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Constructed wetlands and their performance for treatment of water contaminated with arsenic and heavy metals

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Constructed wetlands and their performance for treatment of water contaminated with arsenic and heavy metals

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Constructed wetland models were investigated for removal of As and heavy metals from wastewater. The data on physical, biological and chemical factors in laboratory experiments with river sludge sediment, which resembles natural wetland bed showed that, under anaerobic conditions, As even decreased when the activity of microorganisms was inhibited. Abiotic processes, i.e. adsorption to clay particles, precipitation and co-precipitation with Zn and S^{2-} thus probably enhanced the decrease of As under anaerobic conditions.

In batch models of wetland systems, the performances of heavy metal removal from artificial wastewater varied with the type of the constructed wetland. The subsurface wetland (SSW) and free surface wetland (FSW) removed heavy metals better than hydroponic system (HP) and algae pond (AP). The combination of gravel bed and plants (e.g. *Juncus effusus*) resulted in a high removal rate. The heavy metals mostly accumulated in the plant roots. The remaining amounts were bound to the gravel, precipitated, attached to or incorporated into the cells of microorganisms and adsorbed on sediment to the bottom.

Also a two step constructed wetland system with continuous flow, consisting of HP and FSW was tested for the removal of As, Zn and Cr from an artificial wastewater. A high removal rate was observed in the first phase of the experiment, when a high load of carbon source was supplied, which enhanced anaerobic conditions. Zn, As and Cr probably precipitated with iron and S^{2-}, which were present in the systems. As(V) could precipitate as $FeAsO_4$ or be immobilized on hydrated iron oxides. Under anaerobic conditions, As(V) was reduced to As(III), which could precipitate with S^{2-}. The average removal efficiencies of the HP decreased in the sequence Cr ≈ Zn > As (118, 114 and 18 mg/m^2d, respectively). The characterization of the microbial community showed that there were many types of microorganisms living in the system, which in turn were probably involved in the removal of As, Zn and Cr.

As the toxicity and the environmental behaviour of As strongly depends on the species in which it is present; also the As speciation was investigated in the experiments described above. The data show that methylated arsenic species occurred under reducing conditions. In particular, As(III) was found in compartments with low concentration of oxygen, i.e. near the bottom of the SSW and FSW wetlands, and in the HP of the two-step constructed wetland. Methylated arsenic was also found in the AP due to the appearance of algae which could transform toxic As(V) to other non-toxic As species.

The planted FSW in a field test was highly effective for treatment of acidity and metals from acid mine drainage, with a removal capacity for acidity of about 34-51 mmol $NaOH/m^2d$, for Zn of about 4-10 mg/m^2d and for Fe of about 73-122 mg/m^2d. SSW and HP also remove acidity and metals, although to a lower extent. The hydroponic systems had significantly less capacity for the removal of all parameters than the systems containing soil material.

The plants in the system promoted the neutralization and took up metals from wastewater. In both FSW and SSW, Zn and Fe were accumulated in the roots and at the root surface rather than in the shoots. Soil materials were found to accumulate Fe rather than Zn, especially in the planted FSW.

In conclusion, constructed wetland systems with a combination of gravel/soil matrix and plants have a high removal rate of heavy metals in both lab models and a field test system.

Zusammenfassung

In dieser Arbeit wurde die Eliminierung von As und Schwermetallen aus Abwasser in Modellen für Pflanzenkläranlagen untersucht. Die Ergebnisse aus Laborexperimenten mit Flußsediment, das dem Bodenmaterial aus Pflanzenkläranlagen ähnlich ist, zeigen, dass die As-Konzentration auch dann abnimmt, wenn die mikrobielle Aktivität gehemmt wurde. Abiotische Prozesse, d.h. Präzipitation und Kopräzipitation und Adsorption an Tonmineralien mit Zn und S^{2-} erhöhen deshalb vermutlich die Eliminierung von As aus Abwasser unter anaeroben Bedingungen.

In Batch-Modellen für Pflanzenkläranlagen, hing die Eliminierungsrate für Schwermetalle aus künstlichem Abwasser vom Typ der Anlage ab. Subsurface wetlands (SSW) und free surface wetlands (FSW) eliminierten die Schwermetalle besser als Hydroponiksystem (HP) und Algenteich (AP). Die Kombination von Kiesbett und Pflanzen (*Juncus effusus*) führte zu einer hohen Eliminierungsrate. Die Schwermetalle akkumulierten vor allem in den Pflanzenwurzeln. Der Rest wurde an den Kies gebunden, ausgefällt, an Mikroorganismenzellen gebunden oder in diese aufgenommen oder an das Sediment in den Anlagen gebunden.

Weiterhin wurde ein zweistufiges Pflanzenkläranlagenmodell mit kontinuierlichem Fluß, das aus einem HP und einem FSW bestand, auf seine Eliminierungsleistung für As, Zn und Cr aus künstlichem Abwasser hin untersucht. Hohe Eliminierungsraten wurden in der ersten Phase des Experiments beobachtet, in der eine hohe Fracht einer organischen C-Quelle zugeführt wurde, was die Bildung anaerober Bedingungen förderte. As, Zn und Cr fielen vermutlich zusammen mit Fe und S^{2-} aus, die in dem System vorhanden waren. Unter anaeroben Bedingungen wurde As(V) zu As(III) reduziert, das mit S^{2-} ausfallen kann. Die mittleren Eliminierungsraten des Hydroponiksystems nahmen in der Reihenfolge Cr ≈ Zn > As (118, 114 bzw. 18 mg/m^{2}d) ab. Die Charakterisierung der mikrobiellen Gemeinschaft zeigte, dass viele Mikroorganismenarten in dem System lebten, die vermutlich an der Eliminierung von As, Zn und Cr beteiligt waren.

Da die Toxizität und das Umweltverhalten von As stark davon abhängt, in welcher Form es vorliegt, wurde auch die As-Speziation untersucht. Die Ergebnisse zeigen, dass unter reduzierenden Bedingungen methylierte As-Verbindungen gebildet werden. Insbesondere wurde in Kompartimenten mit geringen Sauerstoffkonzentrationen, d.h. am Boden von FSW und SSW sowie im HP des zweistufigen Pflanzenkläranlagemodells, As(III) gefunden. Methylierte As-Verbindungen wurden auch in AP gefunden, weil dort spontan Algen wuchsen, die As(V) in andere, weniger toxische As-Spezies umsetzen können.

Die bepflanzte FSW in einem Feldversuch entfernte sehr effizient Azidität und Metalle aus einem sauren Grubenwasser. Die Eliminierungskapazität für Azidität betrug etwa 34 - 51 mmol NaOH/m^{2}d, für Zn etwa 4 - 10 mg/m^{2}d und für Fe etwa 73 - 122 mg/m^{2}d. SSW und HP konnten ebenfalls Azidität und Metalle aus dem Abwasser eliminieren, aber in einem geringerem Maß. Die HP-Systeme hatten eine signifikant geringere Kapazität als die Systeme, die Bodenmaterial enthalten.

Die Pflanzen in den Systemen förderten die Neutralisierung und nahmen Metalle aus dem Abwasser auf. Sowohl in FSW als auch in SSW, wurden Fe und Zn eher in den Wurzeln und an den Wurzeloberflächen akkumuliert als in den Sprossen. Das Bodenmaterial akkumulierte mehr Fe als Zn, insbesondere in den bepflanzten FSW.

Insgesamt zeigte sich, daß vor allem die Kombination von Kiesbett bzw. Bodenmaterial und Pflanzen zu hohen Eliminierungsraten in den Pflanzenkläranlagen führte.

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Abbreviation directory

AMD	acid mine drainage
AP	algae pond
As(V)	arsenate
As(III)	arsenite
DMD	dimethylarsinic acid (cacodylic acid)
DNA	deoxyribonucleic acid
DO	dissolved oxygen
Eh	redox potential
FSW	free surface wetland
HP	hydroponic system
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
IC	ion chromatography
IC-ICP-MS	ion chromatography coupled with inductively coupled plasma mass spectrometry
ICP-AES	inductively coupled plasma atomic emission Spectrometry
MMA	monomethylarsonic acid
MPN	most probable number
PCR	polymerase chain reaction
RNA	ribonucleic acid
SSW	subsurface wetland
SRB	sulfate reducing bacteria
SSCP	single strand conformation polymorphism
TMAO	trimethylarsine oxide
bp	base pair
dw	dry weight

1 Introduction

Acid mine drainages (AMD) pose worldwide environmental problems of big dimensions wherever mining occurs. The best documented regions for environmental problems by AMD are Canada and the USA (Reuther, 1995). For Canada, Filion et al. (1990) identified a total area of more than 15000 hectares of acid-generating waste sites associated with both operating and abandoned mines.

In general, contaminated mine water is generated when rock containing sulfidic minerals is exposed to water and oxygen. This results in the production of acidity and elevated concentrations of metals and sulfate in the water (Braun et al., 2001). In this way, the generation of contaminated mine water is a combined chemical and microbiological process (Stumm and Morgan, 1996). Factors influencing the acidity and metal content are sulfide grain size and surface area, porosity and permeability of the deposit, nature of the gangue materials, nature of the sulfide ore, nature of acid-consuming minerals and various environmental factors influencing the activity of micro-organisms (Ritcey, 1989). The generation of the water is highly site specific, and can vary greatly even within a single mine site and the chemical composition may be very different including elements like Al, As, Cd, Cu, Fe, Pb, Mn, Ni, Zn with higher or lower concentrations of sulfate and pH-values ranging from 2.6 to 7.5 (Ritcey, 1989; Wildeman and Laudon, 1989; Dodds-Smith, et al., 1995).

The four main characteristics of contaminated mine water that have the potential to affect the environment are acidity, ferric iron (Fe^{3+}) precipitates, trace metals and turbidity (Kelly, 1988). The importance of each factor varies within and between affected ecosystems and the low pH and the toxicity of trace metals are the most troublesome environmental problems (Kelly, 1988; Wildeman et al., 1991; Connel and Miller, 1984; Kabata-Pendias and Pendias, 1992). Cu^{+} and Pb^{4+} followed by Cu^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+} and other metal ions as well as As(III), As(V) exhibit a broad range of toxicity mechanisms (Connel and Miller, 1984). Moreover, arsenic wastes are also released by many other industries, such as the chemical and electronic industry which use arsenic as a material in their processes, and the production of semiconductors and solar cells, which implies worldwide increasing environmental problems correlated to human health (Gong et al., 2001; Chris Le, 2001; Williams et al., 1996).

In general, the environmental problems by AMD are in the focus of politics and societies in the member states of the EU and in the USA, Canada and Australia. In Africa, Asia and Latin-America environmental protection mostly is considered for new modern mines but

lacking for older or disused mines. The most poorly documented regions are probably the eastern European countries (Reuther, 1995; Braun et al., 2001).

The primary aims of the treatment of contaminated mine water are to neutralize acidity and to remove metals (Braun et al., 2001). In principle, two broad categories of available types of treatment can be used: i) active systems mainly for continuous operation and maintenance and ii) passive systems, which are intended to be self-sustaining after an initial start-up period (Braun et al., 2001). Active systems involve technologies like pH modification, ion exchange, biology-based treatments, adsorption, electrochemical treatment and physical process technologies. These systems are useful in conjunction with operating mines where the scale of the problem is such as to make passive treatment unrealistic or if it is necessary to find short-term or quick-fix solutions (Braun et al., 2001). Passive treatment means i) chemical passive treatment by addition of chemicals like limestone, polymers or others (Braun et al., 2001; Brodie et al., 1993; Ziemkiewicz et al., 1997) and ii) biological passive treatment represented by primary constructed wetlands and secondary algal systems or special bioreactors (Braun et al., 2001; Foster, 1982; Phillips et al., 1994; Bender, et al., 1994; Davison, 1993; Duc et al., 1998). The passive treatment systems and particularly constructed wetlands are advantageous mainly because they cause comparably low costs, are truly self-sustaining and suitable for the treatment of mine water from abandoned mines (Braun et al., 2001).

Constructed wetlands have been used for mine water treatment for about 20 years. However the potential longevity of constructed wetlands for mine water treatment is currently not known and the design concepts and the sizing criteria are still under discussion (Braun et al., 2001).

Moreover, it is necessary to understand the fundamental processes and mechanisms operative in mine water treatment wetlands to realize long-term stable and highly effective removal effects. Because of the very different site-specific wastewater qualities and the very great variability according to sizing, design and fundamental flow characteristics of the constructed wetlands used, it is difficult to compare the efficiencies by literature data, and specific removal rates are often lacking in principle. Till now no fundamental data for comparing the different wetland systems like surface flow, subsurface flow and free water flow constructed wetlands and for evaluating the particular importance of the plants, the soil materials and the micro-organisms are available.

Because of these deficits basic investigations on the functioning of constructed wetlands are necessary. In this way the removal efficiencies for iron, zinc, chromium and arsenic and

the neutralization of mining and synthetic wastewaters were investigated using laboratory-scale and small field constructed wetlands.

1.1 Objectives

1. The removal of arsenic, zinc and chromium in laboratory-scale constructed wetlands was investigated by continuous and discontinuous long-term experiments using synthetic wastewater. Removal rates were calculated, the efficiencies of different wetland systems, represented by a surface flow, subsurface flow, and a hydroponic system were compared, and the specific importance of the soil-bed, the plants, and the microorganisms were evaluated.
2. The removal of iron and zinc as well as the neutralization of an acid mine drainage wastewater in constructed wetlands by continuous long-term field experiments were investigated. The efficiencies of surface flow, subsurface flow, and hydroponic systems, each planted and unplanted, were evaluated and compared.

2 Literature review

2.1 Wetland definition and classification

Constructed wetlands can be divided into various types depending on different characteristics. If the flow path of the water in the systems is of interest, constructed wetlands can be divided into Free Water Surface Wetlands (FSW) where the surface of the water is exposed to the atmosphere as it flows through the bed and Subsurface Flow Wetland (SSW) (Reed, 1991). FSW contain appropriate emergent aquatic vegetation in a relatively shallow bed or channel. In tropical countries, FSW are generally favoured because of their lower capital and operating costs. In SSW the water level is maintained below the surface of the soil materials, which is permeable media or substrate (rock, gravel, sand, etc.). The depth of the soil media is typically 0.3 - 0.6 m. Wastewater flows and contacts a mixture of microbes living in association with substrate and plant roots. A liner is also used to protect groundwater quality.

According to the flow-direction of the water in the systems, wetlands can also be classified as horizontal flow and vertical flow wetlands (Cooper et al., 1996). During the passage through horizontal flow wetlands the wastewater comes into contact with a network of aerobic and anaerobic zones. Vertical-flow wetlands are usually fed intermittently. The wastewater drains down through the bed and is collected by drainage network at the base. The next dose of wastewater traps the air and this together with the aeration caused by the rapid dosing onto the bed leads to good oxygen transfer (Kadlec and Knight, 1996).

2.2 Acid mine drainage (AMD)

Acid mine drainages (AMD) or the wastewaters from mining areas usually have a low pH and high acidity. (That is why it is called acid mine drainages.) AMD contain significant amounts of mineral acidity or sulphuric acid if sulfur, sulfide, or iron pyrites are present. The conversion of these materials produces sulphuric acid and sulfate by sulfur-oxidizing bacteria under aerobic conditions.

$$2S + 3O_2 + 2H_2O \xrightarrow{bact.} 4H^+ + 2SO_4^{2-}$$

$$FeS_2 + 3\frac{1}{2}O_2 + H_2O \xrightarrow{bact.} Fe^{2+} + 2H^+ + 2SO_4^{2-}$$

Salts of heavy metals, particularly those with trivalent metal ions, such as Fe(III), hydrolyze in water and release mineral acidity.

$$FeCl_3 + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+ + 3Cl^-$$

The presence of metals in the solutions is indicated by the formation of a precipitate as the pH is increased during neutralization.

The acid mine wastewaters has influent total iron concentrations of 250 mg/l and above. They must be reduced to less than an average of 3 mg/l (Wieder, 1989). The high concentration of iron may result from natural or artificial sources, typically as seeps of ferrous iron and iron sulfide (pyrites) from anaerobic groundwater and as oxidation of iron sulfide exposed during surface mining (Kadlec and Knight, 1996).

Wetland processes identified as having a potential for removing metals from AMD include: adsorption of metals by ferric oxy hydroxides; plants and algae uptake of metals; complexation of metals by organic materials; and precipitation of metals into oxides, oxy-hydroxides or sulfides. The precipitation as either oxides or sulfides has long-term metal removal potential (Kadlec and Knight, 1996)

Microbially-driven sulfate and iron reduction are processes occurring naturally in wetland sediments which facilitate the removal of metals from acid mine drainage (AMD) through increasing the pH, which in turn results in precipitation of the metals either as hydroxides or as sulfides (Fayson et al., 1994). The sulfate reducing bacteria in the anaerobic zone of wetlands consume acidity, and most of hydrogen sulfide they produce reacts with heavy metals to yield insoluble precipitates (Evangelou, 1998). Adsorption processes, which assist in metal removal, can also be active in wetlands.

2.3 Wetland and the removal of arsenic and heavy metals

Wetlands are widely used and have been reported in a variety of formats and level of detail (Kadlec and Knight, 1996). Wetlands have a capacity for metal removal. Plants can break down, or degrade organic pollutants or stabilize metal contaminants by acting as filters or traps (EPA, 1996).

The performance of constructed wetlands for wastewater treatment depends on many factors, including the type of pre-treatment, influent concentration, flow, wetland type, wetland size, and soil (Brown, 1994).

The pollutants in constructed wetlands are removed through a combination of biological, physical, and chemical processes including assimilation by the plant tissue and microbial transformations. The performance of artificial wetlands shows that suspended solids and readily biodegradable organic matter are generally removed effectively (Brix and Schierup, 1989).

Physical, chemical and biological processes are involved in the removal of heavy metals. The major mechanisms are:

- Adsorption and binding to soils, sediments, particulates, and soluble organics.
- Precipitation as insoluble sulfides, carbonates and oxyhydroxides.
- Uptake and accumulation by plants, including algae, and by bacteria.
- Volatilization as volatile metal species as a result of microbial action or by plant, phytovolatilization.

Phytovolatilization occurs as plants take up contaminated wastewater. The plant roots take up heavy metals and other components through the leaves and the heavy metals are released as volatile species to the atmosphere.

The study of Chen et al. (2000) on phytoremediation of soil contaminated with heavy metals showed that soil contaminated with heavy metals could be remediated with a combination of chemical treatment and plants.

Sobolewski (1996) studied the copper removal from acid mine drainage by constructed wetlands. The two, large and small, constructed wetlands were lined and planted with floating peat mats. Their vegetations were sedge, *Carex rostrata*, and cattail, *Typha latifolia*. It was found that most of the copper was in peat samples near the inlet, predominantly in the organic and exchangeable phases. The mass of Cu recovered from inlet samples represented 68 and 51% of the total mass from large and small wetlands, respectively. It was likely that much of the Cu recovered in the exchangeable phase was actually organic complexes. Moreover, the Cu was recovered in an iron oxide phase, 17 and 25% of total mass of Cu from the large and small wetlands, respectively. Furthermore, it was suggested that the low hydraulic retention time effected to their removal performance.

2.3.1 Physical and chemical factors effecting to the performance of constructed wetland

2.3.1.1 Precipitation and co-precipitation

Precipitation is an important process for metal removal. Removal of metals such as copper or zinc can also take place through sorption or co-precipitation on the surface of iron and manganese oxides (Sobolewski, 1996).

Aerobic processes in the wetland system cause the precipitation of some metals, for example, iron. The oxidation of ferrous iron to ferric iron and the subsequent precipitation of iron oxyhydroxide is a dominant process:

$$4Fe^{2+} + O_2 + 6H_2O \rightarrow 4FeOOH \downarrow + 8H^+$$

In wetlands, formation of sulfide may provide long-term metal removal, and many metals found in mine drainage form highly insoluble precipitates in the presence of dissolved sulfide (Stumm and Morgan, 1996). Sulfide precipitation relies on production of S^{2-} in the sulfate reduction zone of the wetland soil profile. This requires low redox potentials associated with anaerobic conditions. Metals are precipitated from the solution as the insoluble metal sulfide. The basic reaction is as follow:

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

where M^{2+} represents a divalent metal ion, such as Fe^{2+} or Zn^{2+}

The sulfide precipitation also requires a sufficient source of sulfate to match the metal requirement. For instance, precipitation of 1.0 mg/l Cadmium (atomic weight 112.4) requires the reduction of 0.85 mg/l of sulfate (molecular weight 96.1) to obtain the required sulfide. It is a fact that these metal sulfides will remain permanently in wetland sediments as long as they are not re-oxidized or as long as the sediments remain anaerobic (Sobolewski, 1996). Consequently, it is important to induction anaerobic system in wetlands for a high capacity of metal removal.

2.3.1.2 pH and redox potential

In sub-oxic conditions, low redox potential are generated by the biological oxidation of organic carbon and concomitant reduction of oxygen, nitrate, manganese and iron oxides, and sulfate (Hering and Kneebone, 2001).

Both physical and chemical factors, pH and the oxidation/reduction state or redox condition (Eh), are important for the formation and transformation of heavy metals in constructed wetlands. They are the most important factors controlling As and heavy metal speciation and their distribution. The redox condition (Eh) of wetland soil and sediment vary widely from approximately +500 mV (surface soil) to approximately -320 mV (strongly reducing soil). Sediment redox levels can greatly affect toxic metals uptake by plants (Guo et al., 1997). Plant arsenic tissue concentrations and uptake were highest under reduced soil conditions (Marin et al., 1993). Redox condition can affect the degradation and solubility of organic material and then influence the release of heavy metals. Heavy metals can also exist as sulfides under anaerobic conditions, which are susceptible to Eh and pH changes (Gambrell et al., 1980).

Under oxidizing conditions, $H_2AsO_4^-$ is dominant at low pH (less than about pH 6.9), whilst at higher pH, $HAsO_4^{2-}$ becomes dominant ($H_3AsO_4^0$ and AsO_4^{3-} may be present in extremely acidic and alkaline conditions, respectively). Under reducing conditions at pH less than about pH 9.2, the uncharged arsenite species $H_3AsO_3^0$ will predominate (Smedley and Kinniburgh, 2002).

2.3.1.3 Evaporation

Evaporation is the net water loss caused by the evaporation of moisture from the soil surface. It is assumed that, for a wetland system, although the presence of vegetation retards evaporation, by increasing shade and humidity and reducing wind near the surface, transpiration by the vegetation compensates for the difference. It is also influenced by vegetation on the disposal field.

Evapotranspiration can remove high volumes of effluent in the late spring, summer, and early fall, especially if large silhouette and good transpiring bushes are used (EPA, 1998).

2.3.2 Biotic factors in constructed wetlands

There are many factors which have an effect to the performance of wetlands treatment. Importance processes are not only chemical and physical transformations but also biological processes mediated by plants, microorganisms (bacteria, fungi) and algae.

2.3.2.1 Plants

The plants or macrophytes play an important role in constructed wetlands. They act as a temporary storage pool, with most pollutant transformations and sequestering processes occurring in the substrate (Guntenspergen et al., 1989). They provide surfaces and suitable environment for microbial growth and filtration. Special plants (helophytes) work best of all in seminatural wastewater treatment systems (Stottmeister et al., 2003) and have the ability to pass oxygen down through its leaf and stem structure into the rhizomes and out through the roots (Wießner et al., 2002). Emergent and floating leaved species have been preferentially used in pilot studies of constructed wetlands. Potentially useful emergent species include many members of the cattail (*Typha latifolia*), reed (*Cyperus sp.*), rush (*Juncus effusus*), sedge (*Carex rostrata*) and grass families. They have potentially high uptake and production rates.

Most plant species have a restricted translocation of metals and arsenic to the shoots (Stoltz and Greger, 2002) but were found to be root accumulators. Moreover, the plant rhizome provides surface for bacterial growth as well as for filtration of solids. More importantly, plants are known to translocate oxygen from the shoots to the roots (Gersberg et al., 1986). The root zone will offer an oxidized microenvironment in an otherwise anaerobic substrate, which stimulates the decomposition of organic matter and the growth of nitrifying bacteria, which can convert ammonia to nitrate.

2.3.2.1.1 Plant species

Plants are widespread, able to tolerate a wide range of environmental conditions, and can alter their environment in ways suitable for wastewater treatment.

Tanner (1996) indicated that *Juncus effusus* showed the highest mean shoot density (4,534 m^{-2}) of the eight tested species. Above-ground tissue nutrient concentrations were high but there was a low level of biomass production, and it was capable of growth in ammonium-rich organic wastewater, producing a compact stand without major seasonal die-

back. *Juncus effusus* is an evergreen plant which grows very well in advance of the frost-free period, especially spring–bloomers.

Vetiver grass (*Vetiveria zizanioides*) could take up heavy metals from heavy metal contaminated soil. It was found by Chen et al. (2000) that the concentrations of zinc, lead and cadmium in shoots of vetiver grass were 42-67%, 500-1200% and 120-260% higher in contaminated plots than in the control, respectively. Cadmium accumulation by vetiver shoots was 218 g/ha at a soil concentration of 0.33 mg Cd/kg.

Eichhornia crassipes or water hyacinth could detoxify Cr(VI) upon root uptake and transported a portion of detoxified Cr to leaf tissues, and Cr-rich crystalline structures were observed on the leaf surface. Mel et al. (1998) found that the chemical species of Cr in other plants, collected from wetlands that contained Cr(VI)-contaminated wastewater, was also found to be Cr(III). It was suggested that detoxification mechanism for phytoremediation by wetland plants is useful and has potential to be used in detoxification of Cr(VI)-contaminated waste streams.

2.3.2.1.2 Toxicity of arsenic and heavy metals to plants

Toxicity and accumulation of arsenic by plants depends on the plant species, concentration of arsenic and the presence of other ions. At low concentration, arsenic is not essential for plants and appeared not to be involved in specific metabolic reactions; however, it interferes with metabolic processes and inhibits plant growth and sometimes lead to death, at higher concentration (Marin, 1993).

Carbonell (1998) reported that the suitable concentration of P in experiments should be 12.4 mg/l because this concentration can be considered typical of many wetland environments (normal range for extractable P from 10 to 200 mg/kg, with the mean value of approximately 30 mg/kg) and it would allow plants to live for a longer period of time than lower P concentrations, although there might be a competition between phosphate and As, mainly as arsenate.

Arsenic speciation was more important than the As level in solution in determining the phytotoxic effect of As on turnip cultiva. Arsenic chemical form in solution influenced root and shoot dry weights (Carbonell-Barrachina, 1999).

In the case of well-grown plants, arsenic exists mainly in the three-valence state. The main arsenic component in plants with poor growth or which have died was found to be arsenate (Mattusch et al., 2000). Figure 2.1 illustrates the protection mechanism of the pants

from interruption by arsenic. This is an efficient way for plants to protect themselves from interruption of the oxidative phosphorylation by arsenate (Dixon, 1997). After the plant's death, arsenite can be rapidly oxidized again to arsenate by the loss of enzymatic activity.

Mattusch et al. (2000) reported that *Juncus effusus* accumulated moderate total arsenic concentration (around 250-270 µg/kg dry mass) in their shoots, and the predominant arsenic species found in the shoots and blooms was arsenite even though the roots are mainly surrounded by arsenate adsorbed on ferric oxyhydrate.

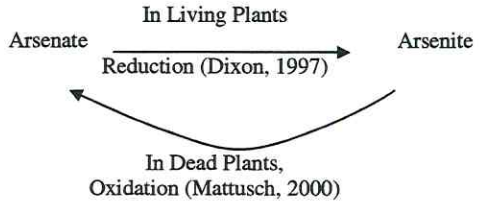


Figure 2.1 Protection mechanism of plants from interruption by arsenic

2.3.2.1.3 Plant accumulation

There is only little data available concerning the accumulation of arsenic in the plant matter. In a reed bed for landfill leachate treatment following arsenic concentrations in comparison to a natural stand were measured [see Table 2.1 (Urbanc-Bercic, 1997)].

Table 2.1 Arsenic concentration in the plant (µg/g dry weight)

	Constructed wetland for leachate treatment	natural stand
Arsenic in roots	3 - 15	0.4
Arsenic in rhizomes	0.4 - 0.9	0.3

Arsenic concentration in different parts of plants depends on its arsenic exposition. Higher values were found by Dushenko et al. (1995) in an aquatic system highly polluted with arsenic from a gold-mine effluent (see Table 2.2).

Phytotoxic symptoms from arsenic to *Typha latifolia* were observed already at concentrations exceeding 300µg/g in sediment, and 400µg/l in the water (Dushenko et al.,

1995). Similar results were obtained with *Spartina patens* whereby the organic form dimethyl arsinic acid showed the highest toxicity (Carbonel et al., 1998).

Table 2.2 Arsenic concentration in shoots and roots of various plant species (μg/g dry weight) exposed to the arsenic from a gold-mine effluent*

Species	Arsenic concentration in shoots	Arsenic concentration in roots
Typha latifolia	17.2	232
Potamogeton pectinatus	1219 (in whole plants)	
Equisetum fluviatile	34	352
Myriophyllum exalbescens	143 (in whole plants)	
Triglochin palustre	40	470
Sparganium sp.	28	133

*Modified from Dushenko et al.,1995

2.3.2.2 Microorganisms

Microorganisms play an important role for metal removal. It has been shown that in the rhizosphere, the zone near the root cells, the density of microorganisms is higher than in the zone far from the roots. The microorganisms can transform heavy metal and arsenic. There are four mechanisms involved with the removal; i.e. adsorption to the cell surfaces, complexion, precipitation and volatilization (Bitton, 1994).

- Adsorption to the cell surface: microorganisms bind metals as a result of interaction between metals ions and the negatively charged microbe surfaces. Gram-positive bacteria are particularly suitable for metal binding. Fungal and algal cells also have a high affinity for heavy metal removal.
- Complexation: microorganisms can produce organic acids (e.g., citric acid), which may chelate toxic metals, resulting in the formation of metalorganic molecules. Metals may also be complexed by carboxyl groups found in microbial polysaccharides and other polymers.
- Precipitation: some bacteria promote metal precipitation by producing hydrogen sulfide, which precipitate metals as their sulfides. Sulfate reducing bacteria (SRB) transform

SO_4^{2-} to H_2S, which promotes the extracellular precipitation of metals from solution. The reactions mediated by SRB are as follows:

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO^{3-}$$

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

where M^{2+} is divalent metal ion (Webb et al., 1998)

- Volatilization: some metals are transformed to volatile species as a result of microbial action. For example, bacterially mediated methylation converts Hg^{2+} to dimethyl mercury, a volatile compound. Some bacteria have the ability to detoxify mercury by transforming Hg^{2+} to Hg^0, a volatile species. This detoxification process is plasmid-encoded and is regulated by an operon consisting of several genes (Gadd and White, 1993).

2.3.2.2.1 Toxicity of arsenic and heavy metals to microorganisms

The toxicity of different metals and the behaviour in the environment depend on their species. A large number of marine animals go further, incorporating methylated arsenic into arsenoribosides, arsenolipids, and other complex organic compounds. Ocean water is 1-2 ppb arsenic, but the methylation and further incorporation of arsenic into complex molecules does not appear to be just a matter of detoxifying it. There appears to be a metabolic reason why these animals produce these compounds. An average lobster dinner contains about 30 mg of arsenic, but arsenic is in a form that is not bioavailable to humans (by comparison, 100 mg of As in the form of As_2O_3 will kill most people).

Treatment of arsenic-loaded sewage with arsenite oxidase-producing bacteria (which catalyse the conversion of As(III) to As(V)) can improve certain arsenic removal methods, since Fe^{3+} more easily precipitates arsenate from wastewater than arsenite. Chromate (CrO_4^{2-}) reducing bacteria, for example, *Enterobactor cloacae*, are resistant to high levels of chromate (10 mM) and can reduce CrO_4^{2-} to Cr(III) anaerobically, precipitating Cr(III) (Gadd and White, 1993).

2.4 Transformation and mobilization of arsenic and heavy metals

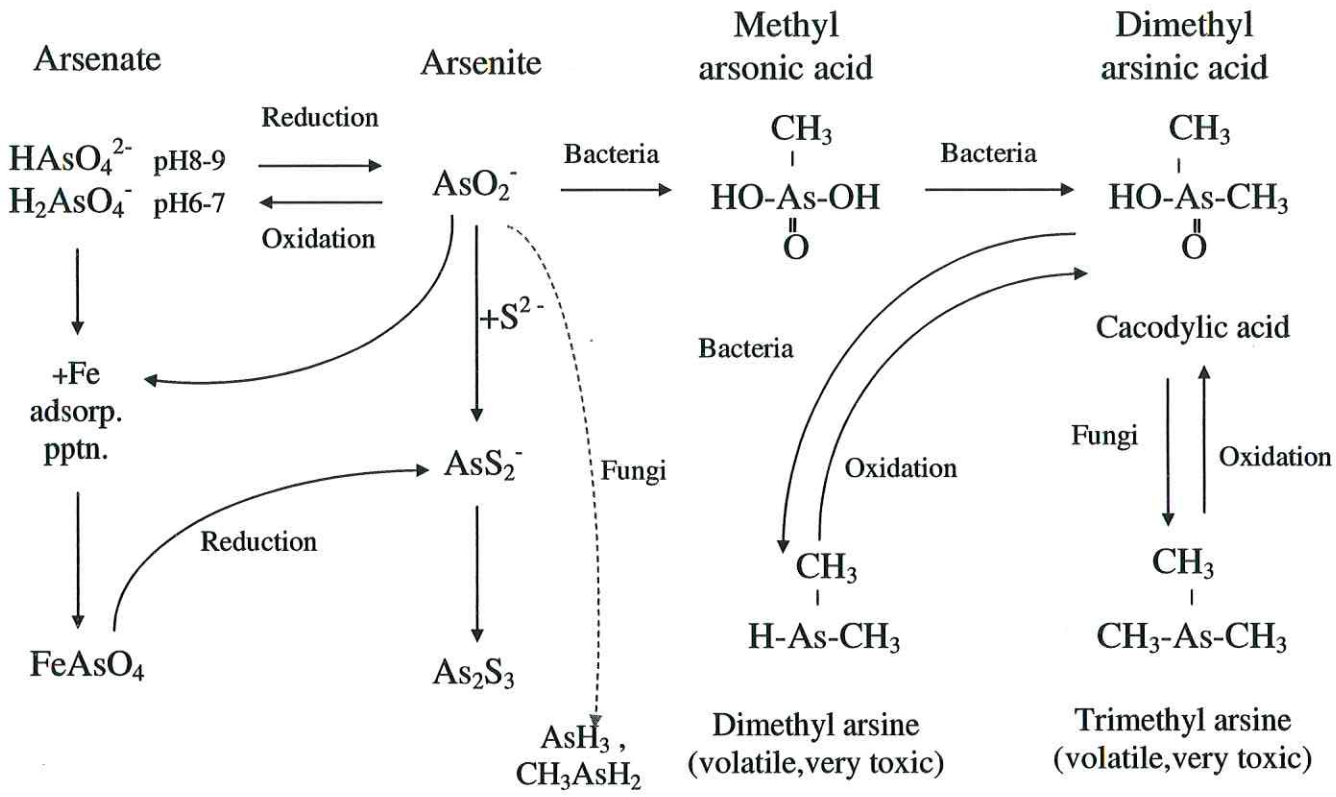
Heavy metals can occur in several forms in water and soils. Interest has increased in sequential extraction techniques to relate the degree of mobility to risk assessment (Mulligan et al., 2001). The speciation and the mobility of metals are important as mentioned by Bourge (1995) because the more mobile the metal is, the more risk is associated with it.

2.4.1 Arsenic

Many arsenic species appear in the environment. Different pathways of arsenic transformation and speciation are illustrated in Figure 2.2. The pathways are either promoted by microorganism or are abiotic chemical reactions. The two major chemical pathways of arsenic or heavy metal transformation are oxidative and reductive pathways depending on the redox state of the environment. Adriano (1986) postulated that the arsenic compound cacodylic acid was metabolized by two pathways; first, an oxidative pathway leading to C-As bond cleavage, second, a reductive pathway leading to alkyl arsine production. Moreover, it was found that 14% to 15% of the arsenic applied in soil could be lost through volatilization of alkyl arsines each year. Oxidation of the methyl substitute to CO_2 occurs in association with microbial oxidation of soil organic matter, producing arsenate.

Many reports revealed that some kinds of microorganisms could transform arsenic into its various forms. The mobility of arsenic commonly increases as reducing conditions are established within sediments or flooded soils. Cummings et al. (1999) reported that the dissimilatory iron-reducing bacterium *Shewanella alga* strain BrY promoted As mobilization from a crystalline ferric arsenate as well as from sorption sites within whole sediments, and *S. alga* cells released arsenate from the mineral scorodite ($FeAsO_4.2H_2O$) as a result of dissimilatory (i.e., respiratory) reduction of Fe(III) to Fe(II).

Toxicity and chemical behaviour of arsenic compounds are largely influenced by the form and speciation of As. As(III) is more mobile and more toxic than As(V). Gaseous arsines are most toxic (see Figure 2.2) whereas arsenobetaine and arsenocholine (mainly found in marine organisms) are non-toxic. As a rule, inorganic arsenicals are more toxic than organic arsenicals and the trivalent oxidation state is more toxic than the pentavalent oxidation state (Adriano, 1986).



(Chengi and Focht, 1979)

Figure 2.2 Chemical forms of arsenic and their transformations

(Source: Modified from Bhumbla and Keefer In Nriagu ed., 1994)

2.4.1.1 Toxicity of arsenic to animals and humans

There are many species and strain differences in the toxicity of As compounds. The purity, physical form and solubility of the compounds also influence toxicity (Stoeppler, 2004).The toxicity of As compounds can be described in the following decreasing order (Stoeppler, 2004, Hindmarsh and McCurdy, 1986): AsH_3 >> As_2O_3 > easily soluble As(III) (e.g. K and Na arsenite) > less soluble arsenite (e.g. Cu arsenite) > As(V) > As-sulfide > metallic arsenic. Examples of As compounds and their toxicity, abbreviation and formula are shown in Table 2.3. The fatal human dose for ingested arsenic trioxide or an alkaline arsenite for an adult is assumed to range from 60 to 300 mg As_2O_3 (Baselt and Gravey, 1995).

Table 2.3 Toxicity of some arsenic compounds to rats (Goessler and Kuehnelt, 2001, Grind and Hanusch, 2002 and Stoeppler, 2004)

Arsenic compounds (IUPAC or common name)	**Abbreviation**	**Formula**	**LD_{50} (mg/kg)**
Arsine	AsH_3	AsH_3	3
Arsenic trioxide	As(III)	As_2O_3	20*
Monomethylarsonic acid	MMA	$CH_3AsO(OH)_2$	700 - 1800
Dimethylarsinic acid	DMA	$(CH_3)_2AsO(OH)$	700 - 2600
Trimethylarsine oxide	TMAO	$(CH_3)_3AsO$	10600
Arsenobetaine	AsB	$(CH_3)_3As^+CH_2$-COO	>10000
Arsenocholine	AC	$(CH_3)_3As^+CH_2$-CH_2-OH	>10000

* For comparison: the LD_{50} for the alkaloid strychnine is 16 mg/kg

2.4.1.2 Methylation of arsenic

Bacteria, algae, fungi, vascular plants, and animals can methylate arsenic like other metalloid substances, such as mercury (Cullen et al., 1979; Frankenberger and Arshad, 2001). There are two series of methylated arsenic compounds. The methylated arsenic(V) compounds include:

- Monomethylarsonic acid (MMA), $CH_3AsO(OH)_2$ and its salts
- Dimethylarsinic acid (DMA), $(CH_3)_2AsOOH$ and its salts
- Trimethylarsine oxide (TMAO), $(CH_3)_3AsO$

These As(V) compounds are produced by algae, cyanobacteria, arthropods, fish, mammals, and other organisms. They are much less toxic than arsenate; each added methyl group decreases the toxicity by a factor of about 10. Since a lethal dose of arsenic in the form of arsenate salts is about 6 g, a lethal dose of arsenic in the form of trimethylarsine oxides is about 6 kg (http://www.cs.umt.edu/GEOLOGY/classes/Geol431/lectur17.htm). The mono-methyl forms are produced by many aerobic organisms as a way of detoxifying arsenic. Arsenate can be eliminated through the kidneys, but it does a lot of tissue damage going through. The methylated arsenates can also be eliminated through the kidneys, but with much less damage.

Hasegawa et al. (2001) reported that methylarsenic(III) species could be produced by phytoplankton in freshwater. MMA(III) and DMA(III) were released as metabolites from the biosynthetic pathway for methylarsenicals by *Closterium aciculare*.

Occurrence of the methylated species monomethylarsonate (MMA) and dimethylarsenate (DMA) and of the reduced inorganic species arsenite in oxygenated surface waters is indicative of algal transformation of arsenic. The methylated species are believed to be detoxification products (Kneebone and Hering, 2000). In most streams, less than 1% of the arsenic is methylated. In lakes, particularly eutrophic ones, over 50% of the arsenic may be methylated. There is definitely a seasonal variation in the amount of methylation that seems to be related to variations in the water's microbial ecology as temperatures change.

Sohrin et al. (1997) studied the seasonal variations of arsenic species in lake water in the mesotrophic northern and eutrophic southern basins of Lake Biwa in Japan. It was found that within the eutrophic zone, arsenite (As(III)) increased in spring and fall, and dimethylarsinic acid (DMA) became the dominant form in summer. Monomethylarsonic acid (MMA) and trivalent methylarsenic species [monomethylarsonous acid, MMA(III), and dimethylarsinous acid, DMA(III)] also appeared, although they were always minor fractions.

2.4.1.3 Volatilization of arsenic and heavy metals

Volatile methyl and hydride derivatives of metal(loid)s are found in gases released from natural environments, such as sediments, wetlands, and hydropogenic springs, as well as from anthropogenic environments such as wastewater treatment plants and waste deposits (Michalke et al., 2000). In aquatic environments, algae and cyanobacteria (blue-green algae) methylate arsenate to monomethyl and some dimethyl As(V), some of which is excreted and some of which is retained. The plankton (e.g. shrimp) consumes the algae and produces a higher percentage of dimethyl (As). The animals higher on the food chain consume the plankton and methylate the arsenic further, producing some trimethylarsine oxide. There is also a series of methylated As(III) compounds:

- Monomethylarsine, CH_3AsH_2
- Dimethylarsine, $(CH_3)_2AsH$
- Trimethylarsine, $(CH_3)_3As$

These compounds are extremely toxic. Arsine itself is highly toxic, and it gains toxicity with each added methyl group. Dimethylarsine is produced by methanogenic bacteria and other anaerobes. Some fungi, such as molds of the *Scopulariosis* genus make trimethylarsine.

Arsine is produced in soil which is contaminated with arsenate, arsenite, monomethylarsonate, and dimethylarsinate, whereas methylarsine and dimethylarsine were produced only from soils which were contaminated with only methylarsonate and dimethylarsinate, respectively. In addition, the resting cell suspension of *Pseudomonas* and *Alcaligenes* produced arsine as the sole product when incubated anaerobically in the presence of arsenate or arsenite (Chengi and Focht, 1979).

Under distinct anaerobic conditions, elements such as As, Se, Sn, Hg etc. can be transformed to volatile forms whereby mainly more toxic methylated compounds are formed. Methanogenic bacteria, for instance, are able to transform inorganic As to volatile dimethylarsine $(CH_3)_2AsH$ (Tamaki and Frankenberger, 1992). It was suggested that this volatilization can be an important process removing arsenic from wetlands.

There are many species of microorganisms which can produce volatile methyl derivatives of metalloids. AsH_3, MMA, DMA, and TMA were produced by *Methanobacterium formicicum* (concentration ranged from 0.5-14.3 ng/70 ml-gas phase), whereas other organisms produced fewer volatile arsenic species and in smaller amounts: *Methanosarcina barkeri* formed arsine and small amounts of an unidentified arsenic-containing compound, whereas *Methanobacterium thermoautotrophicum* produced only arsine. The sulfate-reducing

bacteria and *Clostridium collagenovorans* formed only TMA, and small amounts of arsine were detected in cultures of *Desulfovibrio gigas* (Michalke et al., 2000).

2.4.1.4 Microorganism involved in arsenic transformation

2.4.1.4.1 Arsenate reducing bacteria

Several groups of bacteria are able to use arsenate as an electron acceptor for the dissimilatory reduction to arsenite (Ahmann et al. 1994; Newman et al. 1997a). It was shown that arsenate strongly adsorbed to iron(III) oxyhydroxides dissolved and reduced under anaerobic conditions (Ahmann et al. 1994). This phenomenon is used technically in ex-situ soil washing for the treatment of As-contaminated soils (Legiec et al. 1994).

There are various bacteria that are able to reduce arsenate to arsenite. Recently four new strains of arsenate reducing bacteria were found. They are *Sulfurospirillum barnesii* strain SES-3 (Laverman et al., 1995; Oremland, 1994), *Sulfurospirillum arsenophilus* strain MIT-13 (Ahmann et al., 1994), *Desulfotomaculum auripigmentum* strain OREX-4 (Newman et al., 1997b) and *Chrysiogenes arsenatis* strain BAL-1T (Macy et al., 1996).

- *Sulfurospirillum barnesii* strain SES-3 was found in selenate-respiring enrichment from the Massie Slough marsh in the Stillwater Wildlife Management Area of western Nevada. It belongs to the epsilon subdivision of the Proteobacteria. SES-3 also grows on nitrate but not on sulfate (Laverman et al., 1995; Oremland, 1994).
- *Sulfurospirillum arsenophilus* strain MIT-13 was isolated from arsenic contaminated sediments near the Industry-Plex Site, a superfund site in Woburn, MA. It is in the epsilon subdivision of the Proteobacteria and MIT-13 grows on nitrate, but not on sulfate (Ahmann et al., 1994).
- *Desulfotomaculum auripigmentum* strain OREX-4 is a newly discovered bacterium and was isolated from surface sediments of the Upper Mystic Lake in Winchester, MA. It is a gram-positive bacterium and has a hexagonal S-layer on its cell wall. It grows on scorodite mineral. Moreover, it grows on lactate with arsenate or sulfate as an electron acceptor but does not respire nitrate (Newman et al., 1997b). This bacterium can precipitate arsenic trisulfide (As_2S_3), as a result from the reduction of As(V) to As(III), both intra- and extracellularly. It is suggested that As_2S_3 formation might be important in the biogeochemical cycle of arsenic.

- *Chrysiogenes arsenatis* strain BAL-1T was isolated from a reed bed at the Ballarat Goldfields in Australia. It is gram-negative and appears to be the first representative of a new deeply branching lineage of the Bacteria (Macy et al., 1996).

2.4.1.4.2 Arsenite oxidizing bacteria

Arsenite [As(III)] is more toxic than arsenate, because it inhibits dehydrogenases and some other enzymes due to its ability to react with the functional –SH groups of cystein residues in proteins (Ehrich, 2001; Santini et al., 2001). There are a number of bacteria which are able to oxidize arsenite into the less toxic pentavalent form, arsenate [As(V)].

Bacterial oxidation of arsenite to arsenate was first described in 1918 (Green, 1918). *Bacillus arsenoxydans*, was isolated from an arsenical cattle dip in South Africa by including organic matter in the form of dung extract in the medium.

Turner (1949, 1954) assigned the isolates of 15 arsenite-oxidizing bacterial strains which were isolated by including organic matter in the medium and were therefore heterotrophic arsenite oxidizers. *Pseudomonas arsenoxydans-quinque* was presumably the most rapid oxidizer. This is considered synonymous with *Alcaligenes faecalis* (Ehrlich, 1996).

Pseudomonas arsenitoxidans was found as being able to grow using energy gained from arsenite oxidation. It was isolated from a gold-arsenic deposit and found to grow chemolitho-autotrophically with oxygen as the terminal electron acceptor, arsenite as the electron donor, and carbon dioxide as the sole carbon source (Ilyaletdinov and Abdrashitova, 1981).

Another chemolitho-autotrophic arsenite oxidizer, designated NT-26, is the fastest arsenite oxidizer reported to date with a doubling time of 7.6 hr when grown chemolitho-autotrophically. This organism was isolated from the Granites gold mine in the Northern Territory, Australia (Santini et al., 2000).

2.4.2 Zinc

Zinc is not as toxic as arsenic, however, it is quite often associated with other metals. Source of zinc include brass and bronze alloys, galvanized products, rubber, copying paper, cosmetics, pharmaceuticals, batteries, televisions, tires, metal coatings, glass, paints and zinc-based alloys (Cameron, 1992; Mulltigan et al., 2001).

It can enter the environment from galvanizing plant effluents, coal and waste burning, leachates from galvanized structures, natural areas and municipal waste treatment plant

discharge. Zinc is commonly found in waste as zinc chloride, zinc oxide, zinc sulfate and zinc sulfide.

Soil texture, pH, nature of the parent rocks and organic content all effect the natural content of zinc in the soil. Under acidic conditions, zinc is usually divalent and quite mobile.

At high pH, zinc is bioavailable due to the solubility of its organic and mineral colloids. Zinc hydrolyses at pH 7.0-7.5 and forms $Zn(OH)_2$ at pH values higher than 8. Under anoxic conditions, ZnS can form upon precipitation, whereas the un-precipitated zinc can form $ZnOH^+$, $ZnCO_3$, $ZnCl^+$ and complexes with organics. The sulfide form of zinc is highly insoluble and serves as a sink for zinc in the aquatic environment (Kadlec and Knight, 1996).

Natural levels of zinc in soils are 30-150 mg/kg. Levels of 10-150 mg/kg are normal in plants while 400 mg/kg is toxic.

2.4.3 Chromium

Chromium is one of the toxic metals and has been used by man and introduced into the environment. Chromium is found naturally in the Earth's crust in an average concentration of 100 mg/kg (Hammond, 2002). Chromium is used in chemical industries, metallurgical industries, refractory, wood preservation, metal finishing and tanning (Barcelous, 1999). Dichromate is used as oxidizing agent in quantitative analysis and as mordant in tanning leather. The danger of the environmental contamination depends on the solubility and oxidation state of chromium (Stoecker, 2004).

Chromium typically occurs in the trivalent [Cr(III)] or hexavalent [Cr(VI)] forms in surface waters. The Cr(VI) form is the most toxic and is generally associated with the presence of industrial wastewaters. Cr(VI) is relatively unstable under most environmental conditions and converts to less toxic trivalent form in surface waters, especially when organic matter is present. Trivalent chromium hydroxides and chlorides are relatively insoluble and their formation may significantly reduce chromium availability to biota.

Cr(III) compounds exist as oxides, sulfides, or halides and are soluble at low pH values. At pH 5-6, Cr(III) hydroxide precipitates. However, the stable Cr(VI) complexes can be formed with sulfite ions (SO_3^{2-}) at pH 9 and above provided that an excess of sulfite is present in the solution.

Although chromium is an essential trace element in animals, it does not biomagnify in the food chain. Plants generally have chromium concentrations from 0.01 to 0.1 times the soil concentration. Chromium is transferred from soil and roots to the above-ground plant parts to such a small extent that toxicologically significant concentrations are unlikely (Bolt et al.,

1991). A concentration of 10 mg/kg dry-weight of chromium is considered to be a phytotoxic threshold level in agricultural plants. Chromium(VI) is reported to be toxic to algae at concentrations between <20 and 10,000 µg/l (Nriagu and Nieboer, 1998). Chromium can be transported by some microorganisms through sulfate transport system, for instance *Samonella typhimurium*, *E. coli* and *Pseudomonas fluorescens* (Cervantes et al., 2001).

Phytoremediation has been considered for cleaning up chromium from contaminated soil and water (Gauglhofer and Bianchi, 1991; Barceloux, 1999). *Vallisneria spiralis* L., a root submerged plant, accumulated Cr to about 57.5 mg/kg dry weight of root after 10 days of exposure to 100% tannery wastewater (Sinha et al., 2002). Chromium removal rate of the wetlands receiving municipal wastewater have been measured from 7.92-15.2 kg/ha/yr (Kadlec and Knight, 1996).

2.4.4 Iron

Iron is a metal that may occur at trace to high concentrations in wetland surface waters and sediments. It is frequently required by plants and animals at significant concentrations. In plants, iron is an essential element in chlorophyll synthesis, cytochromes, and in the enzyme nitrogenase. Iron occurs in aquatic plants at a concentration of about 5000 mg/kg. Plant roots contain a higher proportion of iron than stems or leaves (Wetzel, 1975; Kadlec and Knight, 1996).

Iron is a reactive metal, but it is stable in dry air and in water free of carbon dioxide. In biological systems, iron is found in the ferrous [Fe(II)] and ferric [Fe(III)] forms. Oxidation and reduction of iron occurs relatively easily depending on the redox potential. Ferric iron [Fe(III)] is the dominant form under oxidized conditions. Ferrous iron [Fe(II)] is the dominant form under reduced or anaerobic conditions in wetlands and other aquatic environments. Ferric forms stable complexes with a variety of ligands - preferably to their oxygen, nitrogen and sulfur atoms. Ferric ion joins with the hydroxide ion in waters to form reddish-brown ferric hydroxide ($Fe(OH_3)$) or ochre (Kadlec and Knight, 1996; Schuemann and Elsenhans, 2004).

Ferrous iron [Fe(II)] is more soluble than ferric iron and is stable in anaerobic conditions resulting in the release of dissolved iron and associated anion such as phosphate from anaerobic sediment wetlands. The formation of ferrous iron may be controlled by the concentration of sulfide which forms the relatively insoluble ferrous sulfide (FeS) (Kadlec and Knight, 1996).

3 Materials and methods

3.1 Behaviour of arsenic in anaerobic river sediment

The behaviour of arsenic in the presence of river sludge-sediment was studied in anaerobic bottle experiments. The effect of the river sludge-sediment on the decrease of total As and the reduction of As(V) to As(III) was studied through the adsorption and precipitation processes under anaerobic environmental systems.

3.1.1 Preparation of chemical and nutrient solution

Nutrient solution for the experiment contained the following ingredients: 0.5 mg/l of As(V) prepared from the stock solution of 1g/l As(V) (Arsenic stock solution was prepared from $As_2O_5.xH_2O$ dissolved in distilled water), 10 ml/l trace nutrient solution TMS3 (Ingredients of TMS3 are shown in Table 3.1), and the following macronutrients:

- 10 mg/l NH_4^+ (corresponds 29.7 mg NH_4Cl)
- 12.4 mg/l PO_4^{3-} (corresponds 17.75 mg KH_2PO_4)
- 400 mg/l SO_4^{2-} (corresponds 1,026 mg $MgSO_4x7H_2O$)

Table 3.1 Ingredients of trace nutrient solution (TMS3)

Substance	*mg/l*
EDTA-Na / TitriplexIII	100
$FeSO_4x7H_2O$	100
$MnCl_2x4H_2O$	100
$CoCl_2x6H_2O$	170
$CaCl_2x6H_2O$	100
$ZnCl_2$	100
$CuCl_2x5H_2O$	20
$NiCl_2x6H_2O$	30
H_3BO_3	10
$Na_2MoO_4x2H_2O$	10
H_2SeO_3	1
3 ml conc. H_2SO_4	

Note: Modified from Kuschk (1991)

3.1.2 Arsenic sorption in river sludge sediment

The experiments were done in 1-litre bottles. Bottle (A) served as control (without river sludge). The other two bottles (B and C) were filled with 100 ml river sludge and 900 ml nutrient solution (as mentioned in 3.1.1) supplemented with 0.5 mg/l As(V), resulting in 1 litre solution.

In this experiment, 0.1 g/l of phenol was used as carbon source for microorganisms in the river sludge sediment. Sulfate (2g/l) was added in bottle C, as a terminal electron acceptor for microorganisms. All bottles were purged with nitrogen gas for 10 minutes and closed with rubber stoppers to keep them in anaerobic condition. Finally these bottles were incubated at 30°C under dark conditions. Table 3.2 shows the scheme of these experiments.

Table 3.2 Contents in the bottle experiments on arsenic behaviour in anaerobic river sediment

Bottle	Content
A	Nutrient solution, 0.5 mg/l As(V)
B	Nutrient solution, 0.5 mg/l As(V), river sediment
C	Nutrient solution, 0.5 mg/l As(V), river sediment, sulfate

Samples were taken after 24 hours, 2, 3, 7, and 14 days of incubation and they were analysed for the concentration of As(V), As(III) and total arsenic.

3.1.3 Arsenic sorption and effect of biological factor

The experiment was conducted under the same conditions as in the experiment 3.1.2. Additionally, sodium azide was used as biological inhibitor. It was added into the bottle to inhibit microorganisms. If the microorganisms are involved with the arsenic reduction, the reduction should be retarded.

Nutrient solution as described in 3.1.1 was mixed with As(V) and had a final concentration of 0.5 mg/l of As(V). There were four 1 l bottles (see Table 3.3). Bottle A and B served as control, without river sludge. They were filled with 1000 ml nutrient solution. Furthermore, sodium azide was added to bottle B. Bottle C and D were filled with 100 ml river sediment. To bottle D 0.2 g/l sodium azide was added. After that, they were filled up with nutrient solution contaminated with 0.5 mg/l As(V). The final volume of each bottle was 1 litre. The 4 bottles were purged with nitrogen gas to keep the system in an anaerobic condition. Finally, they were closed with rubber stoppers and incubated at 30°C temperature under dark condition. Samples were collected during incubation time; 24 hours, 2 days, 3 days and 7 days, and the concentration of total As was analyzed.

Table 3.3 Ingredients of the different bottles in the experiment on arsenic behaviour in anaerobic sediment

Bottles	Content
A	Nutrient solution with 0.5 mg/l As(V)
B	Nutrient solution with 0.5 mg/l As(V), sodium azide
C	Nutrient solution with 0.5 mg/l As(V), river sediment
D	Nutrient solution with 0.5 mg/l As(V), river sediment, sodium azide

3.1.4 Precipitation of arsenic with sulfide and its co-precipitation with zinc and sulfide

This experiment intended to characterize the behaviour of sulfide, arsenic and zinc under abiotic conditions for forming precipitates.

The first series of this experiment included two experimental flasks. Flask 1 and flask 2 contained 200 ml nutrient solution. 100 mg/l of zinc (in form of $ZnSO_4.H_2O$) and 1 mg/l of As(V) were added to flask 2 whereas flask 1 contained only 1 mg/l of As(V).

In both flasks the solution was adjusted to neutral pH between pH 6.5 to 7. The mixed solution was purged with nitrogen gas for 10 minutes to keep it in anaerobic condition. Sulfide in the form of $Na_2S{\cdot}9H_2O$ (300 mg/l S^{2-}) was then added to both flasks. The flasks were closed with rubber stoppers and shaken for two hours.

The experiment of the second series was performed as a comparatively lower pH to the first series. There were two experimental flasks. The first flask contained 200 ml of nutrient solution. It was amended with zinc 100 mg/l (added in form of $ZnSO_4.H_2O$) and As(V) 1 mg/l. The second flask contained 200 ml nutrient solution. As(V) was added to a concentration of 1 mg/l. The pH of the solution was adjusted to a lower pH, in the range of 4.5 to 5.5.

The mixed solution was purged with nitrogen gas for 10 minutes to keep it in anaerobic condition. An excess amount of sulfide in the form of $Na_2S{\cdot}9H_2O$was applied. The concentration of sulfide (S^{2-}) varied from 1, 10, 100, and 500 mg/l. These concentrations were used in order to determine the effect of sulfide on the precipitation in the system, especially in an extremely high concentration of sulfide (10 g/l $Na_2S{\cdot}9H_2O$). Afterwards, the bottles were shaken for two hours.

The samples of the mixed solution in both experimental series (before addition of $Na_2S{\cdot}9H_2O$) were collected and centrifuged before further analysis. These samples acted as a control.

The samples were collected again after $Na_2S{\cdot}9H_2O$ was added in the mixed solution and shaken for two hours in anaerobic condition. Then they were centrifuged and kept in anaerobic bottles. The redox potential and pH were measured. Finally, the concentrations of total As, S and Zn were analysed immediately by ICP-AES. Arsenic species-compounds and other metal compounds were analysed by IC-ICP-MS.

3.2 General aspects analysis

3.2.1 Arsenic adsorption capacity of gravel

Constructed wetland systems usually contain gravel or other soil material and plants. It is possible that gravel can affect the concentration of metals by adsorption. A high adsorption capacity of gravel could have an effect on the reduction of metals in the water. Therefore, the adsorption capacity of the gravel used in the experiments was analysed. The diameter of the gravel ranged from about 2-8 mm and had an average size of 4 mm.

The method of adsorption capacity analysis of the gravel followed the method for soil adsorption capacity (Fuller et al., 1993). Three different As solutions with concentrations of 10, 50 and 100 µg/l arsenate were prepared from a stock solution. 30 grams of gravel was mixed with 100 ml of each concentration in plastic bottles. Then the bottles were shaken for 24 hours. Solution samples from each bottle were taken at the beginning and at the end of the shaking period. They were filtered with 0.45 µm pore filter-paper and the concentration of total As was analysed by ICP-AES. The adsorption capacity was calculated with the following equation.

$$q = (C_f - C_i)*V/M$$

Where: q is adsorption capacity
C_f is the final concentration
C_i is the initial concentration
V is the volume of the solution
M is the mass of the gravel

The results for the As concentration and adsorption capacity are shown in Table 3.4. It was found that the concentrations of arsenic in solution before and after mixing with gravel did not change significantly.

Table 3.4 Concentrations of arsenic in the solution in different conditions and adsorption capacities of the gravel

Gravel samples	Arsenic in solution(µg/l)		Adsorption Capacity; q (µg/kg)
	Begin(C_f)	End(C_r)	
1	36.4	37.7	-4.3
2	61.9	61.9	0
3	90.8	89.8	+3.3

From these results it was found that the gravel could not adsorb arsenic in significant amounts from the solution. Therefore, this kind of gravel itself has no significant effect on the experiment of arsenic removal in constructed wetlands.

3.2.2 Density of gravel

The density of the gravel was measured based on the water replacement method proposed by Black (1986) and ASTM (1994). The following equation was used to estimate the density of the gravel:

$$\rho = \frac{M.gravel}{V.gravel}$$

Where: ρ is density of gravel; kg/l
M.gravel is the mass of gravel; kg
V.gravel is the volume of gravel; l

The density, volume and weight of the gravel for the FSW were measured. Three replication of measurement was measured at three different depths of gravel (at 0.15, 0.17 and 0.20 m depth; see Table 3.5). From this equation the average apparent density was 2.02 kg/l resulting in an estimated gravel weight used in SSW was about 75.75 kg (Table 3.5).

Table 3.5 Weight of gravel and the apparent density (ρ) in different depths of gravel. Data were measured for FSW and were estimated for SSW.

Replication	Characters of constructed wetlands			
	Gravel depths (m)	Volume (l)	Apparent density (ρ)	Gravel weight (kg)
1 (used in FSW)	0.15	22.5	2.00	45.00[m]
2	0.17	25.5	1.95	49.75[m]
3	0.20	30.0	2.11	63.25[m]
Estimation (used in SSW)	0.25	37.5	2.02*	75.75*

[m] is measured weight
* is the estimated density and the weight of gravel in the SSW

3.2.3 Evaluation of evapotranspiration

Evapotranspiration is a considerable factor, which affects the efficiency of treatment by phytoremediation. It is the sum of evaporation and transpiration of water by plants. It is described by the equation below:

$$ET = E + T$$

where :
ET = Evapotranspiration (l/m^2)
E = Evaporation (l/m^2)
T = Transpiration (l/m^2)

$$I + P = O + ET$$

where:
I = Inlet/ Inflow (l/m^2)
P = Precipitation (l/m^2)
O = Outlet/ Outflow (l/m^2)
ET = Evapotranspiration (l/m^2)

The evapotranspiration was measured both in the lab scale experiments and in the field experiments (in Grosskayna).

3.2.4 Plant biomass

The plant biomass was determined at the beginning of the experiment (fresh weight) and at the end of experiment (fresh weight and dry weight).

The biomass of the algae in the control/algae pond was analysed. Water samples were filtrated by vacuum through filter paper (0.45 μm). Then it was dried for 1 hour at 103-105°C in an oven, left to cool in a desiccator, and weighted. These processes were repeated until the final weight was stable (Standard Methods for the Examination of Water and Wastewater, APHA, AWWA and WEF, 1995).

3.3 Arsenic and heavy metals removal in constructed wetlands

This work intended to study and compare the removal of arsenic and heavy metals in three different types of experimental wetland systems. The first experiment comprised a batch experiment. The second experiment was a study in a laboratory two step wetland model. The third experiment was the treatment of acid mine drainage in six small scale constructed wetlands in the field. The procedures and the systems used in the experiments are explained in the following sections.

3.3.1 Batch experiments simulating important processes occurring in constructed wetlands

3.3.1.1 Preparation and cultivation of the plants

Juncus effusus plants were propagated in hydroponic culture under greenhouse conditions. Three months before starting the experiments the plants were transferred into the model systems that were placed in a greenhouse with a temperature of 25 °C. *Juncus effusus* plants were used in all experiment with constructed wetlands, batch system, a series of continuous flow system and in the system for the removal of acid mine drainage.

3.3.1.2 Experimental design and construction

The experiments were conducted in the greenhouse with controlled air temperature and light intensity, so they were independent from seasonal change. Models of constructed wetlands were set up in small plastic containers with the dimensions of 0.3 x 0.5 x 0.3 m^3 or in a glass column (0.6 m height/ 0.30 m diameter with a total volume of 45 litres) simulating four different wetland/pond systems (see Table 3.6 and Figure 3.1).

- ***Subsurface Wetland (SSW)***

In this model wetland, plants were grown in a gravel bed (grain size 2 – 6 mm). The height of the gravel bed was 25 cm, and the water level was kept 5 cm below the gravel surface, resulting in a water volume of 21 litres. The surface area was 0.15 m^2.

- ***Free Surface Wetland (FSW)***

Juncus effusus was planted in the same type of container as for SSW and the same kind of gravel material was used. The height of the gravel layer was 15 cm and the water level was kept 10 cm above the gravel layer, resulting in a total water volume of 34 litres.

➢ ***Algae Pond (AP)***

Artificial wastewater was put into the container, which was the same type as those for SSW and FSW. The water level was about 22 cm above the bottom (33 litres). There was no vegetation. By time, algae growth started without addition of any inoculums.

➢ ***Hydroponic System (HP)***

A glass column with 0.3 m diameter and 0.07m^2 surface area was used as a pond model. The water level was 60 cm above the bottom and the resulting water volume was 41.5 litres. *Juncus effusus* plants, which covered the whole surface area, were put in and floated in this pond model.

Table 3.6 Characteristics of the containers of constructed wetlands in batch system

Constructed wetlands	Dimension	Gravel weight (kg)	Surface area (m^2)	Water volume capacity(l)	Applied wastewater volume (l)
SSW	0.3x0.5x0.25^{g}	75.75*	0.15	21	14
FSW	0.3x0.5x0.15^{g}	45.00$^{\#}$	0.15	34	29
AP	0.3x0.5x0.22^{w}	-	0.15	33	33
HP	π (0.15)^2 0.60^{w}	-	0.07	42.4	41.5

* Estimated value, $^{\#}$ Measured value, ^{g} gravel height, ^{w} water height

3.3.1.3 Procedure of the experiments

➢ ***Artificial wastewater***

Artificial wastewater was prepared using tap water (containing the following main components (in mg/l): Ca 100; Mg 30; K 10; Na 10; SO_4^{2-} 100; Cl^- 30) to which the following chemicals were added (in mg/l): NH_4Cl 29.7; KH_2PO_4 17.8; SO_4^{2-} 600; additionally 10 ml/l trace mineral solution according to Kuschk (1991) was supplied. Furthermore, $ZnSO_4$ and As_2O_5 was added resulting in a final concentration of 5 mg/l for Zn and 0.5 mg/l for As. Finally, this artificial wastewater was adjusted to pH 4 with H_2SO_4.

It is known that at a concentration of 0.8 mg/l can reduce the plant growth significantly (Carbonell et al., 1998), therefore, 0.5 mg As/l was selected as the concentration used in this study in order not to impair plant growth.

➢ ***Start up of the experiment***

Wastewater was added to the experimental systems, and the first samples were collected after one day. Distilled water was added to each container twice a week to compensate for

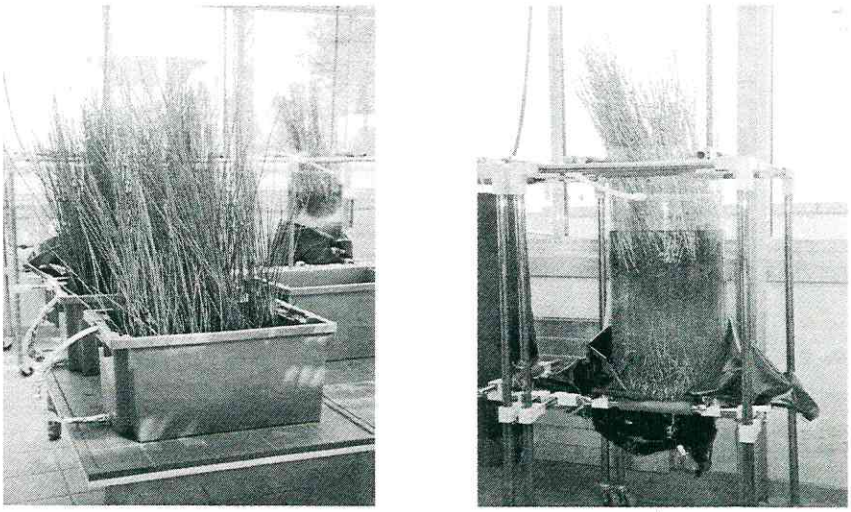
evaporation. The experiments continued for about 90 days and samples of water, plant and gravel were collected and further analysed for physical and chemical parameters.

3.3.1.4 Sampling and analysis

Water samples were taken each week from 3 different depths of the model systems; at the bottom, in the middle, and at the surface of water level.

Total As, Zn and Fe were analysed using inductively coupled plasma atomic emission Spectrometry ICP-AES. Sulfate was measured with ion chromatography using Dionex 100 (AS4A-SC column/AG4A-SC column) with conductivity detection (see 3.6).

Biomass of the plants was analysed both before and at the end of study. Gravel samples were analysed as described in (3.2). The concentration of metals was analysed in exposed and unexposed gravel material.



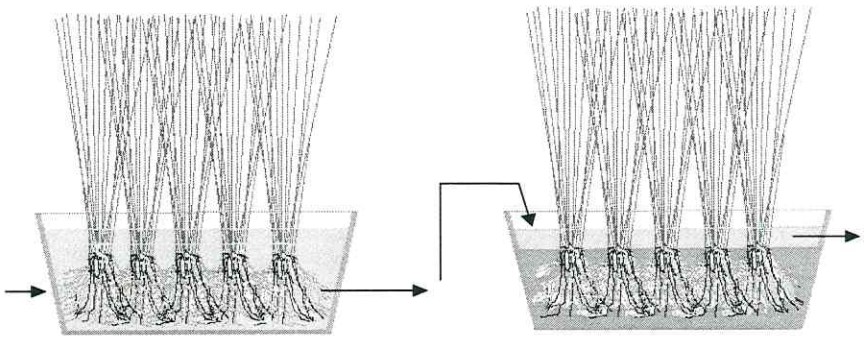
a b

Figure 3.1 Constructed wetlands in the batch experiment; (a) shows the beginning of the experimental set up SSW, FSW, and AP; (b) shows the experimental set up of the hydroponic system at day 65 of being operated with more dense roots in the root zone

3.3.2 Treatment of artificial wastewater containing arsenic and heavy metals in a two step wetland system model

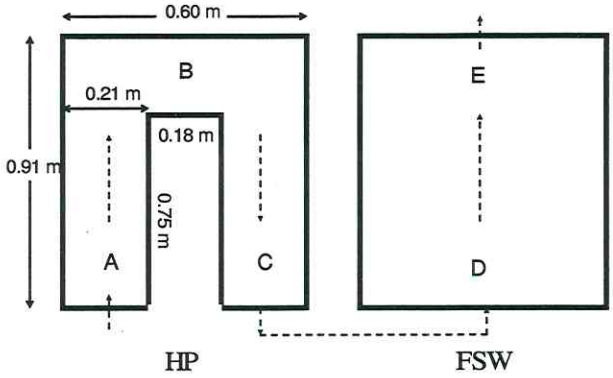
3.3.2.1 Experimental design and operation

The experimental system consisted of 2 containers in a series and the water flowed continuously. The first container was a hydroponic system (HP), which was connected to the second container, a Free Surface Wetland (FSW). The schemes of experiment are shown in Figure 3.2 and Figure 3.3.



Container 1: Hydroponic System Container 2: Free Surface Wetland

Figure 3.2 The experimental scheme of the combination of hydroponic system (HP, with floating plant mats) with free surface wetland (FSW)



Dimension	HP	FSW
Volume (m^3)	0.19	0.10
Surface area(m^2)	0.41	0.55
Height (m)	0.45	0.45

Figure 3.3 Top view scheme and dimension of the model constructed wetland system. HP: Hydroponic system, FSW: Free surface wetland, flow direction and sampling points in the system (from A to E)

The components of synthetic wastewater for this experiment are shown in Table 3.7. Zn, Cr, As (the main contaminants) and other essential nutrients for the plants growth were applied in the same concentration as in the experiment 3.2.1. Sodium benzoate was used as a carbon source for microorganisms and it stimulated an anaerobic condition. Sodium bromide (NaBr) was used as a tracer for hydraulic flow characterization because of its conservative behaviour. Plants or microorganisms do not use bromide; therefore, bromide is a suitable tracer for water flow.

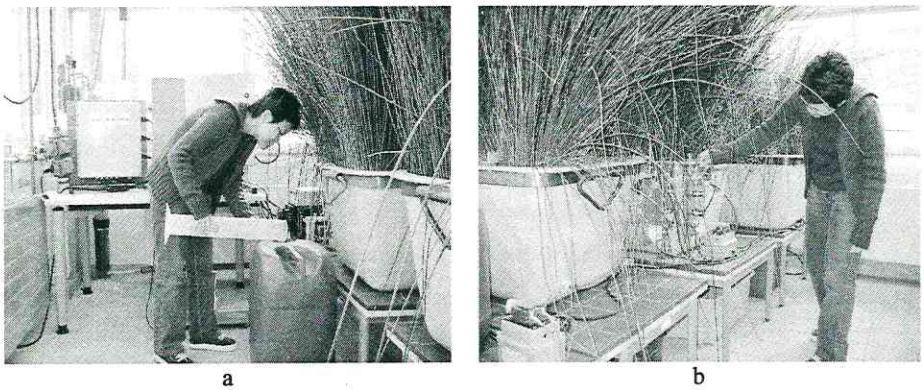
Table 3.7 Composition of the synthetic wastewater used in the model of a two step wetland system

Component	Concentration [mg/l]	Chemical form
As(V)	0.5	$As_2O_5.xH_2O$ Standard, 0.905g/l
Zn	5 (1)	$Zn(CH_3COO)_2.2H_2O$
Cr(VI)	5(1)	$K_2Cr_2O_7$
PO_4^{3-}	5	KH_2PO_4
NH_4^+	200	$(NH_4)_2SO_4$
SO_4^{2-}	684	Tap water, Na_2SO_4
CH_3COOH	100	Acetic acid 96%
C_6H_5COONa	150 (0)	Sodium benzoate
Br^-	5	NaBr

Note: Concentration in bracket was used in the second phase of experiment

Synthetic wastewater was freshly prepared every two days. It was pumped continuously into the HP system (see Figure 3.3 and Figure 3.4). The flow rate of wastewater was adjusted to an average value of 23 l/day which provides a retention time of 5 days. The outlet of the hydroponic system was collected in a glass collector which had a certain volume. This outlet of the hydroponic system was gradually pumped into the FSW. The outlet of the FSW was collected in a glass collector; the volume was measured and the water finally collected in the collector pond. Inlet and outlet of both wetland systems were recorded in a data logger, so that evapotranspiration in both containers could be detected.

The experiment was divided into 2 phases. In first phase (60 days, from 16 July to 13 September 2002) high concentration of the carbon source (sodium benzoate) and of Zn (5 mg/l) and Cr (5 mg/l) were supplied. In the second phase (87 days, from 14 September to 10 December 2002), the carbon source was reduced (without sodium benzoate). Zn and Cr were reduced to 1 mg/l each. The other parameters were kept constant.



a b

Figure 3.4 Preparation of the wastewater (a) and the automatic glass-collector for inlet and outlet volume measurement (b)

3.3.2.2 Sampling and analysis

Water samples were collected from every sampling point at different depths over the time of 5 months. Temperature, dissolved oxygen (DO) and redox potential (Eh) were measured in situ. Zn, As, Cr, Fe, arsenic species and sulfur species of the collected samples were analysed in laboratory later on (see 3.6). Plant, gravel and sediment were sampled and the concentration of metals at the beginning and at the end of experiment was analysed. The community of

microorganisms in the system was investigated at the end of the experiment. Analytical methods are explained in 3.5 to 3.7.

3.3.3 Treatment of acid mine drainage in six small scale constructed wetlands in a field experiment

Model experiments were conducted at the UFZ experimental area, Grosskayna-Beuna, "Recycling Park Beuna", near Merseburg. These field test systems included of 6 small containers, which were a continuously fed with acid mine drainage (AMD) from the gravel pit, Merseburg-Ost. This AMD had a concentration of 2-3 g/l sulfate and its pH was about 3. The chemical characteristic of this AMD is shown in Table C-1 (Appendix C).

3.3.3.1 Experimental set up

The experimental system comprised a storage tank system of AMD and 6 different model constructed wetlands. The experimental design, water volume and surface area of each system are shown in Table 3.8. *Juncus effusus* was used in the planted wetland systems. Container B1 and B2 represented the hydroponic systems. FSW and SSW (B3, B4, B5 and B6) contained a mixture of sand materials, which included sand and fine gravel (0.6-2 mm size, see Appendix C, Table C-2). The water was kept flowing 10 cm below the sand surface of SSW, and 10 cm over the sand surface of FSW. Schemes of the experiment are shown in Figure 3.5.

Table 3.8 Experimental design, water volume and surface area of each container of AMD experiment which were unplanted and planted with *Juncus effusus*

Experimental design	Water volume (l)	Surface area (m^2)
Hydroponic system (HP) B1: planted B2: unplanted	175	0.55
Free surface wetland (FSW) B3: planted B4: unplanted	107.5	0.55
Subsurface wetland (SSW) B5: planted B6: unplanted	57.5	0.55

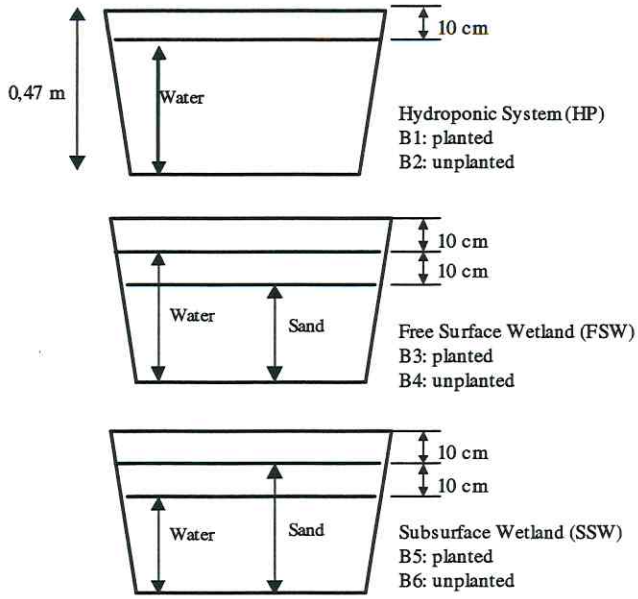


Figure 3.5 Schematic shows the cross section of different type of constructed wetlands for the removal of AMD in the field test systems with a size of 0.47 x 0.60 x 0.91 m^3 and a surface area of 0.55 m^2

3.3.3.2 Operation

The wastewater (AMD) applied in this experiment was transported from the gravel pit Merseburg-Ost a mining area near Grosskayna and stored in a storage tank system. AMD was pumped separately from the storage tank system to the 6 different wetlands. All systems were independent from each other and placed in an open area.

The experiments were operated during spring, summer and autumn of 2001 to 2003. The duration of the experiments was 87 days in 2001 (from September 25 to December 12), 224 days in 2002 (from April 24 to December 4) and 160 days in 2003 (from April 23 to September 30). Since it was very cold and the water froze the system was not operated in the wintertime. Before application, the wastewater was analysed for various parameters, such as metal concentrations and pH. Then, it was fed into each container. The inflow rates of each wetland system during the duration of the experiments are presented in Table 3.9.

Table 3.9 Inflow rate and hydraulic retention time (HRT) of each wetland system during the experimental period

Wetland systems	Year	Duration	Inflow rate [l/h]	HRT [d]
Planted and unplanted hydroponic systems (B 1 and 2)	2001	11.09-06.11	0.365	20.0
		06.11-12.12	0.180	40.5
	2002	15.04-22.05	0.180	40.5
		23.05-03.06	0.270	27.0
		04.06-25.09	0.400	18.3
		26.09-06.12	0.350	20.8
	2003	28.03-30.09	0.350	20.8
Planted and unplanted free surface wetlands (B 3 and 4)	2001	11.09-06.11	0.210	21.3
		06.11-12.12	0.100	44.8
	2002	15.04-22.05	0.100	44.8
		23.05-03.06	0.150	29.9
		04.06-25.09	0.300	14.9
		26.09-06.12	0.250	17.9
	2003	28.03-30.09	0.250	17.9
Planted and unplanted subsurface wetlands (B 5 and 6)	2001	11.09-06.11	0.335	7.2
		06.11-12.12	0.155	15.5
	2002	15.04-22.05	0.155	15.5
		23.05-03.06	0.233	10.3
		04.06-25.09	0.350	6.8
		26.09-06.12	0.300	8.0
	2003	28.03-30.09	0.300	8.0

3.3.3.3 Sampling and analysis

Water samples were collected weekly from inflow and outflow of each system over time. Temperature and pH were measured in situ. Acidity, Zn and Fe of the collected samples were analysed in laboratory later on. At the end of the experiment, exposed plants and sand matrix were sampled and the concentration of Zn and Fe was analysed and compared with unexposed samples (see 3.5 and 3.6).

3.4 Preparation of samples for chemical analysis

3.4.1 Extraction method for total arsenic and heavy metal analysis in plants and sediment

Plant samples were rinsed with distilled water and oven dried at 105-108 °C. Sediment samples were air dried at room temperature. After that, the plant and sediment samples were ground into homogenised powder and digested by microwave extraction. To digest the sample, 2 ml of digestion mixture (HNO_3 : HCl = 4:1) were added to 0.3 g of homogenised sample in a Teflon pressure bomb and heated to 260 °C for 0.5-1 hours. Once the digests has cooled, they were made up to 10 ml with deionised water, filtered using a 0.45 µm Gelman syringe filter. The filtrate solution was analysed for As using hydride generation atomic absorption spectrometry (HG-AAS) and for Zn and other heavy metals using ICP-AES. The detection limit of HG-AAS is 0.3 µg As/l and 0.06 mg As/l, 0.04 mg Zn/l, 0.07 mg Cr/l and 0.05 mg Fe/l for ICP-AES.

3.4.2 Extraction method for arsenic species analysis

In order to analyse for arsenic species the accelerated solvent extraction method was used for plant extraction. Plant, shoots and roots were collected separately and ground using liquid nitrogen. 1-3 g of each ground sample was extracted for 15-20 minutes with 30 ml water. The volume of supernatant ranged from 15 to 20 ml and was filtered with a cellulose filter (pore size 0.45µm). It was diluted to a final volume of 100 ml and the concentration of total arsenic and arsenic species was analysed by ICP-AES and IC-ICP-MS, respectively.

3.4.3 Gravel extraction

The desorption of arsenic from gravel was carried out by immersing the dry gravel sample, about 30 g, in 100 ml of 1M HCl and shaking for 24 hours. Then, the suspension was filtered through medium porosity filter paper and the quantity of arsenic, zinc and iron going into solution was analysed by ICP-AES.

3.5 Chemical and physical parameters analysis

Physical and chemical parameters were analysed with the methods listed in Table 3.10. Temperature, pH, dissolved oxygen (DO) and redox potential (Eh) were measured on site. All other chemical parameters were analysed in the laboratory.

Table 3.10 Analytical methods applied for wastewater characterization

Parameters	Methods
Dissolved Oxygen	DO meter (WTW)
pH	pH meter (WTW)
Redox potential (Eh)	Redox electrode (WTW)
Acidity	Titration DMS-Titrino 716 (Metrohm) with NaOH
Total arsenic	ICP-AES
Arsenic species (As(III), As(V), MMA, DMA)	IC-ICP-MS
Zn, Cr, Fe	ICP-AES
SO_4^{2-}	IC
Sulfur species (S^{2-}, $S_2O_3^{2-}$, SO_3^{2-})	HPLC

IC: Ion chromatography
ICP-AES: Inductively coupled plasma atomic emission Spectrometry
IC-ICP-MS: Ion chromatography coupled with inductively coupled plasma mass spectrometry
HPLC: High performance liquid chromatography

3.5.1 Dissolved oxygen and redox potential

DO and Eh were measured with the SenTix ORP electrode connected to a Multiline P4 (WTW, Germany). To prevent air contact, the electrode was put into a small flow through cuvette. The inlet of the cuvette was connected to a long robust injection needle, which was put into the different depth of the model wetland/pond systems (Figure 3.6). The outlet of the cuvette was connected to a syringe to suck water samples through the cuvette with the electrode. All data were recalculated to the standard hydrogen electrode taking the sample temperature into account.

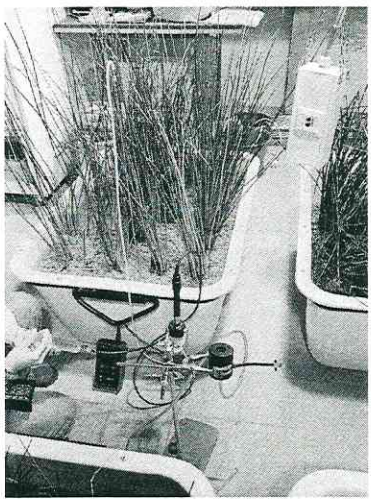


Figure 3.6 The sampling method for physical and chemical analysis.

3.5.2 ICP-AES

An Inductively Coupled Plasma-Atomic Emission Spectrometer (Spectro Cieros) consists of the following components: computer controlled atomic emission spectrometer with background correction, radio frequency generator and argon gas supply, welding grade or better. The detection limits of the operating conditions used are 0.06 mg/l of As, 0.04 mg/l of Zn, 0.07 mg/l of Cr and 0.05 mg/l of Fe. Variations of data from triplicate analysis were within ± 5 % of the average for all elements.

3.5.3 IC-ICP-MS

Ion chromatography coupled with inductively coupled plasma mass spectrometry is a powerful tool to investigate the distribution of arsenic species in plants and corresponding soil extracts (Mattusch et al., 2000). Using a simple species-preserving extraction method involving water, the proposed gradient separation of eight arsenic species is robust and provides long-term stability for the analysis of aqueous extracts of plant material.

The chromatographic system consisted of a LC 250 binary pump (Perkin Elmer), an injection valve with a 200 µL injection loop, an Ion Pac AG7 guard column (all Dionex). The anion-exchange column (250 x 4 mm, 10-µm particles) having alkyl quaternary ammonium exchange sites on a styrene-divinylbenzene copolymer was connected to an Elan 5000 ICP-MS (Perkin Elmer) via a cross-flow nebulizer. The ICP-MS was operated at 1050 W rf-power, 1000 ms dwell time, the data acquisition in the Graphic mode with argon flows of 0.85 l/min (auxiliary gas), 15 l/min (plasma gas) and 0.92 l/min (nebulizer gas). The signal at m/z 75 was monitored. The mobile phase was nitric acid solution with a concentration gradient pumped through the column at 1.0 ml/min.

The gradient consisted of two solvents (A and B). Eluents, solvent A was 0.4 mM HNO_3 and solvent B was 50 mM HNO_3 (Mattusch et al., 2000). The gradient was programmed as follows:

0-2 min	100% A
2-3 min	0-100% B linear gradient
3-8 min	100% B isocratic
8-10 min	100-50% B linear gradient
10-15 min	50% B isocratic
15-15.5 min	50-0% B linear gradient
15.5-20.5 min	100% A isocratic

Concentration of arsenic species is always given as the concentration of elemental arsenic. Stock solutions of arsenic compounds with a concentration of 1000 mg/l were prepared from arsenic trioxide (Fluka), arsenate solution (Titriol®, Merck), and dimethylarsinic acid trihydrate (Merck). Stock solution of mono-methylarsonic acid, arsenobetaine and trimethylarsine oxide were kindly provided by the Institute of Analytical Chemistry, KF-University, Graz, Austria. Stock solutions were stored in the dark at 4 °C and final standard solutions were prepared daily (Londesborough et al., 1999). The detection limits with the optimised chromatographic separation were 0.16-0.60 µg As/l for different species.

3.5.4 HPLC

The inorganic sulfur compounds in the water samples were analyzed by high performance liquid chromatography (HPLC, modified method according to Rethmeier et al, 1997). The sulfur components were derivatized by monobromobimane to yield fluorescent derivatives. This method enables the detection of S^{2-}, $S_2O_3^{2-}$and SO_3^{2-}. The derivatized sulfur compounds were detected by fluorescence emission at 480 nm. The HPLC (Beckman) was equipped with a 250mm*4mm column filled with LiChrosphere® 60 RP select B (5 µm, MERCK). The eluents were 0.25% acetic acid, pH 4 (solvent A) and 100% methanol (solvent B). The flow rate of the eluent was 1 ml/min and the gradient was programmed as follows:

0-5 min	88% A, 12% B isocratic
5-13 min	12-30% B linear gradient
13-16 min	30% B isocratic
16-34 min	30-60% B linear gradient
34-36min	60-100% B linear gradient
36-39 min	100% B isocratic
39-39.1 min	100-12% B linear gradient
39.1-42 min	88% A, 12% B isocratic

The detectable concentration ranges for the sulfur species are 5 µM to 1.5 mM for sulfide, 5 µM to 1.0 mM for sulfite and 1 µM to 1.5 mM for thiosulfate.

3.6 Microbiological analysis

This microbiological analysis was applied to the samples of water, plant roots and sediment collected at the end of the experiment of the two step wetland system (see 3.3.2).

3.6.1 MPN technique

Sulfate reducing bacteria (SRB) were enumerated by the most probable number (MPN) technique using ten-fold dilutions in three parallels of anoxic liquid medium tubes. Sulfate reducing medium (SRB medium) was used as growth medium for this MPN test. The samples were collected from the different points of the hydroponic system and kept under anaerobic condition. Then, the samples were diluted with freshwater medium containing 50 % of lactate as an electron donor (Hard and Babel, 1995; Hard et al., 1996, see Appendix B).

1 ml of each dilution of the water samples was inoculated in individual tubes of 9 ml of SRB medium. The tubes were incubated at 20°C for 100 days. The growth of SRB was detected by the production of FeS precipitated in the tube. Most probable numbers were estimated according to the tables given by APHA, AWWA, and WEF (1995).

3.6.2 Sampling, sample preservation and DNA extraction

The water samples, sediment and the roots of plants were collected at the end of experiment. Sampling points were in the same area where the physical-chemical parameters were measured. All of those samples were kept at –20°C in order to conserve the DNA of the bacterial cells.

DNA was extracted from water samples using DNeasy Tissue Kit (Qiagen). Bacterial cells from root samples were detached by washing in 10 ml sterile saline solution (0.85% NaCl) for 7 min in an ultrasonic water bath. After removal of the root material, the cell suspensions were centrifuged at 4100 g for 30 min. The supernatants were discarded and the pellets were collected. DNA from the pellets was extracted using DNeasy Tissue Kit (Qiagen).

DNA from sediment samples, 500 mg each, was extracted using the FastDNA Spin Kit for soil (BIO 101). All kits were used according to the manufactures' protocols.

3.6.3 PCR amplification of 16S rRNA fragments

Amplification was performed in a total volume of 100 µl with HotStarTaq polymerase, 1x PCR buffer, 5 pmol (0.5µM) primers, 200 µM dNTP, and 4 µl of the DNA extract. For the amplification of the V4-V5 region of small-subunit rRNA genes Com1 and Com2 primers were used (see Appendix B), which hybridize to the target position 519-536 and 907-926, respectively (Peters et al., 2000). PCR was conducted at 94 °C for 3 min for initial denaturation, followed by 35 cycles of denaturation at 94 °C for 60 s, at 50 °C for 60 s, at 72 °C for 70 s, and a final primer extension at 72 °C for 5 min (Tebbe et al., 2001).

After completed amplification PCR products were submitted to 1.7% agarose gel electrophoresis in 1x TAE and run at 100 V. After that, the gel was stained with ethidium bromide for 10 min and exposed in UV light.

3.6.4 DNA profile by single strand conformation polymorphism (SSCP)

PCR products were purified with QIAquick PCR Purification (Qiagen). DNA concentration was measured fluorometrically using Pico Green dye and a fluorescence microplate reader (Wallac Victor). The final concentration of dsDNA for SSCP Gel was 300 ng in a final volume 30 µl. These products were digested with lambda-exonuclease at 37°C for 45 min to obtain single-stranded DNA. The single-stranded molecules were purified with MinElute PCR Purification (Qiagen) to yield a final volume of 10 µl. These samples were incubated at 95°C for 3 min and immediately cooled on ice afterwards.

The electrophoresis was conducted in polyacrylamide gel, 0.625x MDE amended with formamide for mind denaturations (MDE, FMC Bioproducts, Rockland, ME) and carried out in a PROTEAN®II Xi Cell (Biorad). The gel was run at 20 °C, 45 mA, 300 V for 16 h and DNA was visualized according to the silver-staining procedure described by Tebbe et al. (2001).

3.6.5 Identification of SSCP bands

The selected bands of the SSCP gel were cut out with a sterile razor blade and were eluted in 40 µl sterile water at 4°C for 24 h. Reamplification of the extracted DNA molecule was performed with PCR using the same primers and conditions as applied for the above PCR process. The PCR products were purified as described above and a new SSCP gel was run to confirm whether there was only one band of DNA in the PCR products of those cut bands.

3.7 Other methods

3.7.1 Efficiency analysis

The metal removal efficiencies of the constructed wetland systems were calculated by comparing the inflow and outflow loads. The removal efficiency was calculated using the following equation.

$$Efficiency\ (\%) = \frac{Influent\ \ load - Effluent\ \ load}{Influent\ \ load} \times 100$$

3.7.2 Data analysis

The effects of factors, the plants and types of wetlands, on the efficiency for arsenic and metal removal of constructed wetland systems were analyzed by analysis of variance (ANOVA) at 95% confidence level (P=0.05), which has been proposed for any and all possible contrasts between factor means. This method can explain the difference between the efficiency of constructed wetland systems with different plant species or without plants.

4 Results and discussions

4.1 Behaviour of arsenic in anaerobic river sediment

The behaviour of arsenic in the presence of river sediment was studied in anaerobic experiments, in order to understand the effect of the river sludge-sediment on the decrease of total As and the reduction of As(V) to As(III) in anaerobic environmental systems.

It was found that the concentration of As(V), As(III) and total As decreased in all bottles. In the control (A) without river sediment addition, the concentration did not change significantly during the first three days, but it decreased after 7 days of incubation (Figure 4.1a).

In bottle B (nutrient solution with river sediment) the concentration of arsenic decreased significantly (Figure 4.1b). As(V) had decreased more obviously by day 3 and 7 than in the control; As(III) followed the same trend. However, the concentration of As(III) was higher than As(V) after day 3 and 7. In bottle C where sulfate was added as an additional electron acceptor for microbes, it was found that the concentration of total As, As(V), and As(III) decreased over time, too (see Figure 4.1c).

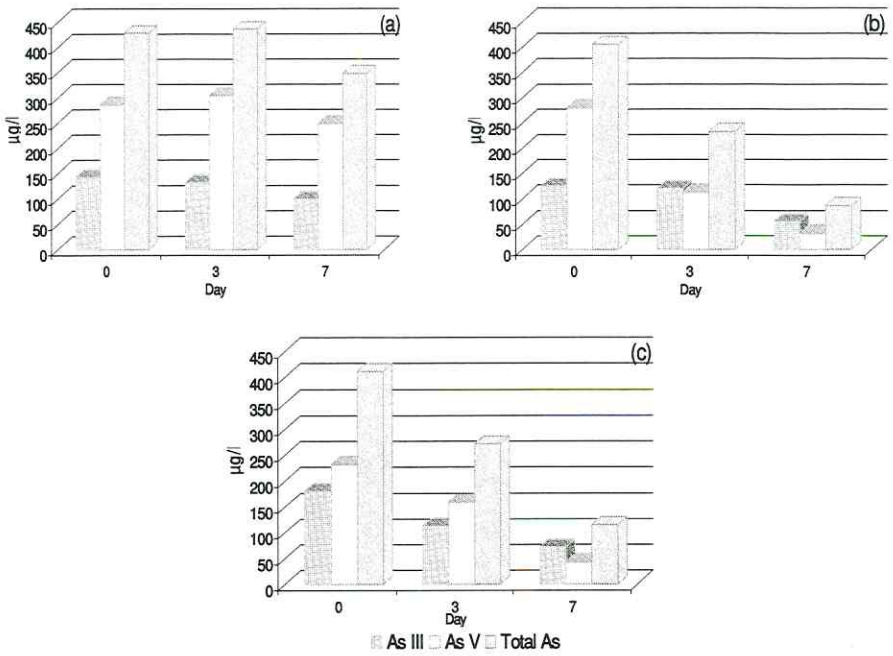


Figure 4.1 The behaviour of arsenic in anaerobic river sediment (bottle experiments) [a: control, b: sediment, c: sediment plus sulfate]

The experiments showed that river sediment had a significant effect on the reduction of As(V) to As(III) and the removal of total As. Sulfate had no significant effect on the reduction of arsenic. Furthermore, it could mean that certain microorganisms in the sediment might have an effect on this.

As biological factors were assumed to have an effect on the reduction and sorption of arsenic in the river sediment, an additional microcosm experiment was performed. Sodium azide was used as a potential inhibitor of microbial activities. River sludge and nutrient solution containing arsenic were used under the same conditions as in the adsorption study, with additional azide but no addition of sulfate.

The concentrations of total arsenic at the beginning of incubation were in the range of 0.4-0.5 mg/l in all 4 bottles (Figure 4.2). After 7 days of incubation, the concentrations of total arsenic in bottle C and D had decreased significantly to 0.12-0.17 mg/l. However, the concentration of arsenic in the both control bottles, A and B, did not decrease.

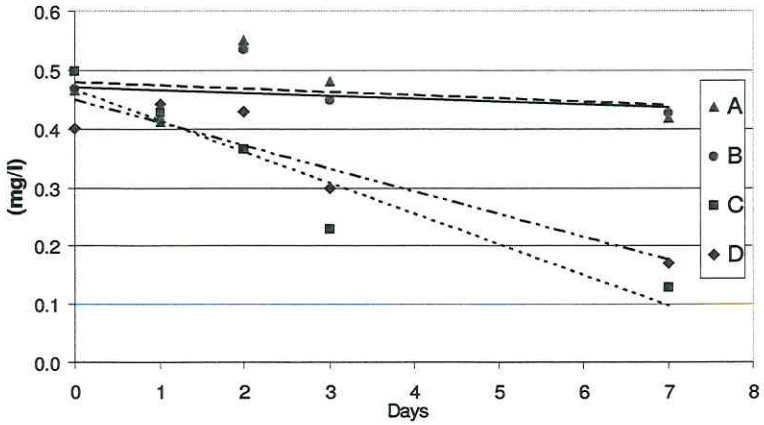


Figure 4.2 Total arsenic concentrations in nutrient solution in different conditions
--▲-- bottle A : no amendments (control), -●- bottle B: with NaN_3 ,
····■···· bottle C: with river sludge, -··-♦-··- bottle D: with river sludge and NaN_3

From these results it can be concluded that microorganisms had no significant effect on the decrease of total As in these bottle experiments. The concentration in bottle D, with added sodium azide to inhibit microbial activities, was decreasing significantly, even though total As in bottle D was slightly higher than in bottle C. It shows that arsenic was fixed in anaerobic condition even if conditions were not suitable for microorganisms or other biological factors.

Therefore, it can be assumed that not only biological factors have an effect on the decrease of arsenic under anaerobic conditions, but also other physical factors.

4.2 Precipitation of arsenic with sulfide and its co-precipitation with zinc and sulfide

At present there are many investigations about the precipitation of arsenic with iron (Belzile and Tessier, 1990; Fendorf et al., 1997). Less is known about its precipitation as arsenic trisulfide (As_2S_3) which was reported in the context of sulfate reducing bacteria (Ahmann et al., 1994; Newman et al., 1997) and the co-precipitation with other metals (Bothe and Brown, 1999; Lumsdon et al., 2001). Therefore, in this study the behaviour of arsenic in the presence of sulfide and the possibility of co-precipitation of arsenic with zinc and sulfide were investigated.

A study of the precipitation of arsenic and zinc as sulfide in anaerobic condition was conducted in two flasks. Flask 1 contained nutrient solution and As and flask 2 contained nutrient solution, As and Zn. The pH, redox potential and the concentration of As and Zn were detected before and after adding 300 mg/l of $Na_2S{\cdot}9H_2O$ to the flasks. The results are shown in Table 4.1.

It was found that the pH of the solution changed from neutral to very highly basic (14), and Eh values were extremely low (-480 to -430 mV) after the addition of $Na_2S{\cdot}9H_2O$. Flask 1, which contained only arsenic, had a higher redox potential or Eh values than flask 2 which contained both arsenic and zinc. After $Na_2S{\cdot}9H_2O$ was added to the solutions, Eh declined rapidly to - 480 mV in flask A (with As) and to - 430 mV in flask B (with As and Zn).

Table 4.1 Precipitation of arsenic and zinc with sulfide at a high pH

Solution in flasks	**Flask 1: with As**		**Flask 2 : with As and Zn**	
	Before adding $Na_2S{\cdot}9H_2O$	After adding $Na_2S{\cdot}9H_2O$	Before adding $Na_2S{\cdot}9H_2O$	After adding $Na_2S{\cdot}9H_2O$
pH	7	14	6.5	14
Redox potential (mV)	242	- 480	220	- 430
Total As (mg/l)	0.89	< 0.09	0.93	< 0.09
Zn (mg/l)	0.64	< 0.03	136.40	0.80
Colour of wet precipitate	-	Grey	White (A small amount)	White
Colour dried precipitate (108 °C)	-	Green	-	Grey

Precipitation could be observed in both flasks. There was a little amount of white precipitate in flask 2 (with As and Zn). It occurred rather suddenly after the addition of Zn and there was a higher amount of precipitate after addition of $Na_2S{\cdot}9H_2O$. In flask 1, there was greyish precipitate occurring after $Na_2S{\cdot}9H_2O$ addition.

The concentrations of Zn and As are shown in Table 4.1. After adding $Na_2S{\cdot}9H_2O$ to the two flasks, arsenic concentrations were reduced from 0.9 mg/l to < 0.09 mg/l. The Zn concentration in flask 1 was reduced almost totally from 136.40 mg/l to 0.80 mg/l. The zinc concentration in flask 2 decreased from 0.64 mg/l to less than 0.03 mg/l when $Na_2S{\cdot}9H_2O$ was added to the solution. The results from this experiment showed that there was a precipitation of arsenic and zinc with sulfide in both systems (systems with and without Zn).

Figure 4.3 shows the chromatogram of arsenic compounds from the solution containing As(V), Zn and S^{2-}. It was found that As(V) was transformed to As(III) and other unknown arsenic species (appearing in the range of retention time 460 – 500 s). For the solution which contained As(V), Zn and S^{2-}, there were 2 large peaks which could not be found in the solution containing only As(V) and S^{2-}. The two peaks occurred in the range of retention time from 650 to 1000 seconds in the chromatogram. These two peaks were assumed to arouse form As/S and As/Zn compounds. However, the peak occurring at a retention time of 650 – 750 seconds was not found in neutral pH solution, before addition of sulfide. They were found only at extremely high pH (pH 14).

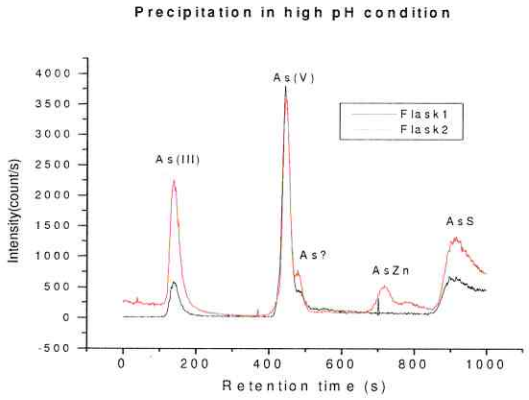


Figure 4.3 IC-ICP-MS chromatogram of arsenic compounds in solution of Flask 1 and Flask 2 [Flask 1: As(V) and Sulfide; Flask 2: As(V), Sulfide and Zn]

A further study on the precipitation of arsenic and other metals with sodium sulfide at lower pH was done. The water samples were adjusted to acidic condition in a pH range of 4.5-5.5. Thereafter, 500 and 1000 mg/l of $Na_2S·9H_2O$ were added. The pH changes are shown in Table 4.2.

Table 4.2 pH values of the solutions in the second experiment, without pH adjustment

Samples	pH	
	Before adding $Na_2S·9H_2O$	After adding $Na_2S·9H_2O$
Nutrient solution+As(V)+Zn	4.5–5.5	9.5-10.5
Nutrient solution+As(V)	5.0–5.5	9.5-10.5

In the solutions from this experiment showing a final pH of 9.5 to 10.5, As(V) and As(III) were detectable. Figure 4.4 shows the chromatogram of As(V) and As(III) and there was no unknown peak found near the end of the chromatogram (at the retention time of about 600-1000 s) as found at higher pH, see Figure 4.3. Those unknown peaks were found only under the extremely high pH conditions.

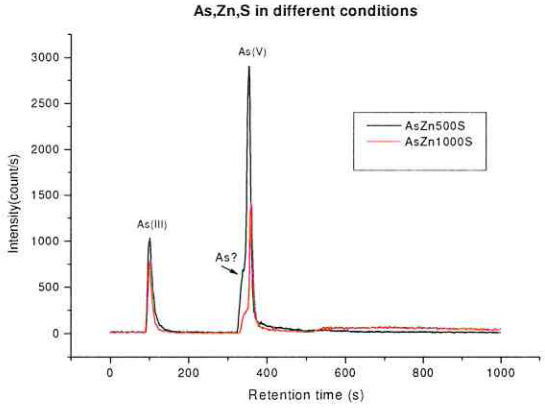


Figure 4.4 IC-ICP-MS Chromatogram of arsenic compounds in solution containing As(V) and Zn with different concentrations of sulfide; 500 mg/l and 1000 mg/l of sulfide

In the experiments with various concentrations of sulfide, pH values were measured. The results are shown in Table 4.3. The concentrations of S, Zn and total As were analysed only in

the experiment with excess amounts of S^{2-}. In Table 4.4 it can be seen that the concentration of As decreased when S^{2-} was added to the system. The concentration of Zn decreased in the same manner as the concentration of As.

Table 4.3 pH values of nutrient solution with various concentrations of sulfide

Concentration of S^{2-} (mg/L)	pH	
	Before adding S^{2-}	After adding S^{2-}
1	6.23	6.32
10	6.21	6.49
100	6.18	9.11
500	6.38	10.98

Table 4.4 Concentrations of Zn and As in the solutions

Samples	Precipitate (colour)	Concentration (mg/L)	
		Zn	Total As
Nutrient solution+As(V)	No	0.68	0.90
Nutrient solution+As(V) + S^{2-}	Yes (grey)	0.04	<0.1
Nutrient solution+As(V)+Zn	No	106.10	0.62
Nutrient solution+As(V)+Zn +S^{2-}	Yes (white)	0.13	<0.1

The concentrations of As and Zn decreased in the presence of a high amount of sulfide and under strongly anoxic condition. The low concentration of metals found in the solution after addition of the excess amount of $Na_2S{\cdot}9H_2O$ (10 g/l) indicated the formation of insoluble metal sulfides. Arsenic has been reported to have a strong affinity for S (Ferguson and Gavis, 1972).

Carbonell-Barrachina et al. (1999) indicated that the As solubility in reduction conditions was perhaps limited by the formation of insoluble As sulfide minerals. After arsenate is reduced to arsenite under reducing conditions, if sulfur is abundant, most of the As reacts with sulfides to form insoluble As sulfide minerals (realgar (AsS), orpiment (As_2S_3)). The behavior of Zn under reducing conditions is similar to that of arsenic, which results in the formation of insoluble zinc sulfides.

4.3 Batch experiments simulating important processes occurring in constructed wetlands

4.3.1 Physical and chemical parameters in water phase

4.3.1.1 pH and redox potential

In both gravel free systems (HP and AP) the pH values were constant at about pH 4. In the gravel bed systems (SSW and FSW) the pH buffered at a higher level of 6–7. No pH gradients within the four systems depending on the depth could be observed (see Appendix A, Figure A-1).

The time pattern of the redox potential was similar to other parameters, such as pH, As and Zn in both gravel free systems (HP and AP). There were no gradients in dependence of the depth (Figure 4.5 and Appendix A). The values stayed relatively constant within oxic conditions, in the range of 430 and 600 mV.

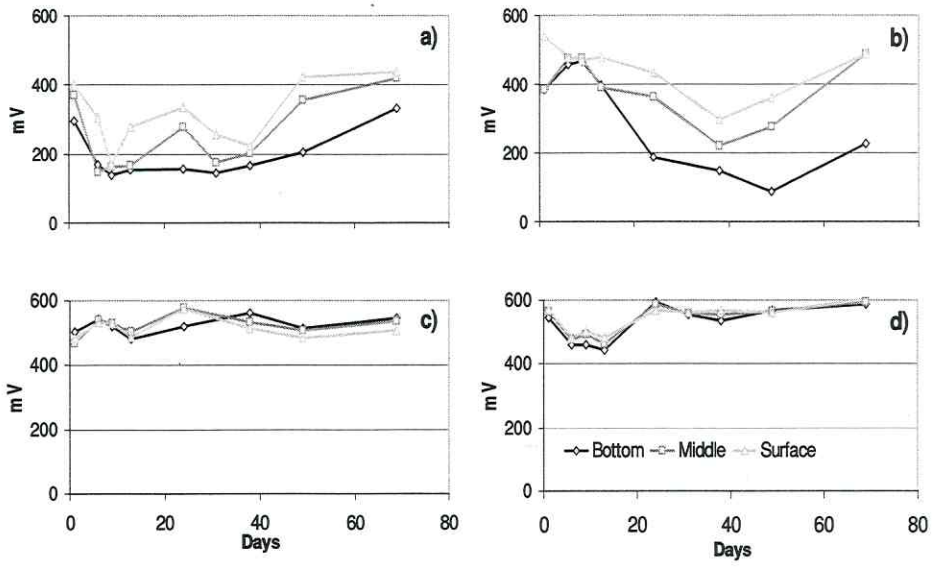


Figure 4.5 Redox potential in different depths in the experimental wetland systems
a) in SSW b) in FSW c) in HP d) in AP

A clear gradient could be seen in the gravel bed systems, SSW and FSW. The minimum value was observed at the deepest sampling points (bottom). Potential differences of up to about 250 mV were measured between the surface and bottom, especially in FSW. However,

measured redox potential in the pore water of both systems was never below 100 mV. This means that no conditions favouring growth of sulfate reducing bacteria prevailed. From these results it has to be concluded that removal of As, Zn and Fe in the model constructed wetlands was realized mainly by other mechanisms than precipitation as their sulfide.

4.3.1.2 Total Arsenic (Total As)

The arsenic concentration was less than 0.5 mg/l at the beginning of the experiment in contrast to a concentration of 0.5 mg/l of artificial wastewater, because the fresh artificial wastewater was diluted with the tap water remaining in the fresh washed wet gravel. Therefore, the As concentrations in the different model systems are illustrated in (Figure 4.6).

The arsenic concentration in SSW and FSW decreased with time of operation. After about 24 days their concentrations had decreased below 0.1 mg/l (detection limits was 0.6 mg/l). Due to the high detection limit of ICP-AES, a new approach of As concentration from IC-ICP-MS method, which represented lower detection limit, has been introduced (see Figure 4.7). Thus, these results indicate the evidence that As was reduced to insignificant value (about 10 µg/l or 0.01 mg/l) in these two planted gravel beds, SSW and FSW. Because the adsorption capacity of gravel for As was very low (in the range of up to 4.3 µg/kg), other processes than direct adsorption must be responsible for the As removal from the water. In contrast to this, the iron content of the gravel was >100 mg/kg. With this surplus of iron in both systems all arsenic could theoretically be bound by iron.

Because the plants themselves did not absorb considerable amounts in the hydroponic system and the adsorption capacity of the gravel is low in the gravel systems, only the combination of both soil and plants, which results in special distinct conditions for As binding, can be the explanation for the phenomenon of best As removal in planted gravel systems.

Theoretically, it can be assumed that by the activity of the roots, organic compounds (rhizodeposition products as the sum of root exudates and dead root matter) are released into the rhizosphere. Some of these compounds can function as iron chelating compounds (Hoffland et al. 1992). Furthermore, these organic compounds can also be used as a carbon source for microorganisms in the soil resulting in the observed decrease of the redox potential. Both, relatively low redox potential and chelating rhizodeposition products stimulate the re-dissolution of crystalline iron(III) which has a low binding capacity for As. In addition, there

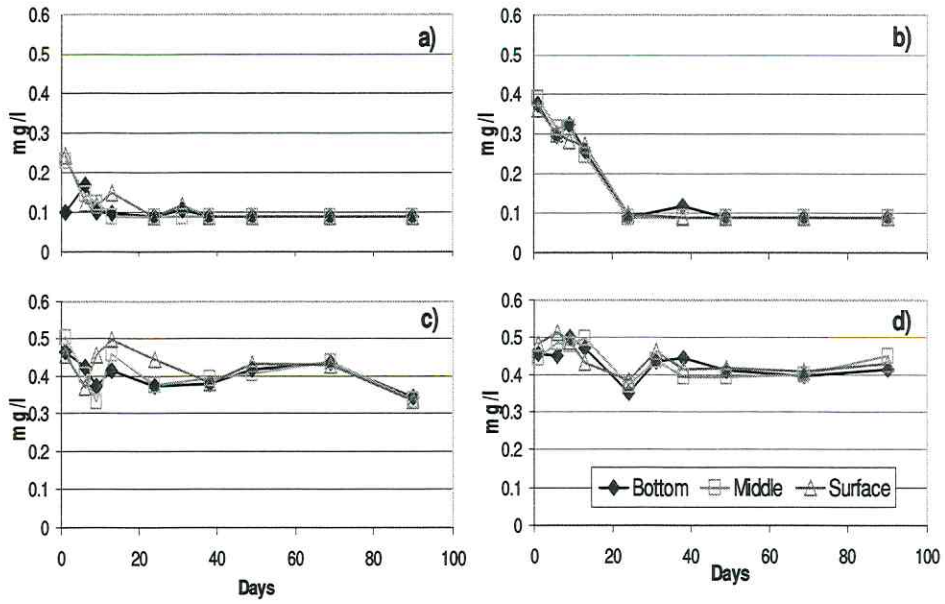


Figure 4.6 Total As concentration in the experimental wetland systems
a) in SSW b) in FSW c) in HP d) in AP

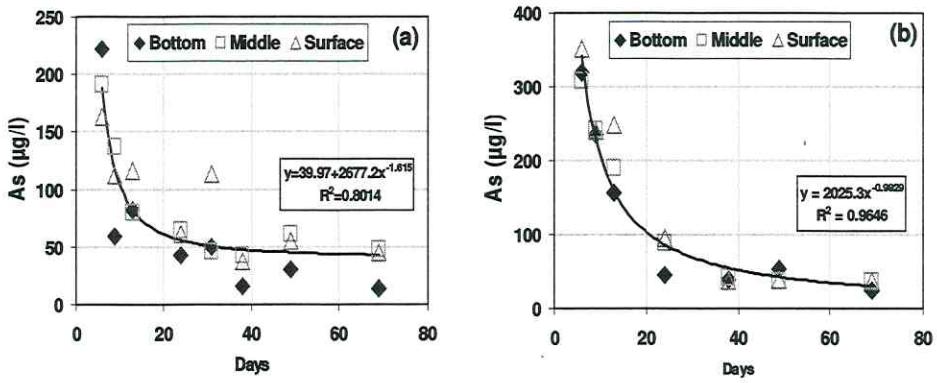


Figure 4.7 Arsenic concentrations which are the sums of the concentration from all kinds of As species (analysed by IC-ICP-MS) in the experimental wetlands (a) SSW and (b) FSW

is the capability of some helophytes to transfer oxygen into their rhizosphere (Grosse and Schröder 1986; Jackson and Armstrong, 1999; Colmer, 2003). In the rhizosphere and especially on the rhizoplane, the oxic conditions can prevail and cause the precipitation of the dissolved iron and co-precipitation of other trace elements especially on the roots forming iron plaques (Wang and Peverly 1996; Doyle and Otte, 1997; ElbazPoulichet et al., 2000; Stottmeister et al., 2003).

In general, a dissolution of crystalline iron and subsequent precipitation by the direct and indirect action of the plants in combination with microorganisms can cause the As removal from the water phase in water logged soils with an apparently low As binding capacity.

The concentration of As in the water phase of HP and AP was nearly constant over time (Figure 4.6c and Figure 4.6d). The average concentration of HP at the end of operation was 0.34 mg/l. Only in HP, As concentration decreased by 35 % during the time of 90 days, whereas in the AP the As concentration in the water stayed unchanged. Both systems were free of a concentration gradient that means the As concentration did not vary in dependence of the depth. Similar behaviour was also observed for pH, redox potential and sulfate and Zn concentration.

4.3.1.3 Zinc (Zn)

The zinc concentration decreased in SSW and FSW. At least 80 % of Zn decreased in both SSW and FSW, which contained both plants and gravel, whereas, the removal rate of SSW was higher and faster than for FSW.

It was found that the average concentration of Zn in HP decreased from 5.5 mg/l to 3.7 mg/l over the period of 90 days (Figure 4.8), with a removal rate of about 30 %. Similar to the total As concentration, no concentration gradient in dependence of the sampling depth could be observed.

Because the concentration of Zn decreased dramatically in both gravel bed systems (SSW and FSW) within a few days and because of the increase of iron concentration over time, while the redox potential did not decrease below 100 mV, the action of sulfate reducing bacteria and resulting sulfide precipitation can be excluded.

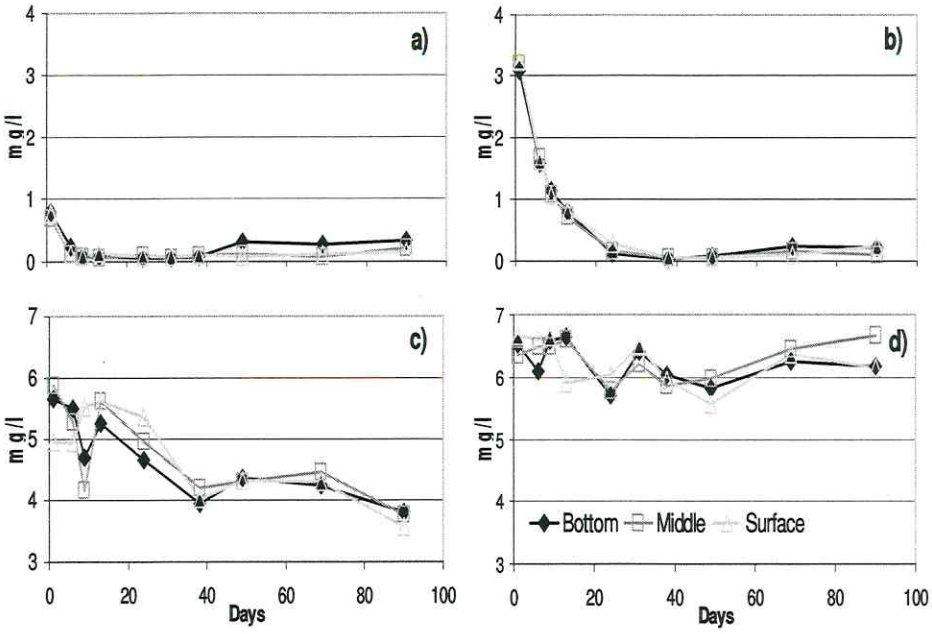


Figure 4.8 Concentration of Zinc in the experimental wetland systems
a) in SSW b) in FSW c) in HP d) in AP

This phenomenon could be explained by the precipitation of heavy metals with substance in the water. In the environmental wetland, both biotic and abiotic factors are involved in the removal mechanisms. The humic substances and hydrous oxides of manganese and iron could transfer heavy metals to the sediments due to adsorption and binding inorganic pollutants to the surface by sediment colloids. Therefore, these occurrences could accelerate the reduction of Zn. A higher capacity of the removal rate was observed in our wetlands which had both plants and gravel. The resulting average pH value in these wetlands was about pH 6. Therefore, the other mechanism that leads to the removal may be flocculation, which is enhanced by high pH and high concentration of suspended solids.

Wood (1990) showed that wetland plants translocate oxygen from the shoots to the root rhizomes through their internal gas space, the aerenchyma. The root and rhizomes in turn leak the oxygen to the reduced environment (Wießner et al., 2002). It is these oxidised conditions that promote precipitation of oxyhydroxides of Fe^{3+} and Mn^{2+}. Furthermore, these precipitated

hydroxides also act as absorption sites for other phytotoxic heavy metals present in the water compartment of the wetland (Wood, 1990; Shrestha et al., 2003).

4.3.1.4 Iron (Fe)

The behaviour of dissolved iron in wetland systems can be seen in Figure 4.9. The low initial concentration of Fe (about 0.2 mg/l) in both gravel bed systems decreased immediately below detection limit (0.05 mg/l with ICP-AES) due to high initial redox potential of about 300-550 mV and in pH range of 6-7. The iron concentration increased over time in the subsurface zones reaching its maximum of up to 6.5 mg/l during days 30-40 in SSW. Afterwards, the iron concentration decreased again connected with an increase of the redox potential (Figure 4.5, Figure 4.9).

The reason for this dissolution of iron from the gravel could be excretion of rhizodeposition products as already discussed above in relation with the dissolved arsenic concentrations. While iron concentrations in HP decreased over time to almost detection limit, in AP after a short period (day 5-15) of extremely low concentrations, iron was still in the range of 0.1-0.2 mg/l. However, it remained relatively constant without depth gradients.

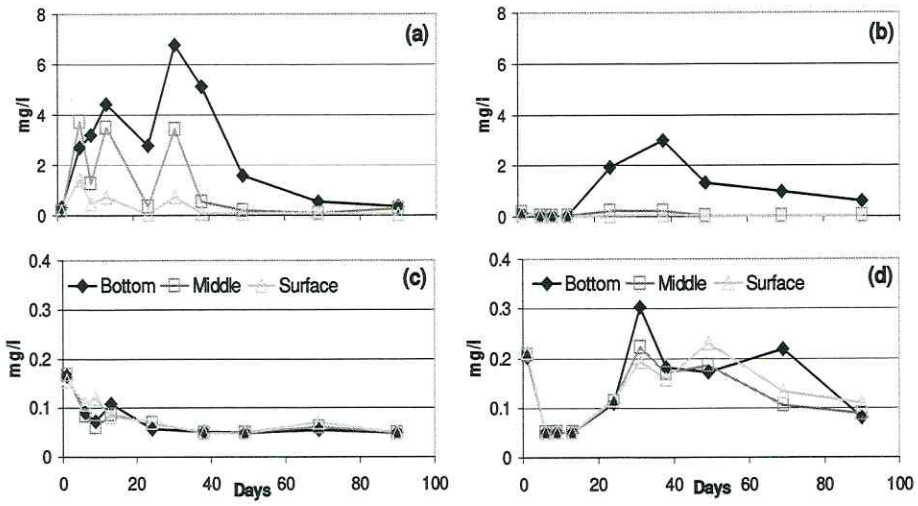


Figure 4.9 Fe concentrations in different wetland systems; a) in SSW b) in FSW c) in HP d) in AP

Iron can precipitated on the root surface and in the rhizosphere of the plants forming a so called iron plaque (Otte et al., 1995; Doyle and Otte, 1997). The precipitation of iron oxyhydroxides in the rhizosphere in turn leads to a concentration gradient of dissolved iron towards the plant roots. The iron oxyhydroxides in turn bind arsenic and zinc, again creating a decreasing concentration gradient of both elements towards the roots. These gradients lead to the diffusion of iron, arsenic and zinc in the direction of the root (Otte et al., 1995).

An increase of arsenic solubility under reduced conditions is associated with dissolution of Fe oxides/hydroxides. A significant correlation has been found between dissolved Fe and As confirming that Fe oxides/hydroxides represent the major sorbing agents for As in soils (Masschelyn et al., 1991; Marin et al., 1993; Fitz et al., 2002).

4.3.1.5 Sulfate

The changes of sulfate concentration are negligible in both systems without gravel (HP and AP). It was constant during the course of the experiment. However, striking changes could be observed in the gravel bed systems SSW and FSW (Appendix A, Figure A-3). In the upper sampling zones of SSW and FSW the concentration decreased over time (addition of distilled water and replacing water loss by evapotranspiration). In contrast, in the deeper zones, especially near the bottom, the concentration increased to considerably high concentrations with values higher than 3 g/l caused by plant transpiration activity. In these high concentrations of water ingredients, some precipitation reactions such as formation of insoluble carbonates of zinc could be stimulated.

4.3.1.6 Arsenic species

At the beginning of the experiment, arsenate (As(V)) was the major species component in all systems (Figure 4.10 to Figure 4.14). In SSW (Figure 4.10), As(V) was changed to other species, mainly reduced to As(III) from day 9. The concentration of As(III) at the bottom of SSW was higher than the concentration in the middle and at the surface. It was found that As(V) of the water sample from the surface was higher than in the middle and at the bottom. On day 31, the changing rate of As(V) to As(III) was accelerated. At each water level, the As(III) concentration increased whereas As(V) decreased simultaneously. Moreover, there were small amounts of other species of arsenic occurring in SSW, shown in the chromatogram (Figure 4.12a).

In FSW the same manner occurred as in SSW that major As(V) species was reduced to other As species (Figure 4.11). Higher concentration of As(III) was found at the bottom (Figure 4.11c). The appearance of other As species was found in less species and smaller concentrations than of SSW because the redox conditions was higher and could not prevail the higher oxidised form of As(V).

In the hydroponic system (HP) As(V) was the major species and was found in a higher concentration (Figure 4.13). An appearance of other As species was found less than other wetland systems.

In the algae pond (AP) there was only wastewater without plants. After some time algae appeared accompanied by the occurrence of numerous As species (Table 4.5, Table 4.6, and Figure 4.14). Different arsenic species like MMA, DMA, and TMAO etc. could be detected in all water depths (Figure 4.14).

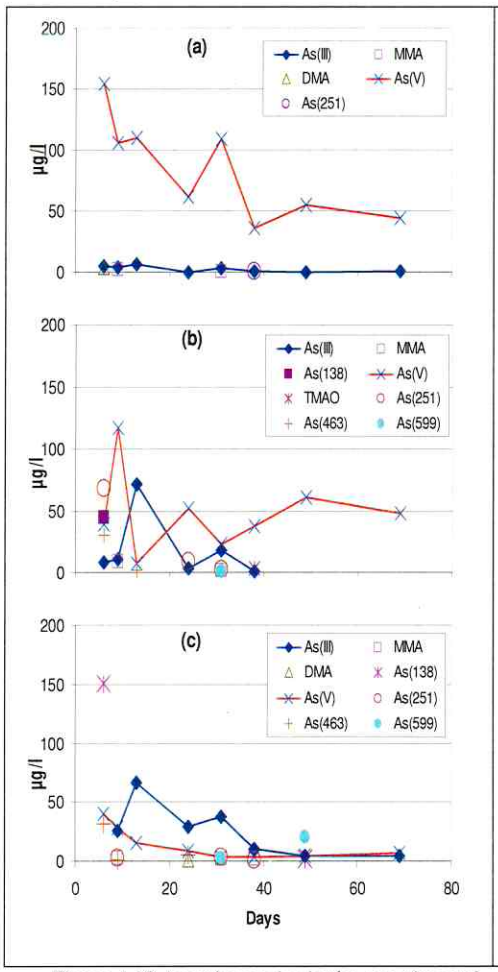


Figure 4.10 Arsenic species in the experimental subsurface wetland (SSW) in different depth gradients a) surface b) middle c) bottom

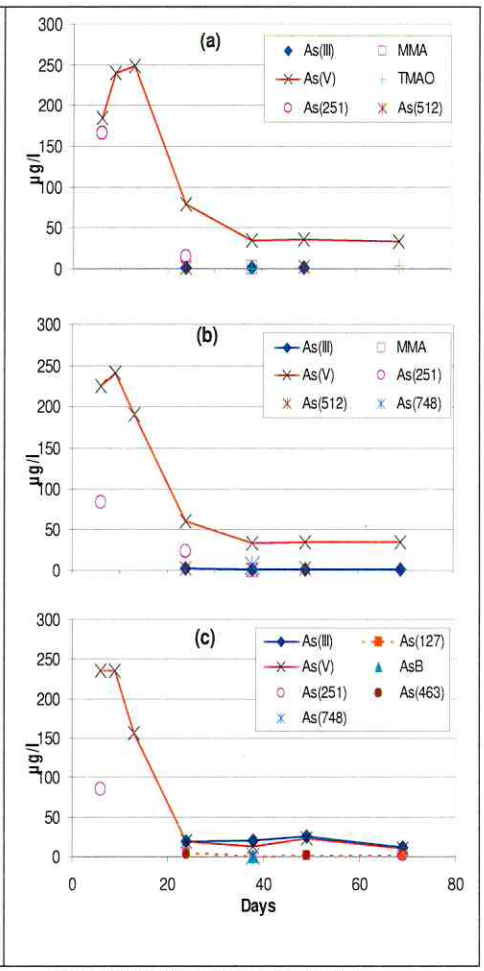


Figure 4.11 Arsenic species in the experimental free surface wetland (FSW) in different depth gradients a) surface b) middle c) bottom

The following Figure 4.12 to Figure 4.14 illustrates the amount of arsenic species found in each wetland on the sampling dates. It indicated that As(V) was the abundance species in an algae pond, although As(V) was transformed to other As species by the appeared algae (for example, at the day 31), which resulting in a high concentration of total arsenic. On the other hand, total As did not decrease significantly.

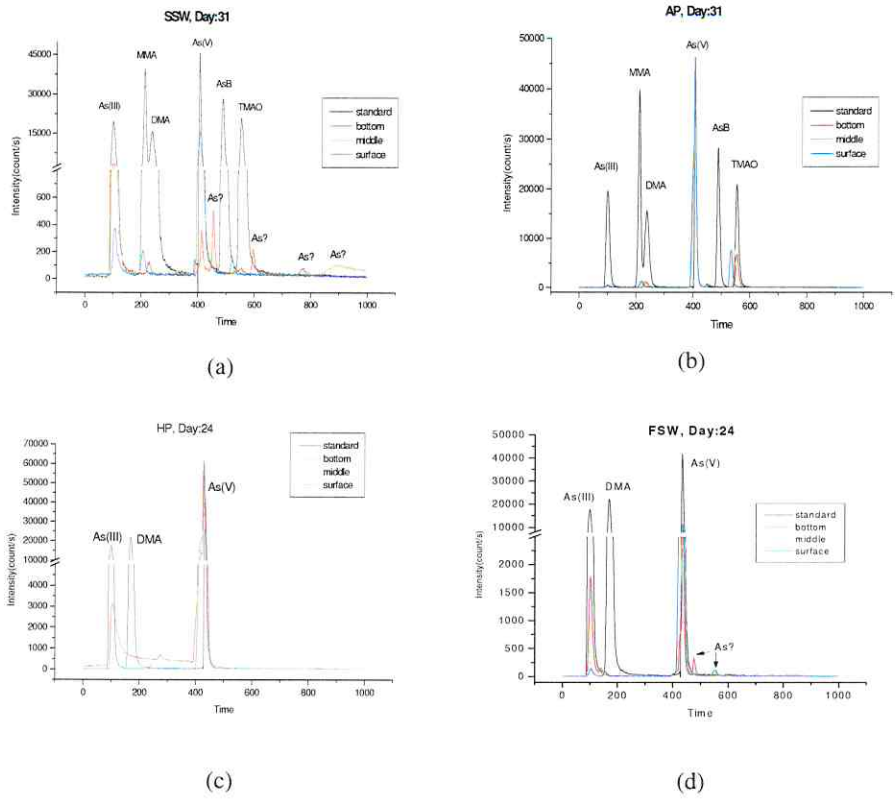


Figure 4.12 IC-ICP-MS Chromatogram of arsenic species in the different experimental wetland systems a) in SSW at day 31, b) in AP at day 31, c) in HP at day 24, d) in FSW at day 24

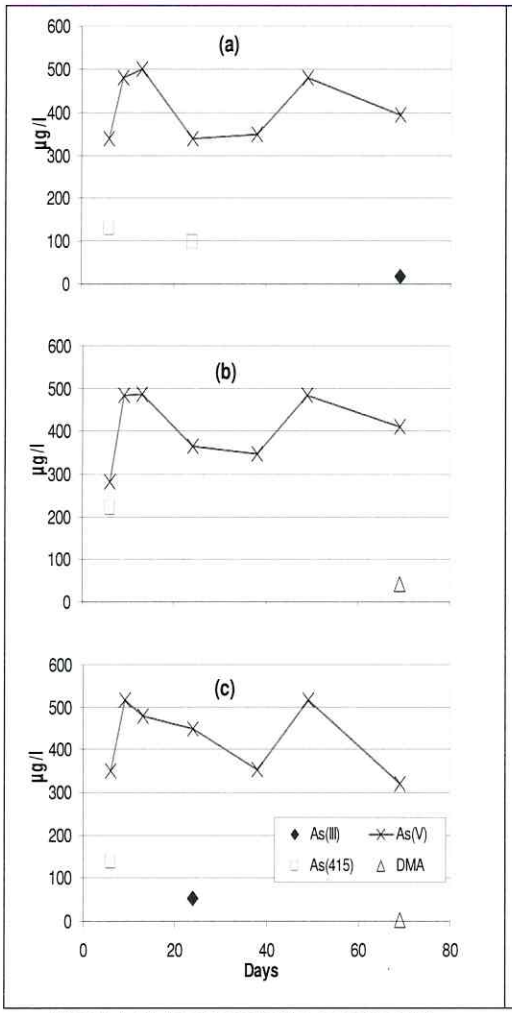


Figure 4.13 As species in the experimental hydroponic (HP) in different depth gradients a) surface b) middle c) bottom

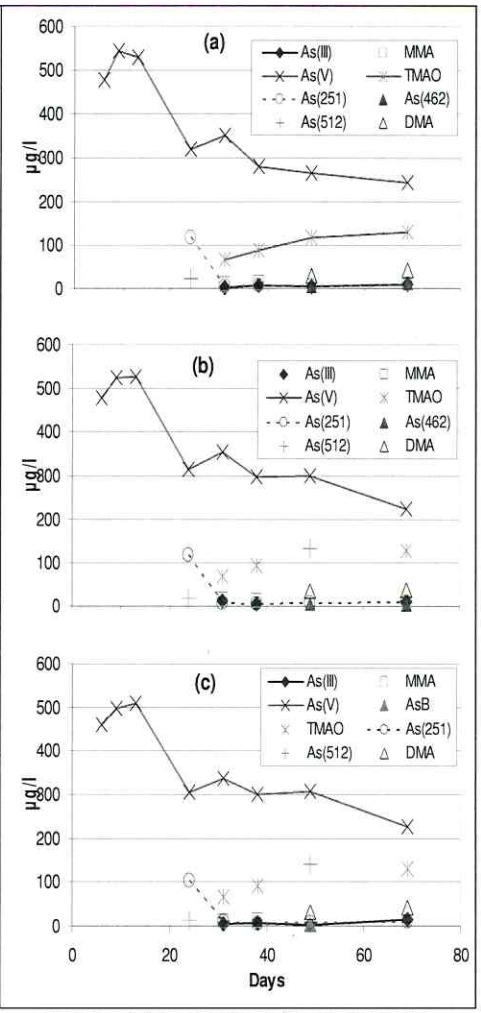


Figure 4.14 As species in the experimental algae pond (AP) in different depth gradients a) surface b) middle c) bottom

Our results indicated that the metabolization and redox reactions of added arsenate were conducted by micro-organisms and/or plants. The occurrence of the different species in the water phase varied with time and with the depth or redox potential in the systems. The transformation of As in SSW occurred earlier (after 5 day) than in HP (after 20 days).

The amount and level of gravel affected the reduction-oxidation state in SSW and FSW. The reasons might be that there was a deficiency of oxygen in the system (average 1.35-2.1 mg/l, facultative condition); accordingly the high gravel level resulted in the lack of oxygen at the bottom of the system (Appendix A, Figure A-2). The lack of oxygen with depth correlated well with the decreasing Eh values. In SSW, the Eh value decreased from day 6 of the experiment in different degrees; at the bottom (140 mV) it was lower than in the middle and at the surface (170 mV), which showed more reducing condition. In the bottom phase of SSW, As(III) became the major As species in solution under reducing conditions. Eh in FSW and SSW was also reduced during the running time of the experiment. Eh of the water at all depths of FSW decreased gradually, especially in the bottom region, to 88 mV on day 49. Therefore, redox values decreased along the depth gradient. The lower redox potential is the higher correlated with the higher amount of water soluble arsenic (Marin et al., 1993).

It was assumed that the algae could transform As(V) into other As species. Hasegawa et al. (2001) reported that methylarsenic(III) species could be produced by phytoplankton in freshwater. MMA(III) and DMA(III) were released as metabolites from the biosynthetic pathway for methylarsenicals by *C.aciculare*. Occurence of the methylated species monomethylarsonate (MMA) and dimethylarsenate (DMA) and of the reduced inorganic species arsenite in oxygenated surface waters is indicative of algal transformation of arsenic. The methylated species are believed to be detoxification products (Kneebone and Hering, 2000).

The mobility of arsenic commonly increases as reducing conditions are established within sediments or flooded soils. Kneebone and Hering (2000) reported about arsenic accumulation in lake sediment via processes which involve the transfer of arsenic from the water column to particulate material and the deposition on the lake floor. Bacteria are also able to methylate inorganic arsenic to aqueous MMA and DMA and also volatile arsine, which are extremely toxic (Takamatsu et al., 1982; Bhumba and Keefer, 1994; Ruokolainen et al., 2000). These processes support our results that many As species occurred in the systems. It is possible that the rest of arsenic is transferred and accumulated in precipitate or microorganisms or transformed into volatile arsenic. Unfortunately, our instruments and experimental setup were not able to analyse the volatile arsenic.

Table 4.5 Arsenic speciation in **SSW** on day 24 and 38 (% Abundance)

Water level	Bottom		Middle		Surface	
Arsenic species	Day 24	Day 38	Day 24	Day 38	Day 24	Day 38
As(III)	31.9	35.8	3.7	19.7	-	2.6
MMA	-	1.9	-	1.7	-	1.0
DMA	1.1	-	-	-	-	-
As(V)	9.3	3.1	57.8	25.3	68.5	92.1
As?(5)*	5.6	7.2	9.5	4.4	-	-

* As?(x) is the sum of unknown species, and (x) is the number of found species.

Table 4.6 Arsenic speciation in **FSW** on day 24 and 31 (% Abundance)

Water level	Bottom		Middle		Surface	
Arsenic species	Day 24	Day 38	Day 24	Day 38	Day 24	Day 38
As(III)	15.9	17.2	2.9	1.3	1.1	1
MMA	-	-	-	0.7	-	0.8
As(V)	16.9	11	67.2	36.3	87.7	38.7
AsB	-	1	-	-	-	-
TMAO	-	-	-	-	-	2.2
As?(5)*	29.8	4.2	19.5	8.5	2.7	

* As?(x) is the sum of unknown species, and (x) is the number of found species.

4.3.2 Arsenic and heavy metals in the plants and other parts of the systems

4.3.2.1 Plant Biomass

The biomass of the plants increased in all experiment, especially in FSW (by 75.95 %, see Table 4.7). The biomass in the pond increased least (30.30 %). There might be an effect of the water level on the plant growth. The water level in FSW was 20 cm above gravel, higher than in SSW where the wastewater was 10 cm below the gravel. Comparing HP and SSW, it was found that % increase of the plant growth in HP was higher.

From these results, it was found that the plants grew very well in places where the water level was high.

Table 4.7 Biomass of the plant in different experimental constructed wetland systems

Constructed wetlands	Begin (kg, fresh weight)	End (kg, fresh weight)	Increase (kg, fresh weight)	% Increase
SSW	4.225	4.680	0.455	10.77
FSW	1.705	3.000	1.295	75.95
HP	1.340	1.746	0.406	30.30

4.3.2.2 Arsenic species in the plants

The concentrations of total As, Fe and Zn in *Juncus effusus* before being exposed to wastewater is shown in Table 4.8. These are the background concentrations of As, Zn and Fe in the plants. It was found that As and metal concentrations in the plants were very low or nearly zero. The results in Table 4.9 show that the plants incorporated arsenic from the wastewater into their biomass and some arsenic species not present in the water could be found in the plants. Arsenic was found in higher amounts in/on the plant roots than in the shoots. There was no significant difference in the amount of arsenic between the plants grown in FSW and SSW.

Table 4.8 Concentration of arsenic and metals in *Juncus effusus* before exposure to wastewater

Juncus effusus	Concentration (mg/kg dw)		
	Total As	**Fe**	**Zn**
Shoots	<2.6	<1.5	<23.0
Roots	<2.9	<1.6	<12.0

Table 4.9 Concentration of arsenic species in *Juncus effusus*

Wetland system	*Juncus effusus*	Concentration (mg/kg fresh weight)				
		As(III) [a]	As(V) [a]	TMAO [a]	As(540) [a]	Total As [b]
SSW	Green shoots	0.16 ± 0.06	0.78 ± 0.21	-	0.05 ± 0.09	0.93 ± 0.13
	Dead shoots	-	0.82 ± 0.19	-	0.08 ± 0.01	
	Roots	0.26 ± 0.08	3.22 ± 1.35	-	-	37.33± 10.40
FSW	Green shoots	0.21 ± 0.01	0.47 ± 0.12	-	-	1.33 ± 0.41
	Dead shoots	0.04 ± 0.04	0.56 ± 0.03	0.05 ± 0.04	0.07 ± 0.01	
	Roots	1.00 ± 0.35	2.03 ± 0.70	0.01 ± 0.02	-	40.86 ± 40.50
HP	Green shoots	0.75	0.44	-	-	4.18
	Dead shoots	0.28	2.12	0.08	0.07	
	Roots	0.14	0.79	0.05	-	6.87

[a] :Arsenic concentration analyzed by IC-ICP-MS
[b] :Arsenic concentration analyzed by ICP-AES

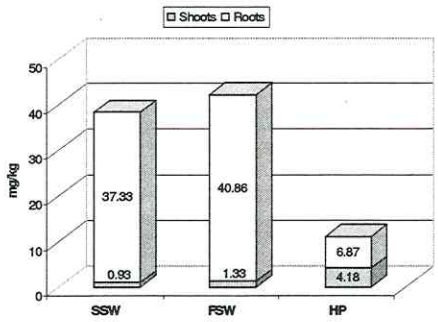
The highest concentrations of As(V) were found in/on the roots, while the lowest concentrations were found in the dead and green shoots. These results agree with the results from Van den Broeck et al. (1998). The plant has a competence to transform As(V) into other species. As(III) was transferred into roots and shoots. TMAO was found in little amounts in the dead shoots and roots. The other unknown species were found in little amounts in green and dead shoots. This unknown arsenic species was detected only in SSW and FSW. The results of this study indicate that plants accumulated more arsenate than arsenite. However, there was more arsenite than arsenate in green shoots which agrees with the observation of Mattusch et al.(2000) and Van den Broeck et al.(1998).

The data about arsenic concentrations in the plants (calculated from the sum of each As species) compared with total As in the water are shown in Table 4.9. It was found that ICP-AES has a higher capability to detect the total amount of arsenic than IC-ICP-MS which is capable to detect the different species of arsenic but not all of the occurring species.

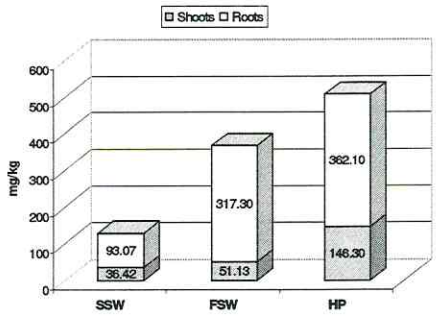
4.3.2.3 As and Zn in the plants and gravel

The amounts of As and Zn in the plants and gravel are shown in Figure 4.15. It was found that As was stored in the root more than in the shoots. Figure 4.15a shows that the concentrations of arsenic in the shoots followed this order: HP>FSW>SSW. When the water level is higher, the accumulation of As in the root decreases. In contrast for Zn, a higher amount was found in

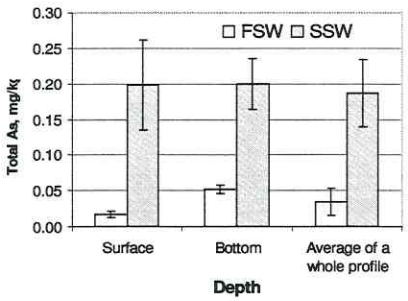
the roots (Figure 4.15b) at the higher water level. However, the accumulated concentration of As in both shoots and roots increased from SSW over FSW to HP. From these results, it can be seen that Zn is stored better in the wetland with a higher water level.



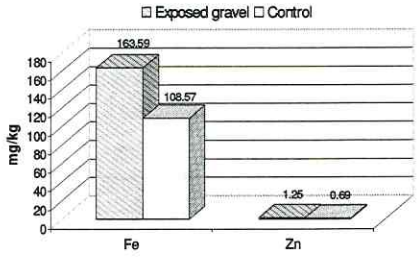
(a) As in plant



(b) Zn in plant



(c) As in gravel



(d) Fe and Zn in gravel

Figure 4.15 Amount of arsenic and zinc in shoots and roots of plant in different types of the experimental wetlands; (a) and (b), the number at the bar indicates the amount in mg/kg dry weight, (c) As adsorbed on the gravel (mg/kg) at the end of experiment, (d) Fe and Zn of exposed and non-exposed gravel with wastewater

Arsenic and zinc were found mostly in the root zone of SSW and FSW, which contained plant and gravel. These Zn contents were found in higher amounts compared to what is normally found in the plants (50 mg/kg), while an iron concentration of about 150 mg/kg was also normally found as a trace element in the plants (Markert, 1994).

In SSW, more As was transferred into the plant than into other parts of the system (Table 4.10). The other mechanisms for the removal of As might be absorption by gravel,

precipitation or metabolization by microorganisms. Figure 4.15c shows the concentration of arsenic extracted from the gravel. Gravel from SSW had a higher arsenic content than that from the FSW, but these amounts are very low compared to what was found in the plants.

The results from the former experiment about absorption by gravel (see chapter 3, section 3.2.1) showed that there was no significant absorption of arsenic by gravel. Therefore, some microorganisms might have had some effect on the transformation of arsenic in the system.

Table 4.10 Arsenic content of the plants (*Juncus effusus*) grown in the laboratory model wetland and pond systems loaded with an artificial wastewater. Data are shown in mg and the data in brackets is shown in per cent.

Constructed wetlands	**Arsenic Input**	**Total As in plants** *Juncus effusus*	**Arsenic in the dead matter**	**Arsenic in the water**	**Other sink for arsenic**
FSW	14.50 (100)	3.67 (25.3)	0.07 (0.5)	0	10.83 (74.7)
SSW	7.00 (100)	7.42 (106)	0.07 (1.0)	0	-0.42 (-6)
HP	20.75 (100)	0.78 (3.8)	0.30 (1.4)	14.07 (67.8)	5.90 (28.4)
AP	16.50 (100)	0	0	14.22 (86)	2.28 (14)

Table 4.11 Estimation of As in the gravel in the experimental wetlands, FSW and SSW

Constructed wetlands	**Gravel weight (kg)**	**Total As (mg/kg)**	**Total As in gravel (mg)**
FSW	45.00	0.03 ± 0.02	1.35 ± 0.90
SSW	75.75	0.18 ± 0.05	13.64 ± 3.79

Table 4.10 shows the estimated mass balance of arsenic after 90 days of the experiment. In all four wetlands, the As accumulation in plant shoots had a small contribution to the mass balance. The small content of As in the shoots in SSW and FSW are 0.93 and 1.33 mg/kg, respectively (Figure 4.15a). Carbonell et al. (1998) studied the arsenic content in *Spartina alterniflora* and found arsenic in the same range, 0.80 – 1.77 mg/kg in leaves and 6.87 – 86.60 mg/kg in the roots. In this experiment, about 24% of As was fixed mostly in or on the roots of FSW. In the pond systems (HP), the roots did not significantly accumulate arsenic.

Table 4.11 shows the amount of As found in or on gravel. The total amount of As found in the gravel of SSW was higher than for FSW.

In this research, the mass balance was estimated for different parts of constructed wetlands, for example, plants, water and gravel. It was found that the amount of total As input in SSW was less than that found in the system. The reason could be that the mass of the gravel in this

system could only be estimated. Errors in this estimate will result in errors in the mass balance. Gravel weight was estimated from its density and volume. In this case, the weight is high when the volume is large, resulting in high estimated amount of As. The other reason could be that gravel sampling from different depths had different concentrations (Figure 4.15c). These different amounts caused higher average values which multiplied by the weight, lead to high values for arsenic. That could be the reason for the very high amount of total arsenic in the gravel.

The higher accumulation of arsenic in roots than in above-ground plant mass correspond with the study on *Typha latifolia, Equisetum fluviatile, Triglochin palustre, and Sparganium sp.* by Dushenko et al.(1995) and on *Spartina alterniflora* by Carbonell et al. (1998), where the accumulation in the roots was 5-14 times higher than in shoots. In HP with the low pH (4-5) and low removal efficiency, the arsenic content in the shoots was considerably higher (about 2 – 5 times) than in the plants in the gravel bed systems (SSW and FSW) where the pH was in the range of 6-7. In addition, a decrease in the arsenic concentration in the water could be observed during the duration of the experiment. The arsenic content in the roots of the plant in HP was significantly lower than in both gravel bed systems. This underlines a possible role of iron plaques for trace element fixation in the root zone of soil and gravel bed wetlands. It can be assumed that because of the higher pH (average pH was 6) in comparison to both pond systems (HP and AP with average pH was 4), and the occurrence of iron in the gravel bed systems, small amounts of the As could be fixed in the iron plaques on the root zone surfaces.

4.4 Treatment of artificial wastewater containing arsenic and heavy metals in a two step wetland system model

After the batch experiments of As and heavy metals removal without additional carbon source, further experiments of the treatment of synthetic wastewater containing As with an additional carbon source were investigated. The experimental system consisted of two different lab-scale wetlands arranged in a series (see Chapter 3). The first wetland was a hydroponic system and the second one a free surface wetland (FSW). This investigation was especially focused on the processes and the efficiency of As removal in the first system, the lab-scale hydroponic system. The helophytes floated and formed a floating plant mat (see Kalin and Smith, 1992). The knowledge about this kind of system is very limited. To ensure an effective removal, the post treatment in a second wetland (free surface wetland) was realized.

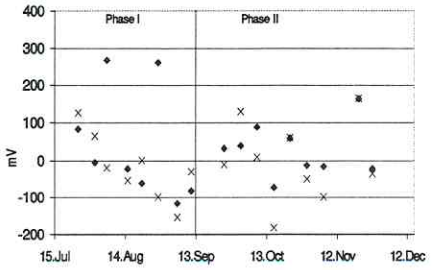
The experiment was divided into 2 phases. In Phase I (60 days, from 16 July to 13 September 2002), the aim was to study the removal of heavy metal in wastewater with a high concentrations of a carbon source (sodium benzoate). In the second phase (87 days, from 14 September to 10 December 2002), the concentrations of the heavy metals Zn and Cr were reduced from 5 mg/l to 1 mg/l because the plant growth was impaired and the carbon source was not further applied.

The concentrations of heavy metals, redox potential (Eh), pH and dissolved oxygen (DO) of the water in the different parts of constructed wetland (in the water phase, plants and the sediments) were analysed.

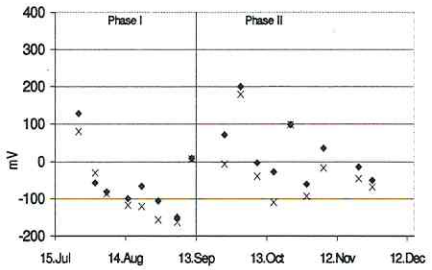
4.4.1 Physico-chemical parameters

4.4.1.1 Dissolved oxygen and redox potential

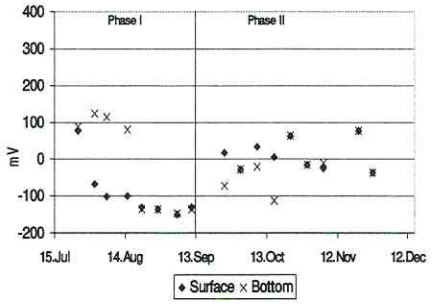
The redox potential (Eh) and dissolved oxygen (DO) in the environment play an important role in determining the mobility of heavy metals. During Phase I (high load), the redox potential in the hydroponic system was decreasing steadily from about 100 mV to less than 0 mV (Figure 4.16). After reducing the load (Cr and Zn from 5 to 1 mg/l; and no carbon source), there was an immediate increase of Eh at the bottom as well as at the surface (in Phase II).



(a) sampling point A

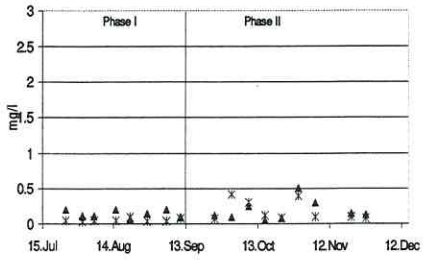


(b) sampling point B

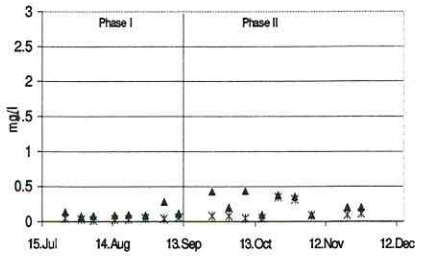


(c) sampling point C

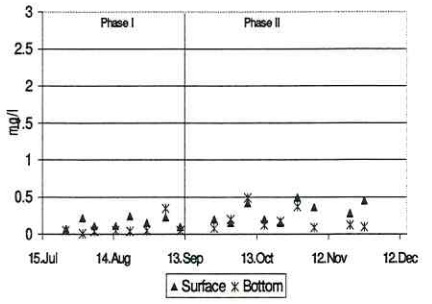
Figure 4.16 Redox potential (Eh-Value) at the bottom and surface; in different points of the hydroponic system (2002)



(a) sampling point A



(b) sampling point B



(c) sampling point C

Figure 4.17 Dissolved oxygen (DO) at the bottom and surface; in different points of the hydroponic system (2002)

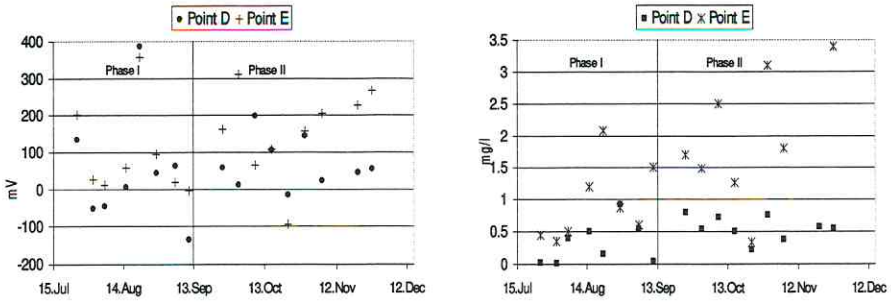


Figure 4.18 Redox potential (a) and dissolved oxygen (b) at the surface of sampling point D (near inflow) and E (near outflow) of the Free surface wetland

Dissolved oxygen was nearly 0 mg/l at all sampling points and at all sampling times (Figure 4.17). This was due to the high amount of organic carbon added to the artificial wastewater. These conditions changed the system into an anaerobic system.

There were some problems in measuring the redox potential and DO at the bottom area of the free surface wetland. Therefore, only the values of the redox potential (Eh) and DO analysed at the surface sampling area are illustrated (Figure 4.18). The patterns of Eh and DO at sampling point D and E of free surface wetland were similar and they were higher than those of the hydroponic system. Eh and DO at sampling point E (near outflow) were higher than at sampling point D (near inflow). The Eh was almost higher than 0 mV and increased to about 300 mV in Phase II. DO at point E increased from nearly 0 to 3.4 mg/l over time. The higher values of Eh and DO indicated the higher oxidizing condition in the free surface wetland than in the hydroponic system.

In the hydroponic system Eh values found at the bottom were lower than those found near the surface. The faster decrease of Eh values at point B and C to about -20 to -170 mV after 55 days (Phase I), indicate a more reducing environment than at sampling point A because of the depletion of organic substrate.

It was proposed that conditions with <120 mV of Eh are termed as a reducing or anoxic conditions (Sposito, 1981). When Eh is less than -120 mV, then there is a highly reduced condition. In the reduced and highly reduced conditions Fe^{3+} and SO_4^{2-} are reduced to Fe^{2+} and S^{2-}, respectively.

The results of Eh and DO in this experiment correlated with the concentration of sulfide (see 4.4.1.2) in the systems. Due to the low level of Eh and DO, which indicates anaerobic conditions, sulfate reduction occurred.

4.4.1.2 Sulfur

Figure 4.19 shows sulfur compounds that occurred in the hydroponic system during the study period. Sulfide (S^{2-}), thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}) could be detected.

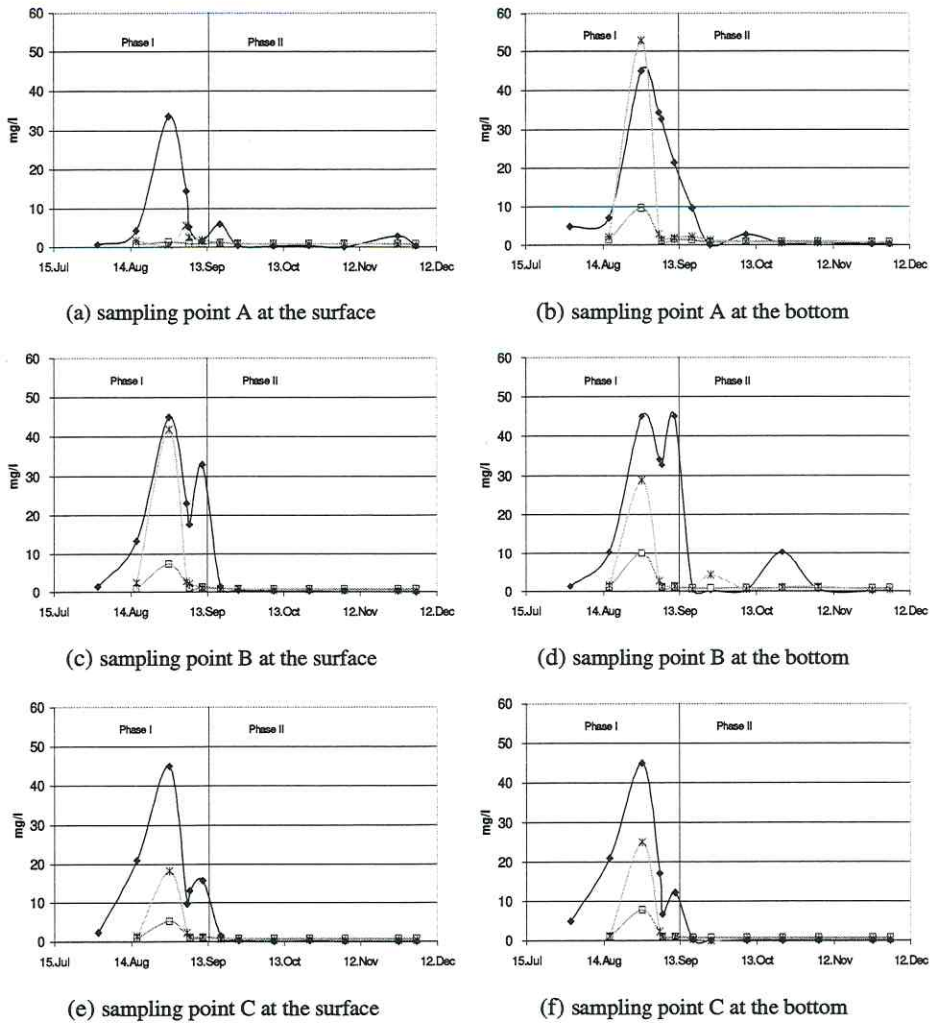
The reduced sulfur compounds were found in higher concentrations in Phase I than in Phase II. The surplus of organic substrate for the microbes resulted in a shortage of available oxygen that results low Eh. In consequence, the dissimilatory sulfate reduction occurred.

Thiosulfate and sulfite have been found to be important intermediates in the sulfur cycle (Jørgensen and Bak, 1991). They are main products of the chemical oxidation of sulfide and can be oxidized to sulfate, or reduced to sulfide.

In Phase I of the experiment, the concentrations ranged from 0.5 to 52.7 mg/l of thiosulfate, 0.8 – 10 mg/l of sulfite and 0.6 - >45 mg/l of sulfide; in Phase II they range from 0 – 4.3 mg/l for thiosulfate, 0 – 1.4 mg/l for sulfite and 0 – 10.2 mg/l for sulfide. The concentrations of sulfur species in Phase I were higher than in Phase II because the carbon source applied only in Phase I stimulated the activities of microorganisms (i.e. sulfate reducing bacteria) in dissimilatory of sulfate reduction.

Wind and Conrad (1995) support the theory that sulfide becomes oxidized and mediated to thiosulfate and other products by the help of the plant roots in deeper zones and by oxygen diffusion near the surface of the system. Furthermore, the decaying roots could provide such environments that make high sulfate reduction potentials reasonable. Despite the aeration of the roots, iron reduction and sulfate reduction are stimulated by the root exudation and deposition products.

There was less input of sulfur and lower concentrations of sulfur compounds found in the FSW, which means that there were less reducing conditions in the system. The concentrations of sulfur species at all sampling points were constant in the range of 0 – 1 mg/l over time (Figure 4.20). The increased redox conditions and oxygen concentration in the FSW are indicative of more aerobic conditions and should inhibit sulfate-reducing bacteria which caused less amounts of reduced sulfur-species.



(a) sampling point A at the surface

(b) sampling point A at the bottom

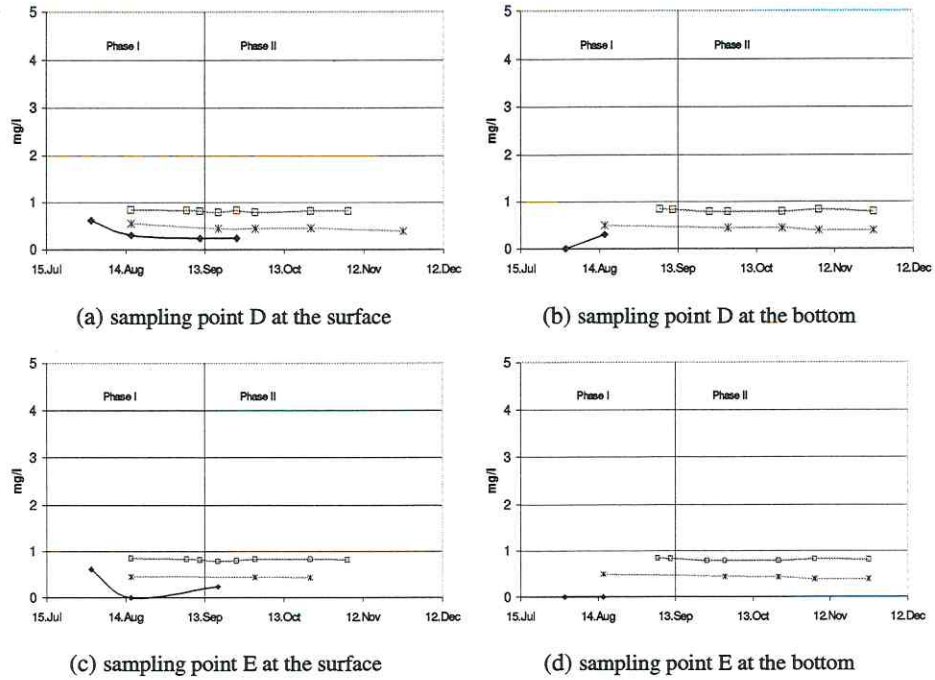
(c) sampling point B at the surface

(d) sampling point B at the bottom

(e) sampling point C at the surface

(f) sampling point C at the bottom

Figure 4.19 Concentrations of sulfur species in different sampling points of the hydroponic system (in 2002) ♦ S^{2-} * $S_2O_3^{2-}$ □ SO_3^{2-}



(a) sampling point D at the surface

(b) sampling point D at the bottom

(c) sampling point E at the surface

(d) sampling point E at the bottom

Figure 4.20 Concentrations of sulfur species in different sampling points of the Free surface wetland (in 2002) ♦ S^{2-} * $S_2O_3^{2-}$ □ SO_3^{2-}

4.4.1.3 Arsenic, zinc and chromium in the water phase

The concentrations of As, Zn and Cr were analysed in water samples collected at different distances from the inflow. The heavy metal loads in the hydroponic system and the FSW are illustrated in Figure 4.21. Iron load was also observed because it is known to affect metal removal mechanisms, e.g. for arsenic and chromium.

The inflow load for the hydroponic system fluctuated in Phase I because of the differing inflow rate. This was due to pump defects.

The inflow As load in the hydroponic system was in the range of 16-32 mg/m^2d (see Figure 4.21a). The As load of the outflow was significantly lower than the inflow. It was nearly constant from 15 August until 3 October (3.8 - 5.3 mg/m^2d). The removal of As found in Phase I was probably due to its precipitation under these highly anaerobic conditions with high S^{2-} concentration. Moreover, some microorganisms may reduce the concentration of arsenic by uptake, accumulation and transformation to other organic arsenic compounds in the

cell. From October until 7 November the outflow load of arsenic in Phase II increased significantly, then, it decreased again to 6 mg/m^{2}d.

In FSW the inflow loads of As ranged between 2.5-11.3 mg/m^{2}d. It was found that the inflow and the outflow of FSW were constant during August to October (as in hydroponic system). The outflow load of FSW was also almost steady and nearly zero at the end of the investigation.

The concentrations of Zn and Cr in the inflow (in hydroponic system) decreased dramatically because of the reduction of a high load (Phase I) to a lower load (Phase II). The inflow load of Zn in the first hydroponic system was in the range of 179.9 – 293.8 mg/m^{2}d in Phase I and 71.1- 38.2 mg/m^{2}d in Phase II. In general, the outflow of Zn was significantly lower than the inflow in both phases. The Zn load of the outflow of FSW was significantly higher during October (81.5 mg/m^{2}d). This may be an effect of high evapotranspiration and low pH. When the pH is low, Zn can be redissolved in the water.

Cr decreased significantly in the hydroponic system. Cr was found in small amounts in the outflow of the hydroponic system; in consequence, it was found in small amounts in both the inflow and the outflow of the FSW (in range of 15 – 0 mg/m^{2}d).

Because the formation of different sulfur compounds was observed, it may be concluded that the decrease of chromium in this experiment is the result of either sulfate reducing bacteria which directly reduce Cr(VI) to Cr(III) or the chemical reduction of Cr(VI) by the biogenic H_2S to Cr(III), and the subsequent formation of $Cr(OH)_3$ precipitates. The reaction between chromium and sulfide results in elemental sulfur (Kim et al., 2001; Vainshtein et al., 2003) and decrease of chromium concentration.

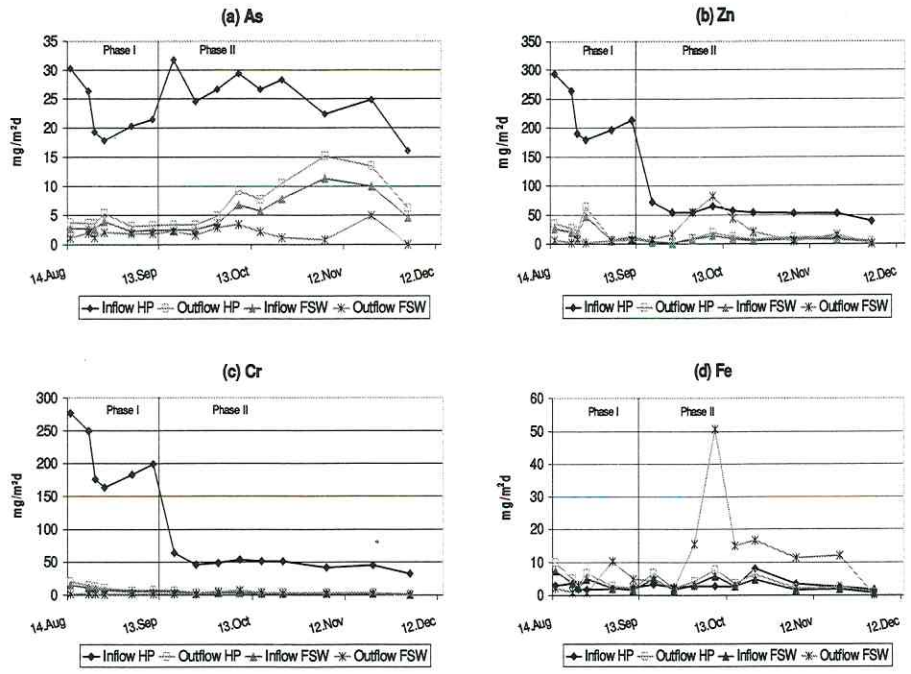
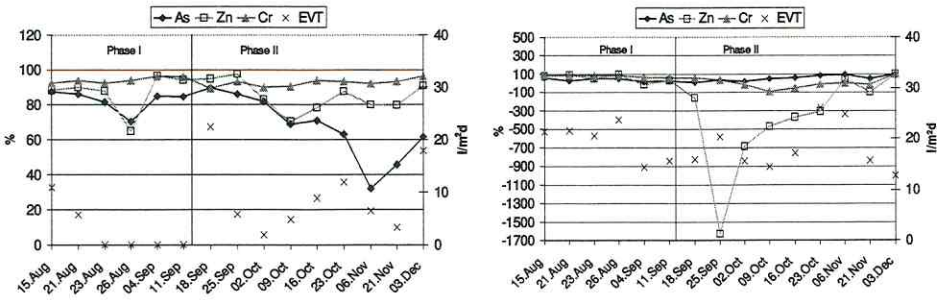


Figure 4.21 Loading rate of heavy metals (As, Zn, Cr and Fe) and the corresponding outflow loads in the hydroponic system (HP) and free surface wetland (FSW)



(a) Hydroponic system (b) Free surface wetland

Figure 4.22 Percent removal of As, Zn and Cr and the evapotranspiration rate (EVT in l/m^2d) in hydroponic system (a) and free surface wetland (b)

Figure 4.22a shows the removal of As, Zn and Cr (in per cent of metal removal) and the evapotranspiration rate in the hydroponic system. Arsenic was removed with a high removal rate of about 86.8 % during Phase I. The removal rate of As began decreasing in Phase II, whereas the input continued as before. The removal rates of As decreased and was lower than 60 % (in November). The removal rates of Zn remained higher at about 70-80 %. In contrast, the removal rate of Cr in the hydroponic system was almost constant at a high value of about 90 %. As already mentioned the inflow load of Zn and Cr continued as before but the amount of organic substrate decreased, which is why it showed less significant difference between the inflow and outflow load.

Figure 4.22b shows the percent removal of As, Zn and Cr in the FSW. It was found that the FSW had a better removal capacity for As, Zn and Cr during Phase I, but the removal capacity for Zn and Cr was very low in Phase II.

Carbonell-Barrachina et al. (1999) proposed that the large increase of As solubility in reducing environment was probably linked to the reductive dissolution of hydrated iron oxides. In our study, the increase of iron concentration is believed to cause the increasing amounts of Zn found in the water phase of the hydroponic system. Iron can form complex with Zn and precipitate. Moreover, the decrease of organic substrate and the low pH of the wastewater may have an effect on the removal mechanism of the heavy metals (Shrestha et al., 2003). The low pH of about 5, which occurred in the system, might induce the dissolution of Zn, Fe and other metals. It also seemed to decrease the bacterial activity to a minimum.

4.4.2 Arsenic zinc and chromium in the plants

Plant samples in the hydroponic system and FSW were collected and separated into roots and shoots. The concentrations of heavy metals in plants from different sampling areas of the wetlands are shown in Figure 4.23. In both shoots and roots, higher concentrations were found in the plants close to the inflow, and concentrations decreased with the distance from the inflow.

Zn, As and Cr were found mostly in the roots rather than in the shoots. The plants were found to accumulate more Cr than Zn and As. A high amount of Cr was accumulated in the root, in the range of 793-4,464 mg/kg, while it was found in the shoots at about 36-386 mg/kg. The maximum accumulated concentration of Zn and As in the plants were 2,188 and 252 mg/kg, respectively (see Figure 4.23a and c). These results agree that most of metals accumulated in the roots than in the shoots or leaves (Kumar et al., 1995; Srivastava et al., 1998; Kleinmann and Cogliatti, 1998).

The plants play an important role for uptake of heavy metals. In the root zone, plant roots provide the area for microorganisms to accumulate heavy metals on the root. Chemical precipitation can happen during this process. Accumulation and precipitation can occur on the plant roots' surface in form of iron oxyhydroxides, which in turn bind other heavy metals (such as As and Cr) and form the iron-plaque attached around the rhizoplane (root surface).

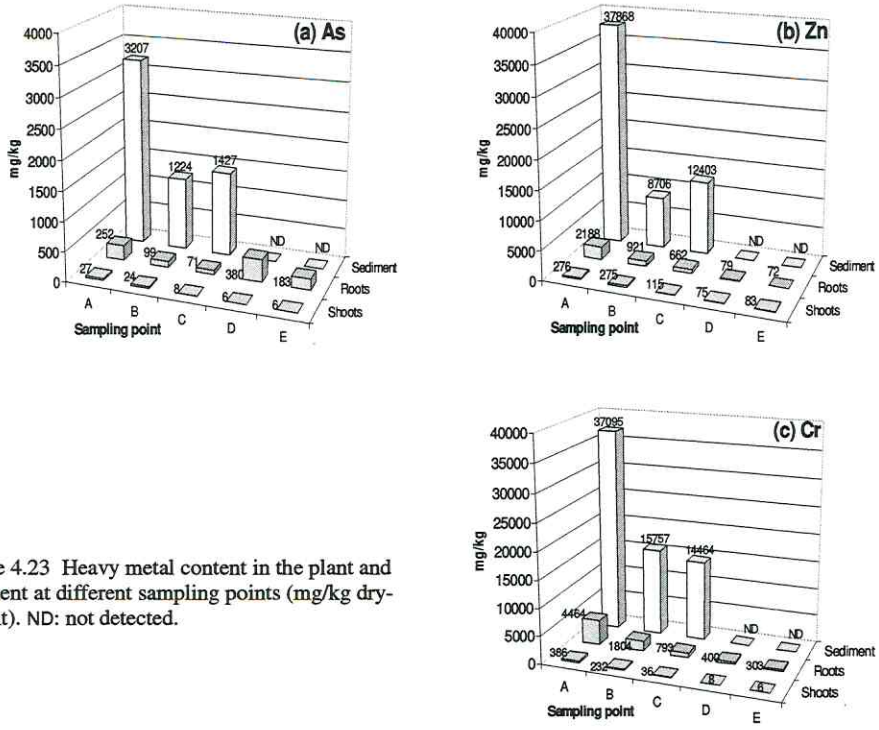


Figure 4.23 Heavy metal content in the plant and sediment at different sampling points (mg/kg dry-weight). ND: not detected.

4.4.3 Arsenic, zinc and chromium in sediment

At the end of the experiment, sediment samples were taken from different distances from the inflow in the hydroponic system. The total amount of sediment in the hydroponic system was 83.7 g dry weight. The accumulations of As, Zn and Cr which higher accumulated by the distance are shown in Figure 4.23. It was found that in the sediment near the inflow of the hydroponic system most of As, Zn and Cr was found. Here as well Cr was accumulated in higher amounts than Zn and As. There were at least 163 mg As, 1,645 mg Zn and 1,878 mg Cr stored in the sediment of the system.

The anaerobic conditions and the productions of sulfur compounds, which occurred in this experiment in Phase I, induced the precipitation of heavy metals with sulfide as ZnS, FeS and As_2S_3 (Gadd and White, 1993) and further adsorption processes. Chromium reduction might occur through chromate reducing bacteria which can reduce CrO_4^{2-} to Cr(III) which precipitates as $Cr(OH)_3$ (Gadd and White, 1993). These results show a similar tendency as found in the plants that the sediment accumulated higher Cr than Zn (see Figure 4.24).

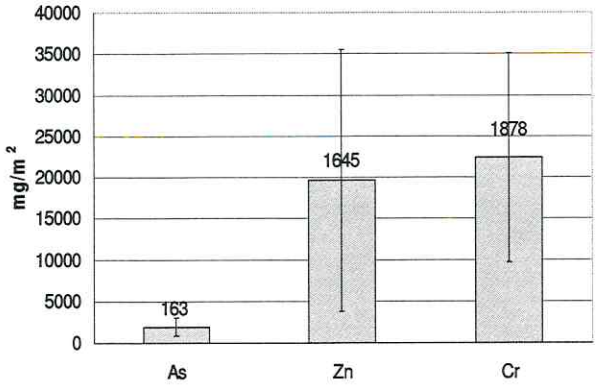


Figure 4.24 Total amount of As, Zn and Cr accumulated in the sediment of the hydroponic system (mg/m^2), the number at the bar indicated the amount in mg

Microorganisms attached to the plant roots can also absorb higher amounts of the heavy metals. As a high concentration of metals was found near the inflow, a high intensity of microbial and chemical process can be assumed. The other coincident mechanisms, such as precipitation, complexion and adsorption, occur near the inflow. The dead microorganisms and other solids settle to the bottom of the wetland. That resulted in the high concentrations of

heavy metals found in the sediment near the inflow rather than in the middle and the outflow. The same decrease in heavy metals depending on the sampling point was found in the plants.

4.4.4 Accumulation of heavy metals in a series of constructed wetlands

The difference between the cumulative inflow and outflow load of heavy metals in the laboratory wetland system shows the amount of heavy metals removed from the water phase over the period of the study. The cumulative inflow load of As increased steadily over time (Figure 4.25a). The concentration of Zn and Cr in the inflow was decreased from Phase I to Phase II, therefore, the increase in cumulative inflow was also decreased (see Figure 4.25b and c).

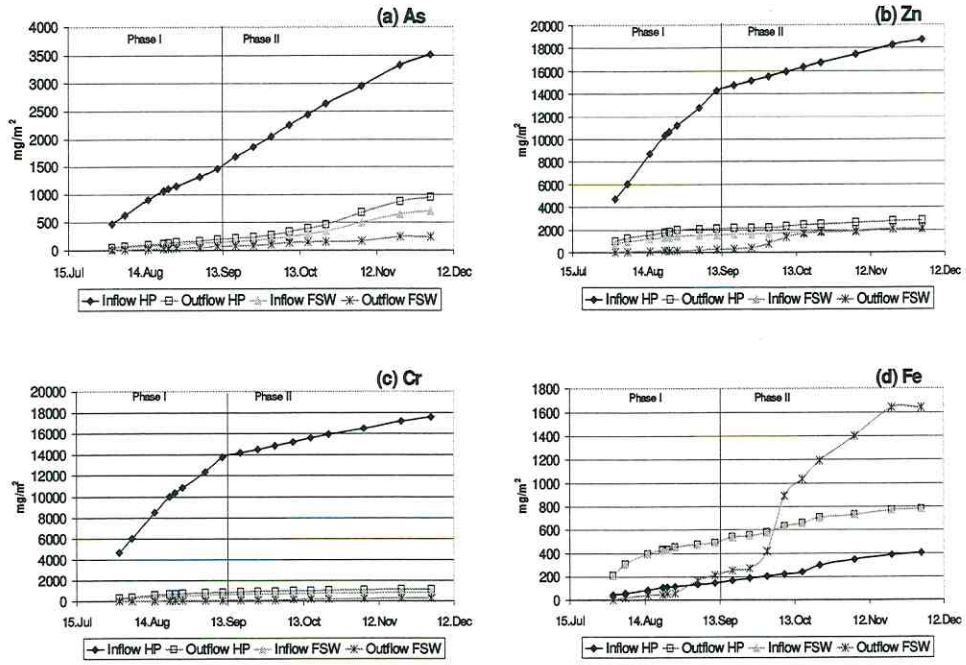


Figure 4.25 Cumulative inflow and outflow load of heavy metals in the hydroponic system (HP) and free surface wetland (FSW)

Arsenic cumulative inflow load in the hydroponic system rose from 476 to 3,512 mg/m^2. In comparison, the cumulative outflow load of As rose from 45 to almost 953 mg/m^2. The resulting removal over this period of 140 days was thus 2,559 mg/m^2, which resulting in a mean removal rate of 18.3 mg/m^{2}d (see Table 4.12).

The final cumulative loads of As in FSW were 708 mg/m^2 in the inflow and 244 mg/m^2 in the outflow, resulting in an average removal rate of 3.3 mg/m^{2}d.

The same behaviour of arsenic accumulation in lake sediment was reported by Kneebone and Hering (2000). Arsenic accumulates in lake sediments via processes that transfer arsenic from the water column to particulate material which is then deposited on the lake floor. These processes include adsorption to manganese and/or iron oxyhydroxides and uptake by phytoplankton with incorporation into algal biomass.

Table 4.12 Cumulative load and the average removal of As Zn and Cr in the experimental hydroponic and FSW after the period of 140 days. Negative value in brackets means the released Fe.

Cumulative load	Hydroponic system			Free surface wetland		
	Inflow (mg/m^2)	Outflow (mg/m^2)	Average removal (mg/m^{2}d)	Inflow (mg/m^2)	Outflow (mg/m^2)	Average removal (mg/m^{2}d)
As	3512	953	18.3	708	244	3.3
Zn	18712	2808	113.6	2093	2079	0.1
Cr	17593	1147	117.5	855	285	4.1
Fe	407	781	(-2.67)	781	1640	(-6.14)

It was found that the cumulative outflow load of Zn was higher than of Cr in both hydroponic and FSW systems. In the hydroponic system, Zn and Cr were accumulated at 15,904 mg/m^2 and 16,446 mg/m^2, respectively. The resulting average removal rates of Zn and Cr in the hydroponic system were similar (113.6 mg Zn/m^{2}d and 117.5 mg Cr/m^{2}d).Their removal rates were higher than that of As (see Table 4.12).

In FSW, Zn was accumulated in the FSW at 14 mg/m^2 with a mean removal rate of 0.1 mg/m^{2}d. Cr was retained at 570 mg/m^2 with an average removal rate of 4.1 mg/m^{2}d. Therefore, hydroponic system had a better removal rate for Cr than Zn, and higher than the removal rate in FSW.

The cumulative loads of iron (Fe) increased over time although there was no additional source. The cumulative outflow load of Fe was higher than the cumulative inflow for both

hydroponic system and FSW (Table 4.12). Iron found in the wastewater originated from the tap water used in the preparation of the wastewater. The reasons for the higher amount of Fe are the reducing environment. Iron can be reduced and dissolved to the wastewater when there are reducing and acidic conditions.

It was found that this series of constructed wetland systems has the capacity to remove As and Cr. Zn was not removed as efficiently because its removal is very dependent on pH. Certain amounts of As and Cr were stored in the plants, sediment and the other parts (Urbanc-Bercic, 1997; Dushenko et al., 1995). It is possible that the undetected amounts of arsenic were transformed into the volatile form by some microbes (Adriano, 1989; Tamaki and Frankenberger, 1992).

In these constructed wetlands heavy metals were analysed in 3 different phases, the water phase, plants and sediment. The heavy metals were found in all phases (see Table 4.13). Because the total amount of heavy metals found in all system was not compensated or equalised the amounts fed in the system, not only the uptake by plants and precipitation are the factors involved in the removal mechanisms, but also other factors of system. Therefore, it is assumed that other parts of the wetlands, such as microorganisms can accumulate heavy metals. Microorganisms should be one of the important factors for the removal. They can take up and transform heavy metals to other forms. Those products could be accumulated in the cells resulting in sludge and the volatile forms of arsenic could be released to the air.

Table 4.13 Heavy metals accumulated in different parts of the hydroponic system

	Arsenic (mg/m^2)	Zinc (mg/m^2)	Chromium (mg/m^2)
Plant	116 ± 6	1120 ± 56	1707 ± 85
Sediment	1953 ± 1091	19659 ± 15877	22439 ± 12709
Sum	2069 ± 1097	20779 ± 15933	24146 ± 12794
Total Input	3512 ± 176	18712 ± 936	17593 ± 880

4.4.5 Arsenic species

Figure 4.26 shows the distribution of As species at the various areas and depths of the hydroponic system. Many As species were found both at the surface and at the bottom area of the hydroponic system. The type of As species was found to be Eh dependent. Under reducing conditions As(V) was transformed to As(III) and other As species. The pattern of concentration of As(III) was opposite to the pattern of As(V), and As(III) and other As species near the bottom area were higher than As(V) in the reducing condition. As(V) decreased with the time and distance from the inflow, while As(III) increased in dependence of time and the reducing conditions. Under oxidizing conditions (see Figure 4.16, especially in October) the mobility of As in the water is related to the presence and behaviour of iron. Arsenic will eventually co-precipitate and become immobilized by the formation of insoluble, hydrated iron oxides (Carbonell-Barrachina et al., 1999; Masschelyn et al., 1991c).

There were some unknown As species found in this experiment. Under highly reducing conditions with abundant carbon sources, microorganisms produce more methylated arsenic species. The methylated arsenic species were only detected at very low concentrations. The high amount of H_2S found in reducing conditions may compete to combine with the reduced arsenic before the methylation mechanism sets in.

The rhizosphere of growing plant roots is a soil compartment where intensive transport processes of water and dissolved substances occur. Therefore, microorganism at living roots can influence the chemical and biological properties of rhizosphere soils (Fischer et al., 1989). This influence is reflected by changing pH or redox potentials. The lower Eh and pH the higher the amount of water soluble arsenic. Although As(III) can become the major As species in water under reducing conditions, some As remains present as As(V) (Marin, 1993).

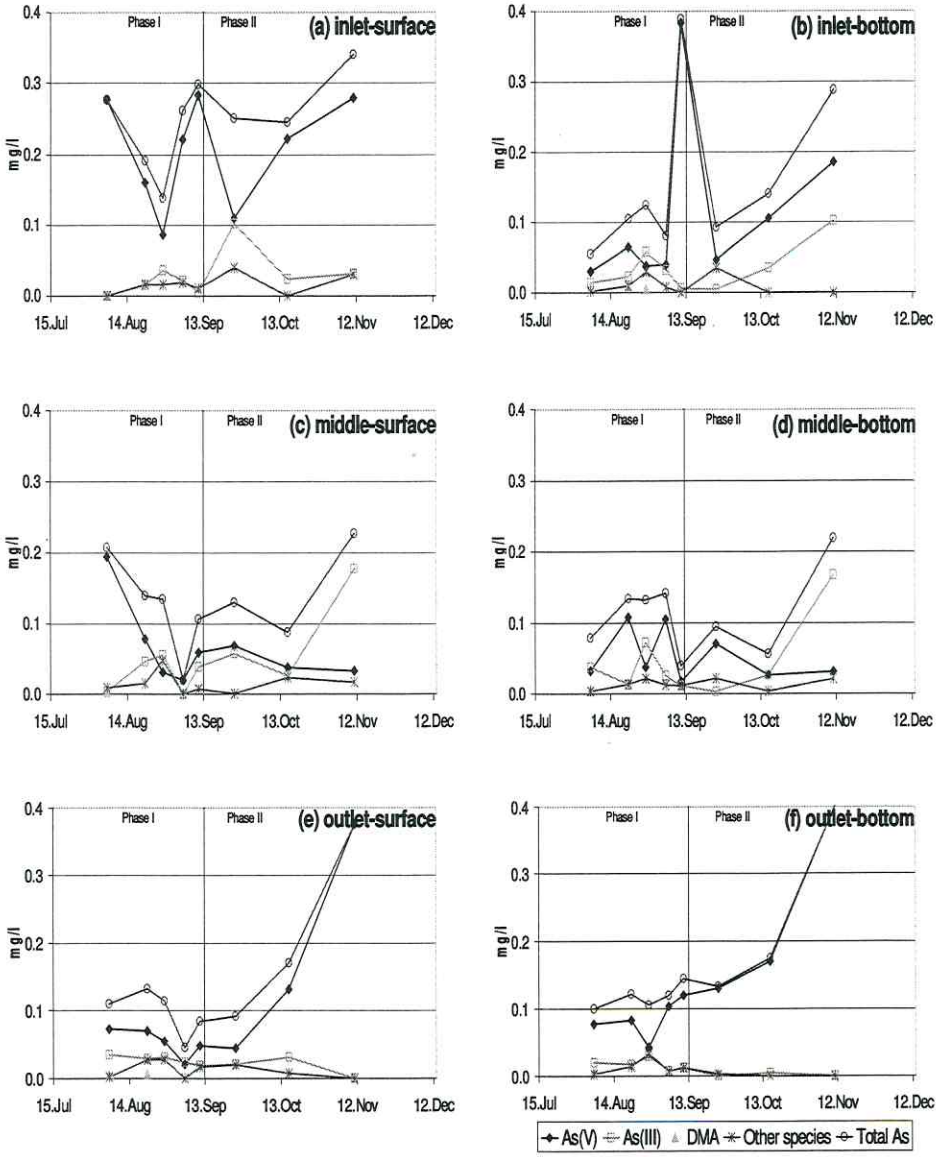


Figure 4.26 Arsenic species in the different sampling sites and depths of the hydroponic system

4.4.6 Characterisation of microbial community in a two step wetland system model by PCR-SSCP (Polymerase chain reaction- single strand conformation polymorphism)

The results from the experimental wetland systems show the removal of different heavy metals. It is known that the physical and chemical processes are generally employed for the removal of heavy metals from wastewater, which include ion exchange, oxidation-reduction, precipitation and many others. Microorganisms also offer an alternative to physical and chemical methods and play an important role for heavy metal removal. The series of experimental wetland was first operated with artificial wastewater with high carbon source, which led to reducing conditions. Some reduced and oxidized sulfur species were found and had an effect on the removal of heavy metals. Therefore, microorganisms should be one of the important factors for the removal. To assure that assumption, the microorganisms were investigated in the reducing conditions. The community of microorganisms can show the possibility of microorganisms in the removal of heavy metals.

4.4.6.1 MPN method for the sulfate reducing bacteria (SRB)

The MPN (most probable number) method was used in testing the amount of bacteria or microorganisms in the samples. In assumption of anaerobic condition with high carbon source and occurrence of sulfate, a sulfate reducing medium was used as growth medium for these microorganisms.

The results of the MPN method show that sulfate reducing bacteria occurred in the inflow zone, in the middle and the outflow of the hydroponic system (Table 4.14).

Table 4.14 Number of SRB in the different sampling points (vary by the distance) of the hydroponic system by the MPN test after 100 days of incubation.

	Near the inflow	In the middle	Near the outflow
MPN (cells/ml)	4.3×10^4	$>2.4 \times 10^3$	$>2.4 \times 10^3$

The number of SRB in the inflow zone was 4.3×10^4 cells/ml after an incubation of 100 days. The water samples from the other distance, in the middle and in the outflow, had at least 2.4×10^3 cells/ml. These results agree with the results of the H_2S found in the water phase of the wetland. The SRB are normally used in the wastewater treatment plants, for instance in the mine water treatment where added organic waste products stimulate their activity.

Oxidation of organic compound (CH_2O) coupled to sulfate-reduction and precipitation of metal ions (Me^{2+}) is summarized below (Christensen et al., 1996).

$$2CH_2O + SO_4^{2-} + H^+ \longrightarrow 2CO_2 + 2H_2O + HS^-$$

$$Me^{2+} + HS^- \longrightarrow MeS + H^+$$

4.4.6.2 DNA study and the polymerase chain reaction (PCR)

To determine the microbial community and SRB, samples of this MPN cultures were analysed using the DNA-PCR technique and compared with the samples taken from many sources of microorganisms, such as water phase, sediment and the roots of plants.

The DNA technology is used to detect microorganisms in wastewater samples. A gene probe or DNA of microorganisms was extracted and isolated from the samples. Then, the PCR technique was applied with this extracted DNA to amplify and get a several million-fold replication of the target DNA, a 400 bp (base pair) fragment. The PCR cycle consists of three steps; DNA denaturation, annealing of primers and primer extension or amplification. Com1 and com2 were used as primers or a short starter sequence.

In all samples from water, sediment and roots a fragment of approximately 400 bp corresponding to the regions of SSU rRNA V4 and V5 was amplified. The results of the PCR products are shown in Figure 4.27. The white band found in the PCR product shows the signal of the DNA in the samples.

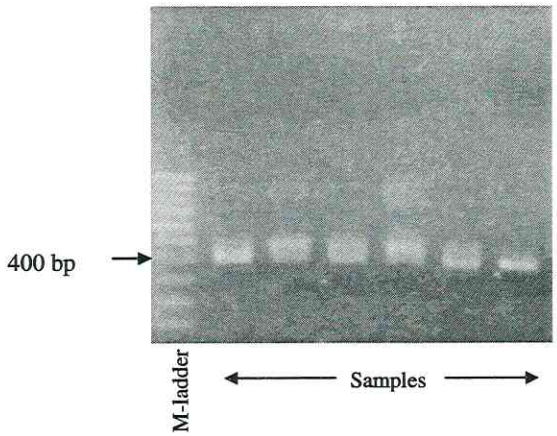


Figure 4.27 PCR gel shows the DNA fragment at 400 base pair (bp) of the bacterial in the samples. Primers; com1 and com2 were used in the PCR process.

4.4.6.3 SSCP profile

The SSCP patterns were generated from the PCR products of bacteria extracted from wastewater and sediments in different sampling sites of the hydroponic system; inflow zone (A), middle (B) and near outflow (C); and the roots sample from point B. Samples from the inflow (D) and outflow (E) of the free surface wetland were also generated for the SSCP gel.

Figure 4.28 shows the SSCP pattern of the bacterial community in the samples compared with the extracted DNA pattern of bacteria grown in the sulfate reducing medium for SRB culture in the MPN test.

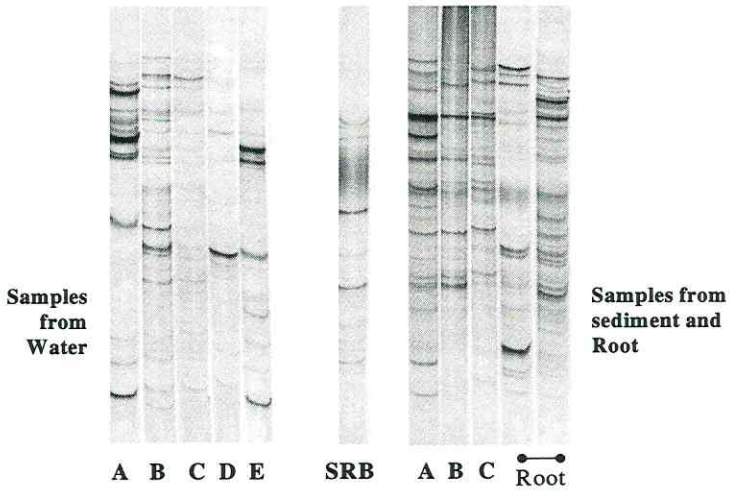


Figure 4.28 SSCP pattern of single-stranded PCR products of samples in the water phase, sediment and root in the different sampling areas; A, B, C, D and E and the samples of the sulfate reducing bacteria (SRB) culture. Roots sample on the left was the living roots, and roots on the right was the dead roots sample (see detail in Chapter 3 Materials and methods)

Comparison of SSCP products of the bacterial community indicated that there was bacterial diversity in the water phase at all sampling points. There are many bands which referred to the variety of microorganism species found in the sulfate reducing medium (SRB). It is possible that there were many different types of sulfate reducing bacteria and some other

kinds of microorganisms which could grow in these conditions. In zone A (inflow), there were some stronger bands, which reflect to the high of bacteria, but diversity was lower in this zone than in zone B (middle) and C (near outflow). The highest bacterial diversity was found in zone B.

It was found that the community of the microorganisms indicated changes in the physical and chemical conditions in the wetland. The changing of pH and redox condition had an effect on the growth of microorganisms. For example, in the hydroponic system, where Eh values decreased from 100 to –150 mV over time in Phase I and it was gradually increased in Phase II, had an effect on the high community of microorganisms (zone A). In contrast, the Eh values in zone D and E in the FWS were higher which results to less microbial community.

In the sediment, the highest diversity of microorganisms was found in zone A. In the study of the community profile in the root zone, more communities were found on the dead roots more than on the fresh roots. The diversity of microorganisms in the root zones is higher than in the water phase. This can be due to the root composition, dead root matter and root exudates which provide suitable growth conditions microorganisms.

4.5 Treatment of acid mine drainage in six small scale constructed wetlands in a field experiment

The experiment simulated the removal efficiency of acid mine drainage by different types of constructed wetlands. Six different small-scale constructed wetland systems (with an area of 0.55 m^2 each) were operated in the field site in Grosskayna, Germany. The experiments were running annually from spring till late autumn for 3 years, from 2001 until 2003. Because the temperature in winter dropped below 0°C and the water froze, the experiments were not operated then.

The results of pH, acidity and the concentration of heavy metals (Fe and Zn) were analysed for three years.

4.5.1 pH

The pH data were collected from July until December 2001, April until December 2002, and from April until September 2003. The pH of the inflow wastewater was about 3. In general, the wastewater had a higher pH after passage through the wetlands.

In the pond system, the pH of the outflow did not increase significantly to about of 3 to 4.

The FSW, which contains soil material and where the water flows over the soil bed, brings better results with a higher pH in the outflow than the hydroponic system. The pH of the outflow of FSW with plants was significantly higher than the outflow of FSW without plants. In 2001, the pH in FSW with plants rose to a maximum of pH 8 (average pH of about 5.9) while the FSW without plants showed an average pH of 3.5 (Figure 4.29d). In 2002, the FSW with plants still showed higher pH than FSW without plants. However, the capacity of FSW with plants for pH treatment decreased compared to the year 2001 to average pH of 4.5. In 2003, the treatment capacity showed the same tendency with an average pH of 4.83 in the outflow. The results of three years show that FSW is capable to increase the pH of wastewater in the beginning but its capacity decreased over time. The results depended on the season and temperature. When the temperature decreased to < 15 °C, the pH in FSW with plants did not increase significantly. This phenomenon could be found during all 3 years of the experiment.

The pH of the outflow of SSW with plants was significantly higher than SSW without plants during the first year of the experiment (2001). The average pH values were 6.4 and 5.2 for SSW with plants and without plants, respectively. Generally, the SSW with plants can neutralize and increase the pH to a higher extent than SSW without plants. However, there was no significant difference of the pH in the outflow between SSW with and without plants in the following years (in 2002 and 2003).

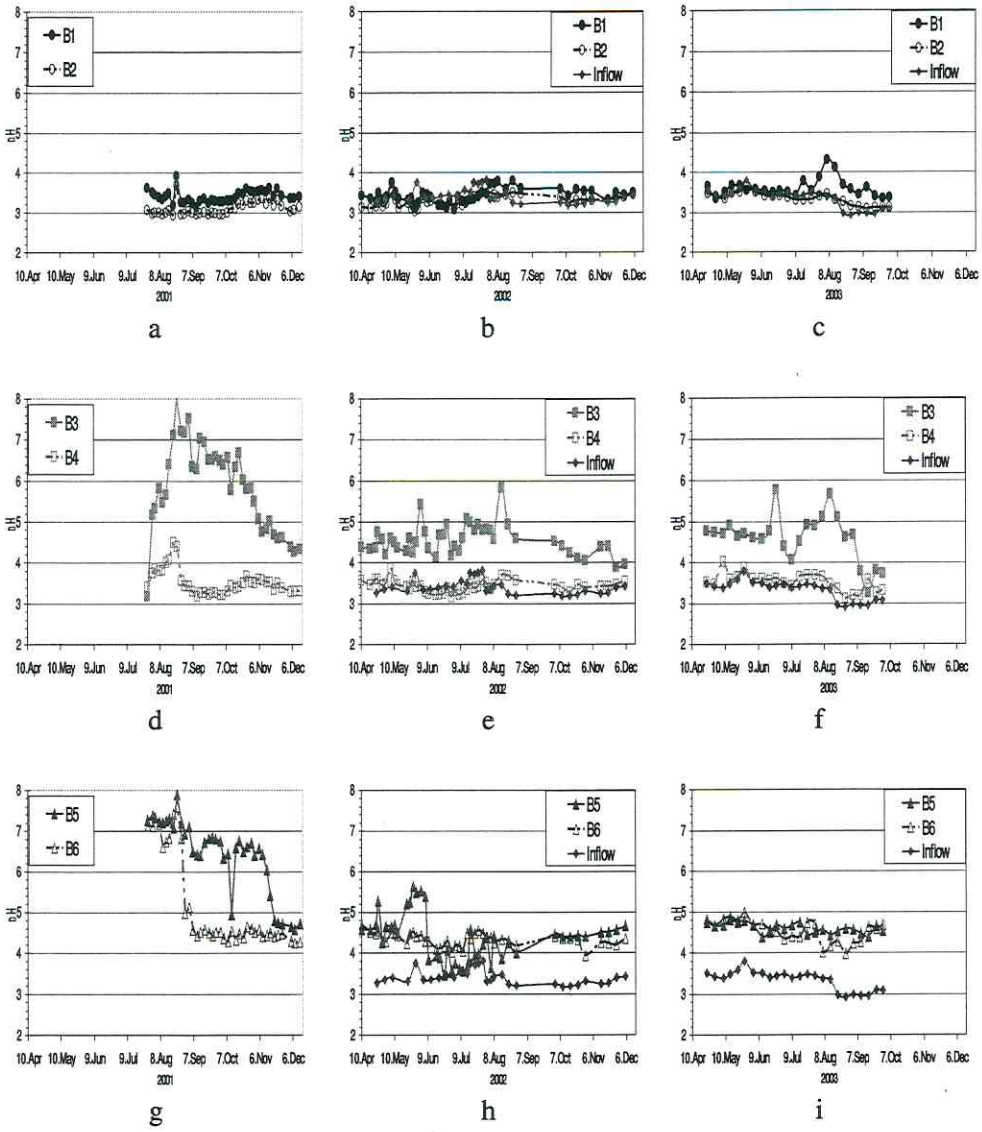


Figure 4.29 pH of the inflow and outflow in different experimental wetlands for AMD treatment (during 2001-2003)

a, b, c : planted (B1) and unplanted (B2) Hydroponic systems
d, e, f : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW)
g, h, I : planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)

From the results of these experiments it can be concluded that the FSW had a higher capacity to increase the pH of the wastewater than other systems. FSW and SSW showed similar behaviour of pH neutralization, because highest capacity was observed during the first period of experiment and the pH decrease overtime occurred in both FSW and SSW. The difference of pH may be due to the decomposition of plant detritus in old planted wetlands. Decomposition of a large amount of detritus results in acid production and a decrease of pH over time. Marschner (1995) stated that roots exudate protons and thus cause acidification to the rhizosphere.

From those results it was found that the soil materials and plants had an effect on pH neutralization. Plants can contribute to the increase as well as to decrease of the pH. Plants release hydrogen ions and are able to decrease the pH by 1 unit in a region of about 2 mm from the root (Muranyi et al., 1994; Greger, 1999). The released H^+ ions can then, by exchange, release metals and thereby the uptake by the plants. While the plants supply significant quantities of oxygen to the root zone, the roots also release oxygen into water and atmosphere during photosynthesis (Reddy et al., 1989, Wießner et al., 2002). The plants assimilate CO_2 also from the water phase during the photosynthesis process which results in an increase of the pH. The pH is usually stabilized by the CO_2-bicarbonate-carbonate-buffering system, which dominates the ionic composition of neutral waters in the range from softwater to hardwater independent of ionic strengths.

It is also possible that the soil materials may contain carbonate that might cause an increase of pH. The carbonate dissolution consumes protons [$CO_3^{2-}(aq) + 2H^+(aq) \rightarrow CO_2(g) + H_2O(l)$], thus decreasing acidity in the water. Soil materials contain probably some neutralizing components which caused the good results in the first year. In the later time this capacity was exhausted.

4.5.2 Acidity

Acidity is defined as the amounts of base, usually sodium hydroxide (NaOH), needed to neutralize the acidic water sample. Less acidity found in the water means less base needed for its neutralization. Acidity results from the production of synthetic organic materials with minerals, metals, and sulfur and iron compounds in the wastewater of mining areas. In the experiments the acidity was reported as acidity load in mmol/m^{2}day (see Figure 4.30 to Figure 4.32).

The acidity load of the inflow was on average 57.25 mmol/m^{2}day during 2001, and 44.3 and 50.2 mmol/m^{2}day in the outflow of the planted and unplanted hydroponic systems (see Figure 4.30 and Table 4.15). The hydroponic systems did not decrease acidity during the first 30 days of the experiment. There was no significant difference of acidity between the planted and unplanted hydroponic systems. Even the acidity of the inflow fluctuated during the second year of experiment (2002), both hydroponic systems showed regularly lower acidity of the outflow. It was found that the planted hydroponic system always had a better efficiency for decrease of acidity. There were the peak acidity loads in July, September and November because of an over load of the outflow, which was due to the high precipitation (rain).

In 2003 acidity decreased less, however, it was higher in the late of 2003 because a higher load of inflow was supplied to all systems.

Free surface wetlands (FSW) contained soil material and the wastewater flew over it. The average acidity load of the inflow of all three years was about 40 mmol/m^{2}day, whereas the average load of the outflow of the planted and unplanted FSW were about 7 and 27 mmol/m^{2}day, respectively. The acidity of the planted FSW was significantly lower than unplanted FSW during the whole period of the experiment (2001 to 2003). This means that the FSW with plants can reduce the acidity better.

In subsurface wetlands (SSW) the behaviour of acidity was similar to the FSW. Acidity decreased significantly in the planted SSW but not in the unplanted SSW. This shows that the plants in combination with soil material are necessary for an efficient removal of acidity. It was found that since June 2003 the SSW without plants could decrease acidity in the same range of the SSW with plants.

Acidity was correlated to the pH in each system. It was found that the planted systems (FSW and SSW) had a higher efficiency in decreasing acidity compared to the other unplanted system. When comparing all systems in 2003, it is interesting that after the input of the high inflow load, FSW with plants continues removing the acidity, whereas the other

systems cannot perform similarly, which makes planted FSW is the most suitable of all systems to decrease acidity. It is more useful because FSW has higher storage capacity of wastewater, also in case of heavy rainfalls.

During the process of sample titration there was a little amount of yellow precipitate in the samples. It is known that salts of heavy metals, particularly those of trivalent metal ions, such as Fe(III), hydrolyze in water to release mineral acidity. This precipitate might be an effect of the extremely high concentration of Al and Fe in the wastewater. These ions formed a white Al precipitate and a yellow Fe precipitate after titration and the pH was higher than 3.

The results of these experiments show that the wetland systems containing soil material had a good effect on neutralization of acid mine drainage. It was probably an effect of calcium carbonate in the soil material which was one of the important factors in the neutralization process. In addition the wetland systems containing plants showed a good neutralization activity depending on the weather or season. In brief the planted FSW has the best capacity in decrease of acidity.

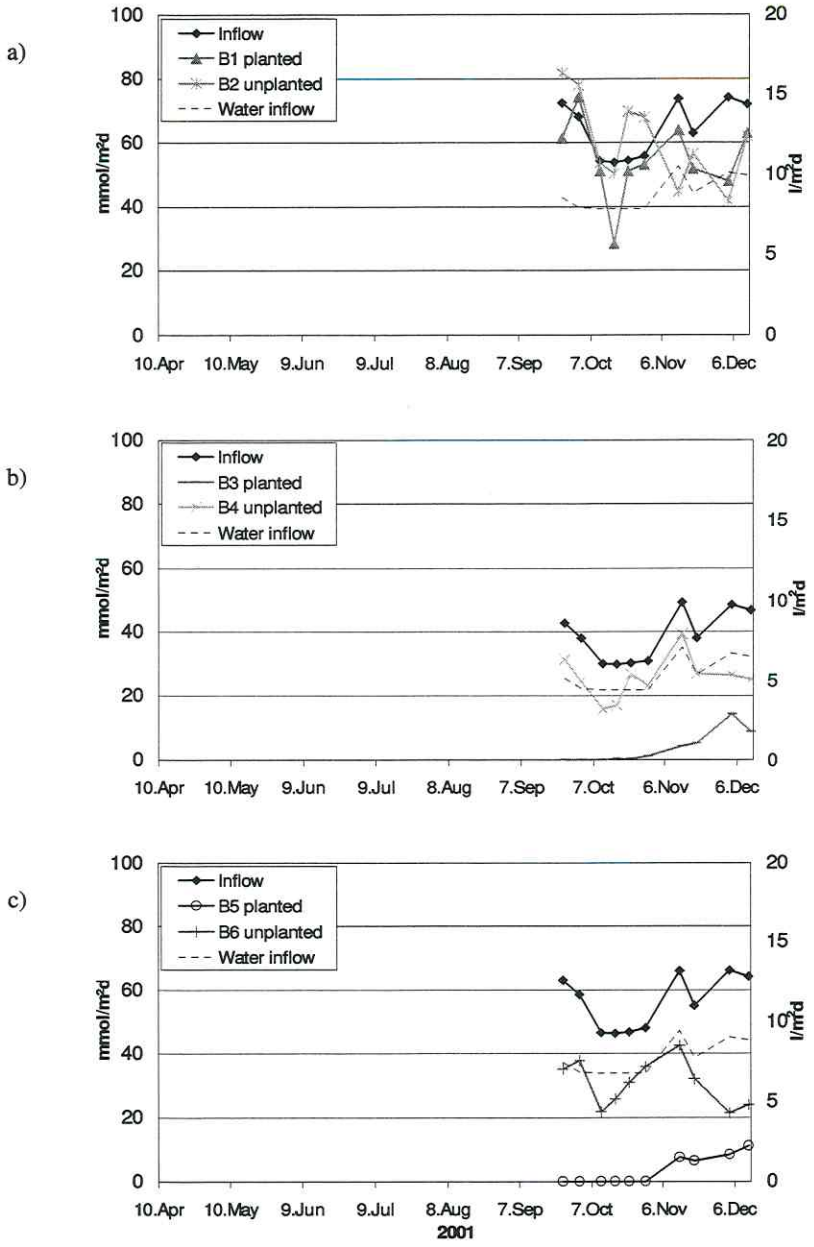


Figure 4.30 Water inflow load and acidity load (mmol NaOH/m^2d) of the inflow and outflow in different experimental wetlands in 2001. a) : planted (B1) and unplanted (B2) Hydroponic systems b) : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW) c) : planted (B5) and unplanted (B6) subsurface Wetlands (SSW)

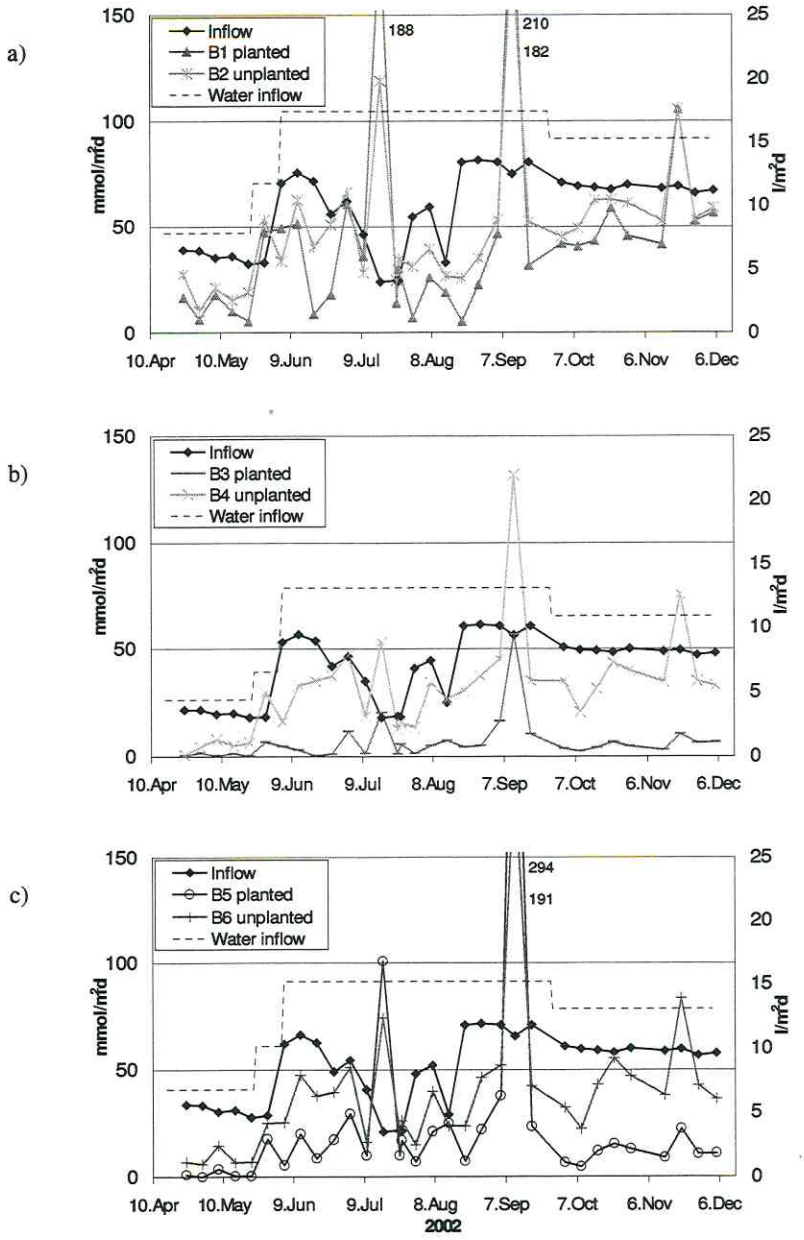


Figure 4.31 Water inflow load and acidity load (mmol NaOH/m^{2}d) of the inflow and outflow in different experimental wetlands in 2002. a) : planted (B1) and unplanted (B2)Hydroponic systems b) : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW) c) : planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)

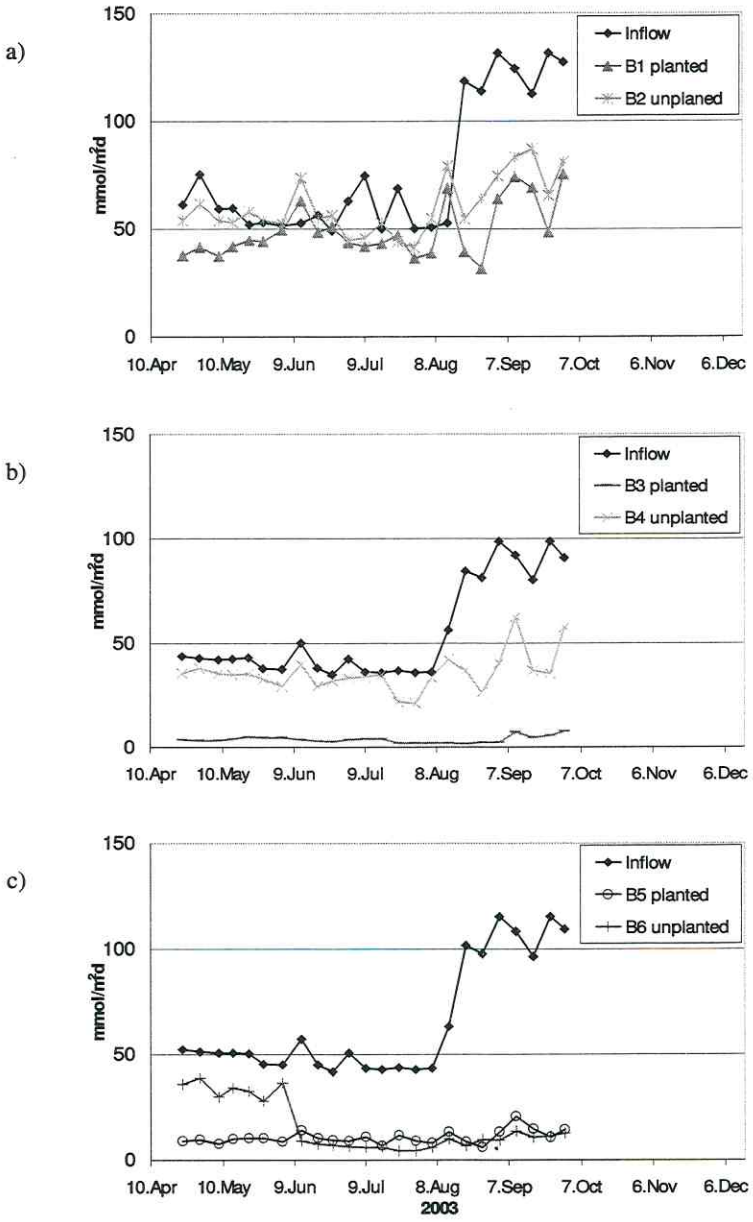


Figure 4.32 Acidity load (mmol NaOH/m^2d) of the inflow and outflow in different experimental wetland systems in 2003. The inflow rate of the water was kept in the same rate for a whole period of this experiment. a) : planted (B1) and unplanted (B2) Hydroponic systems b) : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW) c) : planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)

Table 4.15 Average acidity removal rates and pH in different wetland systems during the operation periods (2001-2003)

Wetland systems	Year	Mean acidity (mmol NaOH /m^2d)		Mean acidity removal rate (mmol NaOH/ m^2d)	pH of the outflow
		Input	Output		
FSW (Planted)	2001	38.47	3.42	35.05	5.86
	2002	40.96	6.65	34.31	4.56
	2003	54.93	3.65	51.28	4.62
FSW (Unplanted)	2001	38.47	25.66	12.81	3.53
	2002	40.96	31.98	8.98	3.44
	2003	54.93	35.76	19.17	3.53
SSW (Planted)	2001	56.08	3.35	52.73	6.42
	2002	49.66	24.5	25.16	4.4
	2003	65.18	10.6	54.58	4.6
SSW (Unplanted)	2001	56.08	30.71	25.37	5.16
	2002	49.66	38.66	11.00	4.33
	2003	65.18	15.66	49.52	4.54
Hydroponic (Planted)	2001	64.08	54.54	9.54	3.41
	2002	57.25	44.29	12.96	3.43
	2003	76.6	49.09	27.51	3.6
Hydroponic (Unplanted)	2001	64.08	62.02	2.06	3.1
	2002	57.25	50.22	7.03	3.29
	2003	76.6	60.16	16.44	3.33

4.5.3 Heavy metals (Zn and Fe) in the water phase

Acid mine drainage causes the increase of the solubility of metals in water leading to elevated concentration of metals like Zn and Fe etc. (Stumm and Morgan, 1981). In the strongly acidic mining wastewater the concentrations of Fe often reach amounts of 1kg Fe/m^3 (Geller et al., 1998). The different species of ferric hydroxides and the ionic Fe(III)-form show a buffering capacity which is comparable with that of carbonate system. This Fe-buffering system stabilizes the pH values between 2 and 4. Because of this important buffering system of the metals in the acid mining wastewater, the concentrations of Zn and Fe were observed in these experiments.

4.5.3.1 Zn

The Zn load of the inflow in the hydroponic systems was nearly constant (8.0-13.5 mg/m^2d) during the year 2001 (see Figure 4.33). The results of Zn loads in the outflow showed no significant difference between the hydroponic system with plants and the one without plants, which was in the range of about 5-17 mg/m^2d.

In the year 2002, the same Zn loads were observed from April to July (Figure 4.34). The inflow loads were higher from late July and decreased again from October. This change of inflow loads means a lower removal capacity. The outflow loads increase even more and were higher than the inflow loads from October till the end of 2002.

Consequently, also in 2003, the hydroponic systems could not remove the Zn from the water (Figure 4.35). There were no significant differences between the inflow Zn loads (average 12.5 mg/m^2d) and the outflow loads of both planted (average 9.73 mg/m^2d) and unplanted (average 9.5 mg/m^2d) hydroponic systems.

The outflow loads of the FSW had a lower Zn load than the inflow during the year 2001. The planted FSW showed lower Zn load in the outflow (in the range of 0.1-5.9 mg/m^2d) than the outflow of without plants (in the range of 2.6-7.9 mg/m^2d). During 2002, the planted FSW still showed higher capacity of Zn removal.

In 2003, the planted FSW had obviously a better capacity for Zn removal. The Zn loads in the outflow of the planted FSW were significantly lower than in the FSW without plants. The average outflow loads of Zn were 2.9 for the planted and 6.4 mg/m^2d for the unplanted FSW.

These results show that the FSW in combination with plants and soil material was effective in Zn removal, and in particular it is more effective than the hydroponic system.

The SSW had only little capacity to remove Zn from the wastewater. The load of Zn in the outflow of the planted SSW (average of 2.5 mg/m^2d) was significantly lower than the

unplanted SSW (average of 8.7 mg/m^{2}d) during the first period of the experiment (2001). The capacity of the planted SSW for Zn removal decreased after the second year of application (2002), as the higher amounts of Zn in the outflow show (average of 17.7 mg/m^{2}d). The Zn load in the outflow was even higher than that of the inflow. This means that Zn was released from the SSW. Nevertheless, the SSW with plants showed a better capacity for the Zn removal again in 2003 (with average of about 9.1 mg/m^{2}d, but less than FSW).

External factors, such as temperature and light, not only influence plants' growth, but also affect metal fixation. Metals uptake has been shown to increase with increasing light intensity (Greger, 1999). It was found that the loads of Zn in the outflow of the planted system were elevated during the winter (November and December, see Figure 4.33 to Figure 4.35) when the plants had less growth. These results agree with the idea that most of the metal uptake by plants is performed during the growth period and by the younger parts of the root where the Casparian strips are not yet fully developed (Hardiman et al., 1984; Marschner, 1995). Metals are largely transported apoplastically in plant tissue. In the xylem vessels metals are probably transcolated in complexed form. Zinc may be transported chelated to organic acids (Greger, 1999; Mench et al., 1988).

There is no correlation between Zn load and the pH in SSW from September to December 2002, while pH also did not decrease. This is contrast to the observation that metal ions can be released to the water phase when the pH is low (Stumm and Morgan, 1981). However, there can be other effects to this phenomenon such as the load of Fe in the system, which affects the dissolution of Zn. The precipitation or the uptake of Zn could be affected by other metals through competition at the uptake sites (Greger, 1999).

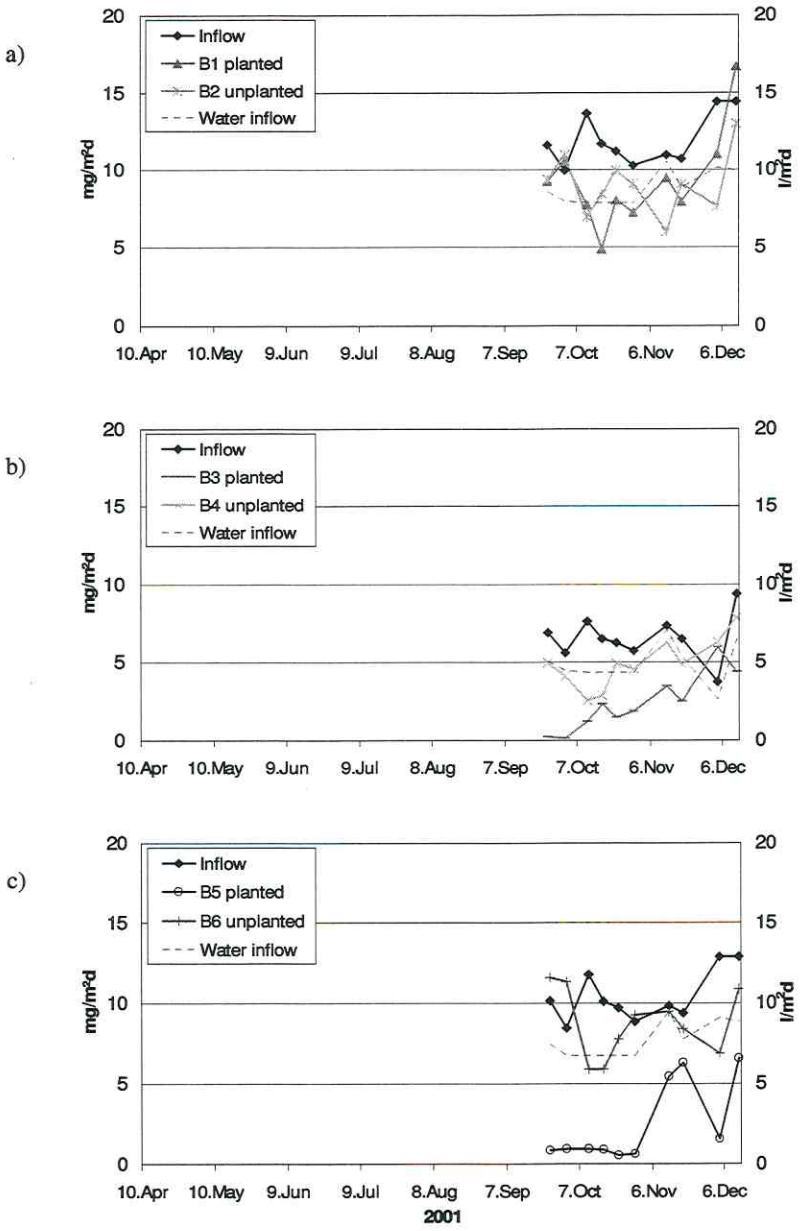


Figure 4.33 Water inflow load and zinc load of the inflow and outflow in different experimental wetlands in 2001. a) : planted (B1) and unplanted (B2) Hydroponic systems b) : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW) c) : planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)

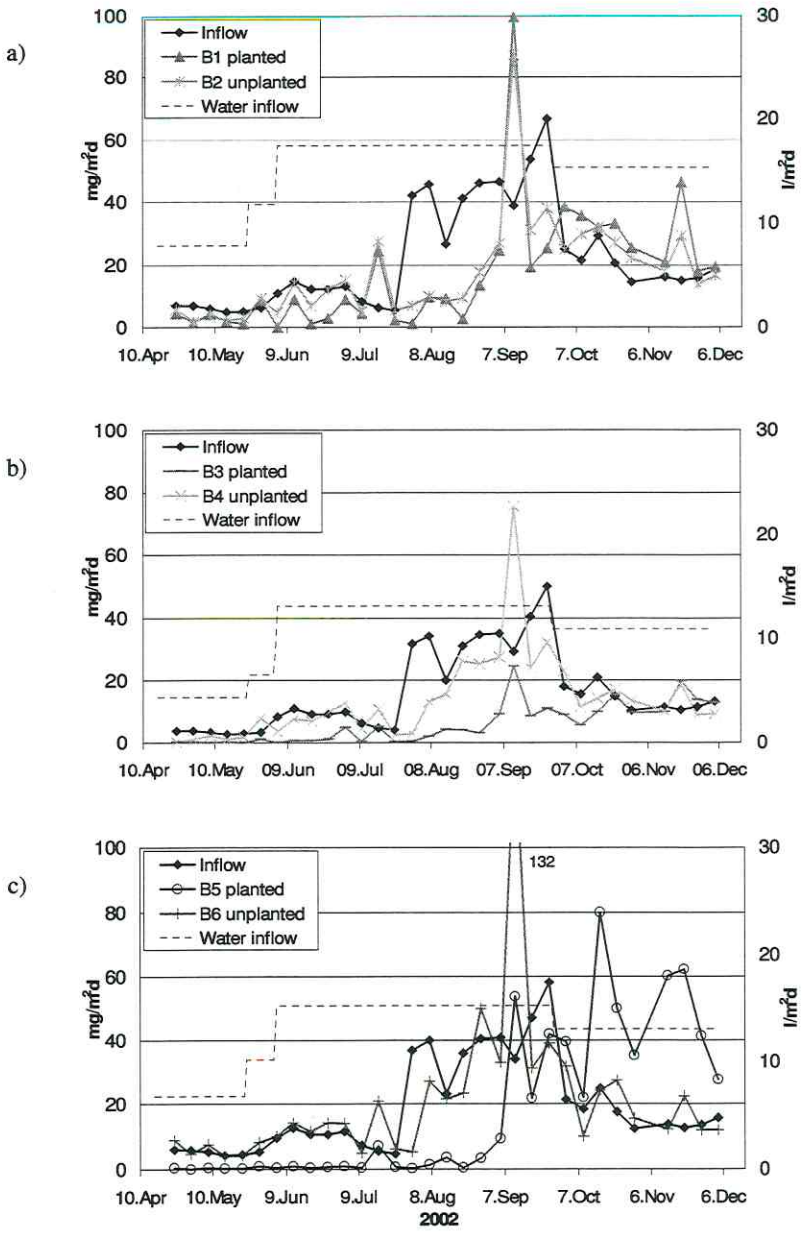


Figure 4.34 Water inflow load and zinc load of the inflow and outflow in different experimental wetlands in 2002. a) : planted (B1) and unplanted (B2) Hydroponic systems b) : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW) c) : planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)

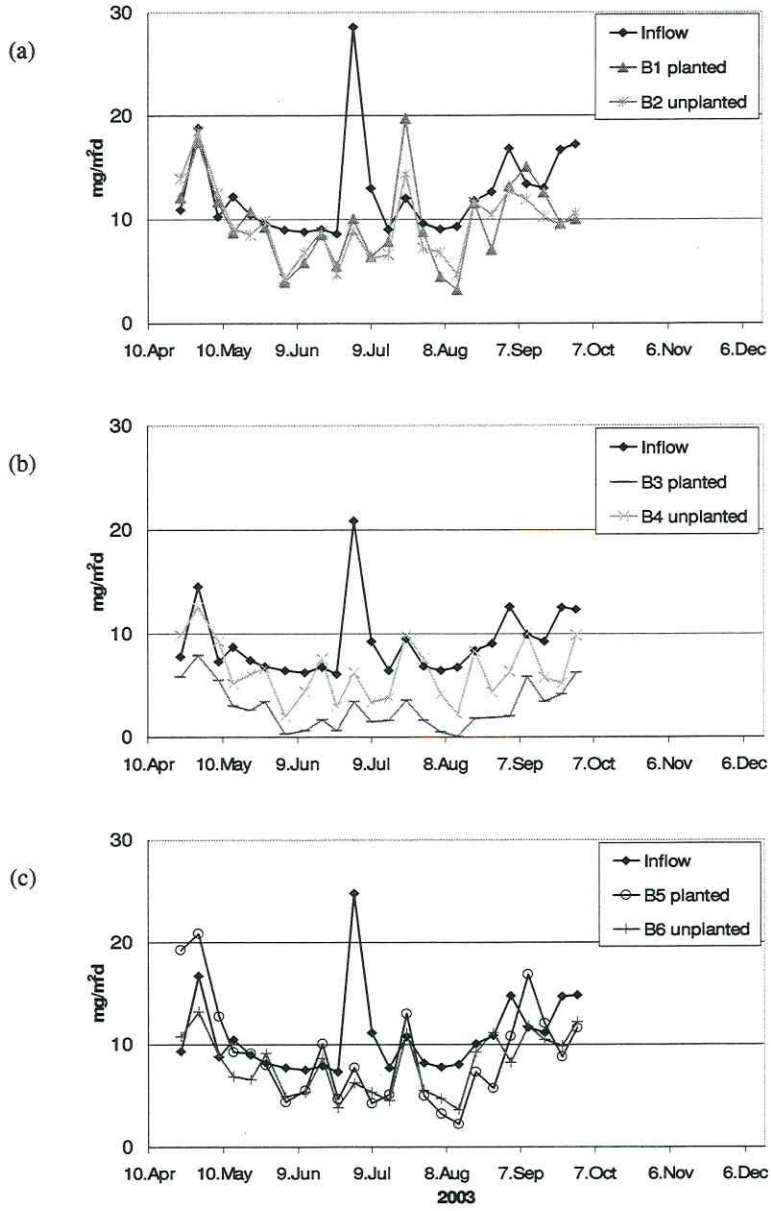
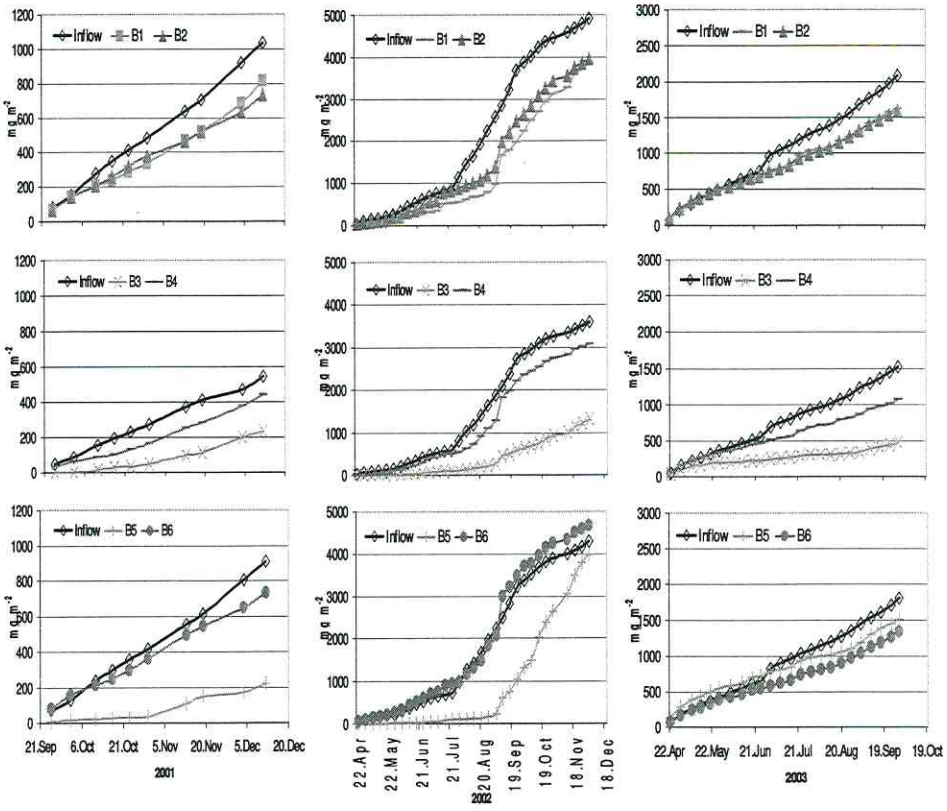


Figure 4.35 Zinc load of the inflow and outflow in different experimental wetlands in 2003. The inflow rate of the water was kept in the same rate for a whole period of this experiment. a) : planted (B1) and unplanted (B2) Hydroponic systems b) : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW) c) : planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)



(a) 2001 (b) 2002 (c) 2003

Figure 4.36 Cumulative zinc load of the inflow and outflow in different experimental wetlands in the year 2001(a), 2002 (b) and 2003 (c)

where : B1, B2: outflow of the planted and unplanted Hydroponic systems
B3, B4: outflow of the planted and unplanted Free Surface Wetlands (FSW)
B5, B6: outflow of the planted and unplanted Subsurface Wetlands (SSW)

Figure 4.36 shows the cumulative zinc load of the inflow and outflow in different experimental wetland systems over the experimental periods of 2001, 2002 and 2003. It was found that the rate of cumulative load was high in the year 2002 with a higher striking capacity. However, the removal capacity of this planted SSW was lower than the planted FSW. The Zn loads of the outflow of the planted FSW and of the planted SSW were lower than Zn loads of the inflow (see Figure 4.36 and Table 4.16). This means the systems containing plants and soil material have higher efficiency in Zn removal.

In conclusion, the planted FSW, with a combination of plants and soil materials, can remove the Zn load (P-value<0.05) in the acid mine drainage significantly better than the other systems even over a long period of 3 years. In contrast, the planted SSW can remove Zn only in the first year of application better but it released Zn again and had less removal capacity after a long running time. The hydroponic system cannot remove significant amounts of Zn from the acid mine drainage.

Table 4.16 Cumulative zinc and iron loads and mean daily removal rates of zinc and iron in the wetland systems during experimental periods of 2001 to 2003

Wetlands	Year	Cumulative Zn (mg/m^2)		Zn: Mean removal rate (mg/m^2d)	Cumulative Fe (mg/m^2)		Fe: Mean removal rate (mg/m^2d)
		Input	Output		Input	Output	
FSW (Planted)	2001[a]	545	237	3.5	7915	168	89.0
	2002[b]	3599	1314	10.2	17570	1399	72.2
	2003[c]	1518	477	6.5	20685	1170	122.0
FSW (Unplanted)	2001[a]	545	439	1.2	7915	3256	53.6
	2002[b]	3599	3086	2.3	17570	8047	42.5
	2003[c]	1518	1068	2.8	20685	4060	103.9
SSW (Planted)	2001[a]	906	224	7.8	12908	4165	100.5
	2002[b]	4282	3973	1.4	21437	17796	16.3
	2003[c]	1799	1511	1.8	24534	768	148.5
SSW (Unplanted)	2001[a]	906	732	2.0	12908	162	146.5
	2002[b]	4282	4659	-1.7	21437	1027	91.1
	2003[c]	1799	1331	2.9	24534	450	150.5
Hydroponic (Planted)	2001[a]	1033	815	2.5	14715	6000	100.2
	2002[b]	4923	3847	4.8	24820	9593	68.0
	2003[c]	2081	1622	2.9	28383	4374	150.1
Hydroponic (Unplanted)	2001[a]	1033	735	3.4	14715	12415	26.4
	2002[b]	4923	3964	4.3	24820	16487	37.2
	2003[c]	2081	1592	3.1	28383	11370	106.3

The duration of the experiments :
a) 25 Sep – 12 Dec 2001, b) 24 Apr – 4 Dec 2002, c) 23 Apr – 30 Sep 2003

4.5.3.2 Iron (Fe)

To remove Fe from the water, iron must be transformed to the insoluble form, such as FeOOH, $Fe(OH)_3$, Fe_2O_3, Fe_3O_4, $FeCO_3$, FeS, and FeS_2, where it precipitates. This process depends on the redox conditions.

The six different experimental wetlands were used to observe their capacity of iron removal. The Fe loads of these 6 different wetland systems during three years of experiment (2001–2003) are shown in Figure 4.37 to Figure 4.39. The behaviour of cumulative iron loads and the daily removal rates of all systems are summarized and illustrated in Figure 4.40 and Table 4.16, respectively.

Fe loads in the outflow of the planted hydroponic system were lower than the inflow during 2001. The Fe removal rate in the hydroponic system decreased in 2002 from 100 mg/m^2d to 68 mg/m^2d. However, the Fe removal rate increased again in 2003, the third year of experiment, with an average Fe removal of 150 mg/m^2d (see Table 4.16).

In the FSW, the Fe loads in the outflow were lower than the inflow during the whole three periods of experiment. Especially the planted FSW had a better removal rate of Fe than the unplanted FSW in 2001 (89 and 53.6 mg/m^2d with an average removal of 98 and 62%, respectively; $P<0.05$; see also Table 4.19). During the second year the planted FSW had a slightly lower removal rate than the first year, however, the removal rate of Fe was higher than of the unplanted FSW. In the third year, the Fe load of the outflow of the planted FSW decreased to nearly 0 mg/l.

The SSW systems showed a better removal Fe in the first and the third year (68% in 2001 and 94% in 2003), but it had a lower capacity in the second year (2002). Generally, it was found that the Fe load in the outflow of the planted SSW was higher than the inflow, whereas the SSW without plants had the better removal capacity in all period of the experiment (during May and August 2002, see Figure 4.38). The Fe removal capacity of SSW was accelerated in 2003 as in the other FSW systems.

The removal capacity of iron from the acid mine drainage by hydroponic systems was improved over a long operation time (see Figure 4.37a, Figure 4.38a and Figure 4.39a). This can be that the consecutive anaerobic processes occurred gradually over time resulting in the precipitation of FeS and FeS_2 (Fe^{2+} with S^{2-}). The plant mats also can provide a dense root zone, which promotes the activities of microorganisms. When the plants die these organic matters decay and induce anaerobic conditions and stimulate the activity of anaerobic bacteria. It could be the reason why the capacity of the planted SSW increased again after the

second year. Santose et al. (2004) supported that an increase in the amount of Fe(III) reduced to ferrous iron or removed from solution can be explained by the presence of a large number of active sites of the biomass, due to the rise of the biomass concentration.

In general, the higher the pH and the clay and/or organic matter load, the more firmly bound are the metals, and the longer is their residence time in soil (Greger, 1999) which is in accordance with the results of the Zn removal in these experiment. In spite of the low pH of about 4.3-4.6 in the SSW (in the second year of the experiment) Fe was removed very well in the SSW without plants, in contrast to Zn. Zn was not removed well in the planted SSW nor the unplanted SSW. It may be that some chemolithotrophic bacteria like *Thiobacillus ferrooxidans* oxidize ferrous iron at low pH-values, thereby, increasing the overall reaction rate drastically (see equation below; Stumm and Morgan, 1996).

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

On the other hand, some bioreactors and artificial wetlands have proved that sulfate-reduction is effective in raising pH and removing metals and sulfate from mine waters (Hammack and Edenborn, 1992; Dvorak et al.,1992; Hedin et al., 1989; Christensen et al., 1996).

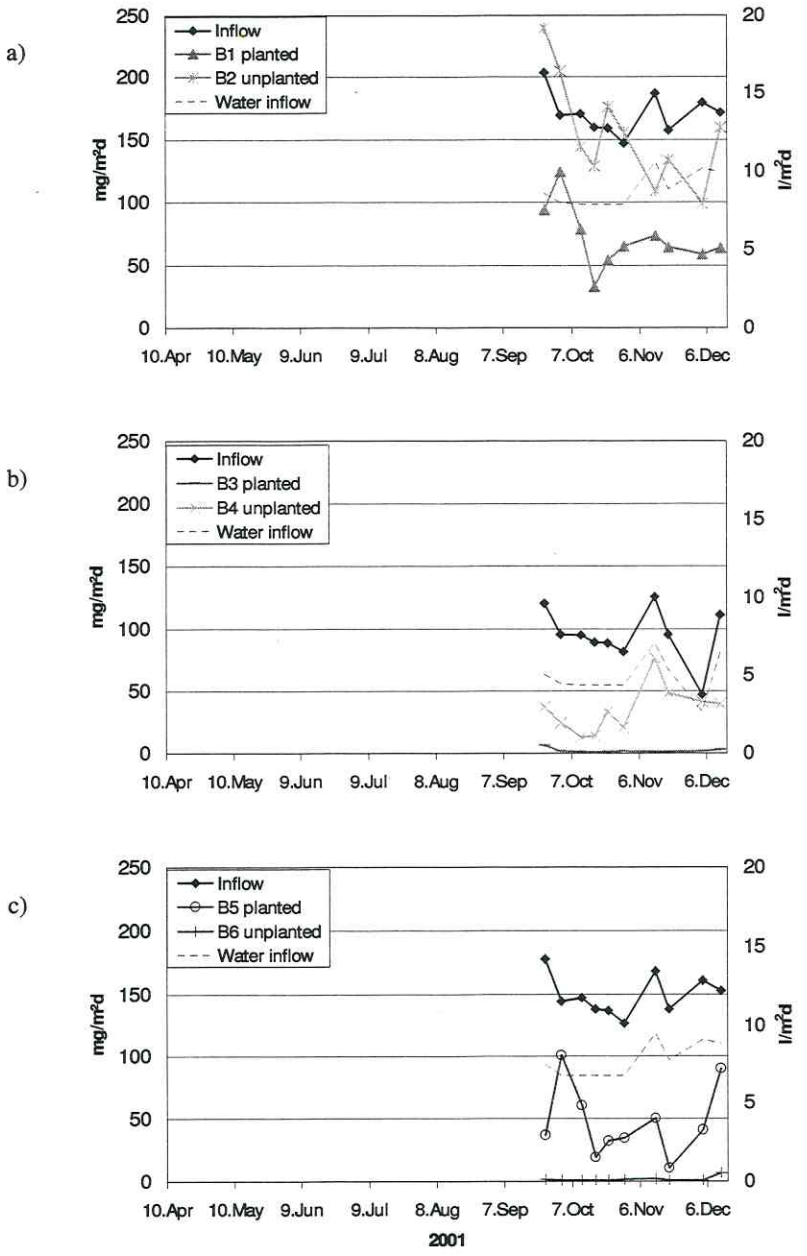
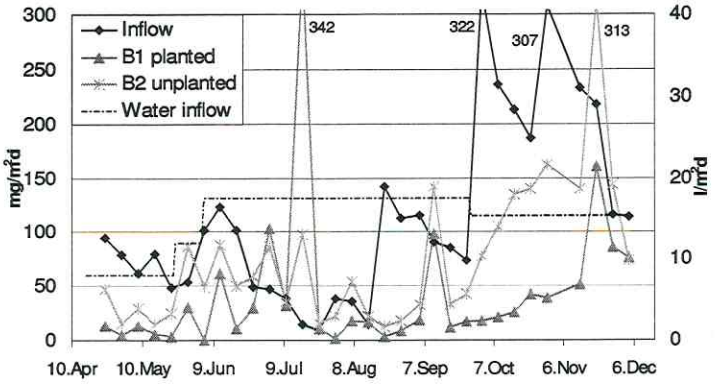
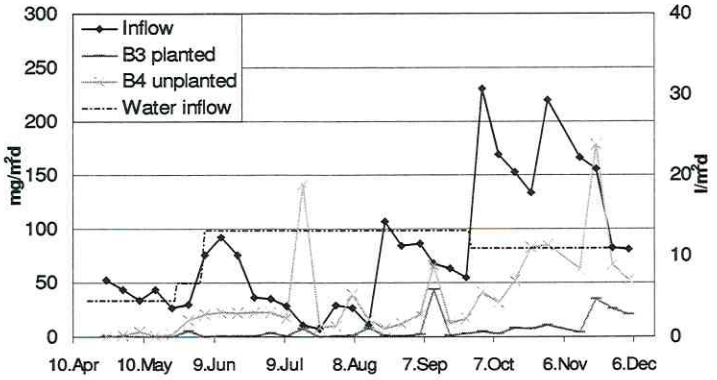


Figure 4.37 Water inflow load and iron loads of the inflow and outflow in different experimental wetland systems in 2001 a) : planted (B1) and unplanted (B2) Hydroponic systems b) : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW) c) : planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)

a)



b)



c)

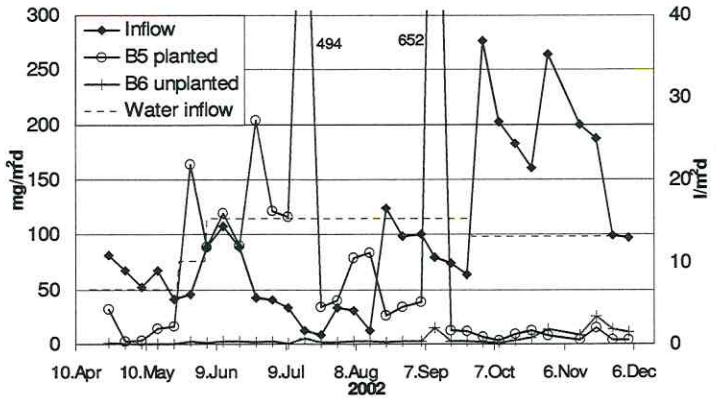


Figure 4.38 Water inflow load and iron loads of the inflow and outflow in different experimental wetland systems in 2002 a) : planted (B1) and unplanted (B2) Hydroponic systems b) : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW) c) : planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)

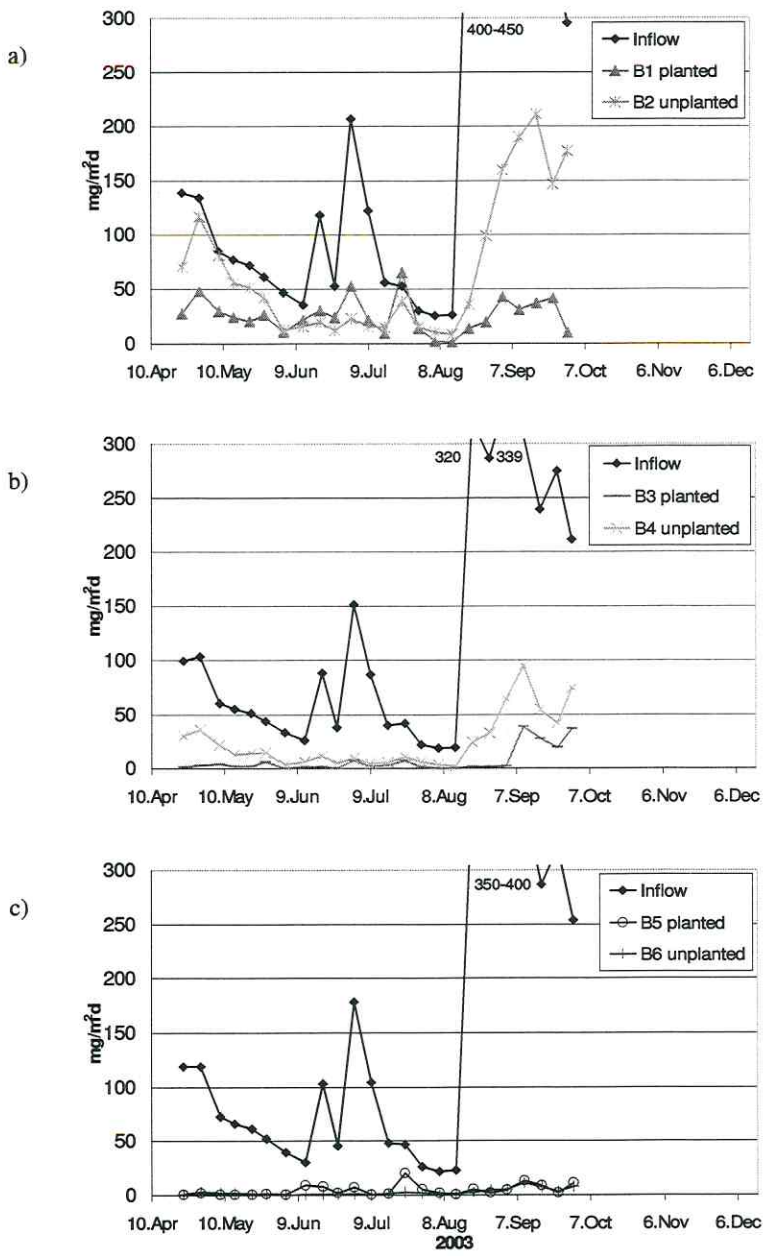
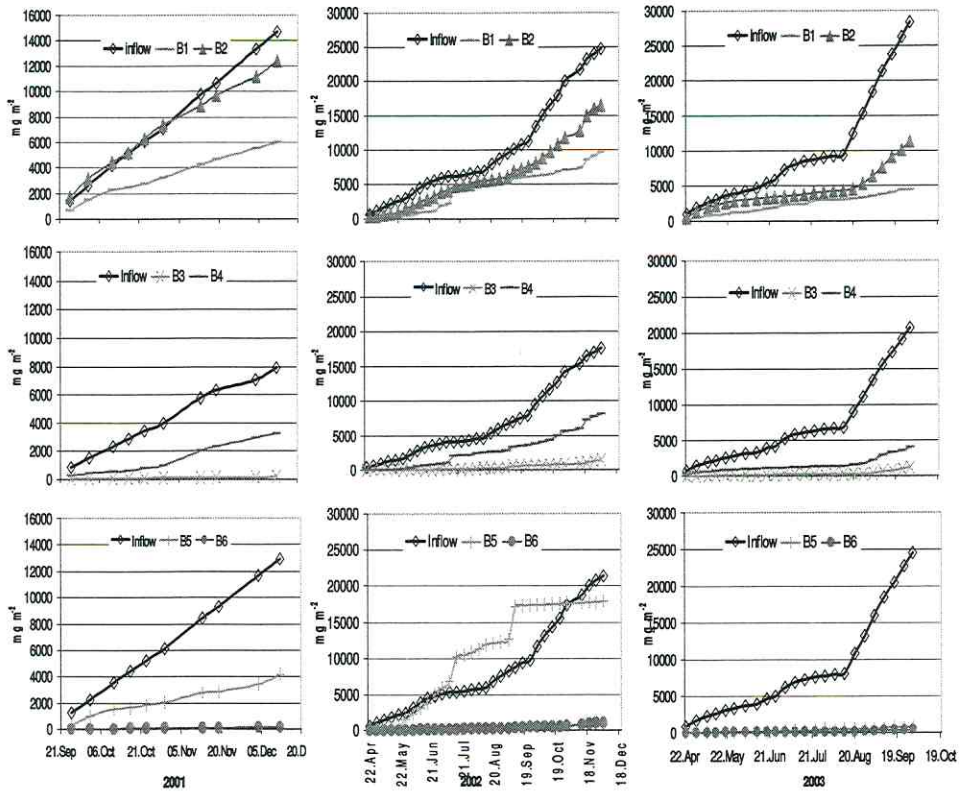


Figure 4.39 Iron loads of the inflow and outflow in different experimental wetland systems in 2003. The inflow rate of the water was kept in the same rate for a whole period of this experiment. a) : planted (B1) and unplanted (B2) Hydroponic systems b) : planted (B3) and unplanted (B4) Free Surface Wetlands (FSW) c) : planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)



(a) 2001 (b) 2002 (c) 2003

Figure 4.40 Cumulative iron load of the inflow and outflow in different constructed wetlands in the year 2001(a), 2002 (b) and 2003 (c)

where: B1, B2: outflow of the planted and unplanted Hydroponic systems
B3, B4: outflow of the planted and unplanted Free Surface Wetlands (FSW)
B5, B6: outflow of the planted and unplanted Subsurface Wetlands (SSW)

4.5.4 Zn and Fe in the plants

The concentrations of Zn and Fe in the plants, which were harvested at the end of the experiment, are shown in Figure 4.41.

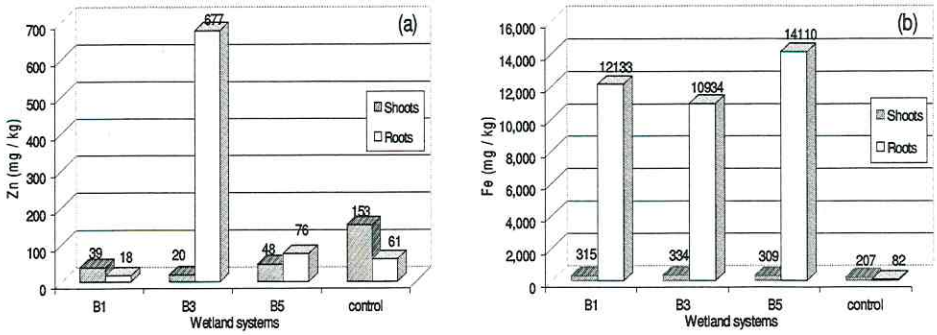


Figure 4.41 Concentration of Zn (a) and Fe (b) in the plants (mg/kg dry weight), in shoots and roots in experimental wetlands for the treatment of acid mine drainage; B1:Hydroponic system, B3:FSW, B5:SSW, control: unexposed plants

The amount of Zn was much higher in the plants of FSW and SSW than in the hydroponic system. It was found that Zn accumulated in the plant roots rather than in the shoots; especially Zn was significantly higher in the roots of the FSW system (of about 677 mg/kg dw) because the soil materials incorporated with plants can induce the rhizosphere processes. Rhizospheric microorganisms and the plant-mycorrhizal colonies adapted to metal-containing biotopes enhanced the Zn uptake by plants (Lambert et al., 1976; Marschner, 1995; Trevors and van Elsas, 1997).

Fe is significantly more accumulated in the roots and at the root surface than in the shoots. These results agree with data of Geller (1998) who showed that the amounts of elements present in the systems associated with the plants can be considerable. Fe is found in a very high amounts compared to Zn. Iron can be translocated from the root to the shoot, which takes places principally through the xylem (Saxena et al., 1999). Regarding Zn and Fe, all the studies support that the oxidation process that occurs in the rhizosphere of plants results in an accumulation of the elements in the direction of the rhizosphere. Moreover, the amount of organic matter in the system seems to be a critical factor determining the accumulation of iron.

Metal accumulation in the root tissues can be accomplished either through deposition of the metal ions along the cell wall and/or inside the vacuoles. Zinc is associated with the cell walls and is primarily sequestered in the vacuole (Hardiman et al., 1984). The plants can take up metal ions via root processes. The processes are root interception of metal ions, entry into the roots through mass flow and diffusion, and translocation of the metals ions from the roots to the shoots (Saxena et al., 1999). It is likely that the metal ions enter the plants either through the symplast (intercellular) or the apoplast (extracellular). Metals are first taken into the apoplast of the roots. Then some of the total amount of the metals is transported further into the cells, some is transported into the apoplast and some becomes bound to cell wall substances (Greger, 1999). Entry of toxic ions into plant tissues may also occur through specific ion transporters, competing with essential ions of similar radii (Cutter and Rains, 1974). Vassil et al. (1998) observed that the uptake of metal-chelate complexes in *Brassica juncea* (Indian mustard) was enhanced by removing Zn and Ca ions from the plasma membrane. In that situation, the physiological barrier (root plasma membrane) to metal uptake was alleviated with a resultant increase in uptake of metals.

4.5.5 Removal efficiency of the constructed wetlands

The ultimate evaluated removal efficiency of the different types of constructed wetlands for acidity, zinc and iron are shown in Table 4.17 to Table 4.19. The per cent removal efficiencies are shown in the brackets. The planted FSW and SSW had very high efficiency for treatment of all parameters (with significant difference to the other treatments, $p<0.05$).

Fe load in the outflow of all planted systems was significantly lower than of the unplanted systems and lead to the conclusion of high efficiency removal. It was also found by Kleinmann (1998) that the hydroponic systems or aerobic ponds are not useful when the water entering the wetland system has a pH less than 4. At such low pH, iron oxidation and precipitation reactions are quite slow and significant removal of iron in the aerobic pond would not be expected.

In the second year of operation the inflow load of Fe was less than in the first year. The outflow loads of each planted system remained lower than the inflow loads except in the planted SSW. Generally, the capacity for the removal of acidity, Fe and Zn decreased in the second year.

The highest mean removal rates were observed in all wetland systems in the third year of experiment (Table 4.19).

Table 4.17 Average removal rate of **Acidity** in different wetland systems (mmol NaOH/m^2d). Data in brackets shows the percentage of removal efficiency. ANOVA is used for the statistical analysis. The experimental periods are: 25 Sep – 12 Dec 2001, 24 Apr – 4 Dec 2002 and 23 Apr – 30 Sep 2003.

Year	HP		FSW		SSW	
	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted
2001	9.54 (15)^{ab}	2.06 (4)	35.05 (92)^{a}	12.81 (33)	52.73 (95)^{b}	25.37 (44)
2002	12.96 (10)^{c}	7.03 (4)	34.31 (83)^{cd}	8.98 (21)	25.16 (45)^{d}	11.00 (20)
2003	27.51 (29)^{eg}	16.44 (13)	51.28 (93)^{eh}	19.17 (29)	54.58 (82)^{gh}	49.52 (71)

a b c d e g h : significant difference (P-value<0.05)

Table 4.18 Average removal rate of **Zinc** in different wetland systems (mg/m^2d). Data in brackets shows the percentage of removal efficiency. ANOVA is used for the statistical analysis. The experimental periods are: 25 Sep – 12 Dec 2001, 24 Apr – 4 Dec 2002 and 23 Apr – 30 Sep 2003.

Year	HP		FSW		SSW	
	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted
2001	2.5 (22)^{ab}	3.4 (23)	3.5 (60)^{a}	1.2 (21)	7.8 (77)^{b}	2.0 (14)
2002	4.8 (9)^{c}	4.3 (4)	10.2 (60)^{cd}	2.3 (9)	1.4 (2)^{d}	-1.7 (-20)
2003	2.9 (20)^{e}	3.1 (21)	6.5 (69)^{eg}	2.8 (27)	1.8 (14)^{g}	2.9 (23)

Note: minus value (-) means releasing Zn

a b c d e g: significant difference (P-value<0.05)

Table 4.19 Average removal rate of **Iron** in different wetland systems (mg/m^2d). Data in brackets shows the percentage of removal efficiency. ANOVA is used for the statistical analysis. The experimental periods are: 25 Sep – 12 Dec 2001, 24 Apr – 4 Dec 2002 and 23 Apr – 30 Sep 2003.

Year	HP		FSW		SSW	
	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted
2001	100.2 (59)^{a}	26.4 (9)	89.0 (98)^{ab}	53.6 (62)	100.5 (68)^{bc}	146.5 (99)^{c}
2002	68.0 (-16)	37.2 (4)	72.2 (89)	42.5 (13)	16.3 (-161)	91.1 (93)
2003	105.1 (73)^{de}	106.3 (55)	122.0 (95)^{d}	103.9 (80)	148.5 (94)^{e}	150.5 (98)

Note: minus value (-) means release of Fe

a b c d e: significant difference (P-value<0.05)

Table 4.20 Amounts of Zn and Fe in the soil materials in FSW and SSW after three years of experiment

Source of gravel	Zn (mg/kg)	Fe (mg/kg)
B3: FSW with plants	9.77	1041
B4: FSW (no plants)	2.38	704
B5: SSW with plant	2.18	467
B6: SSW (no plants)	1.67	504
Unexposed gravel (control)	2.34	624

In the long-term wetland systems will fill up with metal precipitates or the conditions that facilitate contaminant removal. These results can be observed in the third year of operation when the wetlands had a higher removal capacity than in second year. The long term operation could provided more anoxic environment which contains organic substrate leading to the occurring of sulfate reducing bacteria which directly affects the concentrations of dissolved metals by precipitation of metal sulfide afterwards (Kleinmann, 1998; Bender et al., 1989).

The experiments give the results that the wetlands which have plants incorporated with the soil material have a high removal efficiency, especially the planted FSW. The metal contents found in the soil materials indicate that Zn and Fe were stored in the soil material (see Table 4.20). The SSW without plants, which contained only soil material, had a better Fe removal after the second year of operation. Comparing to the planted FSW, it was found that the SSW had less efficiency in acid mine drainage removal, however.

The processes which could involve in these metal removals were metals complex with organic materials, including microorganisms and their organic releases. There were four dominant processes that could trap metals in the wetland systems, such as cation exchange, adsorption, precipitation and co-precipitation, and complexation or chelation. Additionally, the biological component mediated the high dissolved oxygen and redox potential which favour the chemical precipitation of metal oxides and hydroxides (Phillips and Bender, 1998). These oxides and hydroxides, in turn, act as reservoirs for additional metal deposit.

5 Conclusions

This thesis presents the work which has examined the removal behaviour of arsenic and heavy metals from wastewater by different constructed wetland systems. It is necessary to understand the fundamental processes and mechanisms operative in constructed wetlands for mine wastewater to realize long-term stable and highly effective removal. In this way the removal efficiencies according to iron, zinc, chromium and arsenic and the neutralization of mining and synthetic wastewaters were investigated using laboratory-scale and small field constructed wetlands. Physical, biological and chemical factors which affect the efficiency of constructed wetlands for wastewater treatment have been investigated.

In an incubation experiment, added river sludge sediment, which resembles natural wetland bed material, resulted in a >50% decrease of As(V) within 7 days of incubation under anaerobic conditions. Arsenic even decreased when the activity of microorganisms was inhibited. A possible abiotic process is precipitation and co-precipitation of As with Zn and S^{2-} under anoxic conditions. These results were in contrast to the general assumption that As is highly mobile under anaerobic conditions and showed that, in special cases, As was fixed due to a high adsorption capacity of the soil matrix which is not based on the principle of binding to iron.

The first series of experiments, laboratory batch wetland models were characterized for the fate of As and Zn, and their removal efficiency. These wetland systems were simulating a subsurface wetland (SSW), free surface wetland (FSW), hydroponic system (HP), and an algae pond (AP). They were initially loaded with water containing 5 mg/l of Zn and 0.5 mg/l of As as the main contaminants. SSW, FSW and HP were planted with the macrophyte *Juncus effusus.*

AP systems showed almost no changes of all parameters measured. Similarly, no changes could be observed in HP regardless of depth gradients within the system. Nevertheless, the concentrations of total As and Zn in the water compartment decreased slightly during 90 days (about 25% and 30%, respectively). Within the gravel bed systems (SSW and FSW) As and Zn were removed almost completely from the water, and for both parameters the removal process in the SSW was considerably faster.

In both gravel bed systems the iron concentrations and redox potentials showed inversely related changes. During periods of comparatively low redox potential, the iron concentration

of the pore water was rising from 0.1 mg/l up to 3.0 mg/l for the FSW and to 6.8 mg/l for the SSW. In periods of a higher redox potential the iron concentration decreased.

Because the adsorption capacity of gravel for As was very low (in the range of up to 4.3 µg/kg), other processes besides direct adsorption must be taken into account for As removal from the water. Due to the relatively high concentration of iron of the gravel at the end of experiment (estimated to be 109 mg/kg), this surplus of iron in both gravel systems could theoretically bind all As. The plants by themselves in the hydroponic system did not absorb considerable amounts. This fact in combination with the low adsorption capacity of the gravel in the gravel systems, indicated that a combination of both components, i.e. gravel and plants, are required for an efficient removal of As, because only this combination provided the distinct conditions for As binding. Thus, the best As removal was found in planted gravel systems.

It can be assumed that with the activity of the roots, organic compounds (rhizodeposition products as the sum of root exudates and dead root matter) are released into the rhizosphere. Some of these compounds can function as iron chelating compounds. Furthermore these organic compounds can also be used as a carbon source for microorganisms in the soil resulting in a decrease of the redox potential. Both, the relatively low redox potential and the chelating rhizodeposition products, stimulate the redissolution of crystalline iron(III) which has a low binding capacity of As. Nevertheless, because of the capability of some helophytes to transfer oxygen into their rhizosphere, especially on the rhizoplane, the oxic conditions in this compartment can cause the precipitation of dissolved iron and coprecipitation of other trace elements, especially on the roots where iron plaques are formed.

Generally, the dissolution of crystalline iron and the subsequent precipitation, which is caused by the combination of direct and an indirect actions of plants and microorganisms, can result in the As removal from the water compartment in water logged soils with an apparently low As binding capacity.

As the environmental behaviour of As strongly depends on its speciation, we also checked which species were present. Some arsenic species occur in reducing conditions, especially As(III) was found in regions with low concentrations of oxygen, located near the bottom of the SSW and FSW wetlands. Methylated arsenic species were found in low amounts only. Methylated arsenic species were also found in AP because of the appearance of algae which can transform toxic As(V) to other non-toxic As species.

The two step constructed wetland system with continuous flow, consisting of a hydroponic system (HP) combined with a free surface wetland (FSW), was investigated in the laboratory for removal of As, Zn and Cr from an artificial wastewater. High supply of a carbon source (sodium benzoate) as in the first phase of the experiment led to reducing conditions with nearly 0 mg/l of dissolved oxygen and an Eh in the range from 0 to -170 mV. The results showed that the average removal efficiencies of the hydroponic system decreased in the sequence Cr ≈ Zn > As.

The high performance rate depended on the anoxic conditions or the addition of a carbon source. Under these conditions, Cr(VI) was reduced to Cr(III) and precipitated as $Cr(OH)_3$ In this case iron and sulfide can form precipitates with As, Zn and Cr. Under anaerobic conditions, As(V) was reduced to As(III), which precipitated with S^{2-} as As_2S_3. In the presence of Fe, As(V) precipitated as $FeAsO_4$ or immobilized on hydrated iron oxides. During wastewater treatment, additional As species, such as methylated arsenic, were formed in both parts of the wetland model. Because arsenate behaves similar to phosphate, plants and some microorganisms can take up or assimilate arsenate and form organoarsenic compounds, such as arsenosugars. This is a detoxification mechanism to prevent arsenate from inhibiting the growth of microorganisms by interfering with phosphorus processing.

The characterization of the microbial community showed evidence that there are many types of microorganisms living in the system, for example, sulfate reducing bacteria which affected the removal mechanism of As, Zn and Cr. Microorganisms could take up and transform those metals to other forms and accumulate them in the cells resulting in sludge precipitate, or convert the metals into volatile forms e.g. methyl arsine and released them to the air.

The results show that As, Zn and Cr accumulated in plants and sediments along a distance gradient. Higher concentrations of accumulated As, Zn and Cr were found in the plants and sediments near the inflow zone. The accumulated concentrations in the plants were in the order Cr>Zn>As, the same order as the removal efficiency.

Six small scale constructed wetlands were examined for removal of acidity and Zn from acid mine drainage (AMD) in a field test over 3 years. The systems containing both plants and soil material had high removal efficiency. The planted FSW and SSW showed higher capacity of pH and acidity improvement and of Zn removal from the wastewater. Removal capacity for treatment of pH and acidity was high in the beginning of the operation and decreased over time as the capacity of the mechanisms of adsorption to soil material,

precipitation and co-precipitation exhausted. The removal capacity for Zn increased with the duration of operation because reducing conditions were established over time by the activities of plants and microorganisms.

The planted wetlands had better capacity for AMD treatment because the activity of the plants promoted the neutralization of acidity and the plants took up Zn from wastewater. The contaminants were accumulated in the roots and on the root surface rather than in the shoots because the plant roots provide a surface for growth of microorganisms, which are responsible for the removal capacity of contaminated wastewater in the wetland systems. In the field experiments, the seasonal change, rainfall and temperature affected the removal efficiency of the constructed wetlands.

These studies showed that plants played an important role in all types of wetland systems for the treatment of contaminated wastewater. However, not only plants had an effect on the removal of As, Zn, Fe and acidity but also the soil material. The combination of plants and soil materials increased the efficiency in removing the pollutants from wastewater and accelerated the process. These results encourage the implementation of constructed wetlands for remediation of wastewater from mines and industries which emit acidity, As, Zn, Fe and Cr.

The results showed that anaerobic wetlands could offer high performance of heavy metal removal. They provided an environment for sulfate reducing bacteria (SRB) to remove and precipitate contaminants from the wastewater. However, there was a limitation of impairing plant growth when the redox potential was too low and there was the nuisance of smelling production of H_2S. Further studies should investigate more thoroughly the effect and the supplying period of carbon sources in order to maintain the anaerobic conditions while not impairing the plants' activity in the removal of heavy metals.

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Appendix A Physical and chemical parameters in constructed wetland in the batch systems

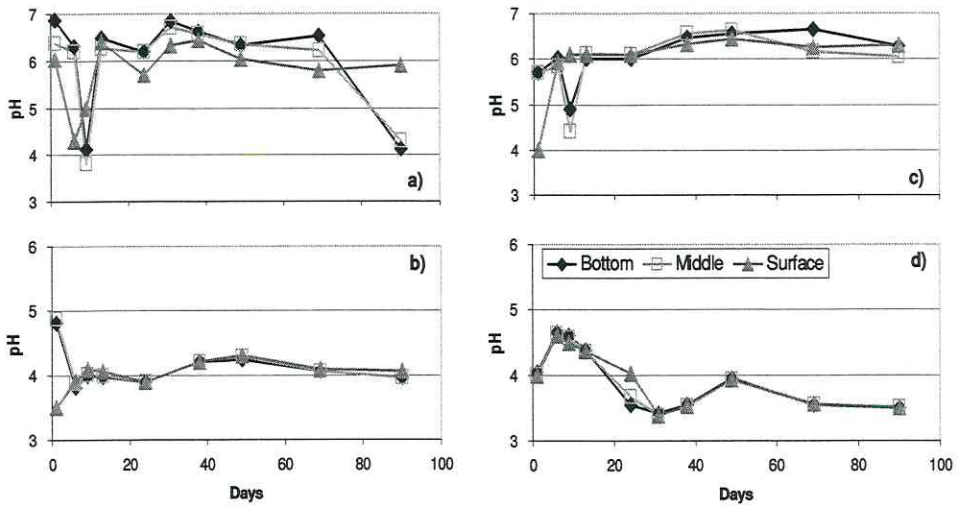

Figure A-1 pH in different wetland system
a) SSW b) HP c)FSW d) AP

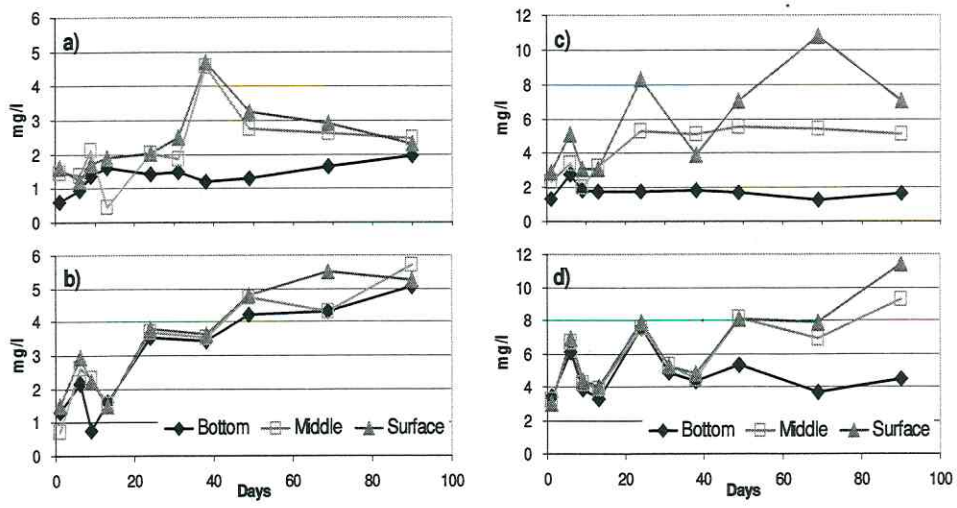

Figure A-2 Dissolved oxygen in different wetland systems
a) SSW b) HP c)FSW d) AP

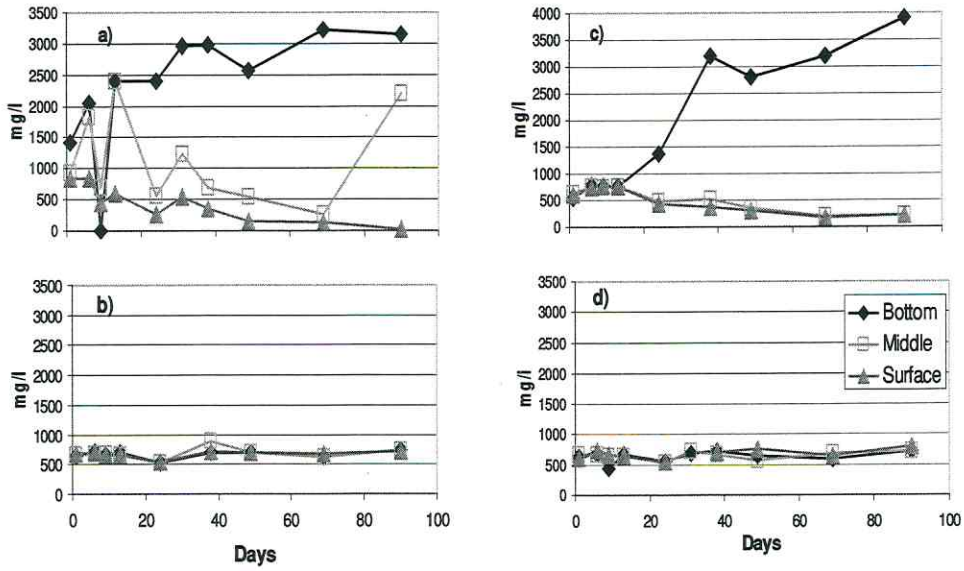

Figure A-3 Concentrations of sulfate in different wetland systems
a) SSW b) HP c)FSW d) AP

Appendix B Treatment of artificial wastewater containing arsenic and heavy metals in a two step wetland system

Table B-1 pH values of the water samples from the different sampling points of the two steps wetland system

Date	Sampling point							
	A		B		C		D	E
	surface	bottom	surface	bottom	surface	bottom	surface	surface
25.Jul.02	6.4	6.3	6.4	6.3	6.5	5.6	6.9	6.8
01.Aug.02	6.1	6.2	6.5	6.5	6.8	6.7	7.1	7.2
06.Aug.02	6.1	6.0	6.7	6.7	6.3	6.2	7.0	7.2
15.Aug.02	6.9	6.7	7.4	7.3	7.1	7.0	7.5	7.8
21.Aug.02	6.3	5.9	6.6	6.6	6.5	6.6	6.7	6.8
28.Aug.02	6.5	6.3	6.8	6.8	6.7	6.7	6.8	6.9
05.Sep.02	7.0	6.9	7.1	7.1	6.8	6.9	6.9	6.9
11.Sep.02	6.6	6.6	6.9	6.7	6.7	7.5	7.0	6.1
25.Sep.02	6.7	6.7	7.2	7.1	6.6	6.9	7.2	5.5
02.Oct.02	7.0	6.7	7.1	7.1	6.7	6.7	6.7	5.4
09.Oct.02	6.5	6.2	6.8	6.7	6.6	6.5	6.2	5.5
16.Oct.02	6.5	6.3	6.6	6.6	6.5	6.6	6.3	4.7
23.Oct.02	6.8	6.3	7.2	6.7	6.5	6.5	6.2	5.0
30.Oct.02	7.2	6.8	7.5	7.2	6.9	6.9	6.5	5.0
06.Nov.02	6.8	6.3	6.8	6.6	6.6	6.5	6.4	4.6
21.Nov.02	6.6	6.1	6.4	6.4	6.4	6.3	6.1	4.6
27.Nov.02	6.8	6.6	6.7	6.4	6.4	6.6	6.2	4.5

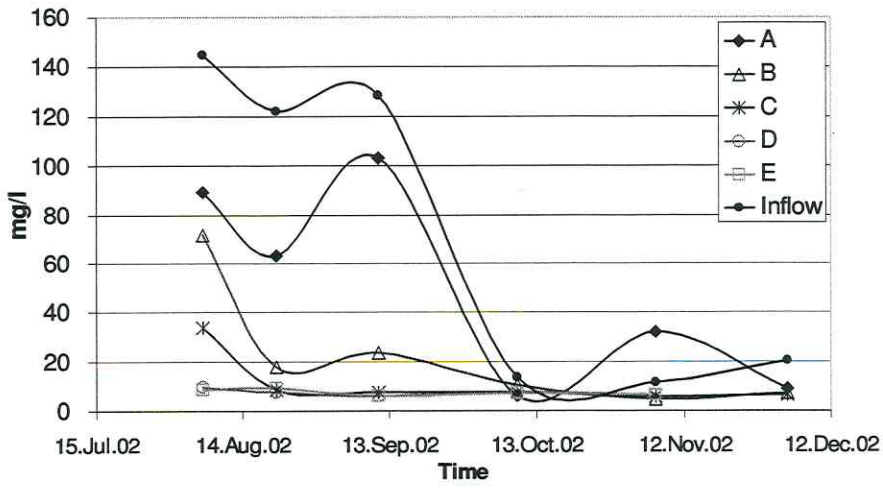

Figure B-1 DOC of the water phase in different sampling points of the two step wetland system

➢ **Sulfate reducing bacteria (SRB) medium**

The medium for growing of sulfate reducing bacteria (SRB) in the most probable number (MPN) method were prepared with the following three solutions (Hard and Babel, 1995; Hard et al., 1996).

Solution 1 :	
0.5 g $FeSO_4$	
Distilled water	10 ml
1M H_2SO_4	1 ml
Purge with N_2	

Solution 2 :	
Reducing solution	
Distilled water	10 ml
Sodium thioglycolate	0.1 g
Ascorbic acid	0.1 g
Sodium dithionite	20.0 mg
Purge with N_2	

Solution 3 :	
Distilled water	1 L
Sodium lactate (50%)	5.5 ml
$CaSO_4$	1.0 g
NH_4Cl	1.0 g
KH_2PO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	2.0 g
Yeast extract	1.0 g

Preparation

1. Autoclave the ingredient of Solution 3 (except lactate), then put lactate in it.
2. Prepare solution 2 under N_2 atmosphere (Sterile filtration)
3. Prepare solution 1 under the atmosphere of N_2 plus 1N of H_2SO_4
4. Mix all three solutions. The mixed medium solution must have a green color of Fe(II) (Hard, B.C. and Babel, W. 1995).

➢ **Primers used for microbial community analysis by PCR-SSCP**

Table B-2 Primers that have been used for microbial community analysis by PCR-SSCP (Tebbe et al., 2001)

Primer	**Primer sequence (5'-3')**	**Targeted microorganisms**	**Target regions of SSU rRNA gene**	**Annealing temperature (°C)**
Com1	CAG CAG CCG CGG TAA TAC	Bacteria	V4 and V5 (~407 bp)	50
Com2	CCG TCA ATT CCT TTG AGT TT	Bacteria		

Appendix C Constructed wetlands for treatment of acid mine drainage in the file test

Table C-1 Chemical characteristic of acid mine wastewater used in the AMD experiments

Element	Concentration [mg/l]	Element	Concentration [mg/l]	Element	Concentration [mg/l]
Ag	<0.0001	Hg	<0.0001	Ru	<0.0001
Al	90	Ho	0.01	Sb	0.0001
As	0.003	I	0.004	Sc	0.025
Au	<0.0001	In	<0.0001	Se	0.01
B	0.3	Ir	<0.0001	Sm	0.06
Ba	0.005	K	3	Sn	0.0001
Be	0.03	La	0.2	Sr	1.3
Bi	<0.0001	Li	0.7	Ta	<0.0001
Br	0.9	Lu	0.003	Tb	0.01
Ca	390	Mg	120	Te	<0.0001
Cd	0.004	Mn	9	Th	0.004
Ce	0.5	Mo	<0.0001	Ti	0.04
Co	0.6	Na	~450	Tl	<0.0001
Cr	0.04	Nb	<0.0001	Tm	0.004
Cs	0.0006	Nd	0.25	U	0.03
Cu	0.15	Ni	1.3	V	0.006
Dy	0.05	Os	<0.0001	W	0.0001
Er	0.027	Pb	0.003	Y	0.3
Eu	0.014	Pd	0.0002	Yb	0.02
Fe	150	Pr	0.06	Zn	1.8
Ga	0.0002	Pt	<0.0001	Zr	0.003
Ge	0.0007	Rb	0.03		
Gd	0.075	Re	0.0001		
Hf	0.0003	Rh	<0.0001		

Table C-2 Soil texture classification of 1500 g soil material applied in the AMD experiments by mean of sieve analysis.

Soil material			
Classification	Soil particle size (mm)	Weight (g)	%
Sand	2.0-0.06	1427	95
Silt	0.06-0.002	58	4
Clay	<0.002	15	1

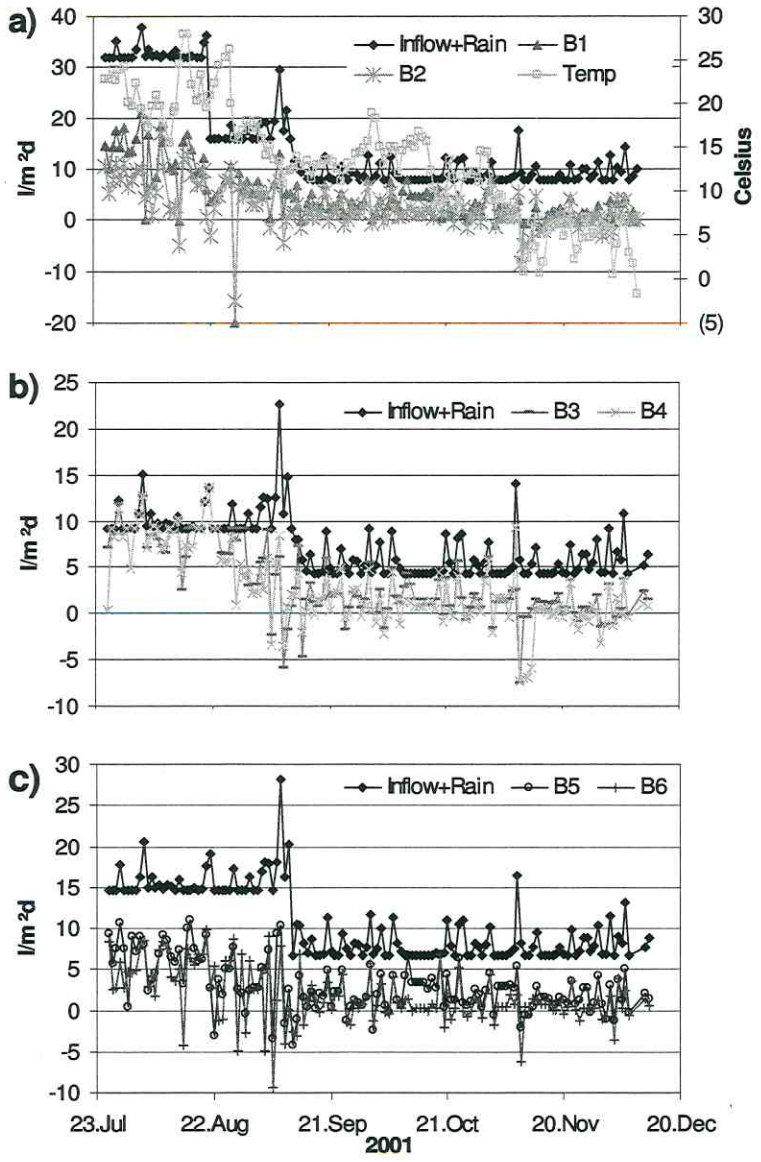

Figure C-1 Water inflow, temperature and evapotranspiration rate in experimental wetlands (2001)
a): planted (B1) and unplanted (B2) Hydroponic ponds
b): planted (B3) and unplanted (B4) Free Surface Wetlands (FSW)
c): planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)

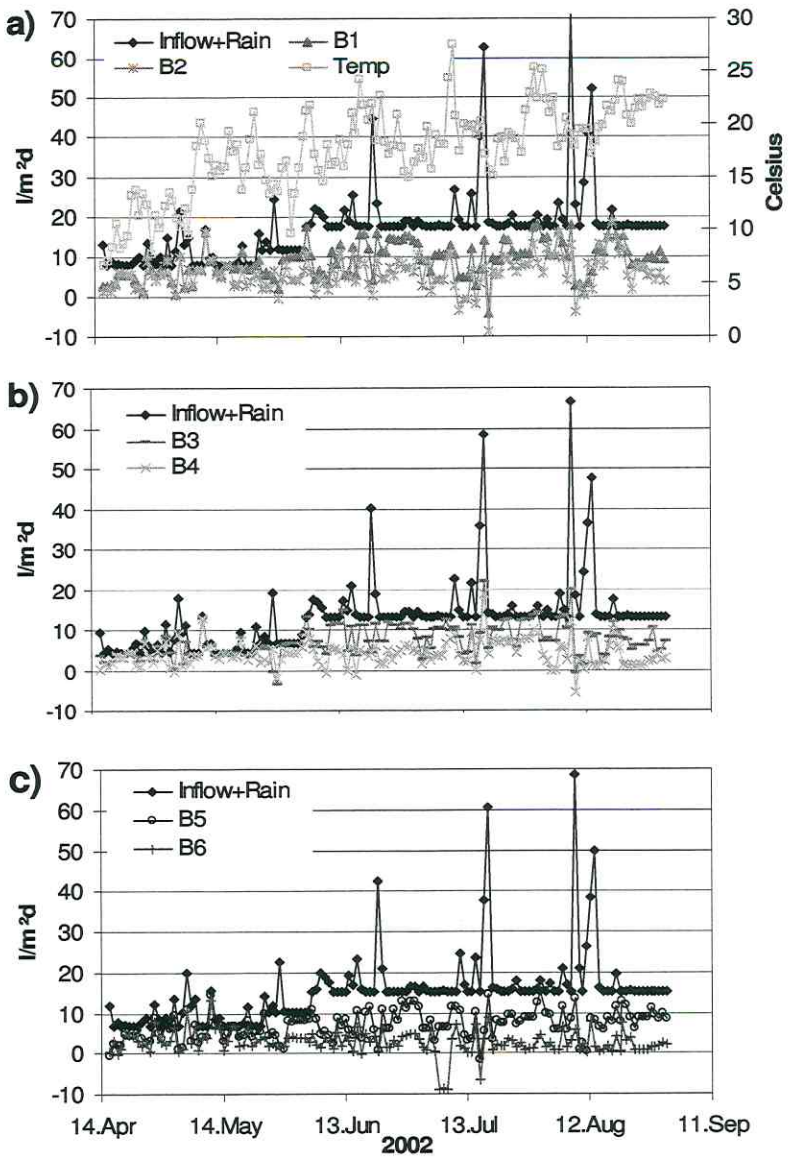

Figure C-2 Water inflow, temperature and evapotranspiration rate in experimental wetlands (2002)
a): planted (B1) and unplanted (B2) Hydroponic ponds
b): planted (B3) and unplanted (B4) Free Surface Wetlands (FSW)
c): planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)

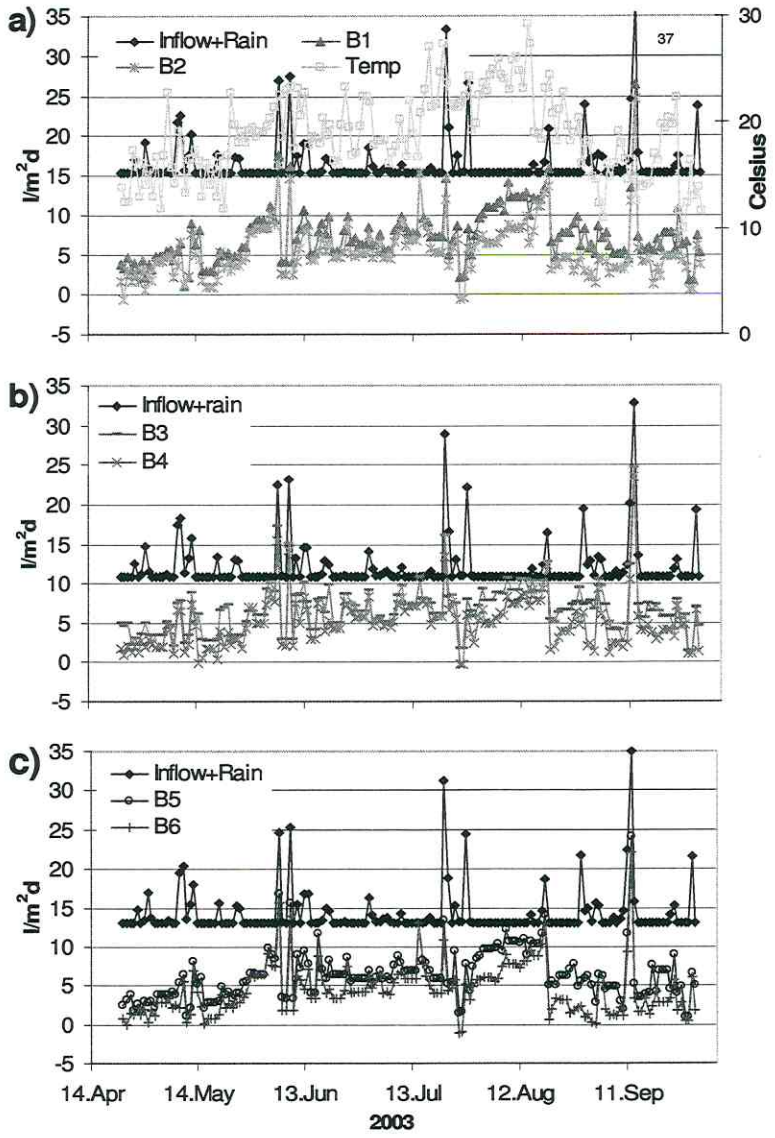

Figure C-3 Water inflow, temperature and evapotranspiration rate in experimental wetlands (2003)
a): planted (B1) and unplanted (B2) Hydroponic ponds
b): planted (B3) and unplanted (B4) Free Surface Wetlands (FSW)
c): planted (B5) and unplanted (B6) Subsurface Wetlands (SSW)