the translocation factors were lower than 1, indicating that metals were mainly retained within the belowground biomass.

(2) Metal concentrations in the belowground biomass decreased with increasing distance from the inlet of the HSSF CW microcosms.

Closest to the inlet of the microcosms, the Cd and Zn concentrations in the belowground biomass of *P. australis* were elevated compared to reed stands in uncontaminated wetlands, whereas Cu and Pb concentrations fell within the range reported in uncontaminated wetlands and CWs and do not indicate excessive concentrations (Table 5.5). Whereas there are a lot of literature reports on metal concentrations in *P. australis*, information about metal concentrations in other wetland plants is less abundant.

Closest to the inlet area, the highest metal concentrations in the belowground biomass of the 4 helophyte species under study were observed in *C. acutiformis* and *I. pseudacorus*. Further along the treatment path, metal concentrations in the belowground biomass of the different helophyte species were similar, evidenced by small variations in the metal concentrations: 0.42 – 0.58 mg kg^{-1} DM for Cd, 9.2 – 12 mg kg^{-1} DM for Cu, 1.6 – 2.6 mg kg^{-1} DM for Pb and 70 – 100 mg kg^{-1} DM for Zn. These concentrations fell within the range of metal concentrations reported in uncontaminated reed stands, sewage-contaminated lakes and HSSF CWs for treatment of domestic wastewater and landfill leachate (Table 5.5).

As the belowground biomass was only rinsed with deionised water before analysis, the metal concentrations in the belowground biomass presumably included metals that were adsorbed and/or co-precipitated on the Fe- (oxy-) hydroxide plaques covering the root surface. Gries and Garbe (1989) and Mays and Edwards (2001) suggested that a major part of the metals were bound as co-precipitates in the Fe- (oxy-) hydroxide plaques covering the root surface.

(3) Metal concentrations in the aboveground biomass were less subjected to spatial variations and did generally not show excessive values.

With the exception of the high Cu and Pb concentrations in the aboveground biomass of the *C. acutiformis* plant located closest to the inlet of the microcosm, spatial variations of these heavy metals were generally small. Concentrations in the aboveground biomasses of the different helophyte species were similar and varied between 1.0 and 7.9 mg kg^{-1} DM and 0.11 – 0.83 mg kg^{-1} DM for Cu and Pb, respectively. Lead and Cu concentrations in the aboveground biomass of the helophytes were in the range of concentrations reported in uncontaminated and metal-contaminated environments (Table 5.6).

Whereas *C. acutiformis*, *I. pseudacorus* and *P. australis* showed the highest Cd concentrations closest to the inlet, a higher range of Cd concentrations was seen in *J. effusus* throughout the entire microcosm, varying between 0.24 – 1.1 mg kg^{-1} DM. Zinc concentrations in the aboveground biomass of *I. pseudacorus* varied between 14 – 58 mg kg^{-1} DM, a range considerably lower than that observed for the other helophyte species. Overall, metal concentrations in the helophytes were comparable to values reported in natural and constructed wetlands and do not indicate excessive accumulation in the aboveground biomass.

Table 5.4

Description of reference numbers in Tables 5.5 and 5.6, together with location and reference

Ref n°	Description	Location	Reference
1	Gravel-based HSSF CW mesocosm for surface runoff	UK	Mungur et al. (1997)
2	Gravel-based CW mesocosm for dairy wastewater	New Zealand	Tanner (1996)
3	Soil-based HSSF CW for domestic wastewater	Germany	Gries and Garbe (1989)
4	Gravel/sand-based HSSF CW for landfill leachate, with natural reed stands as control	USA	Peverly et al. (1995)
5	Gravel-based HSSF CW for domestic wastewater	Belgium	Lesage et al. (accepted)
6	FWS CW for domestic wastewater	Belgium	This work, Chapter 3
7	Uncontaminated and sewage-contaminated lakes	Denmark	Schierup and Larsen (1981)
8	Natural wetland in a submerged mine tailings impoundment	Sweden	Stoltz and Greger (2002)
9	3 lakes that receive untreated municipal sewage	Poland	Szymanowska et al. (1999)
10	Acidified lakes developed in excavations after coal mining	Poland	Samecka-Cymerman and Kempers (2001)
11	Metal-contaminated salt marsh	USA	Windham et al. (2003)
12	Tidal marshes along the river Scheldt	Belgium	Du Laing (2006)
13	Natural wetland and CW mesocosm for effluents of a coal pile basin	USA	Collins et al. (2005)
14	FWS CW for secondary treated domestic wastewater	New Zealand	Chagué-Goff (2005)
15	Metal-contaminated wetlands near Pb, Zn and Cu mines and control wetlands	China	Deng et al. (2004)
16	Gravel-based HSSF CW microcosm for artificial wastewater	Belgium	This study

Table 5.5

Cadmium, Cu, Pb and Zn concentrations in belowground (BG) plant parts of the selected helophyte species, together with values reported in literature (mg kg^{-1} DM). The reference numbers are described in Table 5.4

Helophyte species	**Habitat**	**Plant part**	**Cd**	**Cu**	**Pb**	**Zn**	**Ref n°**
P. australis	HSSF CW microcosm	BG	0.42-7.4	9.2-32	1.7-12	79-372	16
	CW mesocosm	Roots		143-167	27-58	165-264	1
	CW mesocosm	BG		3		80	2
	HSSF CW	Rhizomes	0.06	13	2.4	60	3
		Roots	0.31	19	7.2	460	
	HSSF CW	Rhizomes	0.1	6.5	0.24	12	4
		Roots	0.25	64	12	46	
	HSSF CW	BG	0.2-0.5	6.9-44	1.3-20	32-184	5
	Uncontaminated reed stands	Rhizomes	0.06	4.6	0.3	17	4
		Roots	0.18	23	7.2	70	
	Uncontaminated lake	Rhizomes	0.22	2.1	<1	38	7
		Roots	1.2	18	9.3	245	
	Submerged mine tailings	Rhizomes	0.1	8	8.1	80	8
		Roots	4.6	80	523	1310	
	Sewage-contaminated lake	Rhizomes	0.1	3.1	<1	31	7
		Roots	0.58	15	3.5	158	
	Metal-contaminated marsh	Rhizomes		7.3-13	6.3-8.4	25-67	11
		Roots		55-185	91-142	233-588	
C. acutiformis	HSSF CW microcosm	BG	0.58-27	6.3-96	1.6-24	70-2400	16
C. rostrata	Submerged mine tailings	Roots	1.4	75	305	656	8
J. effusus	HSSF CW microcosm	BG	0.57-4.8	12-56	2.6-12	73-597	16
	CW mesocosm	Roots				53	13
	CW mesocosm	BG		8		340	2
	FWS CW (*Juncus* spp.)	Roots		4	4.5	23	14
	Natural wetland	Roots				87	13
	Metal-contaminated wetlands	BG	<10-50	<20-200	<50-4000	100-2500	15
I. pseudacorus	HSSF CW microcosm	BG	0.53-24	11-151	1.5-17	110-1129	16
	CW mesocosm	Rhizomes		6	8	9	1
		Roots		53-87	22-210	53-64	

Table 5.6

Cadmium, Cu, Pb and Zn concentrations in aboveground plant parts of the selected helophyte species, together with values reported in literature (mg kg^{-1} DM). The reference numbers are described in Table 5.4

Helophyte species	**Habitat**	**Plant part**	**Cd**	**Cu**	**Pb**	**Zn**	**Ref n°**
P. australis	HSSF CW microcosm	Leaves	0.06-0.69	1.0-7.9	0.11-0.64	20-397	16
		Stems	0.04-0.11	1.5-7.7	0.47-1.3	11-45	
	CW mesocosm	Shoots		2		20	2
	HSSF CW	Shoots	0.02	2	1.2	20	3
	HSSF CW	Shoots	0.05	2.9	0.09	17	4
	HSSF CW	Leaves	0.03-0.07	2.2-4.4	0.5-1.1	20-49	5
		Stems	0.03-0.08	0.9-3.3	0.3-0.4	11-39	
	FWS CW	Leaves	0.05	3.7	1.1	51	6
		Stems	0.04	1.3	0.5	72	
	Uncontaminated reed stands	Shoots	0.04	2.6	0.3	18	4
	Uncontaminated lake	Shoots	0.25	5.3	3.7	337	7
	Submerged mine tailings	Shoots	1	6.4	4.1	68	8
	Sewage-contaminated lake	Shoots	0.05	3.3	<1	217	7
	Sewage-contaminated lakes	Leaves	1.3-1.9	2.1-2.5	8.6-11	609-1710	9
	Metal-contaminated lakes	Leaves	1.2-5.0	6.2-7.5	14-22	13-33	10
	Metal-contaminated marsh	Leaves		3.7-11	0.1-3.0	12-79	11
		Stems		1.6-19	0.6-3.2	27-75	
	Tidal marshes	Leaves	0.03-0.22	2.7-8.6	0.5-7.1	11-57	12
		Stems	0.01-0.07	0.5-4.8	0.2-1.0	35-137	
C. acutiformis	HSSF CW microcosm	Shoots	0.09-1.0	2.8-149	0.54-10	31-222	16
C. rostrata	Submerged mine tailings	Shoots	0.4	13	13	90	9
C. remota	Metal-contaminated lakes	Shoots	2.1-2.3	6.6-20	19-27	25-95	10
J. effusus	HSSF CW microcosm	Shoots	0.24-1.1	2.6-6.3	0.44-0.83	54-136	16
	CW mesocosm	Shoots				22	13
	CW mesocosm	Shoots		1		70	2
	FWS CW (*Juncus* spp.)	Shoots		2.5	<2	14	14
	Natural wetland	Shoots				169	13
	Metal-contaminated lakes	Shoots	1.3-4.8	6.3-35	0.5-17	2-39	10
	Metal-contaminated wetlands	Shoots	<5-20	<10-20	<5-150	150-230	15
I. pseudacorus	HSSF CW microcosm	Shoots	0.06-0.79	1.7-5.0	0.38-0.82	14-58	16
	Metal-contaminated lakes	Shoots	2.6	5.8	14	21	10

4.3 Growth and tolerance of the helophytes

The relatively short duration of the experiment and the small scale of the experiment hamper the extrapolation of biomass production to field conditions. Tanner (1996) and Brisson et al. (2006) warned about possible edge effects in small-scale experiments. The exposure of shoots to lateral as well as downward solar radiation, can lead to overestimation of the aboveground biomass production in mesocosm experiments. Moreover, rooting was limited in the HSSF CW microcosms under study, due to the shallowness of the set-up. Overall, it is assumed that within a similar set-up, a comparison between helophyte species can be made and conclusions regarding which species perform better or worse under given conditions can be drawn, as suggested by Tanner (1996).

Spatial variations were observed in the above and belowground biomasses of the helophytes. With a maximum aboveground biomass of 3750 g DM m^{-2}, *P. australis* showed the largest aboveground biomass of the helophyte species under study. Due to its competitiveness and high productivity, *P. australis* is the most common wetland plant in CWs around the world (Tanner, 1996). The mean above and belowground biomasses of *P. australis* in this study were 1302 and 2199 g DM m^{-2}, respectively, which were similar to values of 1750 and 2250 g DM m^{-2} reported by Tanner (1996) in gravel-based mesocosms fed with dairy lagoon effluent. DeBusk et al. (1995) reported above and belowground biomasses of 2300 and 1200 g DM m^{-2} in soil-based mesocosms fed with dairy lagoon effluent. A range of 1650 – 5070 g DM m^{-2} was reported by Vymazal and Kröpfelová (2005) for the aboveground biomass of *P. australis* in 13 gravel-based HSSF CWs for domestic wastewater. Gries and Garbe (1989) and Adcock and Ganf (1994) reported lower aboveground biomasses of respectively 1360 and 788 g DM m^{-2} in soil-based and gravel-based HSSF CWs for domestic wastewater. Similar aboveground biomasses of *P. australis* were reported in natural wetlands of temperate regions by Asaeda and Karunaratne (2000) (2000 g DM m^{-2}), Karunaratne et al. (2003) (1290 g DM m^{-2}) and Soetaert et al. (2004) (587 – 2659 g DM m^{-2}), whereas higher biomasses up to 9000 – 12000 g DM m^{-2} have been reported in warmer climates, such as Australia (Asaeda and Karunaratne, 2000).

Juncus effusus was the least productive of the helophytes under study, with mean above and belowground biomasses of respectively 494 and 627 g DM m^{-2}. Similar above and belowground biomasses of *J. effusus* growing in soil-based mesocosms exposed to dairy lagoon effluent of respectively 500 and 750 g DM m^{-2} were observed by DeBusk et al. (1995). Tanner (1996) reported above and belowground biomasses of respectively 700 and 250 g DM m^{-2} for *J. effusus* grown in a gravel-based mesocosm fed with dairy lagoon effluent, with similar N and P levels as in this study. However, the AG/BG ratio reported by Tanner (1996) was much higher than that reported in this study.

Literature information on growth of *C. acutiformis* in CWs is very scarce. The mean above and belowground biomasses of *C. acutiformis* were respectively 683 and 1004 g DM m^{-2} and were similar to values reported by Aerts et al. (1992) for *C. acutiformis* growing in experimental ponds with a synthetic nutrient solution containing 7.2 mg l^{-1} N_{tot}. Aerts et al. (1992) observed a maximum aboveground biomass of 1140 g DM m^{-2} and an AG/BG ratio of about 1. Lower aboveground biomasses of 160 – 200 g DM m^{-2} were reported for *C. rostrata* growing in a fen, being attributed to the colder climates in northern regions (Saarinen, 1998). The aboveground biomass of *I. pseudacorus* was 532 g DM m^{-2}, whereas a much higher belowground biomass of 1241 g DM m^{-2} was observed. Literature reports on biomass productions of *I. pseudacorus* in CWs are rare. A similar biomass production of 700 – 800 g DM m^{-2} has been reported (APIRS, 2006).

Symptoms of toxicity were not observed during the experiment. The information about metal tolerance in wetland plants is still incomplete, although the suggestion to date is that some helophyte species have an inherent tolerance mechanism instead of evolving tolerance when exposed to a metal-contaminated environment (Taylor and Crowder, 1983a; Ye et al., 1997; Matthews et al., 2004). The suggestion to date is that some helophytes are 'excluders' of metals, restricting the translocation of metals to the shoots up to some extent (Windham et al., 2003). The positive relationship observed between the metal concentrations and the above or belowground biomasses of *P. australis* leads to the assumption that elevated metal concentrations in the above and belowground biomasses did not cause toxicity to *P. australis*. On the other hand, lower biomasses were observed for *C. acutiformis, I. pseudacorus* and *J. effusus* at the position closest to the inlet of the microcosms, where the highest metal concentrations were observed in the belowground biomass. Whereas the translocation of metals to the aboveground biomass was well restricted within *I. pseudacorus* and *J. effusus* in this position, evidenced by low TFs, *C. acutiformis* was less efficient in restricting the movement of Cu and Pb to its aboveground tissues. The accumulation of metals in the belowground biomass of *C. acutiformis*, *I. pseudacorus* and *J. effusus* could have put stress on these plant species and could be a reason for the lower biomasses observed in the position closest to the inlet of the microcosms.

4.4 Synthesis: implications for selection of a helophyte species

Alhough the role of aquatic macrophytes in removal processes has been questioned, they are considered as an integral part of CWs (Brix, 1997, 2004). All helophyte-based microcosms proved highly efficient in the removal of heavy metals from the synthetic wastewater. Overall, the range of metal concentrations observed in the aboveground biomass of the different helophyte species were quite similar and did not indicate excessive accumulation. Helophytes efficiently immobilise metals in the substrate and do not endanger animals that depend on it. This was also demonstrated for reeds growing in CWs for sludge dewatering (De Maeseneer, 1997).

The major factors affecting the choice of a suitable helophyte species are the biomass production and whether a regular harvesting is feasible or not. This microcosm experiment was in line with other literature reports which have shown that *P. australis* is one of the most productive helophyte species. Therefore, when regular harvesting of the biomass is feasible, *P. australis* should be selected over lower biomass producing species such as *C. acutiformis*, *I. pseudacorus* and *J. effusus*. When harvesting is not feasible, lower biomass producing species become more attractive.

Large amounts of biomass require a proper disposal or application when harvested (Stottmeister et al., 2003). Biomass of *Phragmites* has been used throughout history for the production of paper-pulp, roofing materials, forage feed, fertiliser and for heating (Barbraud and Mathevet, 2000; Hansson and Frederiksson, 2004).

Composting the harvested biomass of *Phragmites australis* could present an interesting management strategy. Hansson and Frederiksson (2004) suggested that biomass of *Phragmites australis* should be mixed with other materials with a higher nitrogen content, such as garden wastes, to achieve a C/N ratio between 15 and 30 necessary for an optimal composting process. Composting of green and garden wastes in Flanders is restricted to installations certified by the Flemish Organisation for Composting (VLACO 'Vlaamse Compost Organisatie'). Reuse of the compost as fertiliser or soil-improving substance is regulated by the Flemish regulations relating to waste prevention and management (VLAREA, 1998). Regarding the metals studied, the concentrations in the compost must comply with the following criteria: 6 mg kg^{-1} DM for Cd, 375 mg kg^{-1} DM for Cu, 300 mg kg^{-1} DM for Pb and 900 mg kg^{-1} DM for Zn. Based on the observed concentrations in the aboveground biomass in this study, reuse of compost as fertiliser could be a possible strategy.

Hansson and Frederiksson (2004) studied different strategies in using summer harvested *Phragmites australis* biomass as a nutrient source for crop production in Sweden. Use of the harvested biomass as raw material in biogas production by means of anaerobic digestion and spreading the nutrient rich sludge on the farmland proved to be the best strategy. Moreover, an energy production of 5.19 MJ kg^{-1} DM could be achieved by biogas production. Chopping the biomass and spreading it directly on land, had the disadvantage of nutrient leakage, whereas composting did not produce useful energy.

A recent assessment of Ericsson and Nilsson (2006) showed the large potential of crops as an alternative energy source in Europe. *Phragmites australis* is one of the 30 energy crop species that has been tested more intensively in Europe, but is not yet used on a field scale. The co-combustion of *Phalaris arundinaceae* (Reed canary grass) with bark, wood chips, or peat has proven effective in Scandinavian countries. Next to willow, *Phalaris arundinaceae* is considered as the most promising energy crop in Sweden and Finland (Venendaal et al., 1997).

Solano et al. (2004) and Ciria et al. (2005) recently demonstrated the biofuel-potential of *Typha latifolia* and *Phragmites australis* harvested from pilot-scale gravel-based HSSF CWs for treatment of raw municipal wastewater. Solano et al. (2004) showed high heating values of 17.5 – 20 MJ kg^{-1} DM of the harvested biomass, in the range of values reported for maize, poplar, rice straw and wheat straw. However, operational problems could be encountered in full-scale application of emergent macrophytes as biofuels. The higher ash and silica content lower the efficiency of the combustion by creating low melting point silicates which deposit on the heat exchanger surfaces (Ciria et al., 2005). However, mixing with other biomasses or additives could encompass this problem (Solano et al., 2004). Local small-scale heating plants do not have to rely on one single crop and can use a combination of different biomasses (Venendaal et al., 1997).

Although the harvested biomass could have useful applications and the sustainability of CWs would be further enhanced, plant biomass is usually not harvested from CWs in Europe (EPA, 1993; Stottmeister et al., 2003). When harvesting is not feasible, species with a lower biomass production become more attractive than high biomass producing species such as *Phragmites*. Lower amounts of decomposing plant material will eventually be returned back to the CW after senescence, and thus clogging problems and need for maintenance will be less than when using higher biomass producing species. Moreover, a smaller pool of metals will possibly be released back to the water column during breakdown of the organic matter.

At last, the aesthetic value of the plants is mentioned as a criterion for selection. *Carex acutiformis*, *Iris pseudacorus* and *Juncus effusus* are more attractive than a dense monotypic stand of *Phragmites australis*. The latter plant species has been described to have lower habitat values than other species (EPA, 1993). The aesthetic value of the macrophytes can be more important for example in small systems serving single households or hotels, etc. (Brix, 1997). *Iris pseudacorus* is an attractive ornamental plant that is widely sold by garden and plant dealers (APIRS, 2006). *Juncus effusus* offers potential where low visual impact is sought (Tanner, 1996).

5 Conclusions

The presence of helophytes did not affect the removal efficiencies of Cd, Cu, Pb and Zn from the wastewater. Removal efficiencies were higher than 99 % in both unplanted and planted HSSF CW microcosms. Accumulation of metals in the aboveground biomass of *C. acutiformis*, *I. pseudacorus* and *J. effusus* generally accounted for less than 1 % of the metal removal, whereas a higher contribution was observed for *P. australis*, attributed to its rapid initial establishment during the first 114 days of the experiment. It is suggested that the contribution of *P. australis* in the overall metal removal would be lower if the plants would have been fully established.

Metal concentrations in the belowground biomass of all helophytes were generally higher than in the aboveground biomass, demonstrating the restriction of the translocation process. Metal concentrations in the belowground biomass decreased with increasing distance from the inlet of the HSSF CW microcosms, whereas concentrations in the aboveground biomass were less subjected to spatial variations. Overall, metal concentrations in the aboveground biomass did not show excessive values.

As helophytes were observed to play an insignificant role in the performance of the microcosms and differences in uptake and translocation patterns between species were generally small, other factors determine the selection of a suitable plant species. These factors include management strategies, end-use of the biomass and aesthetic value of the plants. *Phragmites australis* was the most productive of the 4 helophyte species studied, making it the preferable species when regular harvesting and reuse of the biomass, e.g. as a bioenergy source, are feasible. Lower biomass producing species such as *C. acutiformis*, *I. pseudacorus* and *J. effusus* become attractive in situations where harvesting is not feasible, reuse of the harvested biomass is not possible or the aesthetic value of the CW is important.

CHAPTER 6

REMOVAL OF HEAVY METALS IN SUBSURFACE FLOW CONSTRUCTED WETLANDS FOR TERTIARY TREATMENT OF INDUSTRIAL WASTEWATER

1 Introduction

The release of heavy metals into the environment by industrial activities such as mining, land filling, pulp and paper production, electroplating, textile production and petroleum refinement, presents a serious environmental threat (Kadlec and Knight, 1996). Heavy metals can be removed from industrial wastewater by a range of physico-chemical remediation technologies such as precipitation, ion exchange, adsorption, electrochemical processes and membrane processes (Bailey et al., 1999; Kurniawan et al., 2006). Industries in Flanders mainly remove metals from wastewater by precipitation. However, regulatory standards are not always met and in many cases taxes are paid on the load of heavy metals (Diels et al., 2003). Large volumes of secondary treated industrial wastewater containing heavy metals and high levels of sulphates are discharged into the surface water in Flanders (VMM, 2006). Both ecologic and economic factors drive towards a search for additional polishing or tertiary treatment steps to remove the marginal concentrations of heavy metals that can not be removed by classical physico-chemical methods (Brown et al., 2000; Diels et al., 2003). In most developing countries, the lack of or the non stringent nature of environmental pollution control laws and law enforcement causes frequent discharge of municipal and industrial wastewaters into aquatic environments without any level of treatment. Deterioration and scarcity of freshwater reserves are major incentives to search for conservation, remediation and reuse technologies in developing countries (Kivaisi, 2001).

Constructed wetlands (CWs) have been proposed to offer a low cost and low maintenance treatment alternative or tertiary treatment step for industrial effluents (Dunbabin and Bowmer, 1992). Qian et al. (1999) and LeDuc and Terry (2005) stated that CWs are highly efficient in removing low levels of metals from large volumes of wastewater. Constructed wetlands can use natural biogeochemical processes to remove heavy metals from the wastewater. The way in which a CW was constructed largely determines how wastewater treatment occurs and which mechanisms are responsible. Whereas free-water-surface (FWS) CWs are more aerobic, subsurface flow (SSF) CWs are more anaerobic (Kosolapov et al., 2006). The appropriate selection of the hydroperiod, substrate and vegetation, with the specific aim of inducing certain transfer and transformation processes, has been described as ‘integrative design’ by Hawkins et al. (1997) and Gillespie et al. (1999, 2000).

Many authors have described the importance of bacterial sulphate reduction and subsequent precipitation of metal sulphides as long-term removal processes for metals in mine drainage and industrial effluents (Machemer and Wildeman, 1992; Sobolewski, 1996; Chang et al., 2000; O'Sullivan et al., 2004a, b). Under strongly reducing conditions, sulphate reducing bacteria (SRB) convert SO_4^{2-} to S^{2-} by the oxidation of organic carbon (OC). The sulphides can then from stable precipitates with metals and provide a mechanism for long-term immobilisation provided that the substrates remain reduced (Gambrell, 1994). Organic carbon is necessary to stimulate growth of SRB. However, many industrial wastewaters lack OC, and therefore the efficiency of the process largely depends on adding a suitable carbon source. Cheap, widely available and effective organic substrates are desirable (Gibert et al., 2005). Constructed wetlands can incorporate a solid organic carbon source in the substrate, such as hay, straw, peat, compost, etc. (Machemer and Wildeman, 1992; Sobolewski, 1996; Kosolapov et al., 2006). However, substrates with a solid OC source often suffer from compaction and low hydraulic conductivity, which reduces treatment performance and leads to system failure. Using a substrate with good hydraulic properties, such as gravel, and amending the wastewater with a soluble carbon source has therefore been suggested as an alternative (Borden et al., 2001).

This study assessed the tertiary treatment of industrial wastewater in microcosms filled with gravel or a gravel/straw mixture. Straw, a low-value agricultural by-product, was added as a solid OC source. During the first incubation batches, all microcosms received low-strength synthetic wastewater, representative of a secondary treated effluent from a metal-processing industry in Flanders. Although complying with the sectoral discharge standards for wastewater from non-ferro metal industries demanded by VLAREM II (1995), elevated levels of Co, Ni, Cu, Zn and SO_4^{2-} were encountered in the wastewater. The wastewater was alkaline and low in nutrients and OC. High-strength wastewater with 10 to 20-fold higher metal levels, was applied during a second series of incubation batches in order to saturate the substrates. Glucose was fed to the gravel microcosms together with the high-strength wastewater as a soluble OC source. Goals of the experiment were to (1) study the removal kinetics of metals, (2) assess the effects of sorption, sulphate reduction and *Phragmites australis* on metal removal and (3) compare a solid (straw) and soluble (glucose) OC source. *Phragmites australis* (Cav.) Trin. ex Steud., the rooted emergent aquatic macrophyte most commonly found in Flemish treatment wetlands (Rousseau et al., 2004), was selected because of its tolerance to salts, pH variation, metal contamination and reduced conditions (Massacci et al., 2001; Stottmeister et al., 2003).

2 Material and Methods

2.1 Construction of subsurface flow constructed wetland microcosms

Young vegetative propagules of *Phragmites australis* were collected from a local plant nursery and gently cleaned with tap water to remove sediments from the root system. Plants were grown in a 25 % Hoagland nutrient solution (Qian et al., 1999) for a period of 1 month. A schematic presentation of the experimental set-up is presented in Fig 6.1. The experimental set-up consisted of twelve microcosms (40 cm height, 10 cm diameter) which were filled up to 35 cm, six with rinsed gravel (3 – 8 mm diameter) and six with a mixture of gravel and straw (15 % volumetric straw content). Within each substrate type, three columns were left unplanted and three were planted with *Phragmites australis* at a density of 2 plants per column. All microcosms had two tubes (0.5 mm diameter) with pore water sampling ports at a depth of 10 and 30 cm from the surface. Pore water sampling was performed after connecting syringes at the end of the tubes. The dead volume of the tubes accounted for 0.03 or 0.07 ml, depending on the sampling depth. Two redox electrodes (combined platina and gel reference electrode, HI 3090 B/5, Temse, Belgium) were fixed at the same depths in two of the three replicates of each treatment. A flexible tube (5 mm diameter) with its opening fixed at the bottom of each column, served as a siphon to draw out the wastewater at the end of each batch.

2.2 Kinetics of metal removal and dynamics of SO_4^{2-}, OC, Eh, Fe and Mn

A new batch of synthetic wastewater was added every two weeks, from November 2005 until June 2006. Following base composition of the wastewater was used, in mg l^{-1}: 0.01 Fe, 0.01 Mn, 70 Ca, 15 K, 20 Mg, 1500 Na, 2000 SO_4^{2-}, 1000 Cl^-, 100 HCO_3^-, 1 PO_4^{3-}, 7.7 N_{tot} (10 NO_3^- and 7 NH_4^+). Low-strength wastewater, prepared by spiking the base wastewater with Co, Ni and Zn levels of 1 mg l^{-1} and Cu levels of 0.5 mg l^{-1}, was applied during the first series of six batches. During the 2nd and 3rd batch, domestic wastewater of a nearby wastewater treatment plant was mixed with the synthetic wastewater (at a volumetric ratio of 10 %) to stimulate growth of the SRB. High-strength wastewater with metal concentrations of 10 mg l^{-1} was applied during the second series of eight batches. Growth of SRB was further stimulated by adding 50 ml of granular anaerobic sludge, obtained from a potato processing factory (Primeur, Waregem, Belgium) to all microcosms together with the first batch of high-strength wastewater. Glucose was added (500 mg l^{-1} OC) to the gravel microcosms with each batch of high-strength wastewater. The kinetics of Co, Ni, Cu, Zn, Fe, Mn, SO_4^{2-} and OC concentrations in the pore water were studied during the 1st and 6th batch of low-strength wastewater and during the last batch of high-strength wastewater, the 14th batch since the start of the experiment. Plants were exposed to a 14 h light/10 h dark regime. During the 1st and 6th batch, the ambient air temperature varied between 9 and 23 °C. Higher temperatures varying between 15 and 46 °C were observed during the 14th batch. The water level was maintained just below the gravel surface throughout the experiment. Evapotranspiration was corrected by daily addition of deionised water, based on observations of the water level.

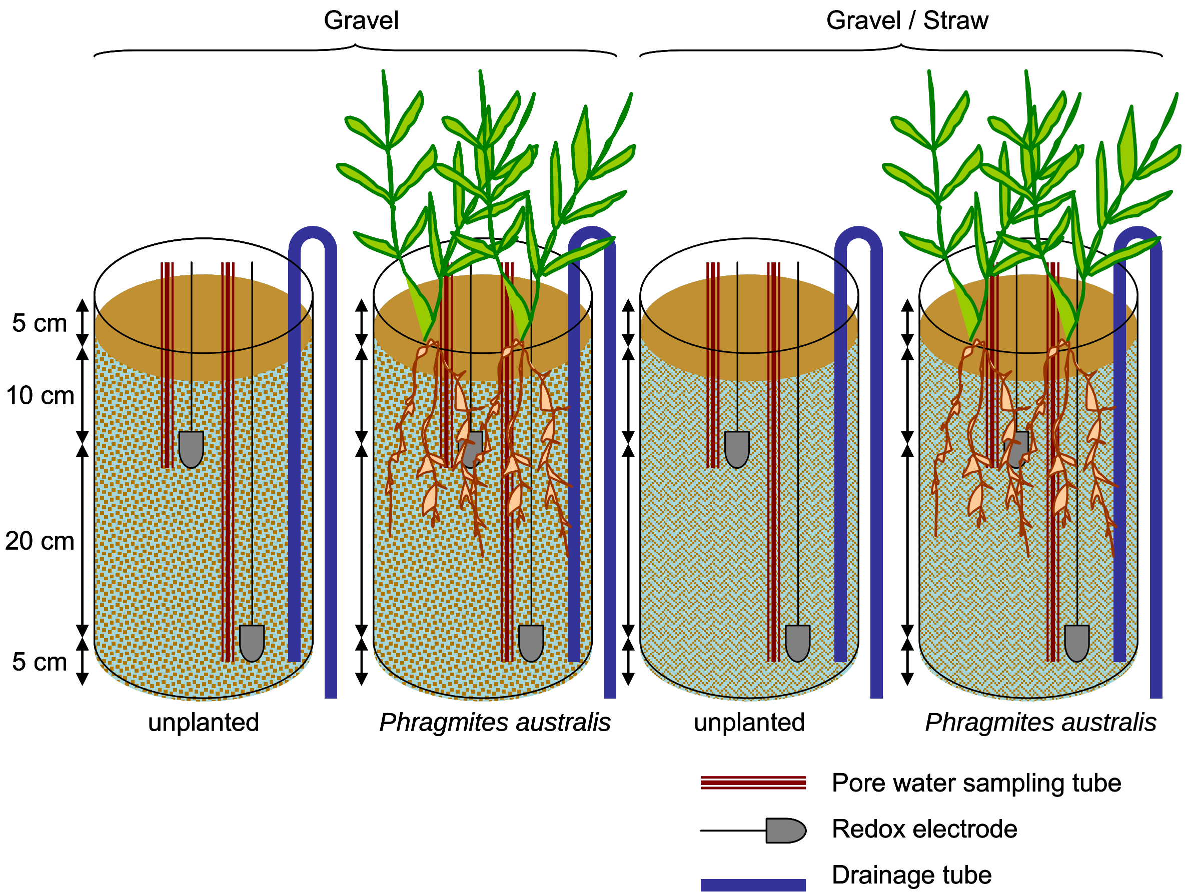

Fig 6.1 Schematic presentation of the experimental set-up.

Samples of 10 ml pore water were collected after 6, 24, 48, 72, 96, 144, 240 and 336 h of addition of a new batch. The wastewater and pore water samples were analysed for SO_4^{2-} with Ion Chromatography IC (Metrohm 764 Compact IC, Herisau, Switzerland) and for OC with a TOC Analyser (TOC-5000, Shimadzu corporation, Tokyo, Japan). Cobalt, Ni, Cu, Zn, Fe and Mn were analysed by means of ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) after acidifying the samples with a drop of 65 % HNO_3. The redox potential (Eh) was monitored at abovementioned sampling times. The wastewater was analysed for pH (electrode, HI 1230B, Temse, Belgium) and EC (electrode, WTW LF 537, Weilheim, Germany) at the start and at the end of each batch.

2.3 Sorption capacity of gravel and straw

Sorption tests were performed with 50 g gravel or 0.5 g straw in 100 ml of mixed metal solution. The base composition of the metal solutions was the same as for the microcosm experiment and was then spiked with increasing levels of Co, Ni, Cu and Zn: 1, 5, 10, 50 and 100 mg l^{-1}. Metals were spiked from a stock solution that contained 1000 mg l^{-1} of each metal, added as sulphate salts. Sorption tests were performed at room temperature with no pH adjustment. Flasks were placed on a shaker for 2 h after which the solution was separated from the gravel or straw by filtration (MN 640 m filter paper, Machery-Nagel, Düren, Germany) and analysed for Co, Ni, Cu and Zn after acidification with a drop of 65 % HNO_3. The concentration sorbed onto the surface of the gravel or straw (q_s, mg kg^{-1} DM) was calculated based on the difference between initial and equilibrium metal concentrations in the solution. The equilibrium concentration in the solution (C_e, mg l^{-1}) and the sorbed concentration q_s were fitted to the Langmuir and Freundlich sorption models:

Langmuir: $$\frac{1}{q_s} = \frac{1}{X_m \times b \times C_e} + \frac{1}{X_m}$$

With X_m (mg kg^{-1} DM) the maximum sorption capacity and b (l mg^{-1}) a measure of sorption energy.

Freundlich: $$q_s = k \times C_e^{1/n}$$

With the empirical constants k ($mg^{1-1/n}$ $l^{1/n}$ kg^{-1} DM) and n (dimensionless).

Control experiments were performed to measure sorption onto the glassware and filter paper. For Co, Ni and Zn sorption was negligible and accounted for only 1.5, 1.6 and 3.3 % of the initial metal concentration. However, the initial Cu concentration was reduced by 9 % by a combination of sorption on the glassware and filter paper. Data were corrected for these sorption effects. Heavy metals were analysed by means of ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA).

2.4 Statistical analysis

Statistical analysis was performed using the S-plus 6.1 software package (Insightful Corp., Seattle, USA). The significance of differences between groups was assessed by means of a Student's t-test for comparison of 2 groups and a one-way ANOVA analysis of variance for comparison of more than 2 groups ($\alpha = 0.05$). Homogenous subsets were determined with a Tukey post-hoc test ($\alpha = 0.05$). Pearson correlation coefficients were determined between Co, Cu, Ni, Zn, Fe, Mn and SO_4^{2-} concentrations in the pore water ($\alpha = 0.05$).

3 Results

3.1 Removal of Co, Ni, Cu and Zn in the subsurface flow constructed wetland microcosms

The metal concentrations in the pore water of all microcosms decreased significantly with time with the largest decrease occurring within the first day after addition of a new batch. The sampling depth and the presence or absence of *Phragmites australis* did generally not affect the metal concentrations in the pore water during the incubation batches with low-strength wastewater (Table 6.1). The major factor affecting metal removal was the substrate type. Fig 6.2 visualises the removal kinetics of Co, Ni, Cu and Zn in the pore water sampled at 10 cm depth of the microcosms. Metal concentrations in the pore water were significantly positively correlated, demonstrating a similar removal pattern ($R \geq 0.93$, $p < 0.001$).

3.1.1 Removal of metals during exposure to low-strength wastewater

The metal concentrations were higher in the pore water of microcosms filled with the gravel/straw mixture than in those filled with gravel during the 1st incubation batch. Whereas Co, Ni, Cu and Zn levels in the pore water of the gravel microcosms decreased to 0.005 – 0.03, 0.01 – 0.05, 0.002 – 0.003 and 0.04 – 0.07 mg l^{-1} after 2 weeks, respectively, their levels in the gravel/straw microcosms were generally one order of magnitude higher: respectively 0.01 – 0.14, 0.09 – 0.22, 0.01 – 0.02 and 0.15 – 0.26 mg l^{-1}. During the 6th batch of low-strength wastewater, lower metal concentrations were observed in the gravel/straw microcosms than during the 1st batch. On the contrary, higher Co and Ni levels were observed in the gravel microcosms during the 6th batch than during the 1st batch, whereas Cu and Zn removal was similar.

3.1.2 Removal of metals during exposure to high-strength wastewater

The increase to metal concentrations of 10 mg l^{-1} in the high-strength wastewater did not negatively affect the removal efficiencies (Table 6.1). A residence time of 1 day was sufficient to reduce Co, Ni and Cu levels below 0.05 mg l^{-1} and Zn levels below 0.1 mg l^{-1} in the unplanted microcosms, corresponding with a removal efficiency of more than 99 %. However, contrary to the incubation batches with low-strength wastewater, an effect of depth was observed in the planted microcosms with generally higher metal levels at 10 cm depth than at 30 cm depth (Table 6.1). At 10 cm from the surface, metal concentrations were generally the highest in the planted gravel microcosms (Fig 6.2). The Co, Ni and Zn concentrations in the planted gravel/straw microcosms initially decreased with time but then increased again towards levels of respectively 0.12, 0.15 and 0.28 mg l^{-1} after 2 weeks, being one order of magnitude higher than in the unplanted microcosms.

Table 6.1

Concentrations of Co, Ni, Cu and Zn in the pore water sampled at 30 cm (a) and 10 cm (b) depth, of the different microcosms after 1 and 14 days, during batch 1, 6 and 14 (µg l^{-1}), together with the associated removal efficiency (%) (n = 3). UG, PG, UGS and PGS denote respectively Unplanted Gravel, Planted Gravel, Unplanted Gravel/Straw and Planted Gravel/Straw

Metal	**Microcosm**	**Batch 1**						**Batch 6**						**Batch 14**					
		1 d			**14 d**			**1 d**			**14 d**			**1 d**			**14 d**		
		a		**b**	**a**		**b**	**a**		**b**	**a**		**b**	**a**		**b**	**a**		**b**
Co	**UG**	40	-	35	6	-	5	101	-	107	40	-	39	6	-	13	3	-	3
	%	*96*	-	*96*	*99*	-	*99*	*90*	-	*89*	*96*	-	*96*	*100*	-	*100*	*100*	-	*100*
	PG	51	-	50	5	-	26	79	-	107	36	-	29	13	-	994	2	-	194
	%	*95*	-	*95*	*99*	-	*97*	*92*	-	*89*	*96*	-	*97*	*100*	-	*89*	*100*	-	*98*
	UGS	125	-	139	139	-	121	19	-	25	5	-	5	21	-	27	4	-	4
	%	*87*	-	*86*	*86*	-	*87*	*98*	-	*97*	*99*	-	*99*	*100*	-	*100*	*100*	-	*100*
	PGS	137	-	160	87	-	11	16	-	21	4	-	4	16	-	27	5	-	118
	%	*86*	-	*83*	*91*	-	*99*	*98*	-	*98*	*100*	-	*100*	*100*	-	*100*	*100*	-	*99*
Ni	**UG**	83	-	65	16	-	10	136	-	130	55	-	52	13	-	20	3	-	7
	%	*91*	-	*93*	*98*	-	*99*	*85*	-	*86*	*94*	-	*94*	*100*	-	*100*	*100*	-	*100*
	PG	113	-	117	20	-	48	153	-	178	57	-	67	25	-	856	4	-	278
	%	*87*	-	*87*	*98*	-	*95*	*83*	-	*81*	*94*	-	*93*	*100*	-	*90*	*100*	-	*97*
	UGS	178	-	196	192	-	183	66	-	109	44	-	44	27	-	41	7	-	8
	%	*80*	-	*78*	*78*	-	*79*	*93*	-	*88*	*95*	-	*95*	*100*	-	*100*	*100*	-	*100*
	PGS	177	-	190	172	-	91	67	-	119	34	-	36	28	-	53	10	-	152
	%	*80*	-	*79*	*81*	-	*90*	*93*	-	*87*	*96*	-	*96*	*100*	-	*99*	*100*	-	*98*
Cu	**UG**	2	-	2	2	-	2	2	-	13	9	-	8	18	-	35	2	-	3
	%	*99*	-	*99*	*99*	-	*99*	*100*	-	*97*	*98*	-	*98*	*100*	-	*100*	*100*	-	*100*
	PG	2	-	4	2	-	3	3	-	3	2	-	2	64	-	284	12	-	137
	%	*99*	-	*99*	*99*	-	*99*	*99*	-	*99*	*100*	-	*100*	*99*	-	*97*	*100*	-	*98*
	UGS	76	-	111	19	-	22	2	-	8	2	-	2	43	-	48	2	-	2
	%	*79*	-	*69*	*95*	-	*94*	*100*	-	*98*	*100*	-	*100*	*100*	-	*99*	*100*	-	*100*
	PGS	71	-	80	16	-	11	4	-	7	2	-	2	33	-	43	2	-	9
	%	*80*	-	*77*	*95*	-	*97*	*99*	-	*98*	*100*	-	*100*	*100*	-	*100*	*100*	-	*100*
Zn	**UG**	40	-	35	45	-	39	38	-	60	12	-	31	59	-	96	6	-	13
	%	*97*	-	*97*	*96*	-	*97*	*97*	-	*95*	*99*	-	*97*	*99*	-	*99*	*100*	-	*100*
	PG	91	-	125	72	-	41	8	-	49	4	-	26	69	-	998	8	-	412
	%	*92*	-	*89*	*94*	-	*97*	*99*	-	*96*	*100*	-	*98*	*99*	-	*90*	*100*	-	*96*
	UGS	175	-	230	154	-	197	297	-	277	51	-	37	75	-	95	15	-	21
	%	*85*	-	*81*	*87*	-	*83*	*74*	-	*76*	*96*	-	*97*	*99*	-	*99*	*100*	-	*100*
	PGS	183	-	244	260	-	166	219	-	231	67	-	62	73	-	91	64	-	277
	%	*85*	-	*79*	*78*	-	*86*	*81*	-	*80*	*94*	-	*95*	*99*	-	*99*	*99*	-	*97*

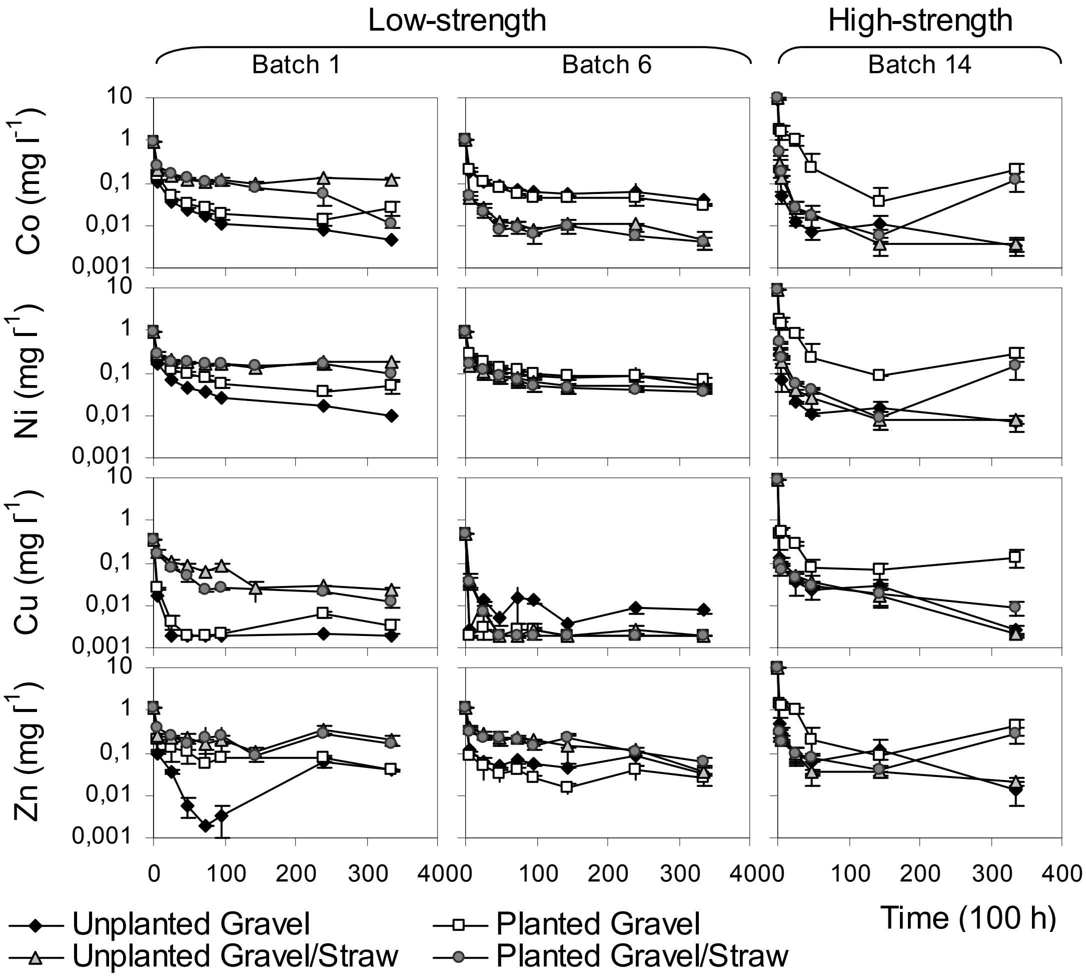

Fig 6.2 Cobalt, Ni, Cu and Zn concentrations in the pore water of 10 cm depth in the microcosms as a function of time after addition of batch 1, 6 and 14 (mg l^{-1}) (n = 3).

3.2 Changes in Eh, SO_4^{2-}, OC, Fe, Mn and pH in the subsurface flow constructed wetland microcosms

The pH was measured at the start and end of each batch. During the 1st batch with low-strength wastewater, the pH decreased significantly in all microcosms, with the highest decrease observed at 10 cm depth (Table 6.2). A minimum pH of 4.8 was observed at 10 cm depth in the unplanted gravel microcosms. Contrary to the first batch with low-strength wastewater, changes in pH were small during the last batches with low- or high-strength wastewater, with a tendency towards neutral values.

Table 6.2

Initial pH and pH after 336 h of the pore water at 10 and 30 cm depth of the different microcosms, during the batches with low-strength wastewater (1 and 6), and the last batch with high-strength wastewater (14) (n = 3). *: difference between initial pH and pH after 336 h is significant at $\alpha = 0.05$; **: significant at $\alpha = 0.001$

Batch	**Initial pH**	**Depth**	**pH after 336 h**			
			Gravel		**Gravel / Straw**	
			Unplanted	**Planted**	**Unplanted**	**Planted**
1	8.0 ± 0.0	10 cm	4.8 ± 0.1 **	6.0 ± 0.2 **	6.2 ± 0.4 **	6.2 ± 0.4 **
		30 cm	6.5 ± 0.0 **	6.8 ± 0.1 **	7.1 ± 0.2 *	7.2 ± 0.1 *
6	8.0 ± 0.1	10 cm	7.9 ± 0.1	7.6 ± 0.2 *	7.5 ± 0.2 *	7.5 ± 0.1 *
		30 cm	7.9 ± 0.1	7.4 ± 0.1 *	7.6 ± 0.2 *	7.5 ± 0.1 *
14	7.4 ± 0.0	10 cm	7.5 ± 0.2	7.2 ± 0.1 *	7.4 ± 0.0	7.1 ± 0.0 **
		30 cm	7.5 ± 0.1	7.2 ± 0.0 *	7.5 ± 0.0	7.3 ± 0.2

Temporal patterns of Eh and concentrations of SO_4^{2-}, OC, Fe and Mn in the pore water sampled at 10 cm depth are presented in Fig 6.3. As for metals, sampling depth did generally not affect the abovementioned parameters during the batches with low-strength wastewater. Dynamics of SO_4^{2-}, Eh, OC, Fe and Mn will be discussed separately.

3.2.1 Dynamics of sulphate

The sulphate concentrations decreased from about 2200 to 1900 mg l^{-1} during the 1st batch, presenting a small but significant reduction of 14 % (Fig 6.3). During the 6th batch of low-strength wastewater, there was no sulphate reduction in the gravel microcosms. However, sulphate concentrations in the pore water sampled at both depths of the gravel/straw microcosms decreased exponentially with time, at a rate of 0.0014 – 0.0019 h^{-1} in respectively the planted and unplanted systems ($R^2 \geq 0.8$). After 2 weeks, sulphate concentrations of 990 and 1200 mg l^{-1} were observed in respectively the unplanted and planted gravel/straw microcosms, representing a decrease of 45 and 54 %.

During the last incubation batch with high-strength wastewater, sulphate concentrations in the pore water of the unplanted gravel/straw and unplanted gravel microcosms amended with glucose, decreased exponentially with time at a similar rate of 0.001 h^{-1}. In the pore water at 30 cm depth of all planted microcosms, a similar sulphate reduction was observed as in the unplanted ones. However, at 10 cm from the surface of the planted microcosms, a small reduction of only 12 % of the initial SO_4^{2-} concentration was observed (Fig 6.3).

3.2.2 Dynamics of the redox potential

During the 1st batch of low-strength wastewater, redox potentials in the gravel microcosms remained positive whereas values of – 200 mV were observed in the gravel/straw microcosms from 144 h onward. After 5 incubation batches with low-strength wastewater, the Eh in the pore water of the unplanted gravel/straw microcosms rapidly evolved to – 100 mV within 6 h and decreased to – 200 mV. A similar Eh was observed in the planted gravel/straw microcosms at 30 cm depth, whereas the Eh varied around 0 mV at 10 cm depth. The Eh measured at 10 cm from the surface was subject to high variability and therefore standard deviations were not presented in Fig 6.3 for reasons of clarity.

During the last batch with high-strength wastewater, the Eh in the unplanted gravel and gravel/straw microcosms decreased to respectively – 200 and – 100 mV at both depths. In all planted microcosms, similar Eh conditions were observed at 30 cm depth as in the unplanted microcosms. However, at 10 cm from the surface of the planted microcosms, the Eh increased towards 300 mV after 2 weeks (Fig 6.3).

3.2.3 Dynamics of organic carbon

A linear increase of OC, at a rate of 1.4 and 1.7 mg l^{-1} h^{-1} was observed in respectively the unplanted and planted gravel/straw microcosms ($R^2 \geq 0.90$) during the first batch of low-strength wastewater (Fig 6.3). After 2 weeks, the OC concentrations in the pore water of the gravel/straw microcosms varied between 500 and 600 mg l^{-1}. An increase of the OC concentration of 2.8 to 15 – 56 mg l^{-1} was also observed in the gravel microcosms during the 1st batch of low-strength wastewater, presumably attributed to dirt present in the gravel pores at start-up. During the 6th batch of low-strength wastewater, the OC concentration in the pore water of the gravel microcosms was negligible and attained a minimum value of 1 mg l^{-1}. Concentrations of OC in the gravel/straw microcosms initially increased but then levelled off between 10 and 20 mg l^{-1} during the 6th batch of low-strength wastewater.

During the last batch of high-strength wastewater, the gravel microcosms were amended with glucose. A rapid reduction of 434 to 50 – 77 mg l^{-1} OC was observed in respectively the unplanted and planted gravel microcosms. The gravel/straw microcosms showed similar OC dynamics as during the 6th batch with low-strength wastewater: an increase followed by an equilibrium OC level of 40 – 45 mg l^{-1}.

3.2.4 Dynamics of Fe and Mn

Iron and Mn concentrations in the pore water of the gravel microcosms remained lower than their detection limits of respectively 0.025 and 0.005 mg l^{-1} during the first 240 h after addition of the 1st batch. At the end of the 1st batch however, higher Mn and Fe concentrations of respectively 0.03 – 2.1 and 0.06 – 0.2 mg l^{-1} were observed. Manganese concentrations in the pore water of the gravel/straw microcosms increased linearly with time ($R^2 \geq 0.97$) to 11 – 12 mg l^{-1} after 2 weeks. An increase of the Fe concentration in the pore water of the gravel/straw microcosms was only observed after 144 h.

During the 6th batch of low-strength wastewater, Mn concentrations in the pore water of all microcosms increased immediately after addition of the new batch and achieved equilibrium concentrations of 0.4 – 1.2 mg l^{-1} from 6 h onward. A similar trend was observed for Fe in the planted gravel microcosms as well, which showed a rapid evolution to equilibrium concentrations of 0.3 mg l^{-1}. On the contrary, negligible Fe concentrations were observed in the unplanted gravel microcosms throughout the 6th batch. Concentrations of Fe in the gravel/straw microcosms achieved a maximum value of 1.2 – 1.7 mg l^{-1} within the first 6 h after which they decreased again.

During the last batch of high-strength wastewater, dynamics of Mn and Fe showed similar patterns. Iron and Mn concentrations generally achieved a maximum value within the first day after addition of the new batch of wastewater, after which concentrations decreased again or levelled of (Fig 6.3).

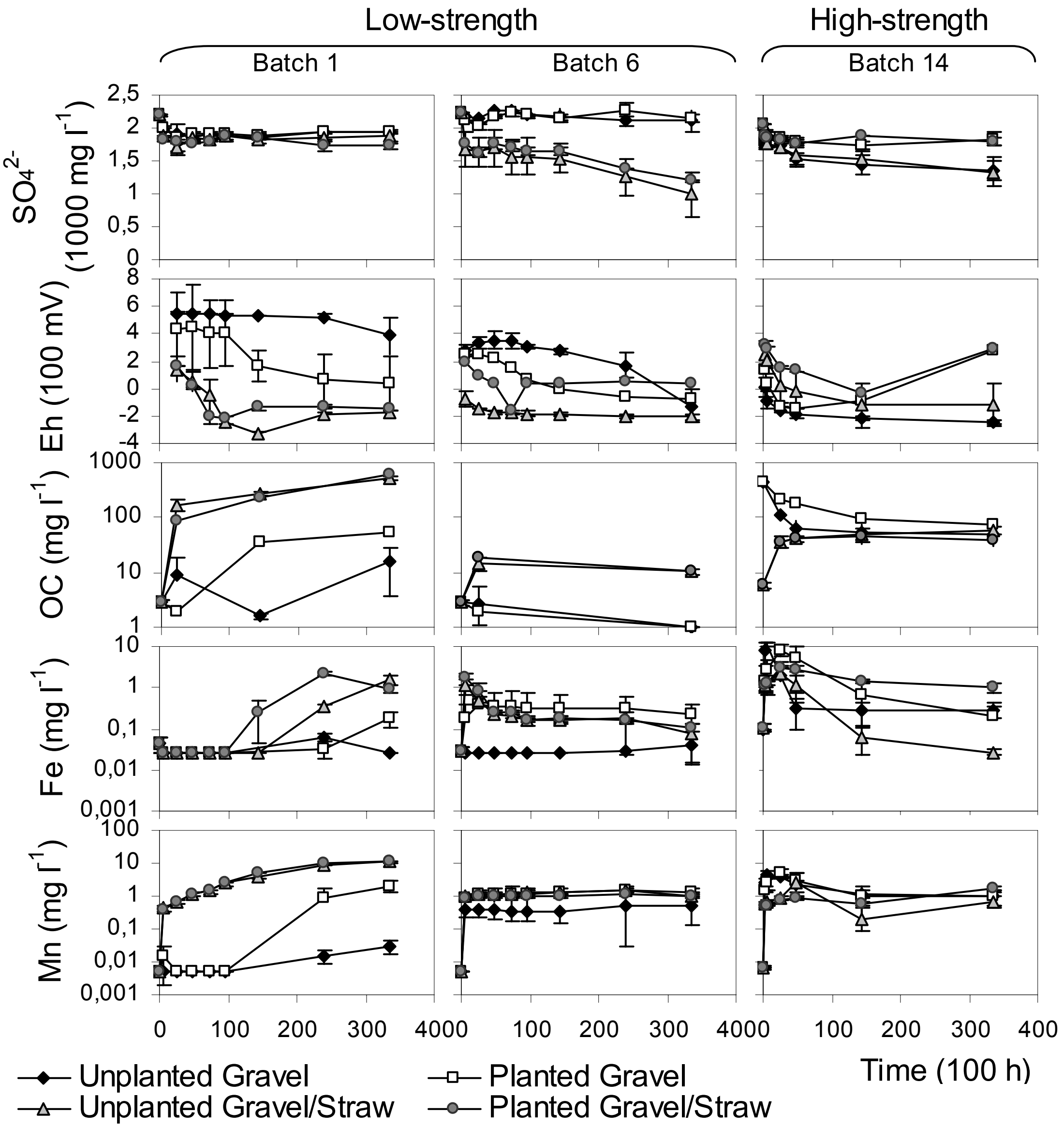

Fig 6.3 Sulphate, OC, Fe and Mn concentrations (mg l^{-1}) (n = 3) and Eh (mV) (n = 2) in the pore water of 10 cm depth in the microcosms as a function of time after addition of batch 1, 6 and 14.

3.3 Sorption capacity of gravel and straw

The sorption properties of gravel and straw were assessed in a separate experiment and results are presented in Fig 6.4. The concentrations of Co, Ni and Zn sorbed onto the surface of gravel and straw (q_s, mg kg^{-1} DM) initially increased with the equilibrium concentration in the solution (C_e, mg l^{-1}) and levelled off in the higher concentration range indicating saturation of the gravel or straw surface. A different trend was seen for Cu of which the sorbed concentration continued to increase with the concentration in the solution (Fig 6.4). For Co, Ni and Zn, the sorption process was well described by the Langmuir model ($R^2 \geq 0.97$ for gravel, R² ≥ 0.93 for straw), whereas the Freundlich model was a better predictor of Cu sorption (R² = 0.93 and 0.99 for respectively straw and gravel). Table 6.3 presents the Langmuir and Freundlich sorption parameters of Co, Ni, Cu and Zn for gravel and straw.

Figure 6.4 also presents the ratio of the Co, Ni, Cu and Zn concentrations in the solution at equilibrium to those in the initial solution versus the sum of the concentrations of the four metals in the initial solution (in mmol l^{-1}). There was competition for sorption sites, with both gravel and straw showing the highest affinity for Cu. In the range of lower total metal concentrations in the solution, following order of decreased affinity was observed for gravel: Cu > Zn > Co ~ Ni. At higher total metal concentrations, the percentage of Co, Ni and Zn that was sorbed by the gravel decreased, whereas Cu sorption remained similar. Contrary to gravel, straw showed a similar affinity for Cu in the lower concentration range as for the other metals. However, upon an increase of the total metal concentration in the solution, straw showed a higher affinity for Cu than for the other metals.

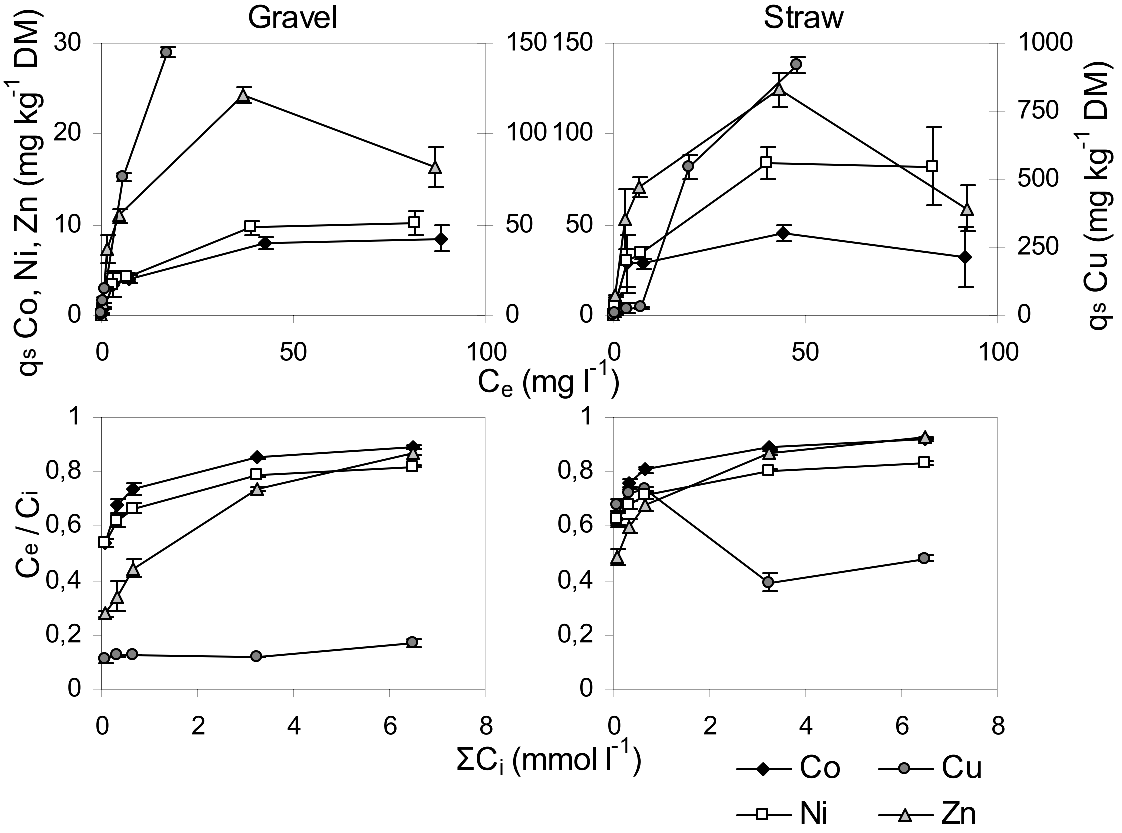

Fig 6.4 The sorbed metal concentrations q_s (mg kg^{-1} DM) on the gravel and straw as a function of the equilibrium metal concentration in the solution C_e (mg l^{-1}); together with the ratio of the concentrations at equilibrium and start C_e/C_i (-) versus the sum of the initial metal concentrations $\sum C_i$ (mmol l^{-1}) (n = 3).

Table 6.3

Langmuir and Freundlich sorption parameters of the gravel and straw

	Gravel				Straw			
	Co	**Cu**	**Ni**	**Zn**	**Co**	**Cu**	**Ni**	**Zn**
Langmuir								
X_m (mg kg^{-1} DM)	9.1	424	11	17	34	-309	93	63
b (l mg^{-1})	0.14	0.032	0.11	2.0	-3.5	-0.017	0.11	-0.37
R^2	1	0.83	1	0.97	0.97	0.47	0.99	0.93
Freundlich								
n	2.2	1.1	1.9	2.3	3.1	0.73	1.8	2.9
k (mg$^{1-1/n}$ l$^{1/n}$ kg^{-1} DM)	1.4	12	1.2	4.0	12	4.7	9.8	24
R^2	0.93	0.99	0.93	0.83	0.71	0.93	0.91	0.64

4 Discussion

4.1 Processes of metal removal in the microcosms

All SSF CW microcosms were very efficient in the reduction of Co, Ni, Cu and Zn from the low- and high-strength wastewater. However, small differences were observed between substrate types during start-up, with the gravel/straw microcosms showing slightly higher metal concentrations in the pore water than the ones filled with gravel. A negative effect of *Phragmites australis* on the removal of Co, Ni, Cu and Zn in the upper layer of all microcosms, was observed during the last batch with high-strength wastewater. In an attempt to elucidate which processes and influences of substrates and plants were responsible for metal removal, the removal patterns of Co, Ni, Cu and Zn were discussed with reference to the dynamics of SO_4^{2-}, OC, Fe, Mn and Eh.

4.1.1 Removal of heavy metals during start-up

During start-up, the substrate type was the major factor affecting metal removal. Straw was added in an attempt to provide an organic carbon source for SRB and ensure long-term removal of heavy metals by precipitation with sulphides. Moreover, straw provided additional sorption sites which was expected to enhance metal removal during start-up. The observation of higher metal concentrations in the pore water of the gravel/straw than the gravel microcosms was therefore unexpected. However, the positive Eh observed during the first half of the experiment and the small reduction of the sulphate levels did not support the presence of bacterial sulphate reduction in the gravel/straw microcosms. Presumably, the decomposition of straw was responsible for higher metal levels in the pore water by complexation with dissolved OC.

The redox potential in the gravel/straw microcosms decreased to – 200 mV within the first 144 h after addition of the first batch of low-strength wastewater. This relatively rapid creation of reducing conditions was attributed to the decomposition of straw. The OC and Mn concentrations in the pore water of the gravel/straw microcosms increased linearly with time, suggesting the release of Mn from the straw. Moreover, the reduction of Mn- (oxy-) hydroxides present on the gravel surface could have contributed to the increase of the Mn concentration in the pore water. An increase of the Fe concentration was also observed after 144 h, corresponding with the creation of reducing conditions. Dynamics of Fe and Mn in the pore water of the gravel microcosms also demonstrated the presence of Fe- and Mn- (oxy-) hydroxides. The small reduction of about 14 % of sulphates within the first day after addition of the wastewater was therefore likely caused by the sorption onto these Fe- (oxy-) hydroxides, a removal pathway for sulphates in oxidised conditions described by Gibert et al. (2004).

The remarkable release of Fe and Mn from gravel upon the creation of reducing conditions has also been observed by Buddhawong et al. (2005). They studied the removal of As and Zn in laboratory-scale FWS and SSF CWs and a pond planted with *Juncus effusus*. Systems were operated discontinuously and fed with synthetic wastewater with 5 mg l^{-1} Zn and 0.5 mg l^{-1} As (pH 4). Removal of As and Zn was highest in the gravel-based SSF CW. As the gravel had a low sorption capacity for As and the plants in the pond did not absorb considerable amounts of As, another removal mechanism must have been present in the planted gravel beds. The authors suggested that organic compounds released by the plant roots favoured a decrease of the Eh, leading to a dissolution of crystalline Fe. In oxic zones, such as encountered in the rhizosphere, this dissolved Fe precipitated again and could co-precipitate other metals. The presence of this removal pathway was supported by dynamics of the Eh and dissolved Fe concentration in the pore water. The Eh remained above 100 mV and there was no evidence of sulphate reduction during the 90 days of their experiment.

Overall, it was concluded that following metal removal mechanisms were present during start-up conditions: (1) sorption onto Fe- and Mn- (oxy-) hydroxides covering the gravel surface in all microcosms and (2) sorption onto the straw in the gravel/straw microcosms. Both mechanisms provide short-term removal of metals as they are subject to changing redox conditions and saturation (Machemer and Wildeman, 1992; Sobolewski, 1996).

4.1.2 Bacterial sulphate reduction as a long-term removal process

After 5 incubation batches with low-strength wastewater, sulphate reduction was observed in the microcosms filled with the gravel/straw mixture. An exponential decrease of the sulphate concentrations in the pore water was observed with time, at a rate of 0.0014 – 0.0019 h^{-1} in the planted and unplanted gravel/straw microcosms, respectively. This small difference in sulphate dynamics was also reflected in the Eh. The Eh in the pore water of the unplanted gravel/straw microcosms rapidly evolved to – 200 mV. A similar Eh was observed in the planted ones at 30 cm depth, whereas it varied around 0 mV at 10 cm depth. The concentrations of OC in the gravel/straw microcosms initially increased but then levelled off at 10 – 20 mg l^{-1}, suggesting a state of equilibrium between input of OC by decomposition of straw and output by sulphate reduction. The dynamics of Fe and Mn in the pore water support the presence of reducing conditions in the gravel/straw microcosms. Manganese and Fe concentrations in the pore water rapidly increased within the first hours after addition of the new batch. Whereas Mn concentrations achieved equilibrium, Fe concentrations in the pore water decreased again, suggesting the precipitation of FeS.

Precipitation with sulphides provided an additional removal pathway for metals in the gravel/straw microcosms, demonstrated by higher removal efficiencies than during start-up. Sulphate reduction was not observed in the gravel microcosms although the decrease of the Eh and the dynamics of Fe and Mn, also suggested the reduction of Fe- and Mn- (oxy-) hydroxides. Removal of Co and Ni was less efficient than during start-up, which could be caused by a combination of saturation of the gravel surface and the reduction of Fe- and Mn- (oxy-) hydroxides and thus a decrease of the number of sorption sites. Removal of Cu and Zn in the gravel microcosms during the 6th batch was similar as during the 1st batch, demonstrating a higher affinity of gravel for these elements than for Co and Ni.

Song et al. (2001) reported similar mechanisms as in this study for the removal of Pb and Zn in laboratory-scale CWs fed with synthetic mine water (34 mg l^{-1} SO_4^{2-}, 0.24 mg l^{-1} Pb and 0.18 mg l^{-1} Zn): (1) adsorption or exchange onto the substrates, (2) sulphide precipitation and (3) co-precipitation with or adsorption onto Fe- and Mn- (oxy-) hydroxides. However, a distinction between removal pathways was not possible based on the influent and effluent data. The laboratory-scale CWs of Song et al. (2001) were filled with different mixtures of aged manure, hay, bark, gravel, peat moss, sand, limestone and aged sludge. The sludge and manure were added to stimulate growth of the bacterial population. Hay, bark and peat moss were added as a carbon source, and gravel, sand and limestone were used to improve hydraulic conductivity. Lead and Zn removal was highly variable in all CWs, but the average percent removal did not differ among different substrate types. Average removal percentages of 90 % for Pb and 72 % for Zn were reported. Sulphates were reduced with 70 %, presumably by bacterial sulphide reduction as evidenced by sulphide detection in the effluent.

4.1.3 Bacterial sulphate reduction: solid versus soluble carbon sources

The increase to metal concentrations of 10 mg l^{-1} in the high-strength wastewater did not negatively affect the performance of all microcosms. Amending the gravel microcosms with glucose successfully enhanced bacterial sulphate reduction at an exponential rate of 0.001 h^{-1}, similar to that in the gravel/straw microcosms. The decrease of the OC concentrations in the gravel microcosms suggests the consumption of glucose by the SRB. As during the 6th batch with low-strength wastewater, OC concentrations in the gravel/straw microcosms increased and levelled off. Iron and Mn concentrations generally achieved a maximum value within the first day after addition of the wastewater, after which concentrations decreased again or levelled off, suggesting their precipitation with sulphides.

This study showed that straw was efficient at creating reducing conditions and stimulating sulphidogenesis. The performance of SSF CWs which rely on the activity of SRB is greatly dependent on the degradability of the substrate. A substrate which supports bacterial sulphate reduction for a prolonged period is preferred. Different organic wastes have been tested as substrates to promote bacterial activity. In their study of four organic substrate types (sheep and poultry manure, oak leaf and compost), Gibert et al. (2004) demonstrated that the lower the lignin content of the substrate, the higher the biodegradability and the capacity for developing bacterial activity. Sheep manure was the most successful organic substrate favouring sulphate reduction, whereas compost was too poor in carbon. Chang et al. (2000) tested five organic substrates (spent mushroom compost, oak chips, spent oak from shiitake farms, sludge from a wastepaper recycling plant and organic rich soil) in their suitability to promote and sustain active sulphidogenesis. The organic rich soil contained less readily degradable organic matter than the other substrates and was the least efficient. The oak chips were initially less efficient than biologically or chemically treated biomass, caused by a release of toxic compounds through the decomposition of raw biomass. However, all substrates showed equally good performances at later stages. The compounds inhibitory to sulphidogenesis released from raw biomass were degraded under anaerobic conditions.

Substrates with a solid organic carbon source have the disadvantage of suffering from compaction and low hydraulic conductivity. Moreover, depletion of the solid organic carbon source will eventually lead to system failure and need for replacement of the substrate. Using a substrate with good hydraulic properties, such as gravel, and amending the wastewater with a soluble carbon source has therefore been suggested as an alternative. Borden et al. (2001) studied the removal processes of Zn in laboratory-scale gravel-based SSF CWs that were either left unplanted or were planted with *Scirpus acutus* or *Typha latifolia*. Systems were batch-fed with synthetic mine-impacted water (24 mg l^{-1} Zn, 200 mg l^{-1} SO_4^{2-} and pH 6.6) and amended with two doses of sucrose (100 and 200 mg l^{-1} C). Borden et al. (2001) showed that an increase of the sucrose concentration successfully enhanced sulphate reduction. However, Zn removal was not affected and the authors suggested the presence of other removal mechanisms including sorption.

4.1.4 Effects of *Phragmites australis* on sulphate reduction and metal removal

In the planted microcosms, similar redox conditions and sulphate reduction were observed at 30 cm depth as in the unplanted ones. However, at 10 cm from the surface of the planted microcosms, the Eh increased towards 300 mV after 2 weeks, corresponding with insignificant sulphate reduction at this depth. Moreover, higher metal levels were observed in the pore water as well, demonstrating the relationship between sulphate reduction and metal removal. Radial oxygen loss of the roots of *Phragmites australis* was suggested to be responsible for the increase in Eh in the top-layer of the microcosms. Presumably, this effect was not observed during the previous batches as the roots of *Phragmites australis* were not fully developed yet.

Plant roots can oxidise the rhizosphere through the process of radial oxygen loss (Armstrong et al., 1994). The latter process is assumed to negatively affect the process of sulphate reduction in the root zone. Relatively few studies have assessed this effect of plants. Borden et al. (2001) showed that both unplanted and planted SSF microcosms were equally efficient in sulphate reduction and Zn removal at 24 °C. However, at a temperature of 4 °C, planted systems showed a greater decrease of sulphate and Zn removal than the unplanted ones.

Reduced performance in unplanted systems was attributed to a decreased activity of SRB during cold temperatures. In addition, increased redox levels further limited sulphate reduction. Reduced internal oxygen consumption due to cold temperatures and dormancy allows greater leakage from roots of some species during the cold season. Stein and Hook (2005) stated that a seasonal variation in oxygen transfer to the root zone can induce a large enough shift in microbial pathways. The efficiency of aerobic respiration over anaerobic respiration is so great that a modest shift toward aerobic conditions could obscure or reverse the effect of temperature on microbial activity.

O'Sullivan et al. (2004a, b) reported the efficient removal of Fe, Pb and Zn by means of bacterial sulphate reduction in 2 experimental FWS CWs planted with *Typha latifolia*, designed to treat effluents from an active Pb/Zn-mine. The FWS CWs had a residence time of 30 days and were filled with a mixture of spent mushroom substrate and inert grit. The processes of bacterial sulphate reduction and precipitation of metals with sulphides were evidenced by (1) efficient removal of SO_4^{2-}, (2) highly reduced conditions in the sediment, in the range of – 300 mV and (3) the detection of metal sulphides in the sediment by means of sequential extraction. O'Sullivan (2004b) reported less sulphate removal during the plant-growing season, and related this to the development of new plant biomass and the negative impact on redox conditions in the substrate.

García et al. (2003) observed higher redox potentials in the surface than in the deeper zones of full-scale gravel-based HSSF CWs for treatment of domestic wastewater that were either planted with *Phragmites australis* or left unplanted. The higher Eh in the top layer was related to the diffusion of oxygen from the atmosphere and radial oxygen loss by plants. However, in this experiment an effect of depth was not observed in the unplanted microcosms. The higher redox potentials observed in the planted microcosms were therefore solely attributed to the radial oxygen loss from the roots of *Phragmites australis*.

The effect of plants on the process of sulphate reduction and thus metal removal remains an interesting topic of debate. However, radial oxygen loss is not the sole process by which plants can affect sulphate reduction and metal removal. Plant roots can excrete a wide range of organic compounds that could serve as a carbon source for SRB. The magnitude of this input of organic carbon is unclear but assumed to vary in the range of 5 – 25 % of the photosynthetically fixed carbon (Brix, 1997). Stottmeister et al. (2003) described the entire process of carbon input as rhizodeposition, consisting of exudates (sugars, vitamins, organic acids, phenol, etc.), mucigels, dead cell material, etc. This quantity of organic carbon compounds released has been estimated at 10 – 40 % of the net photosynthetic production of agricultural crops. Rhizodeposition is only considered significant in CWs that receive wastewater with a low carbon load. However, there are no quantitative studies covering this topic (Stottmeister et al., 2003). At last it is mentioned that large amounts of decaying organic matter can be returned to the system at the end of the growing season (Gessner, 1996; Leuridan, 2004). This pool of organic matter could serve as a carbon source for SRB.

4.2 Saturation of gravel and straw

Maximum sorption capacities of Co, Ni and Zn were respectively 9, 11 and 17 mg kg^{-1} DM for gravel and 34, 93 and 63 mg kg^{-1} DM for straw. Taking into account the masses of gravel and straw, it was predicted that the gravel and gravel/straw microcosms were able to sorb 36 – 40 mg Co, 47 – 50 Ni and 68 – 76 mg Zn. Considering a pore water volume of about 1 l in the microcosms and the almost complete removal of Co, Ni and Zn during the loading with low-strength and high-strength wastewater, it was clear that saturation of the substrates with Co, Ni and Zn was achieved by the 10 – 13th batch of wastewater since the start of the experiment.

Contrary to the other metals, both gravel and straw showed a high sorption capacity for Cu and saturation of the substrates was not reached by the end of the experiment. Machemer and Wildeman (1992) also demonstrated the preferential sorption of Cu over Zn onto fresh mushroom compost in batch sorption studies. A Langmuir sorption maximum of 424 mg Cu kg^{-1} DM was derived for gravel, although the actual maximum sorption capacity could be higher or lower as the process of sorption was not as well described by the Langmuir model. Sorption of Cu on straw was not at all well modelled by the Langmuir isotherm and demonstrated Freundlich characteristics. At a maximum initial Cu concentration of 10 mg l^{-1}, 918 mg kg^{-1} DM was sorbed by straw. Copper removal by sorption processes was important throughout the experiment, corresponding with the fairly constant removal of Cu independent of changes in sulphate reduction. Based on the ‘maxima’ of 424 and 918 mg kg^{-1} DM for respectively gravel and straw observed in the sorption tests, it was predicted that saturation of the microcosms with Cu would be reached after a series of 163 – 186 batches of high-strength wastewater.

4.3 Suggestions for design

Experiments in controlled environments are necessary to understand metal removal processes and influencing factors and lead to the development of better design criteria (Song et al., 2001). This study showed that metals were initially removed by sorption onto Fe- and Mn-(oxy-) hydroxides on the gravel surface and/or sorption onto straw. With time, precipitation with sulphides becomes a dominant removal process.

The last batch with high-strength wastewater showed that a residence time of 24 h was sufficient to lower the metal concentrations below the basic environmental quality standards for surface water of 50 µg l^{-1} for Cu and Ni, and 200 µg l^{-1} for Zn (VLAREM II, 1995). The question arises whether the prolonged residence time of 14 days was necessary. However, the residence time can affect sulphate reduction and long-term performance, and is thus a very important factor in the design of CWs that rely on the activity of SRB.

The importance of the residence time was demonstrated by Gibert et al. (2004). In a column experiment, sheep manure mixed with creek sediment and limestone was packed in columns and fed with synthetic mine water at average residence times of 0.73 to 9 days. Sulphate reduction was not observed at a residence time of 0.73 days. Increasing the residence time and thus lowering the flow rate, resulted in an increase of the sulphate reduction. In another experiment, Gibert et al. (2005) observed negligible sulphate reduction in an upflow column packed with a mixture of municipal compost, calcite and river sediment treating simulated mine water at a short residence time of 16 h. This finding was attributed to the poor OC source to promote activity of SRB under high flow rates. Sobolewski (1996) showed that Cu removal in experimental peat-based CWs planted with *Carex rostrata* that treated wastewater from a former open pit Cu mine, was more efficient in the wetland with the longest residence time (23 versus 12 days), and also suggested the importance of providing a sufficiently long residence time to enhance bacterial sulphate reduction. Based on literature reports and experimental findings, a residence time of 14 days was suggested.

The reduction of 96 g l^{-1} SO_4^{2-} requires 60 g l^{-1} CH_2O. Under the assumption that a quarter of the biomass can be used as a carbon source for bacterial sulphate reduction (Chang et al., 2000), it is derived that 2.5 g l^{-1} straw is necessary for 1 g l^{-1} SO_4^{2-}. Based on the observed sulphate reduction of about 700 mg l^{-1} during 14 days, it was predicted that the straw in the gravel/straw microcosms would be depleted after a total of 26 batches or 1 year.

We extrapolated the experimental findings to field conditions to assess the potential of SSF CWs amended with a solid or soluble carbon source for long-term treatment of a similar type of wastewater. In a first scenario, a large wastewater flow rate of 5000 m³ d^{-1} was considered, representative of effluent flow rates of non-ferro metal industries in Flanders (VMM, 2006). Treatment of a wastewater volume of this magnitude would require a treatment area of 17.5 ha, if a residence time of 14 days would be applied and the system would have a depth of 1 m. The porosity of the gravel/straw mixture was assumed to be 0.4. Under the assumption that 15 vol % straw would be mixed in the substrate and that similar SO_4^{2-} reduction would be achieved as in the microcosm experiment, the straw would be depleted after 1 year. This treatment option is thus not as ‘long-term’ as required. Moreover, a huge surface area is needed. Scenario 1 demonstrates that using a gravel/straw-based SSF CW is not a viable treatment option for large wastewater volumes. Adopting a gravel/straw-based SSF CW would only offer potential for small wastewater flows < 30 m³ d^{-1}, as they require a moderate treatment area of 1050 m². In order to anticipate for removal of the substrate after 1 year, a series of 2 SSF CWs could be constructed. The second CW could then take over activity, when the first CW needs replacement of the filter material. Overall, the depletion of a solid carbon source is a major drawback of this scenario. This, together with possible compaction of the filter (Borden et al., 2001), leads to the suggestion of using a soluble carbon source.

A second scenario considers the viability of using a gravel-based SSF CW that treats wastewater by continuous addition of a soluble carbon source. Considering a large wastewater volume of 5000 m³ d^{-1}, a treatment area of 19.5 ha would be necessary if a residence time of 14 days would be applied and the system would have a depth of 1 m. A gravel porosity of 0.36 was assumed. About 880 kg d^{-1} of OC or 2200 kg d^{-1} of glucose would be needed. At a cost of 1 euro kg^{-1} glucose, a yearly cost of 800 000 euro is derived. This second scenario suggests that gravel-based SSF CWs amended with a soluble carbon source such as glucose, are not economically feasible for treatment of large wastewater volumes in the range of 5000 m³ d^{-1}. Even if a 10 times cheaper carbon source could be applied, such as methanol, operational costs would still be very high. However, mixing the industrial wastewater with domestic wastewater or other types of industrial wastewater that contain a high carbon load, such as wastewater from sugar-processing industries, could offer a cheaper solution. Yearly costs of treating a small wastewater flow rate in the range of 30 m^3 d^{-1} would amount up to 4800 euro if glucose would be used. Overall, this treatment option would be a viable technology for small-scale enterprises and costs would be significantly reduced by mixing with domestic wastewater or applying a cheaper carbon source.

Phragmites australis was observed to negatively affect metal removal by oxygen release in the root zone. However, it is suggested to apply plants as they provide aesthetic value and improve implementation in the landscape (Brix, 1997). An emergent wetland plant or a combination of wetland plants with superficial root systems and less oxygen release, could be selected in order to improve treatment performance and optimal use of the entire depth of the system. For example, *Typha latifolia* was observed to have less effects on the redox potential and Zn removal than *Scirpus acutus* (Borden et al., 2001). *Typha latifolia* was also reported to root at a lower depth than *Phragmites australis* (De Maeseneer, 1997). Harvesting the biomass is not mandatory, as the decaying plant material could act as a carbon source for SRB.

At last it is mentioned that the treatment of metal-contaminated industrial wastewater in CWs that rely on metal sulphide precipitation shifts metals from the wastewater to another environmental compartment and creates a future contamination problem. The question rises whether this technology is the most sustainable treatment option. Moreover, its efficiency requires adequate control of physico-chemical conditions. It is important to realise that metal immobilisation is dependent on redox conditions and thus management of the site. If the CW would be drained, sulphide oxidation can lead to the release of metals, possibly causing a flush of metals out of the CW during rain events. This uncontrolled flush could have devastating impacts on receiving watercourses.

5 Conclusions

During start-up conditions, higher removal efficiencies were observed in the gravel than in the gravel/straw microcosms. Sorption processes were responsible for metal removal during start-up. Straw was chosen as a solid OC source based on its availability and low-cost. However, a disadvantage of straw was the mobilising effect on heavy metals during start-up. Once anaerobic conditions were created and sulphate reduction was taking place, precipitation of metals with sulphides provided an additional removal pathway and higher removal efficiencies. Saturation of the substrates with Co, Ni and Zn was theoretically achieved by the 14th batch, based on Langmuir sorption maxima. Precipitation of metals with sulphides will become the dominant long-term metal removal process.

The relationship between metal removal, sulphate reduction and Eh conditions was demonstrated during the last batch of high-strength wastewater. The addition of glucose to unplanted gravel microcosms successfully enhanced sulphate reduction and metal removal. Amending the wastewater with a soluble carbon source has been suggested as a preferable alternative, as compaction of the filter and depletion of the carbon source are major disadvantages of solid carbon sources.

Phragmites australis negatively affected the precipitation of metals with sulphides in the root-dominated top-layer of all microcosms, evidenced by higher metal levels, less sulphate reduction and higher redox conditions. The selection of a helophyte species with less radial oxygen loss would be preferable.

One has to caution about the use of SSF CWs for the tertiary treatment of metal-contaminated industrial wastewater as metals are shifted to another environmental compartment. Stable redox conditions are required to ensure long-term efficiency. When the site is no longer used, costs for managing the site or treatment of the residues will have to be accounted for in evaluating the overall cost effectiveness of the system.

CHAPTER 7

REMOVAL OF HEAVY METALS FROM INDUSTRIAL EFFLUENTS BY THE HYDROPHYTE *MYRIOPHYLLUM SPICATUM* L.

1 Introduction

The release of heavy metals into the environment by industrial activities such as mining, land filling, pulp and paper production, electroplating, textile production and petroleum refinement, presents a serious environmental threat (Kadlec and Knight, 1996). Heavy metals can be removed from industrial wastewater by a range of physico-chemical remediation technologies such as precipitation, ion exchange, adsorption, electrochemical processes and membrane processes (Bailey et al., 1999; Kurniawan et al., 2006). However, regulatory standards are not always met. Moreover, these technologies are expensive and energy-intensive, driving towards a search of cheaper alternatives in both developing and developed countries (Kivaisi, 2001). Constructed wetlands (CWs) have been proposed to offer a low cost and low maintenance treatment alternative or tertiary treatment step for industrial effluents. Qian et al. (1999) and LeDuc and Terry (2005) stated that CWs are highly efficient in removing low levels of metals from large volumes of wastewater.

The use of aquatic plants, both dead and living, for the removal of heavy metals from wastewater has been investigated by many authors. Helophyte species such as *Phragmites australis*, *Typha latifolia*, *Typha domingensis*, *Schoenoplectus lacustris*, *Iris pseudacorus*, *Carex rostrata*, etc. have been tested for their removal capacities for heavy metals in a number of laboratory-scale experiments (Taylor and Crowder, 1983b; Debusk et al., 1996; Mungur et al., 1997; Stoltz and Greger, 2002; Ye et al., 2003). Full-scale treatment of a range of metal-contaminated industrial wastewaters by CWs planted with helophytes is reported by Peverly et al. (1995), Groudeva et al. (2001) and Batty and Younger (2004). In full-scale CWs, helophytes are accompanied by a gravel or soil-like substrate as a rooting medium. Dunbabin and Bowmer (1992) described the advantages of these combined helophyte-substrate systems in metal removal and reviewed some of the full-scale CWs for the treatment of mine drainage. However, only a minor fraction of metals is removed by the helophytes themselves and can thus be removed by harvest whereas the substrate acts as the main pool for metal accumulation in CWs (Lesage et al., 2004). Moreover, changing environmental conditions such as pH and redox potential can alter the mobility of metals and cause a release back to the water column (Gambrell, 1994). The shift of metals to the substrate and the need for adequate control of physico-chemical conditions have questioned the sustainability of helophyte-based CWs (Lesage et al., 2006).

The shift of metals from the wastewater to the substrate of helophyte-based CWs can be circumvented by using free-water-surface (FWS) CWs in which metals are removed by aquatic macrophytes that are regularly harvested. Floating plants that offer promising results with regard to metal removal from wastewater are *Lemna minor* (Zayed et al., 1998), *Eichhornia crassipes* (Zhu et al., 1999), *Pistia stratiotes* and *Salvinia herzogii* (Maine et al., 2001, 2004). As submerged plants are completely inundated and have the ability to take up metals directly from the water, they are suggested as useful species in reducing metal concentrations in stormwater (Fritioff et al., 2005) and secondarily treated wastewaters (Keskinkan, 2005). The prime potential use of submerged aquatic macrophyte-based wastewater treatment systems is for 'polishing' secondarily treated wastewaters (Keskinkan, 2005). According to Guilizzoni (1991) and Keskinkan (2005), there is scarce study about heavy metal uptake by submerged aquatic plants. The use of submerged aquatic macrophytes for wastewater treatment is therefore still at an experimental stage, with species like *Potamogeton* spp. (Goulet et al., 2005; Fritioff and Greger, 2006), *Ceratophyllum demersum* (Keskinkan et al., 2004) and *Myriophyllum spicatum* (Keskinkan et al., 2003) being tested.

Submerged rooted plants extract metals from the sediment via their root systems and directly from the surrounding water via their shoots. The latter process has been suggested to be predominant when high metal levels are present in the water (Guilizzoni, 1991). Leaves of submerged species are known to accumulate metals to a much greater extent than emergent species (Dunbabin and Bowmer, 1992). Generally, the uptake process of metals in aquatic plants contains two steps: (i) an initial fast, reversible metal-binding step involving physico-chemical processes such as chelation, ion exchange, precipitation and adsorption and (ii) a slow, irreversible ion-sequestration step consisting of biological processes such as intracellular uptake, vacuolar deposition and translocation (Salt et al., 1995). Keskinkan et al. (2003) referred to both processes as biosorption and bioaccumulation. The combination of these processes is responsible for the non-linear removal kinetics of metals from solution.

Uptake of Cd, Cu, Fe, Hg, Pb and Zn by *Myriophyllum spicatum* has been investigated by Keskinkan et al. (2003), Kamal et al. (2004), Sivaci et al. (2004), Yan et al. (2004) and Keskinkan (2005). However, up to now little information is found on the uptake of Co and Ni by *Myriophyllum spicatum*. Concentrations of Co and Ni in *Myriophyllum spicatum* growing in surface water impacted by a Cu smelter and ore processing factory were reported by Samecka-Cymerman and Kempers (2004). However, to our knowledge, the combined study of the removal kinetics and accumulation capacity of Co and Ni by *Myriophyllum spicatum* L. has not been reported in literature.

The objectives of this study were (i) to assess the tolerance of *Myriophyllum spicatum* L. for industrial effluents contaminated with Cu, Co, Ni and Zn, (ii) to assess the removal kinetics and uptake levels in the biomass of the studied metals and (iii) to assess the sorption/desorption properties of the surface of the biomass towards the studied metals and to derive sorption maxima. *Myriophyllum spicatum* L. (Eurasian water milfoil, family of the Haloragaceae) is a submerged, rooted, perennial plant, which reproduces primarily by vegetative fragmentation. The plant is most often found in water of 0.5 – 3.5 m deep. *Myriophyllum spicatum* prefers lakes, ponds and slow-moving streams but can also be found in fast-moving water. About 40 species of *Myriophyllum* are identified and they are present in Europe, Asia and northern America. *Myriophyllum spicatum* is tolerant to a wide range of water quality conditions and can be found in fresh and brackish water. Plants are temperature tolerant and able to overwinter in frozen ponds (APIRS, 2006).

2 Material and Methods

2.1 Free-water-surface constructed wetland microcosms planted with *Myriophyllum spicatum*

Myriophyllum spicatum L. was collected from a local plant nursery and gently cleaned with tap water. Plants were grown for 1 month in a 25 % Hoagland nutrient solution (Qian et al., 1999). After the adaptation period, four replicates of the *Myriophyllum* biomass of about 10 g fresh biomass were analysed for dry matter and metal contents. Metals were analysed by means of ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) after digestion of the dry plant material with 65 % HNO_3 and 20 % H_2O_2 (Du Laing et al., 2003). About 70 g fresh biomass was planted into each of four holding tanks (40 cm height, 20 cm diameter) with a 5-cm layer of gravel at the bottom as a rooting medium. Two unplanted tanks were set up as controls.

Two of the planted and the unplanted microcosms were exposed to 9 l synthetic industrial wastewater. A new batch of synthetic wastewater was added every two weeks, covering a total experimental period of 6 batches or 12 weeks. The following composition of synthetic wastewater was used (in mg l^{-1}): 1 Co, 1 Ni, 0.5 Cu, 1 Zn, 0.01 Fe, 0.01 Mn, 70 Ca, 15 K, 20 Mg, 1500 Na, 2000 SO_4^{2-}, 1000 Cl^-, 100 HCO_3^-, 1 PO_4^{3-}, 10 NO_3^- and 7 NH_4^+. The synthetic (Qian et al., 1999)wastewater had a pH of 8 and an electrical conductivity of 7.7 mS cm^{-1}. The synthetic wastewater is representative for an industrial effluent from a metal-processing industry in Flanders, Belgium. The two other planted microcosms were exposed to a 25 % Hoagland nutrient solution throughout the experiment to study tolerance properties. Fresh nutrient solution was made from a 20 times concentrated solution that was diluted with tap water and was added every two weeks, as for the microcosms exposed to wastewater.

Samples of 10 ml wastewater were collected by syringes after 6, 24, 48, 72, 96, 144, 240 and 336 h of addition of the synthetic wastewater during the first and the last batch, to study the removal kinetics. The initial wastewater added and the samples collected after different times, were analysed for SO_4^{2-} and Cl^- by IC (Metrohm 764 Compact IC, Herisau, Switzerland) and total levels of Co, Ni, Cu and Zn by means of ICP-OES. The wastewater was analysed for pH (electrode, HI 1230B, Temse, Belgium) and EC (electrode, WTW LF 537, Weilheim, Germany) at the start and end of each batch. The composition of the wastewater at the start of the first batch was used as input data of the Visual MINTEQ 2.40b model, in order to assess solubility equilibria (http://www.lwr.kth.se/English/OurSoftware/vminteq/).

Plants were exposed to a 14 h light/10 h dark regime. The ambient air temperature varied between 10 and 19 °C and the relative humidity between 40 and 57 % during the first batch that started in November 2005. During the last batch starting in February 2006, temperature varied between 9 and 23 °C and relative humidity between 27 and 49 %. The water levels in the microcosms were maintained constant throughout the experiment by daily observations. Corrections for evapotranspiration were performed by addition of deionised water. The added volumes of water were monitored and the cumulative evapotranspirations of the microcosms were plotted as a function of time. For all microcosms, a linear relationship was observed between both parameters with the slope representing the daily evapotranspiration. Daily evapotranspiration had a constant value of 1.5 mm d^{-1} in all microcosms, indicating homogeneous ambient conditions throughout the experiment.

Removal rates were calculated based on the concentration profiles of the metals in the wastewater as a function of time. For metal M (Co, Ni, Cu or Zn), in microcosm S (Unplanted, *Myriophyllum* first batch or *Myriophyllum* last batch), within time interval Δt_{i-j} (with i = 0, 6, 24, 48, 72, 96, 144 or 240 h and j being the subsequent sampling time), a removal rate $k_{\Delta ti-j,M,S}$ ($\mu g\ l^{-1}\ h^{-1}$) was calculated as follows:

$$k_{\Delta ti-j,M,S} = \frac{C_{i,M,S} - C_{j,M,S}}{t_j - t_i}$$

With $C_{i,M,S}$ or $C_{j,M,S}$ the concentration of metal M in microcosm S after i or j hours ($\mu g\ l^{-1}$) and t_j and t_i the sampling times (h) that border the time interval. The preceding formula allows the calculation of removal rates within the different subsequent time intervals $\Delta t_{0\text{-}6}$, $\Delta t_{6\text{-}24}$, $\Delta t_{24\text{-}48}$, $\Delta t_{48\text{-}72}$, $\Delta t_{72\text{-}96}$, $\Delta t_{96\text{-}144}$, $\Delta t_{144\text{-}240}$ and $\Delta t_{240\text{-}336}$.

After 12 weeks, *Myriophyllum spicatum* was harvested from the microcosms and total fresh biomass was determined. About 40 g fresh biomass of the *Myriophyllum* plants grown in the 25 % Hoagland nutrient solution was kept for the sorption/desorption experiment. The remainder of the biomass collected from each microcosm was divided into two parts. One part was rinsed 3 times with deionised water for analysis of the total metal content. The other part was rinsed 3 times with 0.1 M HCl to desorb metals from the surface of the biomass. Plants were dried at 50 °C until constant dry weight and analysed for metals according to Du Laing et al. (2003). Analyses were performed in triplicate.

A mass balance was performed in order to assess removal pathways of the metals. The masses of metals removed from the wastewater in the unplanted ($M_{unplanted}$) and planted microcosms ($M_{planted}$) were calculated according to the following formula:

$$M_{(un)planted} = (C_0 - C_{336}) \times V \times N$$

With C_0 the mean initial metal concentration in the wastewater of both batches ($mg\ l^{-1}$), C_{336} the mean metal concentration in the wastewater after 336 h of both batches ($mg\ l^{-1}$), V the volume of wastewater (9 l) and N the number of batches (6).

The mass of metals removed by the *Myriophyllum* biomass ($M_{Myriophyllum}$) was calculated as:

$$M_{Myriophyllum} = (C_e \times DM_e) - (C_i \times DM_i)$$

With C_i and DM_i the initial metal concentration (mg kg^{-1} DM) in the *Myriophyllum* biomass and the biomass (kg DM), and C_e and DM_e the metal concentration (mg kg^{-1} DM) in the *Myriophyllum* biomass and the biomass (kg DM) after 12 weeks.

2.2 Sorption/desorption experiment

Myriophyllum biomass collected from the microcosms that were exposed to the 25 % Hoagland nutrient solution was rinsed with deionised water. Sorption tests were performed with 2 g fresh biomass and 100 ml metal solution, in flasks of 250 ml. The background composition of the wastewater was the same as for the microcosm experiment, with increasing concentrations of Co, Ni, Cu and Zn: 1, 5, 10, 50 and 100 mg l^{-1}. Sorption tests were performed at room temperature with no pH adjustment. Flasks were shaken during a contact time of 2 h, based on the biosorption experiments of Keskinkan et al. (2003) and Yan et al. (2003). The solution was separated from the biomass by filtration (MN 640 m filter paper, Machery-Nagel, Düren, Germany) and analysed for Co, Ni, Cu and Zn by means of ICP-OES.

The concentration sorbed onto the surface of the biomass (q_s, mg g^{-1} DM) was calculated as:

$$q_s = \frac{(C_i - C_e) \times V}{M}$$

With C_i and C_e the metal concentration in the solution at the start and end of the sorption experiment (mg l^{-1}), V the volume (0.1 l) and M the mass of sorbent (0.2 g DM).

The equilibrium concentration in the solution (C_e) and the concentration sorbed onto the surface of the biomass (q_s) were fitted to the following sorption models:

Langmuir: $$\frac{1}{q_s} = \frac{1}{X_m \times b \times C_e} + \frac{1}{X_m}$$

With X_m (mg g^{-1} DM) the maximum sorption capacity and b (l mg^{-1}) a measure of sorption energy.

Freundlich: $q_s = k \times C_e^{1/n}$

With the empirical constants k ($mg^{1-1/n}$ $l^{1/n}$ g^{-1} DM) and n (dimensionless).

The biomass was rinsed with deionised water and used for the desorption experiment. Desorption tests were performed in flasks with 100 ml 0.1 M HCl. Flasks were shaken for 2 h, after which the solutions were filtered (MN 640 m filter paper, Machery-Nagel, Düren, Germany) and analysed for Co, Ni, Cu and Zn by means of ICP-OES.

The concentration desorbed from the surface of the biomass (q_{des}, mg g^{-1} DM) was calculated as:

$$q_{des} = \frac{C_{des} \times V}{M}$$

With C_{des} the metal concentration in the solution at the end of the desorption experiment (mg l^{-1}), V the volume (0.1 l) and M the mass of sorbent (0.2 g DM).

Control experiments were performed to measure sorption onto the glassware and filter paper. For Co, Ni and Zn sorption was negligible and accounted for only 1.5, 1.6 and 3.3 % of the initial metal concentration. For Cu however, 9 % of the initial concentration was removed by a combination of sorption on the glassware and filter paper. Data were corrected for these sorption effects. All sorption and desorption experiments were performed in triplicate.

2.3 Statistical analysis

Statistical analysis was performed using the S-plus 6.1 software package (Insightful Corp., Seattle, USA). Non-parametric Kruskal-Wallis rank tests ($\alpha = 0.05$) were performed to assess the significance of differences of Co, Cu, Ni, Zn, Cl^- and SO_4^{2-} concentrations in the water between different sampling times of the microcosm experiment. Wilcoxon rank tests ($\alpha =$

0.05) were performed to analyse the significance of differences of (i) the pH or EC of the water at the start and end of each batch of the microcosm experiment, (ii) the growth in the wastewater and in the nutrient solution and (iii) the accumulation of Co, Cu, Ni and Zn in the *Myriophyllum* biomass grown in the wastewater and in the nutrient solution.

3 Results

3.1 Removal of Co, Ni, Cu and Zn in the free-water-surface constructed wetland microcosms

A significant decrease ($p < 0.001$) of the metal concentrations in the wastewater with time was observed in all FWS CW microcosms in both batches (Fig 7.1). Removal of Co and Ni was similar and was slower than for Cu and Zn, for which a very rapid decrease was observed. As similar removal rates were observed within certain time frames, the mean removal rates within those time frames were presented in Table 7.1. In the planted microcosms, removal rates were generally the highest in the first 6 h and decreased rapidly towards a constant value for Co and Ni or towards zero for Cu and Zn.

During the first batch, 33 to 34 % of the initial Co and Ni mass were removed from the planted microcosms within the first day (Fig 7.1). The highest removal rates of Co and Ni were observed within the first 6 h (16 – 17 µg l^{-1} h^{-1}) (Table 7.1). Cobalt and Ni levels continued to decrease and reached values of 0.2 mg l^{-1} after 2 weeks, representing a decrease of about 80 % of the initial levels (Fig 7.1). A constant removal rate was observed for Co and Ni from the first day onward. Removal of Co and Ni was slower in the last batch than in the first batch (Fig 7.1). During the last batch, 17 and 27 % of respectively the initial Co and Ni mass were removed from the planted microcosms within the first day. Cobalt and Ni levels decreased to values similar to those observed at the end of the first batch, and this occurred at a constant removal rate of 2 µg l^{-1} h^{-1} from 6 h after the start of the experiment onward (Table 7.1).

Contrary to Co and Ni, removal of Cu and Zn occurred much more rapidly. The initial Zn and Cu level in the planted microcosms was reduced with respectively 38 and 44 % within the first 6 h after contact with the wastewater in the first batch. After 1 day, Cu and Zn concentrations were respectively 0.057 ± 0.009 mg l^{-1} and 0.33 ± 0.00 mg l^{-1}, representing 16 and 28 % of the initial levels. Removal rates decreased with time and approached zero (Table 7.1). From day 2 onward, Cu and Zn levels more or less reached equilibrium and varied between < 0.002 and 0.05 mg l^{-1} for Cu and between 0.007 and 0.19 mg l^{-1} for Zn (Fig 7.1). A similar rapid removal was observed within the first 6 h during the last batch as well, although the levels at equilibrium were higher than during the first batch. Cu levels varied between 0.05 and 0.09 mg l^{-1} from day 2 on, whereas Zn levels reached equilibrium after 144 h and varied between 0.14 and 0.21 mg l^{-1}.

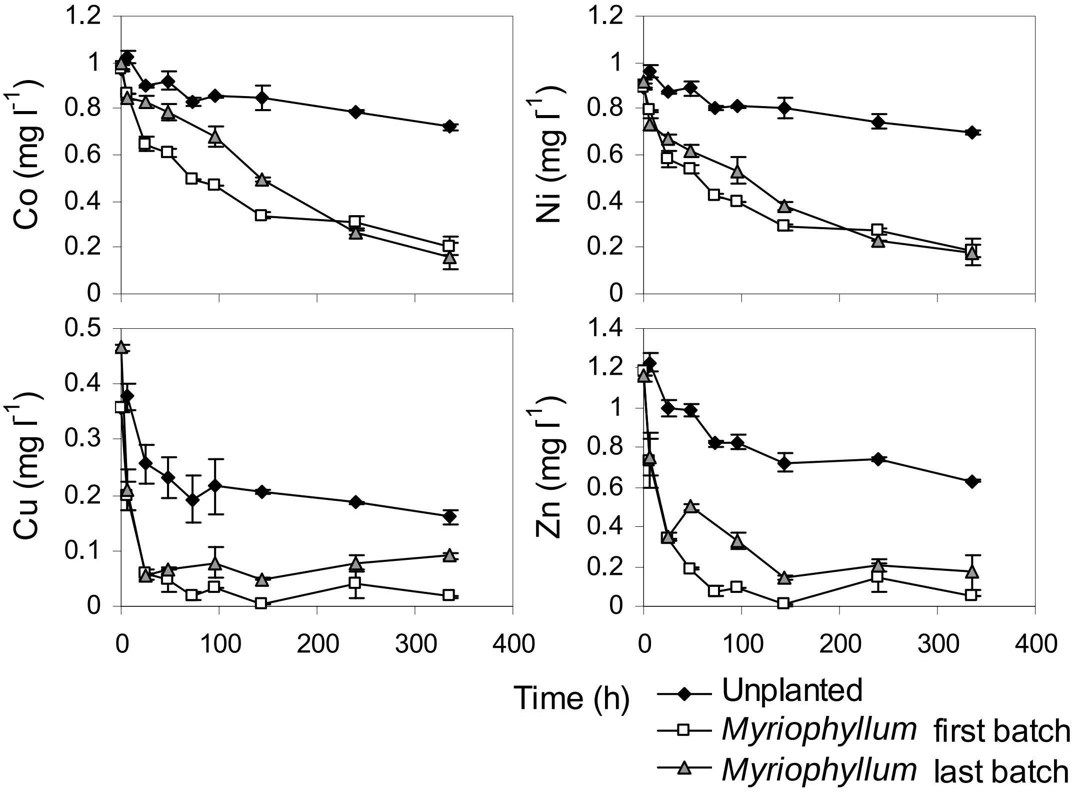

Fig 7.1 Cobalt, Ni, Cu and Zn concentrations in the wastewater (mg l^{-1}) as a function of time (h) of the unplanted controls and microcosms planted with *Myriophyllum spicatum* during the first and last batch (n = 2).

In the unplanted controls, negative removal rates were observed within the first 6 h after contact for all metals under study (Table 7.1). Cobalt, Ni, Cu and Zn levels in the wastewater after 6 h were respectively 6, 6, 8 and 4 % higher than their initial levels. This small increase of the metal levels in the unplanted microcosms within the first hours probably originated from disturbance of the gravel layer at the bottom after addition of the wastewater. The highest removal rate was observed within the following 18 h of the first day, after which the removal rates fluctuated and eventually approached a constant value (Table 7.1).

Table 7.1

Removal rates k (µg l^{-1} h^{-1}) for Co, Ni, Cu and Zn in the unplanted and planted microcosms during the first and last batch, within defined time frames (h)

Metal	**Unplanted**		***Myriophyllum* - first batch**		***Myriophyllum* - last batch**	
	Time Frame	***k* (µg l^{-1} h^{-1})**	**Time Frame**	***k* (µg l^{-1} h^{-1})**	**Time Frame**	***k* (µg l^{-1} h^{-1})**
Co	0-6	-10 ± 5	0-6	17 ± 1	0-6	25 ± 2
	6-24	7 ± 2	6-24	12 ± 2	6-336	2 ± 1
	24-336	1 ± 2	24-336	2 ± 2		
Ni	0-6	-12 ± 5	0-6	16 ± 1	0-6	31 ± 5
	6-24	5 ± 2	6-24	12 ± 2	6-336	2 ± 1
	24-336	1 ± 2	24-336	2 ± 2		
Cu	0-6	-4 ± 4	0-6	26 ± 4	0-6	43 ± 6
	6-24	7 ± 2	6-24	8 ± 2	6-24	9 ± 2
	24-336	0 ± 1	24-336	0 ± 1	24-336	0 ± 0
Zn	0-6	-8 ± 8	0-6	75 ± 23	0-6	68 ± 16
	6-24	13 ± 3	6-24	22 ± 8	6-24	23 ± 5
	24-336	2 ± 3	24-72	5 ± 1	48-144	4 ± 0
			72-336	0 ± 2	144-336	0 ± 1

The pH of the wastewater during the first batch decreased only slightly from 8.0 ± 0.0 to 7.3 ± 0.1 in microcosms planted with *Myriophyllum spicatum* and to 7.5 ± 0.1 in the unplanted controls ($p < 0.01$). However, an important increase to pH 10 ± 0 was observed in the planted microcosms during the last batch ($p = 0.0001$). The EC of the wastewater increased from 7.7 ± 0.0 to 9.0 ± 0.2 mS cm^{-1} in the microcosms planted with *Myriophyllum spicatum* and to 8.3 ± 0.0 mS cm^{-1} in the unplanted controls during the first batch ($p < 0.01$). At the end of the last batch an EC of 7.4 ± 0.1 mS cm^{-1} was observed in the planted microcosms, which was similar to the initial EC ($p = 0.18$). Chloride and SO_4^{2-} concentrations in the wastewater varied around respectively 1200 and 2200 mg l^{-1} with deviations less than 5 %.

3.2 Growth of *Myriophyllum spicatum*

Differences between growth of the two replicates of *Myriophyllum* plants collected from the wastewater were higher than for those collected from the 25 % Hoagland nutrient solution. The initial dry biomass / fresh biomass ratio (DM/FM) was 0.10. The absolute growth of 26 ± 7 g on a fresh biomass basis in the synthetic wastewater, was similar to that in the 25 % Hoagland nutrient solution ($p > 0.05$). About 40 % fresh biomass was formed in both treatments. However, a higher DM/FM ratio of 0.12 in plants harvested from the wastewater than a ratio of 0.095 in those collected from the nutrient solution, was responsible for a higher growth on a dry mass basis in the wastewater. Whereas 30 ± 1 % new dry biomass was formed in the nutrient solution (2.2 ± 0.1 g), 67 ± 16 % dry mass was formed in the wastewater (4.3 ± 0.8 g). Mean growth rates of 0.05 ± 0.01 g DM d^{-1} and 0.31 ± 0.08 g FM d^{-1} in the wastewater were derived under the assumption of linear growth during the experimental period.

3.3 Accumulation of Co, Ni, Cu and Zn by *Myriophyllum spicatum*

Table 7.2 presents the initial total metal levels and the total and sorbed metal levels at the end of the experiment. The sorbed metal fraction was considered as the fraction that was rinsed off the *Myriophyllum* surface by 0.1 M HCl. The sorbed metal concentrations were higher in replicate 2, the replicate with the highest biomass production and the highest surface area available for sorption. Total Co, Ni, Cu and Zn levels in *Myriophyllum spicatum* after 12 weeks of exposure to the wastewater were respectively 3000, 330, 510 and 5 times higher than initial levels ($p < 0.001$) (Table 7.2). These levels were elevated compared to those in plants grown in the 25 % Hoagland nutrient solution ($p < 0.001$).

The microcosms that were exposed to the 25 % Hoagland nutrient solution were included in the experimental set-up for tolerance assessment. Therefore, metal concentrations in the nutrient solution were not monitored during the experiment. However, significant Cu and Zn accumulation was observed in plants grown in the nutrient solution as well (Table 7.2). Dilution of the concentrated 25 % Hoagland solution was performed with tap water. In order to assess the metal levels in tap water, weekly samples of tap water were collected during a period of 6 weeks. The Cu and Zn concentrations in tap water were in the range of respectively 0.04 – 0.14 and 0.52 – 1.10 mg l^{-1}. Cobalt concentrations in tap water were lower than 0.002 mg l^{-1}, whereas Ni concentrations varied between < 0.002 and 0.24 mg l^{-1}.

Seventy four to 81 % of the total metal concentration in the *Myriophyllum* biomass grown in the wastewater, were desorbed by 0.1 M HCl. For plants grown in the nutrient solution, the major part of the total metal level in the biomass was also sorbed on the surface, except for Ni (Table 7.2).

Table 7.2

Total initial metal concentrations (n = 4) and total and sorbed metal concentrations (n = 6) in *Myriophyllum spicatum* grown in the wastewater and in the 25 % Hoagland nutrient solution at the end of the experiment (mg kg^{-1} DM) together with the percentage of the total metal concentration that was sorbed

	Co		**Ni**		**Cu**		**Zn**	
	mg kg^{-1} DM	**%**	**mg kg^{-1} DM**	**%**	**mg kg^{-1} DM**	**%**	**mg kg^{-1} DM**	**%**
Initial	0.5 ± 0.1	0	4.6 ± 0.6		1.5 ± 0.1		612 ± 254	
Wastewater								
Total	1675 ± 195		1529 ± 374		766 ± 206		2883 ± 433	
Sorbed	1243 ± 202	74	1151 ± 382	75	564 ± 209	74	2339 ± 436	81
25 % Hoagland								
Total	12 ± 2		12 ± 3		111 ± 15		1928 ± 340	
Sorbed	8.0 ± 2.2	66	2.2 ± 3.5	19	69 ± 16	62	1576 ± 343	82

In order to assess the removal pathways of the metals, a mass balance was performed (Table 7.3). The difference between the masses of Cu and Zn removed from the water in the planted ($M_{planted}$) and unplanted microcosms ($M_{unplanted}$) was similar to their masses recovered in the *Myriophyllum* biomass after harvest ($M_{Myriophyllum}$). Hovever, 23 and 28 % of respectively the Co and Ni masses removed from the wastewater remained unexplained (Table 7.3).

Table 7.3

Metal masses removed from the wastewater in the unplanted ($M_{unplanted}$) and planted ($M_{planted}$) microcosms, together with the difference between these masses Δ ($M_{planted}$ - $M_{unplanted}$), and the total metal masses recovered in the *Myriophyllum spicatum* biomass after harvest ($M_{Myriophyllum}$) (mg)

Mass fraction	**Co**	**Ni**	**Cu**	**Zn**
$M_{unplanted}$	13 ± 1	10 ± 1	11 ± 1	30 ± 1
$M_{planted}$	41 ± 2	38 ± 1	18 ± 0	61 ± 1
Δ ($M_{planted}$ - $M_{unplanted}$)	28 ± 2	27 ± 1	7.7 ± 0.9	31 ± 2
$M_{Myriophyllum}$	18 ± 3	17 ± 4	8.3 ± 2.4	27 ± 5

3.4 Sorption/desorption properties of *Myriophyllum spicatum*

As the microcosm experiment indicated good metal removal even after a period of 12 weeks, the question arose what were the sorption maxima of *Myriophyllum spicatum* for Co, Cu, Ni and Zn. Fig 7.2 presents the sorbed metal concentrations on the biomass (q_s) as a function of the equilibrium concentrations in the solution (C_e) after a contact time of 2 h. In the lower concentration range, the sorbed Co, Ni and Zn concentrations increased with the concentration in the solution. However, the sorbed concentrations levelled off in the higher concentration range indicating saturation of the surface. A different trend was seen for Cu of which the sorbed concentration continued to increase with the concentration in the solution (Fig 7.2).

For Co, Ni and Zn, the sorption process was described excellently by the Langmuir model ($R^2 \geq 0.99$) whereas the Freundlich model was better suited for Cu ($R^2 = 0.95$). Langmuir sorption maxima (X_m) of Co, Ni and Zn were respectively 2.3, 3.0 and 6.8 mg g^{-1} DM. The measure of sorption energy (b) decreased in following order (in l mg^{-1}): Zn (0.88) > Co (0.32) > Ni (0.25). The sorbed metal concentrations in the blank treatments showed negative values indicating desorption from the surface of the *Myriophyllum* biomass. Following 'sorbed' concentrations were observed for respectively Co, Ni, Cu and Zn, in mg kg^{-1} DM: -2.0 ± 0.2, -0.39 ± 0.06, -3.3 ± 0.7 and -15 ± 6. However, the latter values are negligible compared to the sorbed concentrations reported at higher concentration levels in the solution (Fig 7.2).

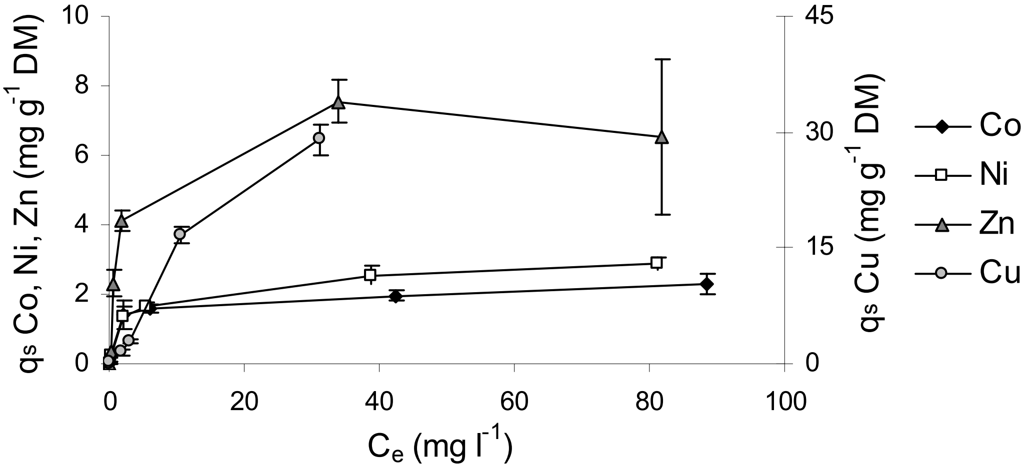

Fig 7.2 The sorbed metal concentrations by *Myriophyllum spicatum* q_s (mg g^{-1} DM) as a function of the equilibrium metal concentration in the solution C_e (mg l^{-1}) (n = 3).

The metal concentrations that were desorbed from the biomass by 0.1 M HCl (q_{des}) as a function of the sorbed metal concentration at the start of the desorption experiment (q_s), are presented in Fig 7.3. A linear relationship was observed between both variables ($R^2 > 0.96$ for all metals). Desorption by 0.1 M HCl did not fully recover the amount of metals sorbed onto the biomass. The percentage that was desorbed varied between 55 % for Cu, 60 % for Ni and 71 – 72 % for Co and Zn. The desorbed Zn concentrations in the lower concentration range largely exceeded the initially sorbed concentrations (Fig 7.3).

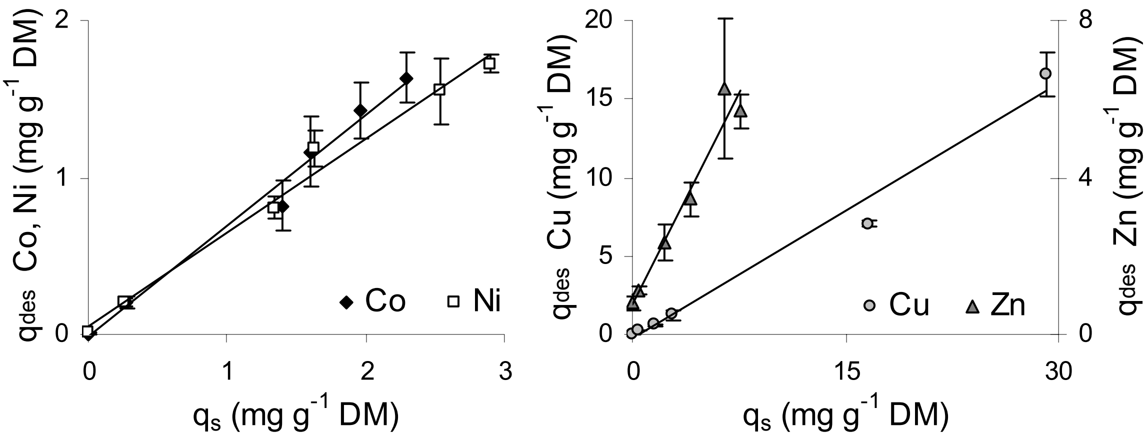

Fig 7.3 The desorbed metal concentration q_{des} (mg g^{-1} DM) as a function of the metal concentration sorbed by the *Myriophyllum* biomass at the start of the desorption experiment q_s (mg g^{-1} DM) (n = 3); together with the linear relationship between both variables.

4 Discussion

4.1 Performance of the free-water-surface constructed wetland microcosms

Myriophyllum spicatum was able to remove Co, Cu, Ni and Zn from the studied industrial wastewater, although differences in the removal kinetics were observed. A relatively long residence time of 2 weeks was necessary to achieve 80 % reduction of Co and Ni. The final Ni level of 0.2 mg l^{-1} is however still in excess of 0.05 mg l^{-1}, the basic environmental quality standard for surface water (VLAREM II, 1995). A shorter residence time of 24 and 48 h was sufficient to achieve respectively 0.05 mg l^{-1} Cu and 0.2 mg l^{-1} Zn, in compliance with basic environmental quality standards (VLAREM II, 1995). Our findings correspond with those of Keskinkan (2005) who reported rapid removal of Cu, Zn and Cd within the first 1 – 5 days and a state of equilibrium in systems with *Myriophyllum spicatum* grown in mixed metal solutions of Cd, Cu and Zn covering a concentration range of 1 – 16 mg l^{-1}. However, Kamal et al. (2004) observed slower removal of Zn and Cu by *Myriophyllum aquaticum*, probably because of higher initial concentrations of 28 and 5.6 mg l^{-1} respectively. Removal efficiencies of 42 and 34 % for respectively Cu and Zn were observed after 3 weeks. Contrary to the concentration dependency of the removal rates of Cu and Zn in our experiment, removal rates observed by Kamal et al. (2004) were constant.

4.2 Tolerance and growth of *Myriophyllum spicatum*

Growth of *Myriophyllum spicatum* was not adversely affected and 67 % new dry biomass had developed in the synthetic wastewater compared to 30 % in the 25 % Hoagland nutrient solution. Guilizzoni (1991) stated that concentration limits for toxicity of submerged plants are less commonly found in literature than for terrestrial and emergent aquatic plants and algae. Stanley (1974) cited in Guilizzoni (1991) derived the following levels at which 50 % inhibition of root growth of *Myriophyllum spicatum* occurred (in mg l^{-1}): 2.5 for Al(III), 2.9 for As(III), 7.4 for Cd(II), 0.25 for Cu(II), 0.46 for Cr(VI), 9.9 for Cr(III), 3.4 for Hg(II), 363 for Pb(II) and 21.6 for Zn(II). Copper appeared to be the most toxic metal, although tolerance to Cu levels of 0.5 mg l^{-1} was observed in our experiment. Kamal et al. (2004) showed that *Myriophyllum aquaticum* had the highest tolerance of 3 aquatic plant species grown in a solution with 28, 5.6, 104 and 0.5 mg l^{-1} of Zn, Cu, Fe and Hg respectively. *Myriophyllum spicatum* tolerated a mixed metal solution of 16 mg l^{-1} of Cd, Cu and Zn in the experiment of Keskinkan (2005). However, inhibition of photosynthesis of *Myriophyllum spicatum* by Cd concentrations of 3.5 and 6 mg l^{-1} has been reported by Stoyanova and Tchakalova (1997).

4.3 Accumulation capacity of *Myriophyllum spicatum*

Metal concentrations in macrophytes are difficult to compare because of differences in wastewater quality, plant species, environmental conditions, analytical procedures, etc. (Guilizzoni, 1991). A first series of factors are related to the wastewater quality: type of metals, concentration and speciation. The latter is affected by abiotic wastewater characteristics such as pH, salinity and the concentration of ligands. Fritioff et al. (2005) described that an increase in salinity caused a decrease in the uptake of Zn, Cd and Cu in the hydrophytes *Elodea canadensis* and *Potamogeton natans*. This is caused by (i) reduced availability of metals in the water due to the formation of complexes between chlorides and metals and (ii) increased competition with sodium ions at uptake sites of the plant surface. Secondly, factors related to the plant are important: plant species, plant tissue and growth stage (Guilizzoni, 1991). Finally, environmental factors such as light and temperature can also affect metal uptake. Fritioff et al. (2005) reported increased uptake of Zn, Cd and Cu from stormwater by two submerged plants with increasing temperature. Higher temperatures caused a higher biomass production and absorption surface, facilitated metal fluxes by changing the lipid composition of the membrane, and increased the metabolism. However, keeping these bottlenecks in performing comparisons in mind, the following section presents a comparison of metal concentrations in *Myriophyllum spicatum* with literature reports in order to obtain a general idea about its accumulation capacity.

Total Co and Ni levels in *Myriophyllum spicatum* after 12 weeks of exposure to the wastewater were respectively 1675 and 1529 mg kg^{-1} DM. These levels largely exceeded Co and Ni levels of respectively 33 – 57 and 18 – 19 mg kg^{-1} DM reported by Samecka-Cymerman and Kempers (2004) in shoots of *Myriophyllum spicatum* collected from a Cu smelter impacted surface water with Co and Ni levels of respectively 0.03 – 0.07 and 0.06 – 0.07 mg l^{-1}. However, the harvestable parts of *Myriophyllum spicatum* were analysed for total metal concentrations without distinguishing between leaves, stems and roots. This complicates comparisons with literature reports, although it can be assumed that the roots are mainly responsible for the high accumulation levels reported in this study, as submerged aquatic macrophytes generally accumulate higher concentrations in their belowground biomass than in their shoots (Guilizzoni, 1991).

Qian et al. (1999) reported Ni concentrations of 1077 and 50 mg kg^{-1} in respectively roots and shoots of *Myriophyllum brasiliense* that grew for 10 days in a 25 % Hoagland nutrient solution spiked with 1 mg l^{-1} Ni. Zhu et al. (1999) reported maximum Ni concentrations of 120 and 1200 mg kg^{-1} DM in respectively shoots and roots of *Eichhornia crassipes* (Water hyacinth) grown for 14 days in a 25 % Hoagland nutrient solution spiked with 10 mg l^{-1} Ni. A similar Ni concentration of 1790 mg kg^{-1} DM was observed in *Lemna minor* grown for 14 days in a 25 % Hoagland nutrient solution spiked with 10 mg l^{-1} Ni (Zayed et al., 1998). However, at a Ni concentration of 1 mg l^{-1} as used in our experiment, accumulation levels in shoots and roots of *Eichhornia crassipes* were respectively 20 and 200 mg kg^{-1} DM, being much lower than the reported concentrations for *Myriophyllym spicatum* in this study. *Lemna minor* also showed very low accumulation of Ni at a concentration of 1 mg l^{-1}. *Myriophyllum spicatum* demonstrated to be a better accumulator of Ni than the floating aquatic macrophytes *Eichhornia crassipes* and *Lemna minor*.

The total Cu concentration in *Myriophyllum spicatum* after 12 weeks of exposure to the wastewater was 766 ± 206 mg kg^{-1} DM. This finding was comparable with levels of 720 – 1040 mg kg^{-1} DM reported by Samecka-Cymerman and Kempers (2004) in shoots of *Myriophyllum spicatum* collected from a surface water with 0.02 – 0.03 mg l^{-1} Cu. Kamal et al. (2004) reported similar concentrations of 549 mg kg^{-1} DM in the shoots of *Myriophyllum aquaticum* grown for 3 weeks in synthetic wastewater with 5.6 mg l^{-1} Cu. Fritioff and Greger (2006) reported Cu levels of 461 and 357 mg kg^{-1} DM in leaves and stems of the submerged plant *Potamogeton natans* grown in the presence of 0.2 mg l^{-1} Cu. Zhu et al. (1999) and Zayed et al. (1998) observed much higher Cu concentrations of 2800 and 3360 mg kg^{-1} DM in respectively roots of *Eichhornia crassipes* and in the biomass of *Lemna minor*, grown in 25 % Hoagland nutrient solutions spiked with 10 mg l^{-1} Cu. However, at a similar Cu concentration of 1 mg l^{-1}, accumulation levels in roots of *Eichhornia crassipes* (600 mg kg^{-1} DM) were in the same range as those reported for *Myriophyllum spicatum*. *Lemna minor* showed a much lower Cu accumulation of 300 mg kg^{-1} at this lower dose.

The total Zn level of 2883 ± 433 mg kg^{-1} DM reported in this study largely exceeded levels of 313 – 515 mg kg^{-1} DM in shoots of *Myriophyllum spicatum* collected from a surface water with 0.16 – 0.20 mg l^{-1} Zn (Samecka-Cymerman and Kempers, 2004) and 304 mg kg^{-1} DM in shoots of *Myriophyllum aquaticum* grown for 3 weeks in synthetic wastewater with 28 mg l^{-1} Zn (Kamal et al., 2004). The initial Zn levels in our study (612 ± 254 mg kg^{-1} DM) were already remarkably higher than these values reported in literature. The total Zn level in *Myriophyllum spicatum* after harvest were more in line with levels in leaves and stems of *Potamogeton natans* grown in diluted Hoagland's medium spiked with 1.3 mg l^{-1} Zn (Fritioff and Greger, 2006).

4.4 Processes of metal removal in the microcosms

Non-linear removal kinetics were observed, demonstrating the prevalence of different removal mechanisms (Salt et al., 1995). Sorption onto *Myriophyllum spicatum* was responsible for the rapid removal of Cu and Zn during the first hours of the experiment, whereas sorption of Co and Ni occurred more slowly. After sorption onto the surface of *Myriophyllum spicatum*, accumulation and translocation within the biomass were responsible for the slower part of the metal removal.

Significant removal of metals was observed in the unplanted microcosms as well. In an attempt to elucidate which processes were at the basis of this finding, the Visual MINTEQ 2.40b model was used. The input data of the model consisted of the nominal concentrations of Fe, Mn, Ca, K, Mg, Na, CO_3^{2-}, PO_4^{3-}, NO_3^- and NH_4^+ and the monitored concentrations of Co, Cu, Ni, Zn, SO_4^{2-} and Cl^- in the wastewater at the start of the first batch. Additional input data were a fixed pH of 8 and a temperature of 25 °C. It was assumed that there was no dissolved organic carbon in the initial wastewater. The model predicted that 70, 97, 66 and 50 % of respectively Co, Cu, Ni and Zn could precipitate as $CoCO_3$, CuO (tenorite), $NiCO_3$ and $Zn_5(CO_3)_2(OH)_6$ (hydrozincite). For Zn, this corresponds quite well with the decrease of 47 % that was observed in the unplanted microcosms. However, the decrease of 25, 55 and 22 % observed for Co, Cu and Ni, respectively, was much smaller than that predicted by the model. Of course, dynamics conditions were encountered throughout the experiment. The model gives an indication of which precipitates may form but is not a strict predictor of the actual metal removal in the unplanted microcosms.

The mass balance showed that the difference between the amounts of Co and Ni removed from the wastewater in the planted and unplanted microcosms were higher than those recovered in the *Myriophyllum* biomass, whereas a correct mass balance was set-up for Cu and Zn. Sivaci et al. (2004) stated that washing the biomass with distilled water may remove electrostatically-bound Cd ions from the plant surface. This might have occurred with Co and Ni that was present on the *Myriophyllum* surface. However, it seems unlikely that Zn and part of the Cu would not have been rinsed of the surface as well. It was therefore assumed that another removal pathway not involving uptake by the plants, but induced by the presence of the *Myriophyllum* plants, was responsible for a significant amount of the Co and Ni removal.

Sulphate levels remained constant with time, thereby excluding the possibility of metal sulphide precipitation as the ‘missing removal process’. A high pH of 10 was observed in the planted microcosms at the end of the last batch. This increase in pH was attributed to the photosynthesis of the plants that occurred at a greater level in the last batch than at the start of the experiment. It was suggested that Co and Ni were partly removed by precipitation with CO_3^{2-} and OH^- as they were removed slowly from the wastewater by uptake of the plants and were thus more susceptible to this third removal pathway than Cu and Zn. Kamal et al. (2004) also reported the importance of a chemical removal pathway, more specifically precipitation as phosphates, in the removal of Cu and Zn from hydroponic systems planted with *Myriophyllum aquaticum*.

4.5 Sorption/desorption properties of *Myriophyllum spicatum*

The sorption process of Co, Ni and Zn onto the surface of the *Myriophyllum* biomass was described well by the Langmuir isotherm. The Langmuir constant *b* is an indicator of the isotherm rise in the region of lower residual metal concentrations and is therefore representative of the 'strength' or 'affinity' of the sorbent for the solute. The Langmuir constants X_m and *b* showed that the surface of *Myriophyllum spicatum* had the highest sorption capacity of Zn, but also had a greater capacity to sorb Zn from dilute solutions than it had for Co and Ni. This finding supports the rapid removal observed for Zn in the above-mentioned experiment versus the slow removal of Co and Ni. Langmuir sorption maxima reported in literature were compared with results of our experiment (Table 7.4).

Blank treatments showed negligible removal of Co, Ni and Zn and 8 % removal of Cu. It was concluded that metal removal mainly occurred through sorption onto the *Myriophyllum* surface. Wang et al. (1996) described pH as a major factor affecting the sorption with the highest removal rates being obtained at initial pH values of 5. Maximum sorption capacities of Ni and Zn at pH 8 in our study are lower than those reported by Wang et al. (1996) and Keskinkan et al. (2003). Zhao et al. (1999) reported a higher Zn removal by *Azolla filiculoides* at increasing pH in the range of pH 3 – 6. It is concluded that too low and too high pH are unfavourable for metal sorption. For Cu, the sorption process was best described by the Freundlich sorption isotherm, contrary to the findings of the authors reported in Table 7.4. Saturation of the surface of the *Myriophyllum* biomass with Cu was not reached in our experiment. The sorbed Cu concentration at the highest residual equilibrium concentration of 31 mg l^{-1} was three times higher than maximum sorption capacities reported in Table 7.4. The Freundlich model assumes that the stronger binding sites are occupied first and that the binding strength decreases with increasing degree of site occupation.

Table 7.4

Maximum sorption capacities X_m (mg kg^{-1} DM) of different metals by *Myriophyllum spicatum* reported in literature and our study, together with the experimental conditions of contact time t (min), solid/liquid ratio S/L (g FM l^{-1}), and initial pH. References: (1) Wang et al. (1996), (2) Yan et al. (2005), (3) Keskinkan et al. (2003) and (4) This study

Reference	**Metal**						**Experimental conditions**		
	Co	**Ni**	**Cu**	**Zn**	**Pb**	**Cd**	**t**	**S/L**	**pH**
1		2400	8200	3600	1300	2800	60	20	3
1		5800	12900	13500	55600	8200	60	20	5
2			10800				120	10	5
3			10370	15590	46490		120	*	5
4	2314	2999	29103**	6780			120	20	8

* Undefined; ** *qs* at the highest *Ce*

Desorption by 0.1 M HCl did not fully recover the metals that were sorbed onto the biomass during the preceding sorption experiment. The percentage that was desorbed varied between 55 % for Cu, 60 % for Ni and 71 – 72 % for Co and Zn. Moreover, there was evidence of leaching, especially for Zn, from within the biomass rather than exclusive desorption from the surface. Rinsing with 0.1 M HCl could have damaged the biomass and it therefore seems unlikely that rinsing with 0.1 M HCl is a suitable strategy to regenerate the *Myriophyllum* biomass. However, different authors have reported the successful cleaning of *Myriophyllum spicatum* with a 'diluted' HCl solution of 3 % (0.8 M) as a preparation step in their biosorption experiments (Keskinkan et al., 2003; Sivaci et al., 2004; Yan et al., 2005). Sinicrope et al. (1992) even rinsed the roots of *Scirpus californicus* with 10 % HNO_3 (1.7 M) in order to remove the adsorbed metals before analysis.

4.6 Suggestions for design

A simple design calculation was performed under the assumption that a daily flow of 5000 m^3 of wastewater is to be treated, representative for effluent rates of a major metal-processing industry in Flanders (VMM, 2006). A depth of 2 m was chosen based on the observed depth range of 0.5 – 3.5 m that can be colonised by *Myriophyllum spicatum* (APIRS, 2006). If Cu and Zn are the target pollutants, a residence time of 48 h is sufficient to reach the basic environmental quality standards for surface water (VLAREM II, 1995). Hence, a FWS CW with a minimum treatment area of 0.5 ha would be needed. A 7-fold higher residence time and surface area would be required to provide an additional 80 % reduction of Co and Ni. Based on the observed growth rates in our experiment, a monthly biomass production of 3.4 ±

0.6 ton DM ha^{-1} could be achieved and the following masses of Co, Ni, Cu and Zn could be extracted from the wastewater by harvest of *Myriophyllum* (in kg ha^{-1} month^{-1}): 6 ± 1, 5 ± 2, 3 ± 1 and 10 ± 2.

The elevated Co, Ni, Cu and Zn concentrations in the harvested biomass, respectively 0.17, 0.15, 0.08 and 0.29 % on a DM basis, do not allow composting and reuse of the biomass as fertiliser or soil-improving substance (VLAREA, 1998). The metals in the biomass could be recovered by ashing the biomass (Zhu et al., 1999). Moreover, the combustion of the biomass could produce useful energy. This has been suggested by Maine et al. (2001, 2004) as a management strategy for harvested biomass of *Pistia stratiotes*.

Zhao et al. (1999) demonstrated the reusability of dried *Azolla filiculoides* biomass for treatment of Zn-contaminated electroplating effluents after acid washing with 0.2 M HCl or 0.1 M H_2SO_4. A high recovery of 86 – 88 % of the sorbed Zn was observed after desorption with HCl, which was slightly less efficient than the near complete recovery with H_2SO_4. An effluent-free treatment technology in which the treated wastewater can be recycled and the recovered Zn solution could be reused in the electroplating process was suggested. However, desorption of the *Myriophyllum spicatum* biomass with 0.1 M HCl was not efficient based on our findings, but other leaching solutions could be tested for their efficiency in metal recovery.

Free-water-surface CWs planted with *Myriophyllum spicatum* offer potential for the polishing of industrial effluents but their full-scale use is limited. It has to be mentioned that *Myriophyllum spicatum* is considered as an invasive weed in several states of the US and Canada and is subject of much control efforts (APIRS, 2006). Some factors described by Kivaisi (2001) that have countered the full-scale use of water hyacinth in developed countries also apply for *Myriophyllum spicatum*: (i) the poor performance in northern-hemisphere winters, (ii) the purchase of land, (iii) the intensive harvesting schemes and (iv) the construction and operation of systems for bioenergy recuperation. However, CWs with floating or submerged plants could offer great advantages in developing countries. First of all, year-round growth and thus treatment efficiency could be provided in warmer climates. Secondly, availability of land is greater. At last it is mentioned that maintenance and harvesting schemes could provide employment.

5 Conclusions

Removal of Co and Ni by *Myriophyllum spicatum* was slower than of Cu and Zn. A residence time of 2 weeks was necessary to achieve Co and Ni levels of 0.2 mg l^{-1}, whereas a shorter residence time of 48 h was sufficient to achieve Cu and Zn levels of respectively 0.05 and 0.2 mg l^{-1}. A removal pathway associated with the presence of the plant that involved precipitation with CO_3^{2-} and OH^- was probably responsible for about one third of the Co and Ni removal. Growth of *Myriophyllum spicatum* was not adversely affected by the wastewater. Total Co, Ni, Cu and Zn levels in *Myriophyllum spicatum* after 12 weeks of exposure accounted for respectively 0.17, 0.15, 0.08 and 0.29 % on a DM basis.

The sorption process of Co, Ni and Zn onto the surface of the *Myriophyllum* biomass was described well by the Langmuir isotherm whereas sorption of Cu was better described by the Freundlich isotherm. Desorption by 0.1 M HCl is not suggested as a useful strategy in recovering the metals and regenerating the biomass.

GENERAL DISCUSSION, CONCLUSIONS AND FUTURE PERSPECTIVES

1 Increasing insights into the removal mechanisms of heavy metals in constructed treatment wetlands

This study dealt with the behaviour of heavy metals in constructed treatment wetlands. Over the last decade, research concerning heavy metals in constructed wetlands (CWs) has increased considerably. In Chapter 1, the current knowledge of the removal mechanisms of heavy metals in CWs was reviewed. Insights into these mechanisms have increased chiefly by the dual approach of (1) monitoring metal accumulation in the compartments of full-scale CWs and (2) studying removal processes and influencing factors by means of laboratory and pilot-scale experiments. This PhD study contributed to the further understanding of removal mechanisms of heavy metals in CWs. Most important findings from the field observations and small-scale experiments with regard to removal mechanisms are summarised in the following section.

1.1 Evidence of removal mechanisms by field studies

Sediment was the most important sink for heavy metals in a free-water-surface (FWS) and horizontal subsurface flow (HSSF) CW for domestic wastewater treatment. Accumulation of heavy metals in the biomass of *Phragmites australis* was of negligible importance (Chapters 3 and 4).

Low redox potentials and high AVS/SEM values suggested a low mobility of heavy metals in the sediment of the FWS CW. Sulphide precipitation was an important removal mechanism for heavy metals in this CW. In the HSSF CW, heavy metals mainly accumulated in the sediment situated nearest to the inlet area. Next to sorption onto organic matter, filtration and sedimentation of suspended solids and associated metals, precipitation as metal sulphides is believed to be an important removal process for metals close to the inlet area of the reed bed.

A quantitative assessment of the importance of the different removal mechanisms was however not possible based on these field observations. Speciation studies of heavy metals in the sediment could deliver useful additional information about the dominant removal processes. Unfortunately, there is currently a lack of speciation methods for sediments in coarse-textured substrates such as gravel. The development of speciation methods for coarse-textured substrates should deserve more attention in future research. Next to these speciation studies, the monitoring in parallel of parameters including Eh, pH and concentrations of dissolved oxygen (DO), SO_4^{2-}, S^{2-}, organic carbon (OC), NO_3^-, Fe and Mn in the pore water would further elucidate the removal mechanisms.

1.2 Elucidation of removal mechanisms by small-scale experiments

The processes of sorption and metal sulphide precipitation were thoroughly studied in gravel-based subsurface flow (SSF) CW microcosms for tertiary treatment of an industrial effluent (Chapter 6). The identification of the prevailing removal mechanisms was possible by study in parallel of parameters including Eh and concentrations of SO_4^{2-}, OC, Fe and Mn.

The following metal removal mechanisms were observed during the start-up conditions: (1) sorption onto Fe- and Mn- (oxy-) hydroxides covering the gravel surface in all microcosms and (2) sorption onto the straw in the gravel/straw microcosms. Both mechanisms provide short-term removal of metals as they are subject to changing redox conditions and saturation (Machemer and Wildeman, 1992; Sobolewski, 1996).

Once anaerobic conditions were created and sulphate reduction was taking place, precipitation of metals with sulphides provided an additional removal pathway and higher removal efficiencies. Precipitation of metals with sulphides will become the dominant long-term metal removal process (Lesage et al., 2006).

2 Transfer of heavy metals from the wastewater to the sediment of constructed wetlands

For a long time, heavy metals were not a topic of high priority in CWs for domestic wastewater treatment. Fortunately, awareness of the persistent nature of heavy metals and possible risks related to their accumulation in CWs for domestic wastewater treatment, have caused an acceleration in research over the last decade (Gschlößl and Stuible, 2000; Obarska-Pempkowiak, 2001; Vymazal, 2003; Chagué-Goff, 2005). The current work presents results of the first in-depth study of the removal of heavy metals in CWs for domestic wastewater treatment in Flanders.

2.1 Performance of Flemish treatment wetlands

Copper and Zn are the most abundant trace metals in the influent wastewater of CWs in Flanders (Chapter 2). Heavy metal concentrations in the influent were generally subjected to a larger variability than in the effluent. Constructed wetlands can thus be considered as effective buffers. The ‘peak-shaving effect’ of CWs has previously been described for organic loading, SS and nutrients by Rousseau (2005).

Total metal concentrations in the effluents were generally far below the basic environmental quality standards for surface water (VLAREM II, 1995). Unfortunately, there are no effluent standards for heavy metals in CWs in Flanders. However, dealing with persistent pollutants, the discharge of heavy metals should be prevented as much as possible to preserve the quality of the receiving surface water (VMM, 2005). The accumulation of metals in sediments of natural watercourses may cause a long-term release of metals back to the water column (De Raeymaecker et al., 2005; Peeters et al., 2005).

The highest removal efficiencies of all metals except Fe and Mn, were observed in the HSSF CW, whereas the tertiary CW was the least efficient of the 4 CWs under study. A positive relationship between influent concentrations and removal efficiencies was observed for most metals, corresponding with findings of Kadlec and Knight (1996).

2.2 Release of Fe and Mn

Manganese and Fe exhibited a particular behaviour within the group of metals studied. They were poorly retained within the 4 CWs (Chapter 2). Manganese and Fe were released from the HSSF CW and this release occurred throughout the year. It was presumably related to the anaerobic conditions in the sediment that led to bacterial reduction of Mn(IV) and Fe(III) and the release of the soluble Mn(II) and Fe(II) species. The tertiary CW, also of the HSSF type, showed a release of Mn but not of Fe, indicating an oxidation reduction state that was intermediate between these needed for reduction of Mn(IV) and Fe(III). This difference between both HSSF reeds beds was presumably attributed to the different type of wastewater that was treated, namely a primary and secondary treated domestic wastewater.

Poor removal of Fe and Mn was also observed in the FWS and VSSF CW. This finding was somewhat unexpected as the design of these types of CWs would generally favour the creation of more aerobic conditions (Stottmeister et al., 2003). In Chapter 3, it was found that the sediment of the FWS CW was in a reduced state (Eh of -141 mV). This could be attributed to the long residence time of about 9 days and the water depth of 50 cm. In the case of the VSSF CW the configuration of a layer of 60 cm of fine gravel above a drainage layer of 30 cm depth, should normally enhance aerobic conditions. However, surface flooding was observed during a visit of the site at the end of May 2006. As mentioned in section 1.1, assumptions regarding the behaviour of Fe and Mn would need to be supported by measurements of e.g. Eh and concentrations of DO and NO_3^-.

It was clearly shown that Fe- and Mn- (oxy-) hydroxides were present on the surface of the gravel in the SSF CW microcosms (Chapter 6). Upon the creation of reducing conditions, a release of Fe and Mn from the gravel substrate was observed. This has recently been observed by Buddhawong et al. (2005) as well. The release of Fe and Mn in the full-scale SSF CWs could therefore also have originated from the gravel itself and not solely from the sediment that had previously accumulated.

2.3 Implications of immobilisation of heavy metals in the sediment

Metals were effectively shifted from the wastewater to the sediments of the CWs (Chapters 3 and 4). Although the FWS CW had been in operation for a period of 16 years, more than 5 times the age of the HSSF CW, the metal contamination level of the sediment of the former was lower than that of the latter one. The contamination level of the sediment of the FWS CW was low to moderate, with mean concentrations of 1.8, 67, 124 and 355 mg kg^{-1} DM for Cd, Cu, Pb and Zn, respectively. These levels are higher than background reference levels, but still much lower than the most stringent Flemish remediation standards for soils of land-use classes I and II, including nature, forestry and agriculture. After 3 years of operation, the sediment in the inlet area of the HSSF CW was significantly contaminated with Cd, Cu and Zn (respectively 2.5, 288 and 934 mg kg^{-1} DM) whereas Pb (162 mg kg^{-1} DM) could form a contamination problem within the near future.

Whether the shift of heavy metals from the wastewater to the sediments of CWs is a sustainable affair should be an important topic of discussion. Few research studies dealt with long-term accumulation of metals in the sediment and with the consequences for operation and end-use of the CWs. Sceptics of the technology attack the immobilising nature of CWs and describe it as a major bottleneck. However, immobilisation and control of metals within a constrained area seems preferred over uncontrolled release into receiving natural watercourses. Sulphide precipitation was postulated as one of the important removal mechanisms for metals in both CWs. As long as reducing conditions are maintained, metals will be effectively immobilised in the sediment.

When a reed bed exceeds its operational lifetime, management of the site will affect the behaviour of metals. If a reed bed would be abandoned or drained, sulphide oxidation and acidification can lead to an initial release of metals. In case of the FWS CW, it was suggested that the buffering capacity of the sediment was sufficiently high to prevent a large increase in acidity after drainage and oxidation. Moreover, other immobilisation processes including adsorption or precipitation with Fe- and Mn- (oxy-) hydroxides, carbonates and complexation with organic matter can prevent a large release of metals. However, due to the complexity of the sediment system and the uncertainty of predictions regarding long-term immobilisation upon changes in oxidation state, it was suggested to the maintain the sediment of the FWS CW in reduced conditions.

In case of the HSSF CW, the situation is somewhat different. Gambrell (1994) cautioned for a release of metals from highly organic sediments upon a change from reducing to oxidising conditions and a decrease of pH. As the nature of the sediment of the HSSF CW is mainly organic, originating from suspended solids applied with the wastewater and decomposing plant litter, significant acidification and consequent metal release are likely to occur upon oxidation. If managers decide not to (immediately) remediate the substrate upon its closure, it is strongly suggested to maintain the site in a waterlogged condition to preserve the immobilisation of heavy metals.

Currently, research studies are carried out to assess the potential of cleaning the gravel of SSF CWs *in-situ* (Behrends et al., 2006; Cooper et al., 2006). Promising techniques under study include sludge fluidisation and pump-out by air and water sparging and the addition of strong chemical oxidising agents such as hydrogen peroxide. However, until these techniques are not fully developed, refurbishment of the substrate of SSF CWs implies digging out, cleaning and/or replacing the gravel. By cleaning the gravel, a waste-stream of sludge is generated. The Flemish regulations relating to waste prevention and management state that sludge should be treated by either anaerobic fermentation, liquid storage, aerobic stabilisation, liming, or thermal drying (VLAREA, 1998). The sludge could also be treated by sludge reed bed facilities. Sludge reed beds can be applied for treatment of activated sludge (De Maeseneer, 1997; Nielsen, 2004, 2006) and faecal sludge from septic tanks (Koottatep et al., 2004a, b). However, such facilities are not present in Flanders. Upon treatment of the sludge, it could then be reused as fertiliser or soil-improving substance provided that the criteria demanded by VLAREA (1998) are fulfilled. Criteria for Cd, Cr, Cu, Ni, Pb and Zn in treated sludge are respectively 6, 250, 375, 50, 300 and 900 mg kg^{-1} DM. The range of metal levels observed in the sediment of the HSSF CW comply with these criteria. The treated sludge could be applied to a soil by means of ploughing or injection.

3 Effects of helophytes on metal removal and implications for design and operation

3.1 Accumulation of heavy metals in helophytes

The following general trends were observed irrespective of the plant species and type of wastewater under study:

- Metal concentrations in the belowground biomass were generally higher than in the aboveground biomass. Overall, metal concentrations in the aboveground biomass were comparable to values reported in uncontaminated environments, natural and constructed wetlands and did not indicate excessive accumulation, a finding recently underlined by Vymazal et al. (2006);
- Metal concentrations in the belowground biomass decreased with increasing distance from the inlet. Metal concentrations in the belowground biomass presumably included metals that were adsorbed and/or co-precipitated on the Fe- (oxy-) hydroxide plaques covering the root surface (Gries and Garbe, 1989; Mays and Edwards, 2001);
- Metal concentrations in the aboveground biomass were less subjected to spatial variations and generally not correlated with concentrations in the sediment and belowground biomass. However, Mn was an exception in the HSSF CW, reflecting the high mobility of this element;
- Overall, low percentages of the mass loadings of heavy metals were accumulated in the aboveground biomass and could be removed by harvest (Chapters 3, 4 and 5).

3.2 Indirect effects of helophytes on metal removal

Chapter 1 reviewed the possible indirect effects of helophytes on metal removal and stressed the need for more research in this area to substantiate these effects. The effects of radial oxygen loss (ROL) by plant roots on the process of sulphate reduction remains an interesting topic of debate. Relatively few researchers have assessed this effect, among which Borden et al. (2001) and O'Sullivan et al. (2004a, b). Chapter 6 contributed to this area of research and clearly demonstrated that *Phragmites australis* negatively affected bacterial sulphate reduction and precipitation of metals with sulphides in the root zone of SSF CW microcosms. Radial oxygen loss of the roots of *Phragmites australis* was responsible for this (Lesage et al., 2006).

However, ROL is not the sole process by which plants can affect sulphate reduction and metal removal. Plant roots can excrete a wide range of organic compounds that could serve as a carbon source and which could stimulate bacterial sulphate reduction. Stottmeister et al. (2003) stated that this carbon input by plants can be significant in CWs that receive wastewater with a low carbon load, such as the industrial wastewater studied in Chapter 6. Moreover, large amounts of decaying organic matter can be returned to the system at the end of the growing season (Gessner, 1996; Leuridan, 2004). This pool of organic matter could also serve as a carbon source for sulphate reducing bacteria (SRB). More research is needed in this area, as there are few quantitative studies covering this topic. Although applying to another process, Rousseau (2005) also related a better denitrification in a HSSF reed bed during the second year of operation to the input of organic matter at the end of the first growing season.

3.3 Selection of helophyte species and implications for design and operation

The negligible contribution of metal accumulation in the plant biomass to the overall metal removal and the negative effect of ROL by plant roots on the process of sulphate reduction (Lesage et al., 2004, 2006), question the use of helophytes in CWs. *"To plant or not to plant",* has been topic of many interesting debates at the conferences of wetland systems for water pollution control of the International Water Association (IWA). Although the role of aquatic macrophytes has been questioned (Mara, 2004), they are considered as an integral part of CWs (Brix, 1997, 2004). Vymazal (2006) recently stated that without plants, the use of the term "constructed wetlands" would no longer be valid.

Although the benefits of macrophytes have been demonstrated, possible differences among plant species are not well established. In their review on experimental studies dealing with the effect of helophyte species selection on the removal of SS, BOD, COD, N and P in CWs, Brisson et al. (2006) clearly observed a positive effect of plants and differences between species. As the observed effects were highly dependent on a variety of factors including the pollutant under study, type of wastewater, climate, experimental design, etc., little generalisation is possible that could guide species selection under any circumstances. There is a need for more research into the mechanisms that make one species more suited than another. The current study suggests that the selection of helophyte species mainly depends on the type of wastewater that is to be treated and the major removal mechanism that is being aimed at. Besides, the end-use of the biomass and aesthetic value also influences the choice of helophyte species.

In the case of CWs for domestic wastewater treatment, a high biomass producing species such as *Phragmites australis* should be selected when regular harvesting is feasible. The biomass could be used for the production of paper-pulp, roofing materials, forage feed, fertiliser or for bioenergy production. However, despite the potential useful applications and the high sustainability of such operation, plant biomass is usually not harvested from CWs in Europe (Stottmeister et al., 2003). As long as harvesting is not the case, species with a lower biomass production become more attractive than high biomass producing species, as discussed in Chapter 5.

In the case of SSF CWs for removal of metals from industrial effluents by inducing precipitation with sulphides, it would be preferable to plant the CWs with emergent wetland plants that have low radial oxygen losses and high carbon availability in the rhizosphere. In this way, integration in the landscape is still being achieved, but without impairing the treatment performance. The need for screening helophyte species towards their effects on rhizosphere conditions in CWs which rely on bacterial sulphate reduction, has very recently been stressed by Stein et al. (2005). Gagnon et al. (2006) showed a higher density and activity of aerobic or facultative bacteria in the rhizosphere of sand-based microcosms planted with *Phalaris arundinacea*, than in those planted with *Phragmites australis* or *Typha latifolia*. They related this to a higher oxygen release from roots of *Phalaris arundinacea*, caused by its rapid colonisation during the experiment, but stressed the need for longer experiments.

At last it is mentioned that the aesthetic value of plants, like *Iris pseudacorus*, can also affect the selection (Brix, 1997). Among the species tested in Chapter 5, *Carex acutiformis*, *Iris pseudacorus* and *Juncus effusus* are more attractive than a dense monotypic reed stand. In his keynote paper on the last IWA conference, Brix (2006) presented the "*Butterfly CW*" located in a tourist area in Thailand as an example of providing adequate wastewater treatment and creating a very appealing plant. Flowers of tropical plants such as *Canna* sp. and *Heliconia* sp. made the treatment plant aesthetically pleasing. Belmont et al. (2004) also underlined the economic benefits of using ornamental plants in CWs in developing countries. They showed that pilot-scale HSSF CWs planted with canna lily (*Canna flaccida*) and calla lily (*Zantedeschia aethiopica*) were equally efficient in removing COD, SS and N from domestic wastewater as systems planted with cattails. Constructed wetlands that are planted with a mixture of plant species also demonstrate a greater potential for wildlife habitat (Mackenzie et al., 2004).

4 Constructed wetlands offer potential for tertiary treatment of industrial wastewater

Whereas CWs are mainly used for treatment of domestic and dairy wastewater in Flanders (Rousseau et al., 2004), they have also been proposed as low cost and low maintenance alternatives or tertiary treatment steps for industrial effluents (Dunbabin and Bowmer, 1992; Qian et al., 1999; LeDuc and Terry, 2005). The potential of CWs for tertiary treatment of a case industrial wastewater was evaluated in two distinct types of CWs (Chapters 6 and 7).

In the gravel-based SSF CW microcosms with the helophyte *Phragmites australis*, heavy metals were effectively removed by immobilisation in the substrate. The FWS CW microcosms planted with the hydrophyte *Myriophyllum spicatum* also proved efficient in removing heavy metals. Sorption onto and accumulation in the plant biomass was responsible for about 45 % of the mass of metals removed. For Co and Ni, precipitation with CO_3^{2-} or OH^- was suggested as an additional plant-induced removal pathway. Chapter 7 showed that *Myriophyllum spicatum* was a better or equally good accumulator as some promising floating aquatic macrophytes reported in literature.

Both types of CWs efficiently reduced metal concentrations in the industrial effluent studied. When exposed to Co, Ni, Cu and Zn levels of respectively 1, 1, 0.1 and 1 mg l^{-1}, lower end concentrations were however achieved in the SSF CWs than in the FWS CWs after a residence time of 14 days. Whereas Co, Ni, Cu and Zn concentrations in the SSF CWs were reduced to below respectively 0.04, 0.07, 0.01 and 0.07 mg l^{-1}, their end concentrations were respectively 0.20, 0.20, 0.05 – 0.10 and 0.14 – 0.21 mg l^{-1} in the FWS CWs. Copper and Zn concentrations in the FWS CWs rapidly attained equilibrium and did not indicate a possible larger removal if the residence time would be extended. For Co and Ni, a constant removal rate of 2 µg l^{-1} h^{-1} was observed after 1 day of addition of the wastewater. When extrapolating this removal rate, it is predicted that a minimum additional residence time of 3 days would be necessary to reduce Co and Ni levels to 0.05 mg l^{-1}, and to be comparable with the ones of the SSF CWs.

4.1 Suggestions for design

Chapters 6 and 7 made some suggestions for design, of which the most important ones are discussed here for both types of CWs.

Gravel-based subsurface flow CWs planted with helophytes:

- Compaction of the filter and depletion of the carbon source are major disadvantages of solid carbon sources. Amending the wastewater with a soluble carbon source has therefore been suggested as the preferable alternative;
- The residence time is an important factor in the design of CWs that rely on the activity of SRB as it can affect sulphate reduction. A residence time of 14 days is suggested. Further research could however determine a threshold residence time;
- As described in section 3.3, the selection of helophyte species with low radial oxygen loss is preferable. Regular harvesting of the biomass is not mandatory;
- Treatment of 5000 m^3 d^{-1} in a gravel-based SSF CW with a depth of 1 m, requires a surface area of about 20 ha and daily carbon load of about 880 kg.

Free-water-surface CWs planted with *Myriophyllum spicatum*:

- Systems with a depth of 2 m were suggested, based on a range of depths reported in literature of water columns that can be colonised by *Myriophyllum spicatum*;
- A minimum residence time of 17 days is required to offer similar performance as the SSF CWs;
- Harvesting of the biomass is mandatory. A monthly biomass production of 3.4 ton DM ha^{-1} could be achieved and 6, 5, 3 and 10 kg ha^{-1} $month^{-1}$ of respectively Co, Ni, Cu and Zn could be extracted. The potential of combusting the biomass for bioenergy production needs more research;
- Treatment of 5000 m^3 d^{-1} requires a surface area of 3.5 ha.

4.2 Selection of a treatment option

The selection of a treatment option depends on a variety of factors, including the availability of land, climate, design and operation costs, potential end-uses of the biomass, etc. Subsurface flow CWs could be a viable technology for small-scale enterprises in both developed and developing countries. They are not suggested for the treatment of large wastewater flow rates where availability of land is low. They are suggested to offer year-round activity in both warm and temperate climates, as their activity is based on immobilisation processes in the substrate. However, a slight reduction of the activity of SRB has been reported by Borden et al. (2001) in winter conditions. Operation costs of the SSF CWs would be significantly reduced by mixing the industrial effluents with other types of wastewaters with a high carbon load or by applying a cheaper carbon source such as methanol. These last options need further research.

Free-water-surface CWs planted with *Myriophyllum spicatum* also offer potential for the polishing of industrial effluents but their full-scale use is limited for different reasons. First of all, as long as there are no disposal or regeneration pathways for the biomass and the biomass can not serve secondary functions, the high productivity of these systems is a major bottleneck. Secondly, there are concerns about accumulation of metals in the food chain by direct consumption or decomposition (Williams, 2002). The open character of these systems requires the implementation of strategies to prevent bioaccumulation. Kadlec & Knight (1996) stated that special care should be exercised not to attract mobile pollutant vectors to open wetland systems. Thirdly, the climate in most developed northern-hemisphere countries does not allow the year-round activity of such systems. At last it is mentioned that although this technology allows for the reduction of metal levels, it does not effectuate the simultaneous reduction of high sulphate levels.

Constructed wetlands could offer great advantages in developing countries (Denny, 1997; Kivaisi, 2001; Belmont et al., 2004; Brix, 2006). First of all, year-round growth of plants is possible in warmer climates. Secondly, availability of land is greater. At last it is mentioned that maintenance and harvesting schemes could provide employment. The construction and operation of systems for bioenergy recuperation could trigger the application of CWs in developing countries.

5 Conclusions

This study contributed to increasing our understanding of the behaviour and removal mechanisms of heavy metals in constructed wetlands. Moreover, it presents the first in-depth study of the removal of heavy metals in CWs for domestic wastewater treatment in Flanders.

In CWs for domestic wastewater treatment, heavy metals are mainly removed by accumulation in the sediment. It is suggested that metals are immobilised by filtration, sedimentation, precipitation and sorption processes. Manganese and Fe were poorly retained in the CWs studied and their dynamics were affected by redox conditions. Small-scale experiments demonstrated that the reduction of Fe- and Mn- (oxy-) hydroxides on the gravel surface could contribute to the release of Fe and Mn observed in full-scale CWs.

Both field surveys and small-scale experiments demonstrated that helophytes do not accumulate excessive concentrations of heavy metals in their aboveground biomass and have a negligible contribution to the overall metal removal. Overall, differences in uptake and translocation patterns of *Phragmites australis*, *Carex acutiformis*, *Iris pseudacorus* and *Juncus effusus* were small. *Phragmites australis* showed the largest biomass production of the 4 helophyte species studied. The species selection will probably be affected by factors including the secondary use of the harvested biomass and the aesthetic value of the plants.

Both SSF CWs planted with *Phragmites australis* and FWS CWs planted with *Myriophyllum spicatum* offer potential for tertiary treatment of industrial wastewaters. However, some practical limitations including land availability and secondary use of the plant biomass currently limit their full-scale application.

6 Future perspectives

Although insights into the removal processes of heavy metals have increased considerably over the last decade, an integrated metal removal model comprising the complex web of biotic and abiotic removal processes is yet to be developed. Modelling efforts should be performed to integrate the current knowledge. There is also a need for more advanced methods for speciation of metals in coarse-textured substrates.

Many research studies report on metal concentrations. However, with the aim of further elucidating the removal mechanisms, it is suggested to include at least the following parameters into future monitoring studies: Eh, pH and concentrations of DO, SO_4^{2-}, S^{2-}, OC, NO_3^-, Fe and Mn.

There is an urgent need for more research about the long-term accumulation of metals in the sediment of the CWs. As the number of CWs approaching life expectancy will be reached in the near future, their refurbishment has received increased scientific attention. Sufficient attention should be given in those studies to the behaviour of metals.

In line with the continuing debate on the role of emergent aquatic macrophytes, indirect effects of plants on metal removal, including radial oxygen loss and carbon input in the rhizosphere will need to be better understood. Eventually, the screening of different plant species for these characteristics will contribute to better design of CWs.

Finally, more research is needed on possible end-uses of harvested plant biomass, especially their potential use as organic fertiliser or bioenergy source. This could trigger their application, especially in developing countries.

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SUMMARY

Constructed wetlands (CWs) are successfully used for the treatment of domestic, agricultural and industrial wastewater, mine drainage, stormwater and landfill leachate, all of which can be contaminated with heavy metals. The removal of heavy metals in CWs is governed by a complex web of physico-chemical and biological processes. Awareness of the persistent nature of heavy metals and their accumulation in CWs have caused an acceleration in research over the last decade. Knowledge of the removal mechanisms has increased by monitoring full-scale CWs and performing small-scale experiments in more controlled conditions.

In a first part of this work, the behaviour of heavy metals in CWs for treatment of domestic wastewater in Flanders was studied. Before the start of this study, heavy metals in the influent and effluent were only monitored to a low-frequent and irregular extent, and their accumulation in the sediments and plants had never been subject of research. In the present study, data from long-term, weekly and short-term monitoring campaigns of influents and effluents of different types of CWs were taken into consideration. Heavy metal concentrations in the influent were generally subjected to a larger variability than in the effluent, demonstrating the buffering capacity of CWs. Total metal concentrations in the effluents were generally far below the basic environmental quality standards for surface water. The highest removal efficiencies of all metals except Fe and Mn, were observed in the horizontal subsurface flow (HSSF) CWs for secondary wastewater treatment. The retention of Mn and Fe was generally poor, a phenomenon presumably related to the reducing conditions prevailing in the sediments.

The study of the distribution of heavy metals in a free-water-surface (FWS) and a gravel-based HSSF CW for domestic wastewater, demonstrated that sediment was the most important sink for heavy metals. Although the FWS CW had been in operation for a period of 16 years, more than 5 times the age of the HSSF CW, the metal pollution level of the sediment of the former was lower than that of the latter one. Contrary to the uniform distribution of metals in the sediment of the FWS CW, the metals were mainly retained in the inlet area of the HSSF CW. After 3 years of operation, the sediment in the inlet area of the HSSF CW was significantly contaminated with Cd, Cu and Zn. Metals are retained by filtration, sedimentation, precipitation and sorption processes.

A second part of this work deals with the role of helophytes in CWs. In both field surveys and small-scale experiments, it was shown that helophytes do not accumulate excessive concentrations of heavy metals in their aboveground biomass and have a negligible contribution to the overall metal removal. In a comparative screening of 4 helophyte species, differences in uptake and translocation patterns of *Phragmites australis*, *Carex acutiformis*, *Iris pseudacorus* and *Juncus effusus* were observed to be small. However, helophytes can also indirectly affect the removal of heavy metals. In an experiment with gravel-based SSF CWs, it was observed that radial oxygen loss from the roots of *Phragmites australis* negatively affected bacterial sulphate reduction and precipitation of metals with sulphides. From the monitored direct and indirect effects, it could be suggested that the selection of helophyte species should mainly depend on the type of wastewater that is to be treated and the major removal mechanism that is being aimed at. Additional criteria for determining the choice of helophyte species are the end-use of their biomass and their aesthetic value.

Whereas CWs are mainly used for domestic and dairy wastewater in Flanders, they also offer potential as tertiary treatment steps for industrial effluents. In a third part of this work, the potential of two distinct types of CWs was evaluated for the tertiary treatment of a case industrial wastewater contaminated with Co, Ni, Cu, Zn and sulphates. The gravel-based SSF CWs with the emergent plant *Phragmites australis* effectively immobilised heavy metals in the substrate. Sorption processes were responsible for short-term metal removal, whereas precipitation with sulphides provided long-term removal. When comparing a solid and soluble carbon source to stimulate the process of sulphate reduction, the soluble carbon source was suggested as the preferable alternative. The FWS CWs planted with the submerged plant *Myriophyllum spicatum* also proved efficient in removing heavy metals by sorption onto and accumulation in the plant biomass. Both types of CWs provide possibilities for tertiary treatment of industrial wastewaters, especially in developing countries.

SAMENVATTING

Aangelegde moerassen kunnen toegepast worden voor de zuivering van huishoudelijk, agrarisch en industrieel afvalwater, effluenten van mijn- en stortactiviteiten, en overstort- en stormwater. Deze wateren kunnen gecontamineerd zijn met zware metalen. De verwijdering van zware metalen in aangelegde zuiveringsmoerassen wordt gewaarborgd door een complex samenspel van fysico-chemische en biologische processen. Een groeiend bewustzijn van de persistente aard van zware metalen en hun accumulatie in zuiveringsmoerassen, hebben tot een versnelling van het wetenschappelijk onderzoek geleid tijdens de laatste tien jaar. De toename aan kennis omtrent verwijderingmechanismen is enerzijds te danken aan het bestuderen van zuiveringsmoerassen op veldschaal en anderzijds aan het uitvoeren van experimenten op kleine schaal in meer gecontroleerde omstandigheden.

Een eerste deel van dit werk handelt over het gedrag van zware metalen in aangelegde moerassen voor de zuivering van huishoudelijk afvalwater in Vlaanderen. Voor de aanvang van deze studie, gebeurde de opvolging van zware metalen in het influent en effluent slechts op onregelmatige basis en met een lage frequentie. Bovendien was de accumulatie van zware metalen in het sediment en de vegetatie geen voorwerp van onderzoek. Data verzameld aan de hand van meetcampagnes op lange-termijn, wekelijkse en korte-termijn van de influenten en effluenten van verschillende types zuiveringsmoerassen, werden in beschouwing genomen in deze studie. Concentraties van zware metalen in het afvalwater werden gekenmerkt door een grotere variabiliteit dan deze in het effluent. Dit wijst op de bufferende werking van deze zuiveringsmoerassen. Metaalconcentraties in de effluenten waren meestal beduidend lager dan de basismilieukwaliteitsnormen voor oppervlaktewater. De wortelzonerietvelden voor secundaire waterzuivering vertoonden de hoogste verwijderingsefficiënties voor alle zware metalen, met uitzondering van Fe en Mn. De matige tot slechte retentie van Mn en Fe werd waarschijnlijk veroorzaakt door de reducerende omstandigheden in het sediment.

In een studie van de distributie van zware metalen in twee moerassen voor de zuivering van huishoudelijk afvalwater, een vloeirietveld en een wortelzonerietveld met grind als filtermedium, werd aangetoond dat zware metalen voornamelijk verwijderd worden door accumulatie in het sediment. De vervuilingsgraad van het sediment van het vloeirietveld na 16 jaar werking was veel lager dan dat van het wortelzonerietveld, ondanks de meer dan 5 maal

jongere leeftijd van dit laatste rietveld. In tegenstelling tot de uniforme distributie van metalen in het sediment van het vloeirietveld, werden metalen voornamelijk geconcentreerd in het sediment van de inlaatzone van het wortelzonerietveld. Een significante contaminatie met Cd, Cu en Zn van dit sediment werd waargenomen na een werkingsperiode van 3 jaar. Metalen worden verwijderd door processen zoals filtratie, sedimentatie, neerslag en sorptie.

Een tweede deel van dit werk gaat dieper in op de rol van helofyten in aangelegde zuiveringsmoerassen. In zowel veldstudies als experimenteel onderzoek werd duidelijk aangetoond dat helofyten geen sterk verhoogde concentraties van zware metalen accumuleren in hun bovengrondse biomassa. Bovendien dragen ze weinig bij tot de verwijdering van metalen. In een vergelijkende studie van 4 helofytensoorten, werden weinig verschillen waargenomen in opname- en translocatiepatronen tussen *Phragmites australis*, *Carex acutiformis*, *Iris pseudacorus* en *Juncus effusus*. Helofyten kunnen echter eveneens op indirecte manier een invloed uitoefenen op metaalverwijdering, bijvoorbeeld door zuurstofafgifte van de wortels. In een experiment met door riet beplante grindfilters, werd een negatief effect van deze zuurstofafgifte waargenomen op de reductie van sulfaten en de neerslag van metalen met sulfiden. Op basis van deze directe en indirecte effecten, werd aangeraden de selectie van helofytensoorten voornamelijk af te stemmen op het te behandelen afvalwater en het beoogde verwijderingmechanisme. Aanvullende criteria die de keuze beïnvloeden zijn o.a. mogelijke toepassingen van de geoogste biomassa en de esthetische waarde van de helofyten.

Aangelegde moerassen worden in Vlaanderen hoofdzakelijk toegepast voor de zuivering van huishoudelijk en agrarisch afvalwater. Nochtans kunnen ze eveneens toepassingen bieden voor de tertiaire zuivering van industriële effluenten. In een derde deel van dit werk werd de mogelijke toepassing van twee verschillende types zuiveringsmoerassen voor een industrieel afvalwater gecontamineerd met Co, Ni, Cu, Zn en sulfaten onderzocht. De zware metalen werden efficiënt geïmmobiliseerd in het substraat van een met riet beplante grindfilter. Verwijdering op korte termijn werd gewaarborgd door sorptie, verwijdering op lange termijn door neerslag met sulfiden. Op basis van een vergelijking studie van een vaste en oplosbare koolstofbron, toegediend om de sulfaatreductie te stimuleren, bleek een oplosbare koolstofbron het meest geschikt. Vloeivelden gekoloniseerd door *Myriophyllum spicatum* waren eveneens efficiënt in de verwijdering van metalen, mede door sorptie en plantopname.

Beide types aangelegde moerassen hebben toepassingsmogelijkheden voor de tertiaire zuivering van industrieel afvalwater, in het bijzonder in ontwikkelingslanden.

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Van de Moortel, A.M.K., **Lesage, E.**, Tack, F.M.G. and De Pauw, N. (2005). Evaluation of the role of the root surface of aquatic macrophytes in the removal of heavy metals in constructed subsurface flow wetlands. In: Book of abstracts of the 1st International Symposium on Wetland Pollutant Dynamics and Control, September 4-8, 2005, Ghent, Belgium, 289-290.

Lesage, E., Tack, F.M.G., De Pauw, N. and Verloo, M.G. (2006). Advanced treatment of industrial wastewater by the submerged aquatic plant *Myriophyllum spicatum*. In: Book of abstracts of the 6th International Workshop on Nutrient Cycling and Retention in Natural and Constructed Wetlands, May 31 - June 4, 2006, Třeboň, Czech Republic.

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ATTENDED SYMPOSIA, CONFERENCES, AND WORKSHOPS

8th PhD Symposium on Applied Biological Sciences, Ghent, Belgium, October 9, 2002.

1st International Seminar on the Use of Aquatic Macrophytes for Wastewater Treatment in Constructed Wetlands, Lisbon, Portugal, May 8-10, 2003.

6th International Symposium on Environmental Geochemistry, Edinburgh, Scotland, September 7-11, 2003.

8th International Symposium on Biogeochemistry of Wetlands, Ghent, Belgium, September 14-17, 2003.

Education Day ‘Small-scale wastewater treatment’ (Technological Institute – Environmental Technology), Antwerp, Belgium, October 9, 2003.

9th PhD Symposium on Applied Biological Sciences, Leuven, Belgium, October 16, 2003.

52th Post-University Education Day ‘Biomonitoring of the environment: different measurement is more knowledge?’, Ghent, Belgium, December 3, 2003.

NecoV Winter Meeting ‘Ecotechnics and Nature Development (Netherlands-Flemish Ecological Society), Ghent, Belgium, January 14-15, 2004.

9th International Conference on Wetland Systems for Water Pollution Control (IWA specialist group), Avignon, France, September 26-30, 2004.

9th International Symposium on Biogeochemistry of Wetlands, Baton Rouge, USA, March 20-23, 2005.

1st International Symposium on Wetland Pollutant Dynamics and Control, Ghent, Belgium, September 4-8, 2005.

Education Day ‘Heavy metals in the environment: new insights, new laws?’ (Katholieke Universiteit Leuven), Heverlee, Belgium, September 23, 2005.

Education Day ‘Small-scale wastewater treatment by means of plant systems’ (Hogeschool West-Vlaanderen), Kortrijk, Belgium, March 1, 2006.

6th International Workshop on Nutrient Cycling and Retention in Natural and Constructed Wetlands, Třeboň, Czech Republic, May 31 – June 4, 2006.

12th Symposium on Applied Biological Sciences, Ghent, Belgium, September 21, 2006.

10th International Conference on Wetland Systems for Water Pollution Control (IWA specialist group), Avignon, France, September 23-29, 2006.

SUPERVISION OF SCRIPT STUDENTS

Sarah Roggeman, MSc. Environmental Science and Technology (2002-2003). Tolerance of helophytes applied for rhizofiltration of wastewater contaminated with heavy metals.

Ingeborg Leuridan, Bio-engineer Environmental Technology (2003-2004). Complexation of copper by decomposition residuals of *Phragmites australis* in surface flow constructed wetlands.

Annelies Van de Moortel, Bio-engineer Environmental Technology (2003-2004). Distribution and removal of heavy metals in helophyte filters.

Kim De Block, Bio-engineer Environmental Technology (2004-2005). Accumulation of metals in reed beds for the treatment of domestic wastewater.

Maja Šimpraga, MSc. Environmental Sanitation (2004-2005). Metal accumulation in a constructed surface flow wetland.

Mathias Vandamme, Bio-engineer Environmental Technology (2004-2005). Plant-based systems for tertiary treatment of industrial wastewater.

Soetkin Gardin, Isabelle Joos, Karolien Scheerlinck and Nina Van Den Bilcke, project report (2005-2006). Small-scale wastewater treatment by means of reed beds: future blackpoints?

Charity Mundia, MSc. Environmental Sanitation (2005-2006). The use of constructed wetlands for removal of heavy metals from industrial wastewater (Sidmar environmental prize).

JURY MEMBER OF SCRIPT STUDENTS AT GHENT UNIVERSITY

Sebastiaan De Smedt, Robert Gruwez, Wouter Sysmans and Renke Vandeweyer, project report (2004-2005). Ecology of ‘green’ tertiary (wastewater) treatment systems. Promotor: Prof. dr. N. De Pauw.

Melanie Franck, MSc. Environmental Science and Technology (2004-2005). Ecology of treatment wetlands: assessment by means of plankton and macrophytes. Promoters: Prof. dr. N. De Pauw and ir. D. Rousseau.

Abigail Marie L. Torres, MSc. Environmental Sanitation (2004-2005). Subsurface flow constructed wetlands (SSF CW): pilot-scale experiments and applicability in the Philippines. Promoters: Prof. dr. N. De Pauw and ir. D. Rousseau.

Hannelore Six, MSc. Environmental Science and Technology (2005-2006). Mobility of nutrients and heavy metals in sediments of a combined sewer overflow detention basin. Promotor: Prof. dr. ir. F. Tack.

REVIEW ACTIVITIES FOR INTERNATIONAL JOURNALS

Aquatic Ecology (1)
Science of the Total Environment (2)

ORGANISATION OF INTERNATIONAL CONFERENCES

Member of the organising committee of the 1st International Symposium on Wetland Pollutant Dynamics and Control (WETPOL), Ghent, Belgium, September 4-8, 2005.

AWARDS

3rd poster price at the NecoV Winter Meeting (Netherlands-Flemish Ecological Society), Ghent, Belgium, January 14-15, 2004.