

**Effects of helophyte species and system types in treatment of
artificial acid mine drainage under carbon-deficient and
sulphate-reducing conditions in laboratory-scale horizontal flow
constructed wetlands**

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List of Abbreviations and Acronyms

AMD	Acid Mine Drainage
BOD	Biochemical Oxygen Demand
CWs	Constructed Wetlands
DO	Dissolved Oxygen
DRS	Dissimilatory Sulphate Reduction
Eh	Electrical Potential/Redox Potential
EvT	Evapo-Transpiration
FPRM	Floating Plant Root Mat
HHRMF	Horizontal Hydroponic Root Mat Filter
HPLC	High-Performance Liquid Chromatography
HSSF	Horizontal Subsurface Flow
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectrometer
ORP	Oxidation-Reduction Potential
PRBs	Permeable Reactive Barriers
PRMF	Plant Root Mat Filter
ROS	Reactive Oxygen Species
SF	Surface Flow
SRB	Sulphate Reducing Bacteria
SSF	Subsurface Flow
TMS	Trace Mineral Solution
Tot. Fe	Total Iron
TS	Total Sulphur
TSS	Total Suspended Solids
TSS	Trace Salt Solution
VSSF	Vertical Subsurface Flow

Summary

Surface water has been used by mankind as the main source of water for domestic agriculture and industrial use in numerous countries around the world. However, water quality can be affected by pollution due to mining activities generating acid mine drainage which influences the natural environment, human health and aquatic ecosystem. The major pollutants in acid mine drainage are high amount of sulphate and dissolved heavy/toxic metals. Constructed wetlands are promising in situ water treatment methods which helps in the remediation process by stimulating microbial growth within the rhizospheric zone of the plants. The sulphate in constructed wetlands are removed by dissimilatory sulphate reduction in strict anaerobic conditions which in turn helps in the removal of metals as metal sulphides.

The objective of this work was to improve the basic knowledge about acid mine drainage treatment (sulphate transformation processes which in turn leads to metal removal process) in constructed wetlands. Therefore, experiments for the investigation of the sulphate and heavy metal removal in lab based/model constructed wetlands have been performed. Accordingly, the main aim of this research is to investigate sulphate and metal removal pathways in constructed wetlands treating acid mine drainage. For this, several approaches were applied: (a) various kinds of model wetlands were used to investigate different removal processes (b) intensification of treatment processes with stimulation of the dissimilatory sulfate reduction in an autotrophic way by the use of hydrogen gas.

The application of hydrogen gas (as external electron donor) combined with common physico-chemical and biological parameters helped us to intensify the sulphate reduction process and removal of metals in constructed wetlands. This helped us to understand comprehensively the importance of an electron donor in the treatment process.

In the lab-scale wetland systems, the performances of heavy metal removal from artificial acid mine drainage varied with the type of the constructed wetland. The horizontal subsurface flow constructed wetland (HSSF CW) and horizontal hydroponic root mat filter constructed wetland (HHRMF CW) removed sulphate and metals better than horizontal surface flow constructed wetlands (HSF CW) and unplanted horizontal subsurface flow constructed wetland (Unplanted HSSF CW).

The combination of gravel bed and plants (e.g. *Juncus effuses*/*Phragmites australis*) resulted in the highest removal rate in HSSF CW. The heavy metals mostly precipitated to the bottom of the wetland as metal sulphides.

Though under carbon limited and anoxic conditions, a high mean removal efficiency of sulphate and heavy metals was observed in the experiments with HSSF CW so it is vital to take the importance of the/an electron donor into consideration. With hydrogen gas as electron donor the performance efficiency was increased by almost 12% for sulphate removal and almost 6% increase in aluminium removal efficiency.

In conclusion, this research exhibits that the combination of physico-chemical measurements along with hydrogen gas as an electron donor is an efficient tool for investigation of sulphate and metal removal processes in constructed wetlands. Such information is not only beneficial for understanding the processes taking place in these wastewater treatment facilities but also necessary for future technological improvement of constructed wetlands.

Keywords: Acid mine drainage, constructed wetlands, dissimilatory sulphate reduction, horizontal subsurface flow constructed wetland, horizontal surface flow constructed wetlands, horizontal hydroponic root mat filter constructed wetland, sulphate, sulphide.

Zusammenfassung

In zahlreichen Ländern der Welt wird Oberflächenwasser von der Menschheit als Hauptwasserquelle für die heimische Landwirtschaft und industrielle Nutzung verwendet. Die Wasserqualität kann jedoch durch Verunreinigungen, verursacht durch Bergbauaktivitäten, beeinträchtigt werden, die die natürliche Umwelt, die menschliche Gesundheit und das aquatische Ökosystem beeinflussen. Die Hauptschadstoffe in sauren Grubenwässern sind ein hoher Anteil an Sulfaten und gelösten Schwermetallen/toxischen Metallen. Pflanzenkläranlagen bieten vielversprechende in situ Wasseraufbereitungsmethoden, die beim Reinigungsprozess helfen, indem sie das mikrobielle Wachstum in der Rhizosphäre der Pflanzen stimulieren. Das Sulfat in Pflanzenkläranlagen wird durch dissimilierende Sulfatreduktion unter strengen anaeroben Bedingungen entfernt, was bei der Entfernung von Metallen wie Metallsulfiden hilft.

Das Ziel dieser Arbeit bestand darin, das Grundwissen über die Aufbereitung von sauren Grubenwässern (Sulfatumwandlung und Metallabbauprozesse) in Pflanzenkläranlagen zu verbessern.

Dazu wurden Versuche zur Untersuchung von Sulfat- und Schwermetallabbauprozessen an labor- und modellbasierten Pflanzenkläranlagen durchgeführt. Demzufolge besteht das Hauptziel dieser Arbeit in der Untersuchung von Sulfat- und Metallabbauprozessen in Pflanzenkläranlagen zur Aufbereitung der sauren Grubenwässer.

Dazu wurden mehrere Ansätze angewendet:

(a) die verschiedenen Modelltypen von Pflanzenkläranlagen wurden verglichen, um unterschiedliche Abbauprozesse zu erforschen (b) die Intensivierung der Verarbeitungsprozesse mit Stimulation der dissimilatorischen Sulfatreduktion auf autotrophe Weise durch die Verwendung von Wasserstoffgas.

Die Anwendung von Wasserstoffgas (als externer Elektronendonator) in Kombination mit gemeinsamen physikalisch-chemischen und biologischen Parametern hat dazu beigetragen, den Prozess der Sulfatreduktion und der Entfernung von Metallen in Pflanzenkläranlagen zu verbessern. Dies hat uns ermöglicht, die Bedeutung des Elektronendonators für den Aufbereitungsprozess umfassend zu verstehen.

In den Pflanzenkläranlagen im Labormaßstab variierte der Anteil der entfernten Schwermetalle aus künstlichen sauren Grubenwässern abhängig vom Konstruktionstyp der Pflanzenkläranlagen. Die horizontal unterirdisch durchströmte Pflanzenkläranlage (horizontal subsurface flow constructed wetland (HSSF CW)) und der horizontal hydroponische Grundmetallfilter der Pflanzenkläranlage (horizontal hydroponic root mat filter constructed wetland (HHRMF CW)) entfernten Sulfate und Metalle besser als die horizontale oberirdisch fließende Pflanzenkläranlage (HSF CW) und die nicht bepflanzte horizontale unterirdisch fließende Pflanzenkläranlage (unbepflanzte HSSF CW). Die Kombination aus Kiesbett und Pflanzen (*Phragmites australis*) führte zu der höchsten Reinigungsleistung in der horizontal unterirdisch durchströmten Pflanzenkläranlage (HSSF CW). Die Metalle wurden größtenteils als Metallsulfide auf dem Grund der Pflanzenkläranlagen abgelagert.

Obwohl unter kohlenstoffbegrenzten und anoxischen Bedingungen eine durchschnittlich hohe Effizienz bei der Entfernung von Sulfat und Schwermetallen in den Experimenten mit HSSF CW beobachtet wurde, ist es wichtig, die Bedeutung des Elektronendonators zu berücksichtigen. Mit Wasserstoffgas als Elektronendonator wurde die Leistungseffizienz bei der Sulfatentfernung um fast 12% und bei der Aluminiumentfernung um fast 6% gesteigert.

Abschließend zeigt diese Arbeit, dass die Kombination von physikalisch-chemischen Messungen zusammen mit Wasserstoffgas als Elektronendonator ein effizientes Werkzeug zur Untersuchung von Sulfat- und Metall abtragenden Verfahren in Pflanzenkläranlagen ist. Diese Informationen sind nicht nur für das Verständnis der Prozesse in diesen Kläranlagen von Vorteil, sondern auch für die zukünftige technologische Verbesserung von Pflanzenkläranlagen insgesamt.

Schlüsselworte: saure Grubenwässer, Pflanzenkläranlage, dissimilierte Sulfatreduktion, Pflanzenkläranlage mit horizontalem unterirdischen Fluss (horizontal subsurface flow constructed wetland (HSSF CW), Pflanzenkläranlage mit horizontalem oberflächlichen Fluss (horizontal surface flow constructed wetlands (HSF CW), Pflanzenkläranlage mit hydroponischem Wurzelmattefilter (horizontal hydroponic root mat filter constructed wetland (HHRMF CW), Sulfate, Sulfide.

1. Introduction

Acid mine drainage (AMD) is perhaps the most detrimental result of hard rock mining on water quality throughout the world. AMD is the result of a chemical reaction that takes place when mineral deposits containing sulfides are exposed to oxygen and water during the mining process (Gray, 2005).

Acid mine drainage (AMD), characterized by low pH and high concentrations of sulfate and heavy metals (Fig. 1.1 and 1.2), is an important and widespread environmental problem related to the mining industry. (Neculita et al., 2006). Water infiltrating through the metal sulphide minerals, effluents of mineral processing plants and seepage from tailing dams becomes acidic and this acidic nature of the solution allows the metals to be transported in their most soluble form (Sheoran and Sheoran , 2005).

Mine wastes are the largest volume of materials handled in the world (ICOLD, 1996). The generation of acidic drainage and the release of water containing high concentrations of dissolved metals from these wastes is an environmental problem of international scale. Acidic drainage is caused by the oxidation of sulfide minerals exposed to atmospheric oxygen. Although acid drainage is commonly associated with the extraction and processing of sulfide-bearing metalliferous ore deposits and sulfide-rich coal, acidic drainage can occur wherever sulfide minerals are excavated and exposed to atmospheric oxygen. Engineering projects, including road construction, airport development, and foundation excavation are examples of civil projects that have resulted in the generation of acidic drainage (Bl owes et al., 2003).



Figure 1. 1- Pollution of ground and surface water from acid mine drainage and contaminated dust and soil from mine dumps (Environment News South Africa, 2016)

Chapter 1

When the mining process exposes the sulfides to water and air, together they react to form sulfuric acid. This acid can dissolve other harmful metals and metalloids (like arsenic) from the surrounding rock.

Acid mine drainage can be released anywhere on the mine where sulfides are exposed to air and water including waste rock piles, tailings, open pits, underground tunnels, and leach pads. (Johnson and Hallberg, 2005).

Broadly there are two main sources of acid mine drainage namely;

(1) Primary sources (2) Secondary sources (Akcil and Koldas, 2006)

1) Primary sources

- Mine rock dumps
- Tailings impoundment
- Underground and open pit mine workings
- Pumped/nature discharged underground water
- Diffuse seeps from replaced overburden in rehabilitated areas
- Construction rock used in roads, dams, etc.



Figure 1. 2- Ohio valley mushroom farm site in North Lima, Ohio contaminated with acid mine drainage (AMD) resulting from surface mining conducted in the 1980's and an AUM from the late 1800's (Wikimedia Commons, 2014)

2) Secondary sources

- Treatment sludge pounds
- Rock cuts
- Concentrated load-out

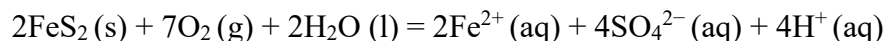
- Stockpiles
- Concentrate spills along roads
- Emergency ponds

In many environmental settings, the consequence of AMD is considered moderate to severe, mostly independent of pH and acidity. The primary factors that determine the rate of acid generation are (Ata Akcil and Soner Koldas, 2006):

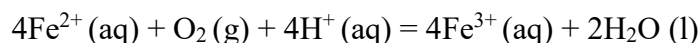
- pH
- Temperature;
- Oxygen content of the gas phase, if saturation is less than 100%;
- Oxygen concentration in the water phase;
- Degree of saturation with water;
- Chemical activity of Fe_3C ;
- Surface area of exposed metal sulfide;
- Chemical activation energy required to initiate acid generation; and
- Bacterial activity.

The chemistry of oxidation of pyrites, the production of ferrous ions and subsequently ferric ions, is very complex, and this complexity has considerably inhibited the design of effective treatment options (Blodau, 2006).

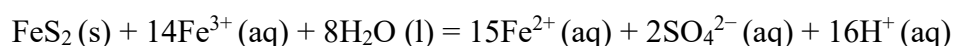
Although a host of chemical processes contribute to acid mine drainage, pyrite oxidation is by far the greatest contributor. A general equation for this process is:



The oxidation of the sulfide to sulfate solubilizes the ferrous iron (iron (II)), which is subsequently oxidized to ferric iron (iron (III)):



Either of these reactions can occur spontaneously or can be catalyzed by microorganisms that derive energy from the oxidation reaction. The ferric cations produced can also oxidize additional pyrite and reduce into ferrous ions:



The net effect of these reactions is to release H^+ , which lowers the pH and maintains the solubility of the ferric ion (Blodau, 2006).

As soon as AMD begins, metals are released into the surrounding area which can have a negative effect on all surrounding biology. Metals are released into the environment and begin to precipitate in water. Acid mine drainage creates acidic metalliferous conditions in water and is responsible for physical, chemical, and biological degradation of stream habitat. The three most common heavy metals resulting from mine drainage are iron, manganese, and aluminium. In addition to dissolved metals, precipitated iron (Fe) or aluminium hydroxide may form in surrounding waterways. As ferric and aluminium hydroxides form, the availability of oxygen decreases. This effect is detrimental to aquatic life. Iron hydroxides and oxyhydroxides may

physically coat the surface of stream sediments and streambeds destroying habitat, diminishing availability of clean gravels used for spawning and reducing available food, such as benthic macro invertebrates. Also, the scouring of iron flocculent may increase turbidity in the water and increase suspended solids, which can hinder the feeding habits of fish (Chestnut et al., 2011).

Of the three major metals present in mine drainage (Fe, Mn, and Al) aluminium has the most severe negative effect on aquatic life. The EPA has recommended a Secondary Maximum Contaminant Level (SMCL) of 0.05–0.2 mg/L for aluminium in drinking water (ATSDR, 2008). The presence of aluminium ions coupled with low pH is highly detrimental to plants as well as aquatic life form (Earle & Callaghan, 1998). The interaction with hydrogen ions decreases sodium uptake and increases sodium loss in the blood and in tissue which can increase calcium levels. High calcium concentrations can potentially reduce mortality. Streams most susceptible to degradation from elevated aluminium, however, normally have low concentrations of calcium (Chestnut et al., 2011). Research by the Pennsylvania Department of Environmental Protection shows stream investigations have “indicated that a combination of pH less than 5.5 and dissolved aluminium concentration greater than 0.5 mg/L in local waters will generally eliminate all fish and many macro invertebrates” (Earle & Callaghan, 1998).

Manganese can be present in a many different forms and compounds. The main problem with manganese is the difficulty by which it is removed from discharge. The pH must be raised to a level of 10.0 and/or higher before manganese will precipitate. The high pH requirement for precipitation allows manganese to travel long distances downstream from its original source (Chestnut et al., 2011). Fish and invertebrates are more sensitive to Mn toxicity at low pH, especially if water hardness is low. Under high-pH conditions algae are the most sensitive, regardless of water hardness conditions (Peters et al., 2011). The Food and Nutrition Board of the Institute of Medicine (IOM, 2002, WHO, 2011) set adequate intake levels for manganese at 2.3 mg/day for men and 1.8 mg/day for women. The IOM also set a tolerable upper intake level at 11 mg/day for adults, based on a recent review (Greger, 1999; IOM, 2002, WHO, 2011)

In human excess copper is known to cause cancer, neurological disorders and chronic disease like diabetes, cardiovascular disease and atherosclerosis (Jamova and Valko, 2011). The daily demand for Cu is 1.5 to 3 mg per day in human being and any amount excess in drinking water is harmful to health (Minnesota Department of Health, 2005). Copper (Cu) is one of the micronutrients needed in very small quantities by plants. The normal range in the growing medium is 0.00005 -0.0005 µg/L (PRO-MIX, Premier Tech Horticulture, 2018). Excess dissolved copper (Cu) in an aquatic system can have a dramatic effect on normal species function by disrupting sodium (Na) potassium (K) exchanges during osmoregulation (US Environmental Protection Agency, 2007). Acute and chronic Cu toxicity is a function of not only dissolved copper concentration, but also a range of additional physiochemical conditions within surface waters including, but not limited to, temperature, alkalinity and hardness. Generally speaking, as the hardness increases, the toxicity of Cu decreases. With a measured hardness of 50 mg/L, acute Cu toxicity values for many fresh water genera fall between 17 µg/L and 10 mg/L while chronic values for several species range from 3.9 µg/L to 60µg/L (Burton & Pitt, 2001).

In humans, cadmium damages kidneys, liver, pancreas and lungs. Cadmium-induced testicular damage and testicular necrosis have been documented by many reporters (Dalton et al., 2005). Cadmium is a potent human carcinogen causing preferentially prostate, lung and gastro-

intestinal (kidney and pancreas) cancers (Jamova and Valko, 2011). Cadmium (Cd) is perhaps the most toxic metal identified by the Colorado Department of Public Health and Environment (CDPHE) as it relates to the 303(D) listing for the main stretch of Clear Creek. The estimated lethal oral dose for humans is 350–3500 mg of cadmium; a dose of 3mg of cadmium has no acute effects on adults (Krajnc et al., 1987, WHO, 2011). Studies of Cd bioaccumulation have shown that the gills are a primary target organ in fish and long term exposure can lead to Cd accumulation within, and subsequent diminished function of, the liver, kidneys and intestines (Rashed, 1999). Hematological effects of Cd exposure include diminished red blood cell count and reduced blood and tissue metabolite levels (Gill & Pant, 1985). The toxic effects of As on living organisms are well documented (Niragu, 1994), with each valence state having distinct toxic properties. Much of the toxicity of As(III) is associated with the ability of this trivalent oxyanion to form bonds with functional groups of proteins. Chronical effects include bronchitis, myocardial infarction, arterial thickening, peripheral neuropathy, hyperkeratosis, hyper-pigmentation, the so-called “black foot” disease (necrosis, mainly on palms and soles, first identified in Taiwan), skin (Col et al., 1999; (Tsuruta et al., 1998), lung, bladder (Hopenhayn-Rich et al., 1996a), liver and kidney cancer, as well as teratogenic effects (inorganic As can cross the placenta), mutagenic changes, and genotoxicity (Carson et al., 1986; Florea et al., 2004; Mandal and Suzuki, 2002). In fishes this element is able to enter cellular metabolism and in some cases it enhances the production of free radicals. The latter can modify virtually all cellular constituents, including membranes. The chain may be ended by cell death via necrosis or apoptosis and the discrimination between these both ways depends on many circumstances (Lushchak VI, 2011).

Both lead (Pb) and zinc (Zn) also have the capacity to alter both species morphology and physiology. Chronic concentrations of Zn and Pb can lead to increased opercular rates coinciding with decreased oxygen consumption while acute levels can lead to severe gill damage as well as severely diminished liver and kidney physiochemical function (Skidmore, 1964). It also result in neurological disorders, cognitive impairments, hypertension and other disorders (Patrick, 2006a). Lead concentrations in drinking water should be below 0.015 mg/l (National Center for Environmental Health, Division of Emergency and Environmental Health Services, 2016) and toxicity levels have been seen to occur at ingestion of greater than 225 mg of zinc. The daily requirement of zinc for adult humans is 15–22 mg/day (WHO, 1996).

In human excess cellular iron that is not used by other ferroproteins accumulates in ferritin, however its iron-binding capacity is limited (Ganz, 2003). Iron overload is a condition typical for patients suffering from hemochromatosis that causes widespread organ damage (Jamova and Valko, 2011). As a precaution against storage of excessive iron in the body, JECFA established a provisional maximum tolerable daily intake (PMTDI) in 1983 of 0.8 mg/kg of body weight, although iron concentrations of 1–3 mg/l can be acceptable for people drinking anaerobic well-water (WHO, 1996).

As AMD is recognized as one of the more serious environmental problems in the mining industry, its causes, prediction and treatment have become the focus of a number of research initiatives commissioned by governments, the mining industry, universities and research establishments, with additional inputs from the general public and environmental groups (Akcil and Koldas, 2006).

Preventing the formation or the migration of AMD from its source is generally considered to be the preferable option, although this is not feasible in many locations, and in such cases, it is necessary to collect, treat, and discharge mine water. There are various options available for

remediating AMD, which may be divided into those that use either chemical or biological mechanisms to neutralize AMD (Fig.1.3) and remove metals from solution. Both abiotic and biological systems include those that are classed as “active” (i.e., require continuous inputs of resources to sustain the process) or “passive” (i.e., require relatively little resource input once in operation) (Johnson and Hallberg, 2005).

A more useful subdivision is between those remediation technologies that rely on biological activities and those that do not. Within these major groups, there are processes that may be described as either “active” or “passive” (Johnson and Hallberg, 2005).

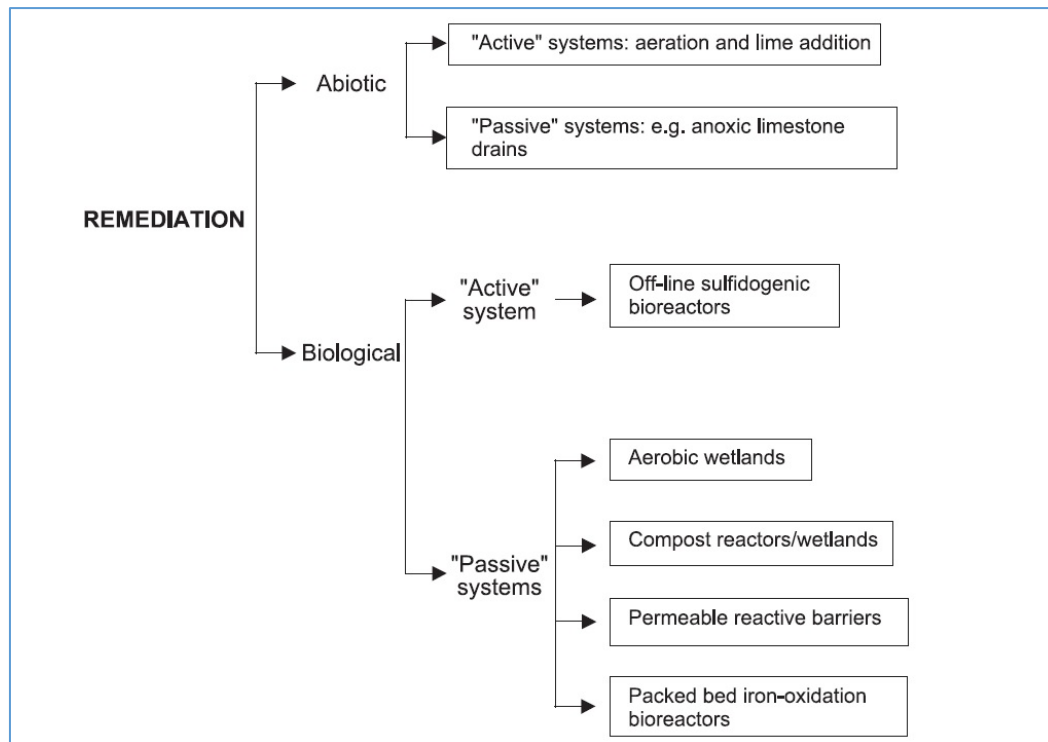


Figure 1. 3- Biological and abiotic strategies for remediating acid mine drainage waters (Johnson and Hallberg, 2005).

1.1. Abiotic remediation strategies

1.1.1. Active technologies

Most common method used to mitigate acidic effluents is an active treatment process involving addition of a chemical-neutralizing agent (Coulton et al., 2003b). Addition of an alkaline material to AMD will raise its pH which in turn accelerates the rate of chemical oxidation of ferrous iron (for which active aeration, or addition of a chemical oxidizing agent such as hydrogen peroxide, is also necessary), and cause many of the metals present in solution to precipitate as hydroxides and carbonates. Although active chemical treatment provides effective remediation of AMD, it has the disadvantages of high operating costs and problems with disposal of the bulky sludge that is produced (Johnson and Hallberg, 2005).

1.1.2. Passive technology

An alternative approach for addition of alkalinity to AMD is the use of anoxic limestone drains (Kleinmann et al., 1998). The objective with these systems is to add alkali to AMD while maintaining the iron in its reduced form to avoid the oxidation of ferrous iron and precipitation of ferric hydroxide on the limestone (armoring), which in turn severely reduces the effectiveness of the neutralizing agent. Although anoxic limestone drains produce alkalinity at a lower cost than constructed compost wetlands, they are not suitable for treating all AMD waters. In situations where the AMD contains significant concentrations of ferric iron or aluminium, the short-term performance of anoxic limestone drains may be good, but the buildup of hydroxide precipitates gradually decreases drain permeability, which may cause failure of the drain within some months of construction (Johnson and Hallberg, 2005).

1.2. Biological remediation strategies

1.2.1. Active biological systems: sulfidogenic bioreactors

Off-line sulfidogenic bioreactors represent a radically different approach for remediating AMD (Johnson, 2000; Boonstra et al., 1999). Sulfidogenic bioreactors utilize the biogenic production of hydrogen sulfide to generate alkalinity and to remove metals as insoluble sulfides, which is one of the processes that occurs in compost bioreactors and permeable reactive barriers (PRBs). However, off-line sulfidogenic bioreactors are constructed and operated to optimize production of hydrogen sulfide (Johnson and Hallberg, 2005).

1.2.2. Passive biological

1.2.2.1. *Aerobic wetlands*

Aerobic wetlands are generally constructed to treat mine waters that are net alkaline. This is because the main remediative reaction that occurs within them is the oxidation of ferrous iron and subsequent hydrolysis of the ferric iron produced, which is a net acid generating reaction. In order to maintain oxidizing conditions, aerobic wetlands are relatively shallow systems that operate by surface flow. Macrophytes are planted for aesthetic reasons to regulate water flow (e.g., to prevent channeling) and to filter and stabilize the accumulating ferric precipitates (ochre). They also provide additional surface area for precipitation of solid phase ferric iron compounds and minerals. In addition, by causing oxygen flow from aerial parts to their root systems, some aquatic plants may accelerate the rate of ferrous iron oxidation (Johnson and Hallberg, 2005).

1.2.2.2. *Anaerobic wetlands/compost bioreactors*

In contrast to aerobic wetlands, the key reactions that occur in compost bioreactors used to mitigate AMD are anaerobic. The microbially catalyzed reactions that occur in compost bioreactors generate net alkalinity and biogenic sulfide and therefore, these systems may be used to treat mine waters that are net acidic and metal-rich, such as AMD from abandoned metal mines. Again, in contrast to aerobic wetlands, the reductive reactions that occur within compost wetlands are driven by electron donors that derive from the organic matrix of the compost itself oxidation (Johnson and Hallberg, 2005).

1.2.2.3. *Composite aerobic and anaerobic wetlands*

Passive bioremediation systems that utilize a combination of aerobic and anaerobic wetlands have been used for full-scale treatment of AMD (Johnson and Hallberg, 2005).

1.2.2.4. *Permeable reactive barriers*

Permeable reactive barriers (PRBs) are being used increasingly to treat a wide range of polluted groundwater. Those that have been installed to bio remediate AMD operate on the same basic principles as compost Bioreactors (Benner et al., 1997). Reductive microbiological processes within the PRB generate alkalinity (which is further enhanced by dissolution of limestone and/or other basic minerals) and remove metals as sulfides, hydroxides, and carbonates (Johnson and Hallberg, 2005).

1.2.2.5. *Iron-oxidizing bioreactors*

The oxidation of ferrous iron to ferric in acidic ($\text{pH} < 4$) mine waters is greatly accelerated by iron oxidizing prokaryotes (bacteria and archaea), many of which are autotrophic (i.e., like green plants, they fix inorganic carbon and have minimum nutritional requirements) (Johnson and Hallberg, 2005).

1.3. Wetlands (Natural and Constructed Wetlands)

Due to very limited financial budgets simple methods/systems for wastewater treatment like ponds or wetlands are often preferred (Kadlec and Knight, 1996a; Al-Malack et al., 1998; Mbuligwe, 2004; Davidson et al., 2005; McCardell et al., 2005). The wastewater treatment in constructed wetlands is a relatively new emerging technology with some advantages: no energy for aeration is needed – roots of special plants (helophytes) allow the transport of oxygen to the rooted soil (Armstrong, 1990a). Numerous studies have shown the effectiveness of constructed wetlands in terms of sulphur, carbon and nitrogen removal (Kadlec et al., 2000b; Vymazal, 2002; Stottmeister et al., 2003; Sousa et al., 2003; Mashauri et al., 2003; García et al., 2004; Kaseva, 2004, Wiessner et al., 2005a). Sulphate in wetlands initiates Eh and pH changes, C-transformation and, indirectly the mobilization of nutrients (Feng and Hsieh, 1998; Lamers et al., 1998) – all these processes incorporated with heavy metals and influences on S, C, N-removal under dynamic gradient redox conditions in constructed wetlands are not yet well understood.

Wetlands are land areas that are wet during a part or all of the year because of their location in the landscape, as they are frequently transitional between uplands (terrestrial systems) and continuously flooded (aquatic) systems (Kadlec & Wallace, 2008). Constructed wetlands (CWs) are the wastewater treatment technology or system designed to employ ecological processes found in natural wetland ecosystems. According to Kadlec and Wallace (2008), modern CWs are man-made systems that have been constructed to emphasize specific characteristics of wetland ecosystems for improved treatment capacity. They are characterized by low capital costs, low operation and maintenance costs, and their perceived value for beautification and wildlife habitat improvement (Cole, 1998). CWs are widely used in wastewater and groundwater treatment due to their low energy requirements and easy operation (Garcia et al., 2010). Wetlands, both constructed and natural, are promising in-situ water treatment method thank to enhanced microbial growth within the plants' rhizosphere, which creates an effective contaminant degradation zone (Kadlec & Wallace, 2008). While

microorganisms play the primary role in pollutant elimination, plants enhance the microbial activity to remove pollutants (Stottmeister et al., 2003). Due to the mosaic of aerobic and anaerobic zones within the root zone of the plants, contaminants can be removed by a variety of processes, aerobic as well as anaerobic.

Constructed wetlands are an exciting application of technology that is very effective at improving water quality. While they don't solve all water quality problems, they hold much promise as a new type of water treatment system that combines low cost and high efficiency. Those attributes alone make them attractive systems, especially to small and medium-sized cities and many industries.

Constructed wetlands (CWs) are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and the associated microbial assemblages to assist in treating wastewaters (Kadlec and Knight, 1996). These systems can be used for secondary or tertiary treatment of wastewater from households and/or municipalities, a function they have in common with natural wetlands. Unlike natural wetlands, treatment in constructed wetlands is performed under more controlled environments, which allow for greater treatment efficiency and constancy of wetland functions across the entire bed (Vymazal et al., 1998). They are designed to take advantage of many of the same processes that occur in natural wetlands, but do so within a more controlled environment.

Although originally introduced as a municipal or domestic wastewater treatment method, CWs have been successfully used to treat a variety of contaminated waters (Brix, 1994a; Chen et al., 2006; Cooper, 2009). Some of the examples are –

1. Domestic wastewater: This process is good for single households or small dwellings but it has its own drawback as it sends oxidizing nitrogen to the ground water (Kadlec & Wallace, 2008).
2. Animal wastewater: CWs are used for treatment of waste water generated from farm and ranch operations. Here the treatment level may be categorized as mostly primary (Kadlec & Wallace, 2008).
3. Acid Mine Drainage Treatment: The performance of wetland treatment system depends upon the input water quality and the specific mechanism of metal removal processes. If the primary removal mechanism is anaerobic systems, it works efficiently as long as sulphate reducing bacteria reduce sulphate to sulphide thus precipitating metals as metal sulphides and generating alkalinity (Sheoran and Sheoran, 2005). Sulphate reduction would be more likely to offer long-term treatment (Hedin et al., 1994).
4. Industrial wastewater: Wetlands are very efficient in treating the industrial waste water which have very high concentration of biodegradable organic and nitrogen content (Kadlec & Wallace, 2008).
5. Leachate and remediation: Both subsurface flow wetland and surface flow wetland is used for the treatment of landfill leachate and it is one of the rapidly developing technologies (Mulamoottil et al., 1998).
6. Urban stormwater treatment: the use of constructed wetlands, usually with accompanying ponds, is now a best management practice for controlling the quality of runoff (Kadlec & Wallace, 2008).

7. Agricultural wastewater: Wastewaters from various feedlot operations are commonly treated with free water surface constructed wetlands with series of lagoons as pretreatment step (Kadlec et al., 2000). HF constructed wetlands are used where inflow concentrations are much lower as compared to raw wastewaters because of intensive pretreatment (Vymazal, 2009).

The pollutants are removed from the inflowing water by a combination of processes (chemical, physical and biological) within the wetland, such as sedimentation, precipitation, adsorption to soil particles, assimilation by plant tissue and microbial transformations. Heavy metals in a wetland system may be sorbed to wetland soil or sediment, or may be chelated or complexed with organic matter. Metals can precipitate out as sulphides and carbonates, or uptake by plants. Macrophytes can enhance pollutant removal within the system by either assimilating them directly or by providing an environment for surface microbial attachment to transform and uptake pollutants. The rhizosphere of aquatic plants is also a primary site for pollutant uptake and transformation as it is a zone of oxygen transfer between the plant and sediment which is a requisite for sediment microbial activity and pollutant oxidation (Brix, 1994a).

High productivity results from having high availability of light, nutrients, and water, and from the plant's morphological and physiological ability to take advantage of this environment. High levels of activity also occur at the microbial level resulting in the decomposition of organic matter and other substances. For these reasons, aquatic ecosystems (wetlands, in particular) have been considered as alternatives and/or supplements to a variety of water treatment and recycling processes (Bavor et al., 1995; Wood, 1995; Brix, 1994b; Cullen, 1989). Under anaerobic conditions, sulphate-reducing bacteria (SRB) oxidize simple organic compounds by utilizing sulphate as an electron acceptor and generate sulphide (S^{2-}) and alkalinity. This biogenically produced sulphide can react with dissolved metals to form metal sulphide precipitates since the solubility of most toxic metal sulphides are generally very low (Kim et al., 1999).

1.3.1. Some advantages of constructed wetlands are:

- i) Low cost of construction and maintenance when compared to the costs of treatment plants
- ii) Low requirements for energy
- iii) Flexibility
- iv) Nature-like technology
- v) High process stability (buffering effect) and
- vi) Optimal aesthetic appearance

1.3.2. The disadvantages include:

- i) Requirement for large amounts of land, depending on their use
- ii) Seasonal variability in their effectiveness
- iii) Temperature and fluctuations in flow affect their function and display inconsistent contaminant removal rate

- iv) Aging problem may contribute to a decrease in effectiveness.

1.3.3. Types of constructed wetlands

CWs have basic classification based on the type of macrophytic growth, further classification is usually based on the water flow regime (Vymazal, 2007). CWs can be designed in a variety of hydrologic modes. Constructed wetlands for wastewater treatment may be classified according to the life form of the dominating and emergent macrophytes into systems with free-floating, rooted emergent and submerged macrophytes (Kadlec, 1989; Brix and Schierup, 1989), but the design of the systems in terms of media as well as the flow regime varies (Fig.1.4). However, nowadays the two main types of CWs are distinguished as surface flow (SF) and subsurface flow (SSF) CWs (Kadlec & Wallace, 2008). SF CWs are vegetated systems with open water surface and typically have water depths of less than 0.4 m. In SSF CWs, no free water is visible because the water flows through a porous medium planted with emergent water plants (helophytes). SSF CWs are further subdivided into horizontal flow (HSSF) and vertical flow (VSSF) systems depending on the direction of water flow through the porous soil (usually sand or gravel). Moreover, hybrid systems which combine different types of CWs are also used (Vymazal, 2010). This chapter compares the different variants of CWs.

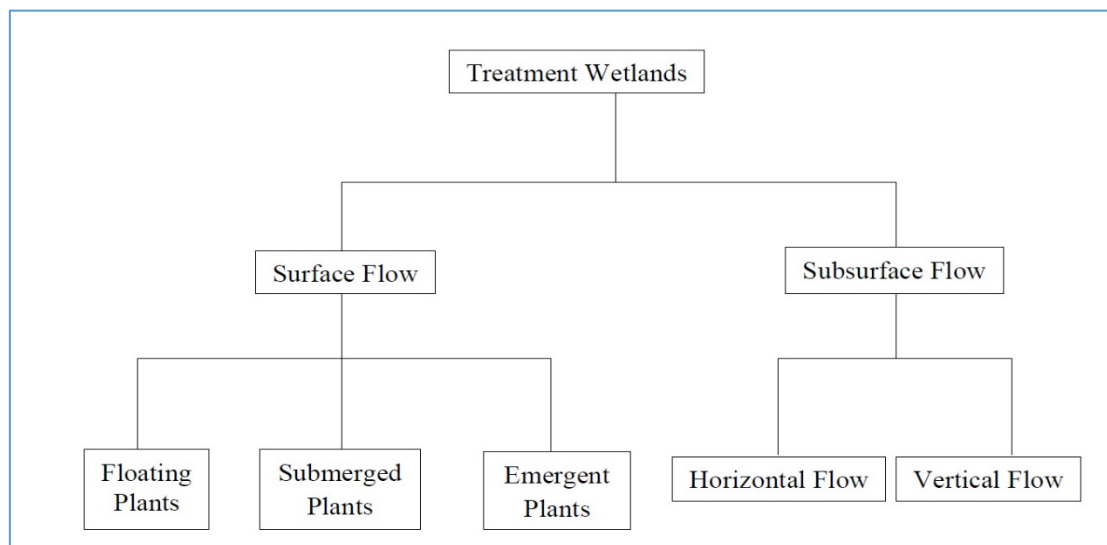


Figure 1. 4 - Classifications of constructed wetlands (Kadlec & Wallace, 2008)

1.3.3.1. *Surface flow constructed wetlands*

A typical SF CW (Fig. 1.5) with emergent macrophytes is a shallow sealed basin or sequence of basins, containing 20 - 30 cm of rooting soil, with a water depth of 20 - 40 cm. Dense emergent vegetation covers a significant fraction of the surface, usually more than 50 %. Besides planted macrophytes, naturally occurring species may be present (Kadlec 1995). SF CWs have advantage of being closely mimic natural wetlands. CWs with SF are frequently used in North America (Kadlec & Wallace, 2008) and Australia (Merz, 2000).

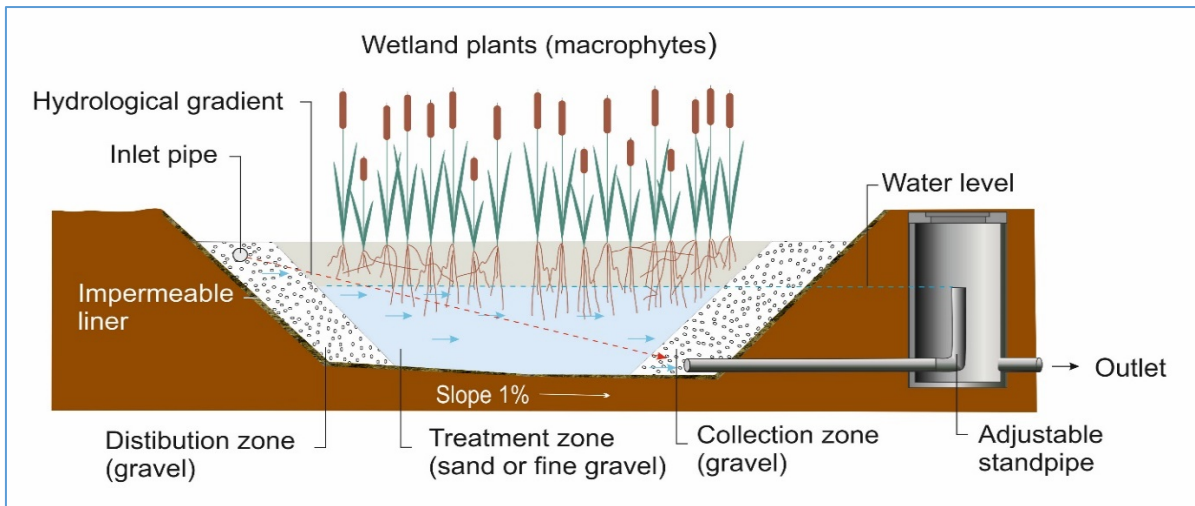


Figure 1. 5- Functional schematic of a free-water surface constructed wetland (Modified from Wildeman et al. 1993, Kadlec & Wallace 2008, GTK 2014).

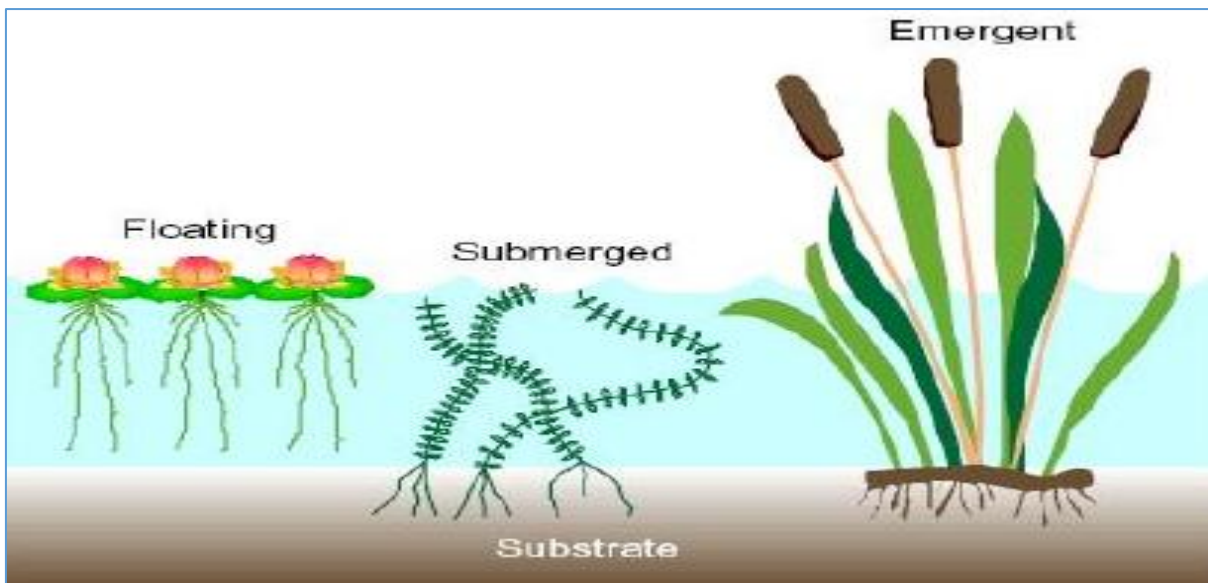


Figure 1. 6- Plants for free-water surface flow constructed wetlands (SA'AT, 2006).

Besides municipal wastewater, SF CWs with emergent vegetation have been used to treat various types of wastewaters. They are the most commonly used for advanced treatment of effluent from secondary or tertiary treatment processes. SF wetland systems offer low construction cost, but they generally have a lower contaminant removal efficiency compared with SSF systems. There has been a recent attempt to develop an open-water zone, without vegetation, to improve the N removal efficiency, promote better inflow flux, and provide wildlife habitats (Jang et al., 2007).

SF CWs are efficient in removal of organics through microbial degradation and settling of colloidal particles. Suspended solids are effectively removed via settling and filtration through the dense vegetation. Attached and suspended microbial growth is responsible for removal of soluble biochemical oxygen demand (BOD). The major oxygen (O₂) source for these reactions is re-aeration at the water surface. N is removed primarily through nitrification/denitrification

and NH₃ volatilization under higher pH values caused by algal photosynthesis. SF CWs also provide removal of phosphorus, but at relatively slow rates because of limited contact of water with soil particles which adsorb and/or precipitate phosphorus. Plant uptake represents only temporal storage because the nutrients are released to water after the plant decay (Vymazal, 2010).

1.3.3.2. *Subsurface flow constructed wetlands*

1.3.3.2.1. *Horizontal subsurface flow constructed wetlands*

Horizontal subsurface-flow wetlands (Fig.1.7) are the most widely used concept of constructed wetlands in Europe. This design was pioneered in Germany by Seidel in the 1950s and developed further in the 1970s (Brix, 1994b). The design typically consists of a shallow rectangular bed with gravel or other medium to support the roots of vegetation, planted with the macrophytes, lined with an impermeable membrane and water control structure that maintains a shallow depth of water. Mechanically pre-treated wastewater is fed in at the inlet and passes slowly through the filtration medium under the surface of the bed in a more or less horizontal path until it reached the outlet zone where it is collected before discharge via level control arrangement at the outlet. Water level always remains below the surface of the wetland bed and during the passage of wastewater through the reed bed the wastewater makes contact with a network of aerobic, anoxic and anaerobic zones (Vymazal, 1999).

During the passage of the wastewater through the rhizosphere, the wastewater is cleaned by microbiological degradation and by physical and chemical processes (Brix, 1987; Cooper et al., 1996).

HSSF CWs are typically designed to treat primary effluent prior to either soil dispersal or surface water discharge. The design typically consisted of a rectangular bed planted with the common reed (*Phragmites australis*) and lined with an impermeable membrane. Mechanically pre-treated wastewater is fed in at the inlet and passes slowly through the filtration medium under the surface of the bed in a more or less horizontal path until it reaches the outlet zone where it is collected before discharge via level control arrangement at the outlet. Typical arrangement of HSSF CW has the depth of filtration bed usually 0.6-0.8 m in order to allow roots of wetland plants and namely *Phragmites* to penetrate the whole bed and ensure oxygenation of the whole bed through oxygen release from roots. Roots and rhizomes of reeds and all other wetland plants are hollow and contain air-filled channels that are connected to the atmosphere for the purpose of transporting oxygen to the root system. The majority of this oxygen is used by the roots and rhizomes themselves for respiration, but as the roots are not completely gas-tight, some oxygen is lost to the rhizosphere (Brix, 1994a; Brix, 1997).

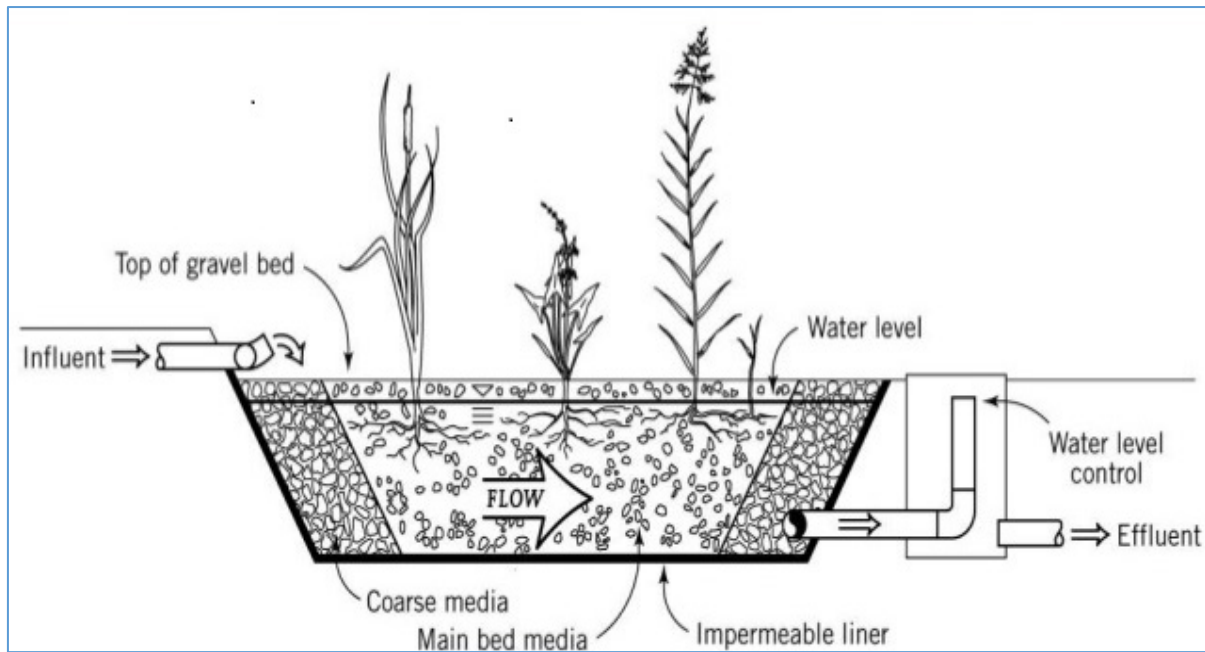


Figure 1. 7- Horizontal subsurface flow constructed wetlands, (In SlideShare, 2014).

According to the working principle of HSSF CWs, the amount of oxygen released from roots and rhizomes should be sufficient to meet the demand for aerobic degradation of oxygen consuming substances in the wastewater as well as for nitrification of the NH_3 . However, many studies have shown that the oxygen release from roots of different macrophytes is far less than the amount needed for aerobic degradation of the oxygen consuming substances delivered with sewage and that anoxic and anaerobic decomposition play an important role in HSSF CWs (Brix, 1990). As a result organic compounds are degraded aerobically as well as anaerobically by bacteria attached to plant underground organs (i.e. roots and rhizomes) and media surface and the removal of organics is generally very high in HSSF CWs (Vymazal, 2005).

Two important functions occur through this system as a result of:

- Oxygen is supplied to the heterotrophic organisms in the rhizosphere, and hydraulic flow through the medium is increased and stabilized.
- Organic matter and suspended solids are removed effectively via these systems but the removal of N and P varies greatly depending on the loading rate of the wastewater, type of substrate, and the type and composition of the wastewater. The flow rate is an important factor as high input resulting in surface flow has to be avoided as this prevents the wastewater coming into contact with the sediment and the rhizosphere (Moshiri and Brix, 1993).

HSSF wetland systems are generally more expensive than SF wetlands, although maintenance costs remain low compared to alternatives. They are commonly used for secondary treatment for single-family homes or small cluster systems (Wallace & Knight, 2006) or for small communities (Kadlec & Wallace, 2008). However, there are many other applications to specialty wastewaters from industry. In general, HSSF wetlands have been utilized for smaller flow rates than SF wetlands, probably because of cost and space considerations. These systems are capable of operation under colder conditions than SF systems, because of the ability to insulate the top. A key operational consideration is the propensity for clogging of the media. HSSF wetlands do not provide the same opportunities for ancillary benefits that SF systems do. Unlike SF wetlands, because the water is not exposed during the treatment process, the risk

associated with human or wildlife exposure to pathogenic organisms is minimized (Kadlec & Wallace, 2008).

1.3.3.2.2. *Vertical subsurface flow constructed wetlands*

Vertical subsurface-flow (Fig.1.8) systems allow for improved hydraulic conditions and water/rhizosphere contact. These systems composed of a flat bed of gravel topped with sand, with macrophytes growing at the same sort of densities as like horizontal subsurface flow system. This design provides percolation flow with intermittent loading, flooding the surface which improves soil oxygenation when compared to horizontal subsurface-flow systems. The liquid then gradually drains vertically down through the bed and is collected by drainage network at the base. Therefore, during the loading period, air is forced out of the soil and during the percolation phase the surface soil dries out drawing air back into the soil pore spaces. This process therefore provides alternating oxidizing/reducing conditions in the soil promoting alternating nitrification and de-nitrification reactions and P adsorption. Vertical flow, and more significantly, vertical up-flow systems are currently being developed for Freshwater Ecology and preliminary findings appear to indicate that these systems are promising as single-use, low load systems such as household treatment systems, particularly for P removal (Breen and Chick, 1995; Chick and Mitchell, 1995).

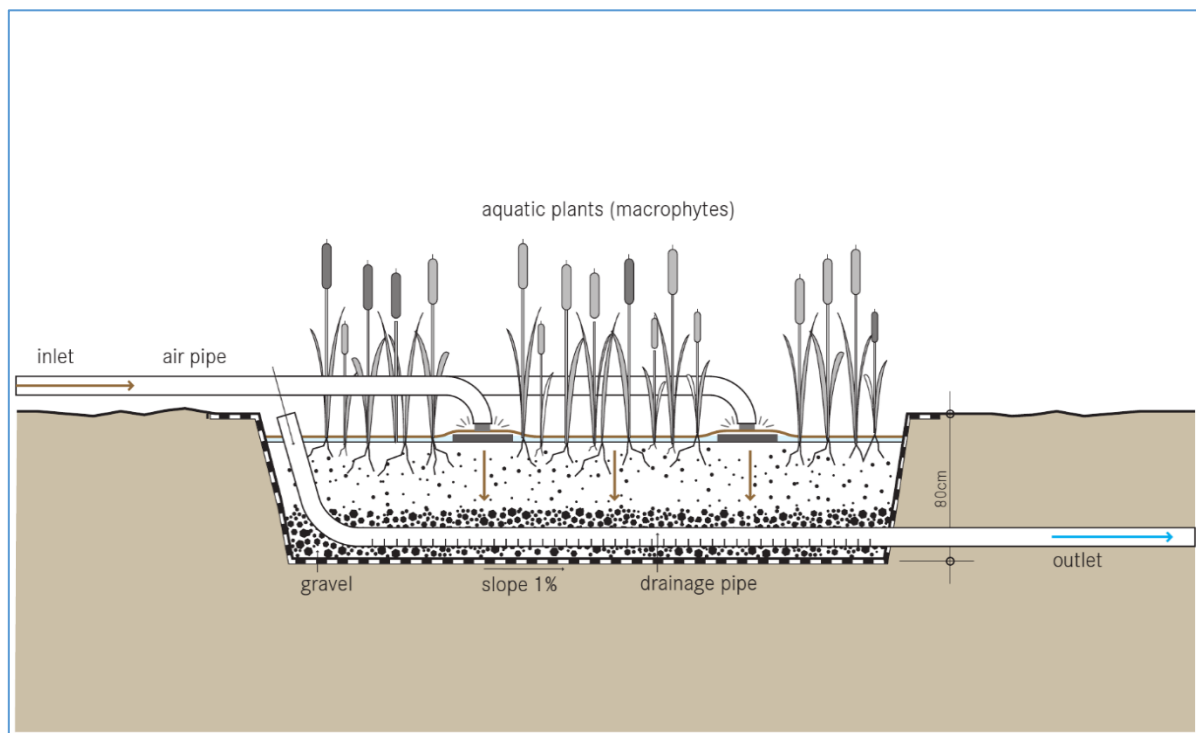


Figure 1. 8- Vertical flow constructed wetland, (TILLEY et al., 2014).

They were originally introduced by Seidel (1965) to oxygenate anaerobic septic tank effluents. However, the VSSF CWs did not spread as quickly as HSSF CWs probably because of the higher operation and maintenance requirements due to the necessity to pump the wastewater intermittently on the wetland surface (Vymazal, 2010). VSSF CWs are very effective in removing organics and suspended solids as well as pathogens (EEC, 1998). Removal of

phosphorus is low unless media with high sorption capacity are used (Vymazal, 2009). Bed clogging might be a problem, particularly if the beds are operated without a resting period (Platzer & Mauch, 1997). In order to achieve a good performance and at the same time to prevent clogging, it is important that the bed medium allows the passage of the wastewater through the bed before the next inflow arrives while at the same time holding the liquid back long enough to allow the contact with the bacteria growing on the media (Brix & Arias, 2005).

As compared to HSSF CWs, VSSF CWs require less land (Brix & Arias, 2005). Such CWs are able to clean contaminated waters with very high concentrations of contaminants even during cold winters (Brix et al., 2002). The ability of VSSF wetlands to oxidize NH_4^+ has resulted in their use in applications with higher NH_4^+ than municipal or domestic wastewater. Landfill leachates and food processing wastewaters can have NH_4^+ levels in the hundreds of milligrams per liter, and the key to reduction is the ability to nitrify. Successful VSSF wetlands therefore have formed part of the treatment process for those wastes (Kadlec, 2003).

1.3.3.3. ***Floating plant root mat / non floating plant root mat filter***

A free-floating macrophyte system (Fig.1.9 and 1.10) typically consists of basins or channels, with a natural or constructed subsurface barrier of clay or impervious geotechnical material to prevent seepage, soil or another suitable medium to support the emergent vegetation, and water at a relatively shallow depth flowing over the soil surface. The shallow water depth, low flow velocity, and presence of the plant stalks and litter regulate water flow and, especially in long, narrow channels, ensure plug-flow conditions (Reddy et al., 1984).

Floating root mat (FPRM), where the wetland plants are growing on the water surface or touching to the rooting proof bottom of the water body, and the root mat can function as a biofilter for the contaminated water. Generally, a floating root mat involves the growth of helophytes, usually rooted into the soil, but in this case converted into artificially macrophyte root mats floating in a pond or canal. These plants form a dense floating mat of roots and rhizomes, and by means of this a preferential hydraulic flow in the water zone between the root mat and the non-rooted bottom can be expected. The water is forced to flow through the root mat which operates as a filter when this root mat occupies the whole water body and touches the bottom of the pond or canal (Chen, 2012).

Floating plant root mat (FPRM) and non-floating plant root mat filter (PRMF) are hybrids of helophytes-containing soil free ponds and conventional soil based CWs. Because of their specific structure, FPRM combines benefits from ponds and CWs, and is therefore used for the treatment of different types of wastewaters and removal of different pollutants such as suspended solids, nutrients, metals, and organic contaminants. The removal efficiencies are dependent on different factors such as the climatic conditions and the type of water. FPRM and PRMF are similar to ponds as they have an open water body, and are also similar to conventional soil based CWs as both of them use helophytes, but ponds are usually dominated by phytoplankton (Kadlec, 2005). In the field of water treatment, FPRM was probably first used in eutrophicated lakes and rivers, for example, in Germany (Hoeger, 1988). The development of a dense root mat by plant roots is important for the start-up of FPRM and PRMF (VanDuzer, 2004). The submerged macrophyte system uses plants which have their photosynthetic tissue entirely submerged. The diversity of plants available for use is great and includes low productivity oligotrophic water species (*Lobelia dortmanna*), commonly occurring species in freshwater systems (*Potamogeton* spp., *Ceratophyllum* spp., and *Myriophyllum* spp.) and high productivity species that thrive in eutrophic waters (*Elodea canadensis*; *Hydrilla verticillata*). Emergent plants partly submerged under water and partly

above the wetland surface in case of bed with emergent plants. These plants can assimilate nutrients directly from the water but only grow well in oxygenated waters. Therefore, these systems are not suitable for receiving wastewater with a high loading of organic matter.



Figure 1. 9- Floating plant root mat, (SPEL Environment, 2017).



Figure 1. 10- Floating plant root mat, (Environmental monitor, 2011).

Their primary use could be to polish treated water, whether derived from a secondary treatment system or a low pollutant effluent source. Emergent water plant species like *Phragmites australis*, *Typha latifolia*, *Typha angustifolia*, *Juncus effusus* have the potential to grow as floating mats/islands, where most of them can form self-buoyant FPRM in nature or grow successfully on rafts with the potential to remove water contaminants (VanDuzer, 2004).

The advantages of FPRM are direct uptake of nutrients from the water through the plant roots, shade preventing algal proliferation, easily adjusting with varying water levels, extensive root network ensuring physical filtration and providing a large attachment surface for microorganisms and an ecological value/shelter for fauna. Disadvantages are seasonally dependent removal efficiencies and a relatively long start-up period. Unlike the facultative and aerated ponds, FPRM depend on the presence of macrophytes to achieve an enhanced removal of pollutants. In case of accumulation of too much sediment, the plant root mat can be easily shifted aside to allow its removal (Chen, 2012).

The water/sediment interaction and associated microbial activity is the driving force behind water purification processes and therefore a sink for nutrients in both constructed and natural wetlands. Another significant factor which determines the effectiveness of a wetland as a water treatment system is the amount of time that the water stays in contact with the wetland, and this is related to the size of the wetland and the amount of water it receives.

In order to overcome the overland flow, wetland systems were designed with a low aspect ratio (length to width ratio). It resulted in a very wide beds and short passage length (Brix, 1998). However, the design with a very long inlet trenches caused problems with water distribution and, therefore, the inlet trench was subdivided into two or more separate units that could be loaded separately in order to get better control on the distribution of water (Brix, 1998).

The following equation, first proposed by Kickuth (1977), has been widely used for sizing of horizontal subsurface-flow systems for domestic sewage treatment:

$$Ah = Qd (\ln C_{in} - \ln C_{out}) / K_{BOD}$$

Where, Ah is the surface flow of bed (m^2), Qd the average flow ($m^3 d^{-1}$), C_{in} the influent BOD_5 ($mg l^{-1}$), C_{out} the effluent BOD_5 ($mg l^{-1}$) and K_{BOD} is the rate constant ($m d^{-1}$).

1.3.4. Technological aspects/ removal mechanisms

The removal of contaminants from wastewater in biological treatment systems can be impacted by various physical, chemical and biological processes. Among them plants and microorganisms play the key role.

1.3.4.1. Role of plants in CWs

The choice of plants is an important issue in constructed wetlands, as they must survive the potential toxic effects of the wastewater and its variability. Horizontal subsurface-flow systems are planted with the common reed (*Phragmites australis*) (Vymazal, 2005), although other plant species, such as cattails (*Typha spp.*) bulrushes (*Scirpus spp.*) and reed canarygrass (*Phalaris arundinacea*) have been used for both domestic and industrial wastewater treatment (Mbuligwe, 2005; Vymazal, 2005; Vymazal and Kropfelova', 2005; Shepherd et al., 2001).

The choice of different plant species (Table 1.1) should take into account some factors such as the rooting depth, plant productivity and tolerance to high loads of wastewater (Brix, 1994). The main emergent macrophyte species used in CWs in the Mediterranean countries are *Canna*

spp., *Iris spp.*, *Cyperus spp.*, *Typha spp.*, *Phragmites spp.*, *Juncus spp.*, *Poaceae spp.* and *Paspalum spp.* (Korkusuz, 2005).

Moreover, plant roots provide a large surface area for the settlement of bacteria communities as well as for adsorption of contaminants (García et al., 2010). In the root zone of CWs the rhizosphere is established. The rhizosphere is the zone that surrounds a plant root and is influenced by compounds exuded by roots (Farrar et al., 2003; Narula et al., 2009). At the rhizosphere, microorganisms feed on these compounds resulting in complex biological and ecological processes (Bais et al., 2006). Root exudates also named “rhizodeposits” are a mix of several substances including, carbohydrates, organic acids and amino acids (Stottmeister et al., 2003). The amount of these carbon inputs from plants to the substrate is related to plant growth (Lu et al., 2002; Jones et al., 2004). Depending on plant species and growth stage, on average 10–25% of C assimilated by photosynthesis is translocated to the roots and exuded to the adjacent substrate (Kuzyakov et al., 2001). Root exudates are a primary driver of microbial growth and elevated microbial activities, and can also affect nutrient acquisition by both microbes and plants (Jones et al., 2004). These rhizodeposits serve microorganisms as electron donors supporting the removal processes in CWs (Imfeld et al., 2009). Near the rhizospheric zone of HSSFCWs anoxic/anaerobic conditions prevail due to the carbon from the plant’s rhizodeposits (Farrar et al., 2003). Wetland plants like *Phragmites australis*, *Typha latifolia* and *Juncus effusus* are suited to survive under anoxic/anaerobic conditions and they do release oxygen into the subsurface layer of the wetlands (Brix, 1997). The root oxygen release rates of *Phragmites* estimated by different techniques vary between 0.02 g m⁻² day⁻¹ (Brix 1990), 1-2 g m⁻² day⁻¹ (Gries et al. 1990), and 5-12 g m⁻² day⁻¹ (Armstrong & Armstrong 1990). The oxygen release by the wetland plants serves as electron acceptor for microorganisms and so influencing biodegradation processes. According to the working principle of horizontal subsurface-flow constructed wetlands (HF CWs), the amount of oxygen transferred over the surface by either diffusion or convective transport and the oxygen released from roots and rhizomes should be sufficient to meet the demand for aerobic degradation of oxygen consuming substances in the wastewater as well as for nitrification of the ammonia. However, many studies have shown that the oxygen release from roots of different macrophytes is far less than the amount needed for aerobic degradation of the oxygen consuming substances delivered with sewage and that anoxic and anaerobic decomposition play an important role in HF CWs (Brix, 1990; Brix and Schierup, 1990). As a result organic compounds are degraded aerobically as well as anaerobically by bacteria attached to plant underground organs (i.e. roots and rhizomes) and media surface and the removal of organics is generally very high in HF CWs.

In the rhizosphere, fluctuations of the redox conditions at micro-gradient scale occur from aerobic to strict anaerobic (Wießner et al., 2005). These micro-gradients influenced by changes of temperature and light, permit the establishment of different biological routes influencing the removal of the contaminants (Soda et al., 2007). The rhizosphere is considered the most active region of the wetland and plays a fundamental role in the contaminant removal (Imfeld et al., 2009).

Anaerobic degradation is usually a multi-step process. In the first step, the primary product of fermentation are fatty acids, such as acetic, butyric and lactic acids, alcohols and the gases CO₂ and H₂ (Mitsch and Gosselink, 2000; Vymazal, 1995; Vymazal et al., 1998b). Strictly anaerobic sulfate reducing bacteria and methane-forming bacteria then utilize these intermediate products of fermentation and, in fact, depend on the complex community of fermentative bacteria to supply substrate for their metabolic activities. Both groups play an

important role in organic matter decomposition (Valiela, 1984; Grant and Long, 1981; Vymazal, 1995).

The acid-forming bacteria are fairly adaptable but the methane-formers are more sensitive and will only operate in the pH range 6.5–7.5. Over-production of acid by the acid-formers can rapidly result in a low pH value. This stops the action of the methane-forming bacteria and will result in production of odorous compounds from the constructed wetland. Anaerobic degradation of organic compounds is much slower than aerobic degradation. However, when oxygen is limiting at high organic loadings, anaerobic degradation will predominate (Cooper et al., 1996).

Factors influencing the exudation from the plants are (ii) type of plants, (iii) age of the plants, (iv) water composition, (v) redox environment, (vi) hydraulic conditions and (vii) temperature and light (Wießner et al., 2002, 2005; Lynch and Whipps, 1990; Münch et al., 2007). Many of these factors interact with each other in CWs, influence the physiological status and therefore the contaminant removal mechanisms (Kuschek et al., 2003; Hijosa-Valsero et al., 2010).

The most common plants used in HSSFCWs are *Phragmites australis*, *Typha latifolia* and *Juncus effusus* (Vymazal and Kröpfelová, 2008). They are used because of their high tolerance to contaminants, salinity and toxic compounds. The physiology of wetland plants is characterized by the *aerenchyma* which serves to exchange gases (i.e. oxygen) between the shoot and the root by either diffusion or convective flow (Brix, 1994). In addition, wetland plants “pump” water from the subsurface to the leaves through *stomata* (Kadlec and Wallace, 2009), inducing the uptake, translocation and metabolization of the contaminants from the water phase (Dordio and Carvalho, 2010). Moreover, CWs lose water to the atmosphere from the water and subsurface (evaporation), and from the plants (transpiration). The combination of the two processes is named *Evapotranspiration* (Kadlec and Wallace, 2009). Evapotranspiration is determined by climatic parameters and is partially compensated by precipitation (Kadlec and Wallace, 2009). Moreover, evapotranspiration is an important issue in CWs that causes increase of contaminant concentrations in the water phase as well as variations of the hydraulic retention time (Lim et al., 2001). The effect of evapotranspiration needs to be considered for computing removal efficiencies (Bojcevska and Tonderski, 2007). In dependence on seasonal variations under different climatic conditions, the performance of CWs varies depending on the plants growth periods (Kuschek et al., 2003).

With the decay of biomass from fall to winter period, transpiration and plant-microbial interactions are reduced (Kadlec and Wallace, 2009). In the winter time, plants show no green leaves as a result of the cold season. Moreover, lower water temperatures at the subsurface of the CW are expected as well as lower rates of root exudation (Farrar et al., 2003). In summer, the maximum growth of the plants and therefore the higher plant-microbial activity takes place. In spring, the sprouting period of the plants begins. Young plants emerge next to the old ones and gradually recover their biomass. The biological activity of the plants start to increase (e.g. the amount of rhizodeposits and oxygen), microbial communities get more active and evapotranspiration gradually increases, too (Münch et al., 2007). At sub- and tropical regions, planted HSSF CWs can work continuously during the whole year without having a large variation of the plant status.

Although, several investigations on wastewater treatment by CW have compared removal efficiencies winter/summer (e.g. Hijosa-Valsero et al., 2010; Reyes-Contreras et al., 2012), still poor attention has been given to the intermittent variation of the physiological status of the plants along the year and their role on the wastewater treatment efficiency. Instead, the changes in the seasons have served to explain the lower removals of contaminants during the winter

and the higher removals in summer. In HSSF CWs, the spatial and temporal growth of the plants is expected to influence the microbial activity and the redox condition in the saturated zone of the wetland at both spatial and temporal scale and therefore to influence the treatment efficiencies. The temporal redox condition dynamics as a result of the plant response to daylight has been observed in laboratory scale reactors (Wießner et al., 2005). In addition, in pilot scale CWs, it has been associated to be involved in the pollutant removal (Kuschik et al., 2003).

The macrophytes growing in constructed treatment wetlands have several properties in relation to the treatment processes that make them an essential component to the design. The most important effects of the macrophytes in relation to the wastewater treatment processes are the physical effects that the plant tissues give rise to erosion control, filtration effect and provision of surface area for attached microorganisms. The macrophytes have other site-specific valuable functions, such as providing a suitable habitat for wildlife and giving systems an aesthetic appearance. The major roles of macrophytes in constructed treatment wetlands are summarized in Table 1.1.

Table 1. 1- Summary of the major roles of macrophytes in constructed treatment wetlands (Brix, 1997).

Macrophyte property	Role in treatment process
Aerial plant tissue	<ul style="list-style-type: none"> • Light attenuation → reduced growth of phytoplankton • Influence on microclimate → insulation during winter • Reduced wind velocity → reduced risk of resuspension • Aesthetic pleasing appearance of system • Storage of nutrients
Plant tissue in water	<ul style="list-style-type: none"> • Filtering effect → filter out large debris • Reduce current velocity → increase rate of sedimentation, reduces risk of resuspension • Provide surface area for attached biofilms • Excretion of photosynthetic oxygen → increases aerobic degradation • Uptake of nutrients
Roots and rhizomes in the sediment	<ul style="list-style-type: none"> • Stabilizing the sediment surface → less erosion • Prevents the medium from clogging in vertical flow systems • Release of oxygen increase degradation (and nitrification) • Uptake of nutrients • Release of antibiotics

The general requirements of plants suitable for use in constructed wetland wastewater treatment systems include (Tanner, 1996):

- Ecological acceptability; i.e., no significant weed or disease risks or danger to the ecological or genetic integrity of surrounding natural ecosystems; tolerance of local

climatic conditions, pests and diseases; ready propagation, and rapid establishment, spread and growth; and

- High pollutant removal capacity, either through direct assimilation and storage, or indirectly by enhancement of microbial transformations such as nitrification (via root-zone oxygen release) and denitrification (via production of carbon substrate).

The hydraulic retention times, including the length of time the water is in contact with the plant root, affects the extent to which the plant plays a significant role in the removal or breakdown of pollutants. Whereas plants significantly affect the removal of pollutants in horizontal subsurface systems with long hydraulic retention times used to clean municipal wastewater, their role is minor in pollutant removal in periodically loaded vertical filters, which usually have a short hydraulic retention time (Stottmeister et al., 2003).

Emergent and floating leaved species have been preferentially used in pilot studies of constructed wetlands. Potentially useful emergent species include many members like common reed (*Phragmites australis*), cattail (*Typha latifolia*), reed (*Cyperus sp.*), rush (*Juncus effusus*), sedge (*Carex rostrata*) and grass families. They have potentially high uptake and production rates. 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 (4534 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.

1.3.4.2. *Role of microorganisms in treatment process*

Microbial processes are vitally important for the proper functioning of constructed wetlands. Because of the presence of ample water, wetlands are typically home to a variety of microbial and plant species. The diversity of physical and chemical niches present in wetlands results in a continuum of life forms. This biological diversity creates interspecific interactions, resulting in greater diversity, more complete utilization of energy inflows, and ultimately to the emergent properties of the wetland ecosystem.

In constructed wetlands, the main process in the transformation of nutrients and mineralization of organic pollutants is carried on by microorganisms (Sim, 2003). 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.

Depending on the oxygen input by helophytes and availability of other electron acceptors, the contaminants in the wastewater are metabolized in various ways. In subsurface flow systems, aerobic processes only predominate near roots and on the rhizoplane (the surface of the root) (Faulwetter et al., 2009; Stottmeister et al., 2003). In the zones that are largely free of oxygen, anaerobic processes such as denitrification, sulphate reduction and/or methanogenesis take place (Faulwetter et al., 2009; Stottmeister et al., 2003).

Under aerobic conditions, ammonium is oxidized by microorganisms to nitrate, with nitrite as an intermediate product. Two different groups of bacteria play a role in the nitrification step: ammonium oxidizers and nitrite oxidizers. Recently, a new pathway was discovered by Mulder et al., (1995) that anamox bacteria can use nitrite as an electron acceptor and anaerobically convert ammonium and nitrite to nitrogen gas. In contrast to the traditional nitrification-

denitrification route, Anamox is an autotrophic process. The microorganisms use bicarbonate as a carbon source.

Jackson and Myers, (2002) reported that sulphate reducing bacteria were present throughout the free-water surface pilot wetland soil and water. The water chemistry suggested that conditions were well suited for these organisms to thrive in all parts of the wetlands. The high concentration of sulphate in the produced water ensured that there was a ready supply of substrate for sulphate reducing bacteria.

Phosphorus removal is provided through a complex of physical, chemical and microbiological processes, although adsorption and precipitation to the soil has usually been considered to be the main removal process (Kadlec & Wallace, 2008, Kröpfelová, 2008); microbiota uptake and plant uptake may also play a role in the removal of phosphorus (Kröpfelová, 2008).

1.3.4.3. ***Total suspended solids and carbon removal:***

Solids that are not removed in pre-treatment system are effectively removed by filtration and settlement (Cooper et al., 1996; Vymazal et al., 1998b). Most of the suspended solids are filtered out and settled within the first few meters beyond the inlet zone. The accumulation of trapped solids is a major threat for good performance of HF CWs systems as the solids may clog the bed. Therefore, the effective pretreatment is necessary for HF CWs systems (Vymazal et al., 1998b). Organic compounds can be broken down for consumption by microorganisms in a wetland system. This biodegradation removes the organic compounds from water as they provide energy for the organisms. Organics can also be degraded when taken up by plants. They can also sorb to surfaces in the wetland, usually to plant debris. The primary removal mechanisms for BOD and TSS are flocculation, settling, and filtration. As the wastewater slowly flows horizontally through the wetland bed, it acts as a horizontal gravel filter, thereby providing opportunities for TSS separation by sedimentation, physical straining and capture, and adsorption on biomass attached to the gravel and root system (US EPA, 2000).

Removal of suspended solids and BOD are very high in all types of CWs. While in SF and VSSF CWs the microbial degradation processes are mostly aerobic, in HSSF CWs anoxic and anaerobic processes prevail. The treatment efficiency is similar for SF and HF CWs, while for VSSF CWs the percentage efficiency is higher due to higher inflow concentrations. However, the outflow concentrations are comparable for all types of CWs (Vymazal, 2010).

1.3.4.4. ***Sulphur removal:***

Wetlands can function as sulphur sink through their internal production and release of hydrogen sulphide as a gas, release of elemental sulphur or methyl sulphide gas, precipitation of elemental sulphur, and precipitation and burial of insoluble metallic sulphides. Physical transport processes and biogeochemical reactions, many of them driven by aquatic plants, may result in the extensive sulphur cycling between oxidizing and reducing conditions. Oxidation of sediment sulphide produces oxidized sulphur species (i.e. SO_4^{2-} , S^0) and may release associated metals or metalloids to the water column (Simpson et al., 1998).

In constructed wetlands, especially subsurface horizontal flow systems, very little attention has been paid to the sulphur metabolism. In the case of an industrial wastewater loaded with SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ (area-specific load of $1.1 \text{ g S m}^{-2}\text{d}^{-1}$), Winter (1985) showed that constructed wetlands can act as a sink for sulphur. Two percent of the load was retained in the soil, 31 % as S^0 , 25 % as organic S (mainly in humic matter), 15 % as sulphate, 11% as sulphide and only a small fraction was released by volatilization to the atmosphere or taken up by plants (1%). Both microbial and abiotic processes are responsible for these transformation processes.

1.3.4.5. ***Nitrogen removal:***

Nitrogen transformation in constructed wetlands has already been the subject of several papers. The main removal mechanism is microbial nitrification-denitrification; in contrast, incorporation into the plant biomass is only of minor importance (Cooper and Maeseneer, 1996; Laber et al., 1999; Urbanc-Bercic and Bulc, 1995; Bayley et al., 2003). Constructed wetlands for the treatment of domestic sewage usually cause the removal of ammonia due to nitrification and also the removal of nitrate and nitrite owing to denitrification (Brix, 1994; Börner, 1992).

The major removal mechanism of nitrogen in HF CWs is nitrification/denitrification (Vymazal, 1999). Field measurements have shown that the oxygenation of the rhizosphere of HF CWs is insufficient and, therefore, incomplete nitrification (i.e. oxidation of ammonia to nitrate) is the major cause of limited nitrogen removal. Zhu and Sikora (1994) pointed out that no obvious nitrification could be observed when dissolved oxygen concentration is lower than 0.5 mg l^{-1} . In general, nitrification which is performed by strictly aerobic bacteria is mostly restricted to areas adjacent to roots and rhizomes where oxygen leaks to the filtration media. On the other hand, prevailing anoxic and anaerobic conditions offer suitable conditions for denitrification but the supply of nitrate is limited as the major portion of nitrogen in sewage is in the form of ammonia. In addition, mineralization of organic nitrogen (ammonification) which proceeds both under aerobic and anaerobic conditions actually adds ammonia to the system. Volatilization, adsorption and plant uptake play much less important role in nitrogen removal in HF CWs (Cooper et al., 1996; Vymazal, 1999; Vymazal et al., 1998a). Volatilization is limited by the fact that HF CWs do not have free water surface. Hence, algal activity is negligible in these systems and, therefore, pH values do not increase. The adsorption capacity of the commonly used media (gravel, crushed rock) is very limited.

1.3.4.6. ***Pathogens***

Pathogens are present in untreated domestic wastewaters as well as in runoff waters from animal sources. CWs, especially those that have long residence times (greater than about ten days) provide some disinfection. The extent of removal is strongly dependent on the hydraulic efficiency of the wetland. Empirical evidence is available that demonstrates that a significant die-off of indicator organisms and pathogenic species occurs in CWs. The most common indicator group is fecal coliforms, and in a review of 130 SSF CWs the median global removal was a $1.82 \log_{10}$ reduction (Kadlec & Wallace, 2008).

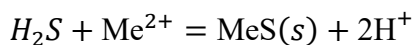
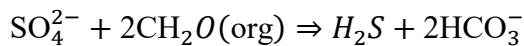
1.3.4.7. ***Greenhouse gas production***

The aerenchyma tissue also plays a role in the methane emission through helophyte plants in wetlands which were estimated at $940 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ for a cattail wetland (Yavitt and Knapp, 1995). Thomas et al., (1996) summarized and cited other papers in which helophytes are responsible for 50-90 % of the total methane flux from wetlands. Tanner et al., (1997) estimated methane emission from constructed wetlands used to treat agriculture wastewater to account for around 2-4 % of wastewater carbon loads in vegetated wetlands and 7-8 % of loads in un-vegetated systems.

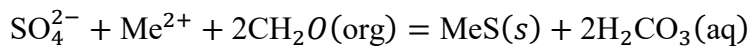
1.3.4.8. *Removal of heavy metals*

The benefits of using wetlands to remove a wide range of water-borne contaminants have been long recognized, especially for heavy metals (Sobolewski, 1999; Zhu et al., 1999; Kadlec and Knight, 1996). For metal removal in CWs, precipitation and co-precipitation of metals are two of the most important mechanisms (Lesage, 2006). The anoxic environment and organic matter production in wetlands promote chemical and biological processes enhancing metal removal from the impounded waters (Hansen et al., 1998). Metal removal in CWs is mostly focused on biological reduction of sulfate resulting in metal sulfides. Most metal sulfides are insoluble and remain stable in reduced conditions, resulting in retention in the wetlands.

Sulfate-reducing bacteria (SRB) mediate dissolved sulfate reduction to hydrogen sulfide and metals are immobilized by the resulting sulfide according to the simplified reactions:



Where, Me is a divalent metal cation



In general metal sulfides are less soluble than their carbonate or hydroxide counterparts, achieving more complete precipitation and stability over a broader pH range (Blais et al., 2008). Sulfate is most often present in industrial wastewater at much greater concentration than metals, and the production of excessive H_2S (or HS^-) may not only adversely affect the environment due to toxicity and odor, but also may increase metal mobility (Lewis, 2010).

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

- Adsorption and binding to soil and gravel matrices, sediments, particulates, algae, bacteria and oxide minerals
- Precipitation as insoluble sulphides, carbonates and co-precipitations with Fe oxyhydroxides
- Uptake and accumulation by plants and microbial biomass
- Volatilization as volatile species as a result of microbial action or by plant, phytovolatilization

Phyto-volatilization occurs as plants take up contaminated wastewater. Plants take up heavy metals, metalloids and other components through their roots and shoots and the heavy metals or metalloids are released as volatile species to the atmosphere. The relative importance of and removal by these mechanisms will vary from wetland to wetland, based upon: media selection, influent water composition, and biological activity in the wetland.

1.3.5. **Physico-chemical factors effecting performances of constructed wetlands**

1.3.5.1. *Physical processes/factors*

1.3.5.1.1. *Settling and Sedimentation*

Sedimentation is crucial for removal of TSS from wastewater in CWs. Low water velocities, coupled with the presence of plant litter (in SF CWs) or sand/gravel media (in HSSF and VSSF CWs), promote settling and seizure of TSS. In SF CWs, the presence of dense wetland vegetation causes retaining of TSS by filtration. HSSF wetlands are very effective in trapping and retaining TSS from the wastewater, however, the accumulation of TSS material reduces the hydraulic conductivity of the wetland, often to a significant degree, and causes bed clogging (Kadlec & Wallace, 2008).

Settling and sedimentation achieve efficient removal of heavy metals associated with particulate matter in the acid mine water (Horner, 1995; Kadlec and Knight, 1996; Hammer, 1997; ITRC, 2003). Once a heavy metal is in a wetland, whether the water is stagnant or mobile, a number of dynamic transformations may occur (Johnston, 1993; Matagi et al., 1998). Metals may be transported from water to the substrate or biota or vice versa. The presence of metals in AMD can be easily filtered and retained in wetlands. In calm waters the particles, which are denser than water, will settle out (Sheoran and Sheoran, 2006).

Sedimentation rates can be expressed in terms of vertical accretion (cm/year) or mass accumulation ($\text{g/m}^3/\text{year}$). Mats of floating plants in wetlands may serve, to a limited extent, as sediment traps as surface water typically moves very slow or is calm through wetlands due to the characteristic broad sheet flow and the resistance provided by rooted and floating plants (Sheoran and Sheoran, 2006). Efficiency of suspended solids removal is proportional to the particle settling velocity and the length of wetland (Johnston, 1993). For particles, which are light or less dense than water, sedimentation becomes possible only after flocculation. Flocs generally settle more rapidly in a wetland than do individual particles. Flocks may also adsorb other types of suspended particles including heavy metals. In wetlands flocculation is enhanced by high pH, concentration of suspended matters, ionic strength and high algal concentration (Sholkovitz, 1978; Hakanson and Jansson, 1983; Matagi et al., 1998).

Sedimentation has long been recognized as the principle process in removal of heavy metals from waste water in natural and constructed wetlands. It is not a simple straightforward physical reaction. Other chemical processes like precipitation and co-precipitation have to occur first. Sedimentation is a physical process after other mechanisms aggregate heavy metals into particles large enough to sink (Walker and Hurl, 2002). In this way heavy metals are removed from waste water and trapped in the wetland sediments, thus protecting the ultimate receiving surface and subsurface water bodies i.e. aquatic ecosystem.

ITRC, (2003) has reported filtration of metals suspended on solids. Acid mine water may contain suspended solids including metals which are easily filtered and retained in wetlands. Sinicrope et al., (1992) and Noller et al., (1994) reported the removal of cadmium, lead, silver and zinc by filtration. The removal rate was reported to be 75–99.7% cadmium, 26% lead, 75.9% silver and 66.7% zinc. Hares and Ward, (2004) in 39-month study also postulated removal of heavy metals by sedimentation and filtration in the high reed biomass wetlands. Such processes may be important for mine drainage.

1.3.5.2. *Chemical process*

1.3.5.2.1. *Adsorption*

Among the main mechanisms responsible for contaminant removal in CWs, physico-chemical processes as sedimentation, adsorption and precipitation at the water-sediment, root-sediment and plant-water interfaces are of significant importance. As substrates may remove wastewater constituents by ion exchange/non-specific adsorption or specific adsorption/precipitation, a

choice of substrate is crucial. Different substrates also may differ in their sorption capacities. Sorption may be reversible or irreversible due to mineralization of sorbed materials or to the formation of very strong chemical bonds (Kadlec & Wallace, 2008). For example, the performance of various substrates for heavy metal removal was estimated for CWs treating industrial wastewater (Scholz & Xu, 2002). No statistically significant performance increases of lead and copper reduction were observed when using more expensive adsorption filter media. Besides, the good overall filtration performance not only for lead and copper, but also for BOD₅ and turbidity removal was reached during the set-up phase of 10 months. This implies that irreversible adsorption or precipitation onto the soil surface is a significant removal mechanism for pollutants (Chen, 2012). Through sorption NH₃ can be stored for a short-term in CWs during drawdown periods until oxidized.

Removal of heavy metals (As) under oxic condition is perhaps best explained by the adsorption of heavy metals (As) with algae, bacteria, plant roots, organic substrates, and/or adsorption onto oxide minerals and subsequent co-precipitation specifically with Fe (III) oxyhydroxides. Reasons for the immobilization of heavy metals (As) under oxic condition were similarly explained by other authors (Wilkie and Hering, 1996; Bednar et al., 2005, Hering et al., 1996; Driehaus et al., 1998).

1.3.5.2.2. Oxidation and hydrolysis of metals

Iron, aluminium and manganese can form insoluble compounds through hydrolysis and/or oxidation that occur in wetlands. Thus leads to formation of variety of oxides, oxyhydroxides and hydroxides (Moffet and Zika, 1987; Wieder, 1989; Karathanasis and Thompson, 1995; Tarutis and Unz, 1995; Batty et al., 2002; Woulds and Ngwenya, 2004). Iron removal depends on pH, oxidation–reduction potential and the presence of various anions (ITRC, 2003). Fe³⁺ may be removed simply by raising pH to 3.5 with sufficient retention period. Stark et al., (1994) reported Fe removal to be nearly 100% after 8 years of operation at SIMCO wetlands. Whereas Fe²⁺ is highly soluble in water that have low dissolved oxygen up to pH 8. Thus first the Fe²⁺ needs to be oxidized to Fe³⁺ at pH less than 4 or 5 when bacteria also plays a role of catalyst so as to oxidize ferrous to ferric iron (Robbins and Norden, 1994).

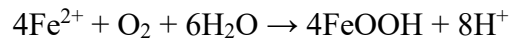
Aluminium is purely governed by pH (Hedin et al., 1994). It can precipitate as aluminium hydroxides around pH close to 5. Manganese removal is the most difficult to be achieved because its oxidation takes place at a pH close to 8 (Stumm and Morgan, 1981). Bacteria play an important role in the oxidation of Mn since they accelerate the oxidation of Mn²⁺ to Mn⁴⁺. Aluminium removal by oxidation and hydrolysis in wetland treating AMD at Kentucky has also been reported by ITRC, (2003). The removal rate was 33% whereas in natural wetland.

1.3.5.2.3. Precipitation and co-precipitation

Precipitation is an important process for metal removal. Precipitation can refer to the reaction of phosphate ions with cations of metals such as Fe, Al, Ca, or Mg. For P removal, absorption and precipitation by wetland soils are generally considered more important than uptake by plants (Richardson & Craft, 1993). However, wetland soils become soon saturated under any long-term increase in phosphorus loading (Kadlec & Wallace, 2008). Sorption is important for phosphorus during the start-up period for a CW. 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 and concomitant co-precipitations of several metals or metalloids, for example, iron and arsenic.

The oxidation of ferrous iron to ferric iron and the subsequent precipitation of iron oxyhydroxides is a dominant process:

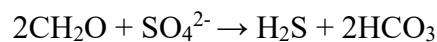


1.3.5.2.4. *Metal sulphides*

In wetlands, formation of sulphide may provide long-term metal removal, and many metals found in mine drainage form highly insoluble precipitates in the presence of dissolved sulphide (Stumm and Morgan, 1996). Sulphide precipitation accomplish on production of S^{2-} in the sulfate reduction zone of the wetland soil profile. This requires low redox potentials associated with anaerobic conditions.

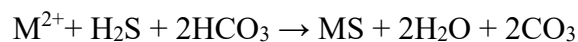
Anaerobic conditions enhance microbial sulphate reduction where sulphate-reducing bacteria fulfill their energy needs by using sulphate as electron acceptor coupling with anaerobic oxidation of organic matter during their respiration (Fig. 1.11 and 1.12). This process promote alkalinity production and potentially generate sufficient sulphide (S^{2-}) which might lead to the heavy metals (As, Fe) precipitation as sulphides (As_2S_3 , FeS_2) with low solubility (Moore et al., 1988; Kim et al., 1999). The metal- sulphide (As-sulphide, FeS_2) phases have been suggested as important sinks for metals.

Wetlands with appropriate substrate promote the growth of sulphate reducing bacteria in anaerobic conditions. In acid mine water which is rich in sulphates, these bacteria will generate hydrogen sulphide. Most of the heavy metals reacts with hydrogen sulphide and leads to formation of highly insoluble metal sulphides (Stumm and Morgan, 1981).

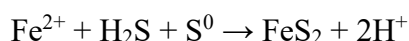


Where, CH_2O represents organic matter.

Bacterial sulfate reduction results in the precipitation of dissolved metals as metal sulfide solids:



Where, M represents metals. For Fe, pyrite formation is also possible:



The precipitation of metal sulphides in an organic substrate improves water quality by decreasing the mineral acidity without causing a parallel increase in proton acidity. Protons released by H_2S dissociation ($\text{H}_2\text{S} \rightarrow 2\text{H}^+ + \text{S}^{2-}$) are neutralized by an equal release of HCO_3 during sulphate reduction.

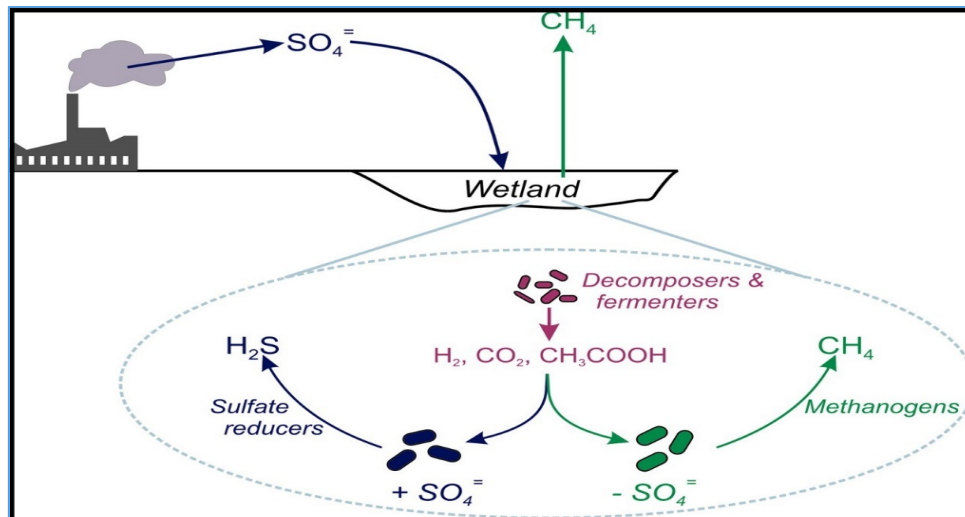


Figure 1. 11- A schematic diagram of dissimilatory sulphate reduction in wetlands (Ecologist, 2010)

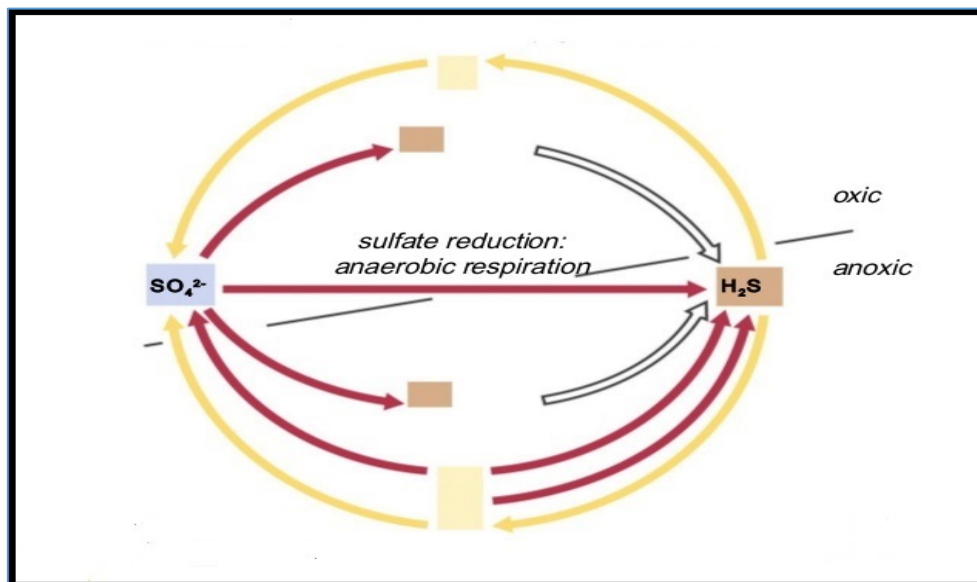


Figure 1. 12- A Schematic diagram of dissimilatory pathway by sulphate-reducing bacteria (Brock biology of microorganisms).

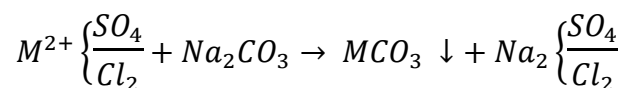
The substrate in wetland which plays a very important role in acid mine water treatment positively influences sulphate reduction (Groudev et al., 1999; Gibert et al., 2004). Sulphide minerals have been reported in a number of wetland sediments (Debusk et al., 1996; Sobolewski, 1999; DeBusk and DeBusk, 2000). Metals such as copper, lead, zinc, cadmium, arsenic etc. forms highly insoluble sulphide compounds in contact with low concentration of hydrogen sulphide (ITRC, 2003). Mungur et al., (1997) reported the removal rate of copper from AMD to be 81.7–91.8% in laboratory scale wetland based on the design of a constructed wetland, whereas, lead removal rate was reported to be 75.8–95.3% and for zinc it was 82.8–90.4%. Similarly Schiffer, (1989) also reported removal rate of lead in natural wetland by precipitation as insoluble sulphides to be 83.3%. Hawkins et al., (1997) reported the removal efficiency of copper, lead and zinc to be 33%, 79% and 85% respectively. Collins et al., (2004) reported the removal rate of aluminium, iron and zinc to be 93–99%. As sulphide is generated copper and zinc are completely removed. The field results suggest that upon startup of a constructed wetland, the adsorption of dissolved metals onto organic sites in the substrate

material will be an important process but over time sulphide precipitation becomes dominant process for metal removal from AMD (Machemer and Wildeman, 1992). Murray-Gulde et al., (2005) also reported sulphides to sequester available metals in the system.

The Metal-sulfide precipitation also requires a sufficient source of SO_4^{2-} to match the requirements. It is a fact that these metal or metalloid sulphides 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 prevail anaerobic conditions in wetlands for a higher capacity of arsenic and heavy metal removal.

1.3.5.2.5. *Metal carbonates*

Heavy metals may also form carbonates when the bicarbonate concentration in water is high. Although carbonates are less stable than sulphides, but can still perform a significant role in initial trapping of metals (Sheoran and Sheoran, 2005). Sobolewski, (1996) reported that there are few cases of carbonate retention in wetlands. Carbonate formation can take place when bacterial production of bicarbonate alkalinity in wetland sediments is substantial or when limestone occurs in flow path of acid mine drainage (ITRC, 2003). Sobolewski, (1999) reported significant quantities of copper and manganese carbonates accumulated in some natural wetlands. Carbonate precipitation is especially effective for the removal of lead and nickel (Lin, 1995):



Where, M represents metal. Schiffer, (1989) reported the removal rate of manganese and nickel in wetland and in marsh receiving fresh water to be 79% and 25% in form of metal carbonates.

1.3.5.2.6. *Redox potential*

Redox conditions and pH have been shown to be crucial factors influencing the release and transformation of heavy metals from contaminated sediments (Mok and Wai, 1989; Masscheleyn et al., 1991). These are important factors controlling heavy metal speciation and their distribution. The redox state of the environment is the result of an energy demand from both aerobic and anaerobic microbes that can mediate heavy metals transformation towards thermodynamic equilibrium. Therefore, evaluation of the redox state may serve as a quantitative measure for heavy metals mobility under various redox conditions. The redox condition (Eh) of wetland soil and sediment vary widely from approximately +500 mV (surface soil) to approximately -320 mV (strongly reducing soil).

Anaerobic conditions are distinguished by a combination of the absence of O_2 and a redox potential (Eh) lower than +400mV (Rowell, 1981). Eh values below 400mV indicate activity of denitrifying bacteria and Eh below 100mV reduction of Fe^{3+} ions, respectively. Eh lower than -100mV indicates reduction of sulphates and organic substances (fermentation) and Eh below -200mV indicates activity of methanogenic bacteria, respectively. However, if microbial processes are intense, the Eh can decrease temporarily to values which are lower than those expected for the redox couple functioning in the system. It is known that in such conditions more than one type of microbial process can take place at the same time (Dušek et al., 2008).

1.3.5.2.7. *Evapo-transpiration*

Evapo-transpiration is the net water loss caused by the evaporation of moisture from the wetland surface and also by the transpiration of wetland plants due to their physiological activity. In case of high water loss from the wetland systems, concentrations of the contaminants substantially increases and thus sometimes prevents to achieve required effluent concentrations and hence area specific mass loading rates are calculated by taking water loss into considerations. 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.

Evapo-transpiration 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).

1.3.5.3. *Biotic factors affecting heavy metals removal in constructed wetlands*

1.3.5.3.1. *Plant biomass*

Biological removal is perhaps the most important pathway for heavy metal removal in the wetlands. Probably the most widely recognized biological processes for metal removal in wetlands is plant uptake. Denny, (1987) and Greenway, (1997b) recognized number of categories of plants widely; emergent, surface floating, or free floating rooted leaves, submerged macrophytes and trees. Denny, (1980, 1987) and Greenway, (1997a, b) further noted that plants play a very important role in pollutant removal. The main route of heavy metal uptake in wetland plants was through roots in emergent and surface-floating, where as in the case of plants with either completely submerged leaves or both floating and submerged leaves or free floating take up metals through leaves and roots. Submerged rooted plants have some potential for the extraction of metals from sediments as well as water, while rootless plants can extract metals only from water (Cowgill, 1974; Matagi et al., 1998; Sriyaraj and Shutes, 2001). The rate of metal removal by plants varies widely, depending on plant growth rate and concentration of the heavy metals in plant tissue. The metal uptake rate per unit area of the wetland is often much higher for herbaceous plants, or macrophytes such as cattails. In the case of foliar absorption of heavy metals, this is a passive movement in aqueous phase through cracks in the cuticle or through the stomata to the cell wall and then the plasmalemma (Price, 1977; Everard and Denny, 1985). Arisz, (1961) while locating the sites of mineral uptake in plants found that, mostly by exchange of cations, a passive process, most of the ions penetrated the plants. Briggs and Robertson, (1997) while working on *Vallisneria spiralis L.* confirmed that the cation exchange sites were located in the cell wall. Sharpe and Denny, (1976) confirmed this location by electron microscopic studies of *Potamogeton pectinatus* leaf cells. Grill et al., (1985) identified these sites in the cell wall and named them as phytochelatins. Phytochelatins are heavy metal complexing peptides composed of different aminoacids (α -glutamic acid—cysteine) n -glycine $n = 3-7$, which are involved in detoxication and homeostatic balance of heavy metals in plant cells. Excess heavy metals are bound to cell wall in a process called metathiolate formation through mercaptide complexes.

Briggs et al., (1961); Denny, (1987); Denny et al., (1995) while working on submerged plants *Potamogeton crispes L.* and *Potamogeton pectinatus L.* reported that lead was taken from the sediments by minimal translocation to leaf tips, dead regions and in lower older leaves and initially accumulated into cell by non-metabolic force flow of solute into the apparent free

space by electron micrographs of tissues. Uveges et al., (2002) reported invasive and native species *Lythrum salicaria* (purple loosestrife) as quite tolerant species in lead contaminated waste water.

Sharpe and Denny, (1976) and Welsh and Denny, (1979) specified that the uptake of lead into *P. pectinatus* is a physical equilibrium with ionic or particulated lead binding to immobile sites in the cell wall free space and not necessarily associated with any specific exclusion mechanism. Golterman et al., (1975) concluded that as copper is an essential trace element in photosynthesis especially in the photosystem I and cytochrome biochemical processes, high copper concentrations were observed in active growing sites like stem apices and young leaves which acted as sinks for copper deposition (Matagi et al., 1998).

Sharpe and Denny, (1976) and Welsh and Denny, (1979) reported that most of the metal uptake by plant tissue is by absorption to anionic sites in the cell walls and the metals do not enter the living plant. Edroma, (1974) reported that because of much of the metal uptake by plant tissues is by absorption to anionic sites in the cell walls, wetland plants can have very high magnitudes of up to 200,000 times of heavy metal concentration in their tissues compared to either surrounding environments. Dolar et al., (1971) has shown the accumulations of mercury by *Myriophyllum spicatum* when grown in sediments containing either organic or inorganic mercury compounds.

Cheng et al., (2002) reported that constructed wetlands with well grown *Cyperus alternifolius* and *Vallisneria spiralis* is an effective tool in phytoremediation of cadmium, copper, manganese, zinc and lead. About one-third was absorbed predominantly by lateral roots while rest was removed in the top layer of the sediment. Weis and Weis, (2004) reported most of the marsh plant species as the sites for phytoremediation of metals. *Phragmites australis*, an invasive species in the northeast US sequesters more metals than the native *Spartina alterniflora*. Primarily maximum uptake of metals was observed in roots. These roots have been reported to be the most beneficial for phytostabilisation of the metal contaminants. Barley et al., (2005) also reported that metal uptake was higher in the roots.

Denny et al., (1995) further reported that mostly the roots of *Cyperus papyrus* trapped heavy metals. Greenway and Simpson, (1996); Greenway, (1997a, b); Polprasert et al., (1996) and Scholes et al., (1998) reported that roots of the wetlands play very important role in wastewater purification followed by stem and leaves. Edroma, (1974) reported that plants that grow near the heavy metal contaminated areas showed good degree of heavy metal tolerance. Gregory and Bradshaw, (1965); McNeilly and Bradshaw, (1968) and Mashauri et al., (2000) reported that this tolerance is genetically determined and occurs through natural selection.

Sediment pH, organic matter context and plant genotype, can have marked effects on metal uptake. The bioaccumulation of metals is based on root uptake but also plants can accumulate relative amounts of metals by foliar absorption of atmospheric deposits on plant leaves (Moore and Ramamoorthy, 1994; Greenway and Simpson, 1996; Greenway, 1997a, b).

Emergent macrophytes play very important role in heavy metal recycling in wetlands (Greenway, 1997b; Mashauri et al., 2000; Brix, 1997; Pip and Stepaniuk, 1992; Mays and Edwards, 2001; Scholz, 2003). While sediments of wetlands form primary sinks for heavy metals (Gray et al., 2000), macrophytes may absorb heavy metals through roots and also shoots. Collins et al., (2005) while working on remediation of metal laden acid mine run off from coal storage pile reported that shoot elemental concentrations differed between plants of deep and shallow wetlands. With higher zinc, aluminium and iron concentrations in shallow wetlands and higher sodium, manganese and phosphate concentrations in plants in deep wetlands.

During the growing season the macrophytes can contain a substantial metal load (Wood and McAtamney, 1994; Matagi et al., 1998; Nelson et al., 2002).

Some macrophytes like *Typha angustata*, *T. elephantiana*, *Desmostachya bipinnata*, *Saccharum bengalense* etc. can tolerate high concentration of several metals in their body mass without showing negative effects on the growth (Sheoran, 2004). Dunbabin and Bowmer, (1992); Skousen et al., (1992); Greenway, (1997a, b); Groudev et al., (2001a, b, c) and Ye et al., (2001) found that macrophytes are more tolerant than others. Although the mechanism of metal tolerance and uptake is poorly understood, pH, water and sediment chemistry, organic matter (substrate) and temperature also play an important role (Sheoran, 2004).

Phytoplanktons have also been reported to be quite efficient in heavy metal removal from acid mine water in wetlands (Phillips et al., 1994; Bender et al., 1989, 1991, 1993; Bender and Phillips, 1993; Sheoran and Bhandari, 2005). Kalin et al., (2004) reported that algae like *Chara*, *Nitella* provide a simple, long term means to remove uranium and other radio nuclides from uranium mining effluents.

1.3.5.3.1.1. Toxicity of heavy metals on plants

Like all living organisms, plants are often sensitive both to the deficiency and to the excess availability of some heavy metal ions as essential micronutrient, while the same at higher concentrations and even more ions such as Cd, Hg, As are strongly poisonous to the metabolic activities. Researches have been conducted throughout the world to determine the effects of toxic heavy metals on plants (Reeves and Baker, 2000; Fernandes and Henriques, 1991).

The phytotoxicity of Zn and Cd is indicated by decrease in growth and development, metabolism and an induction of oxidative damage in various plant species such as *Phaseolus vulgaris* (Cakmak and Marshner, 1993) and *Brassica juncea* (Prasad et al., 1999). Cd and Zn have reported to cause alternation in catalytic efficiency of enzymes in *Phaseolus vulgaris* (Van Assche et al., 1988; Somasekharaiah et al., 1992) and pea plants (Romero-Puertas et al., 2004). High levels of Zn in soil inhibit many plant metabolic functions, result in retarded growth and cause senescence. Zinc toxicity in plants limited the growth of both root and shoot (Choi et al., 1996; Ebbs and Kochian, 1997; Fontes and Cox, 1998). Zinc toxicity also causes chlorosis in the younger leaves, which can extend to older leaves after prolonged exposure to high soil Zn levels (Ebbs and Kochian, 1997). Another typical effect of Zn toxicity is the appearance of a purplish-red color in leaves, which is ascribed to phosphorus (P) deficiency (Lee et al., 1996).

The regulatory limit of cadmium (Cd) in agricultural soil is 100 mg/kg soil (Salt et al., 1995). Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and finally death (Sanita di Toppi and Gabbrielli, 1999; Wojcik and Tukiendorf, 2004; Mohanpuria et al., 2007; Guo et al., 2008). In general, Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants (Das et al., 1997). Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoots (Hernandez et al., 1996).

Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (Lewis et al., 2001). Exposure of plants to excess Cu generates oxidative stress and ROS (Stadtman and Oliver, 1991). Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus et al., 2001).

High level of Hg^{2+} is strongly phytotoxic to plant cells. Toxic level of Hg^{2+} can induce visible injuries and physiological disorders in plants (Zhou et al., 2007). Hg^{2+} can bind to water channel proteins, thus inducing leaf stomata to close and physical obstruction of water flow in plants (Zhang and Tyerman, 1999). High level of Hg^{2+} interfere the mitochondrial activity and induces oxidative stress by triggering the generation of ROS. This leads to the disruption of bio membrane lipids and cellular metabolism in plants (Messer et al., 2005; Cargnelutti et al., 2006).

Chromium (Cr) compounds are highly toxic to plants and are detrimental to their growth and development. It affects the germination process of seeds. Chromium stress is one of the important factors that affect photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation and enzyme activities (Clijsters and Van Assche, 1985). Chromium stress causes alteration in the production of pigments, which are involved in the life sustenance of plants (e.g., chlorophyll, anthocyanin) (Boonyapookana et al., 2002) and also causes increased production of metabolites (e.g., glutathione, ascorbic acid) as a direct response to Cr stress, which may cause damage to the plants (Shanker et al., 2003b).

Lead (Pb) is one of the ubiquitously distributed most abundant toxic elements in the soil. It exerts adverse effect on morphology, growth and photosynthetic processes of plants. Lead is known to inhibit seed germination of some plants and also causes inhibited root and stem elongation and leaf expansion. High level of Pb also causes inhibition of enzyme activities (Sinha et al., 1988a, b), water imbalance, alterations in membrane permeability and disturbs mineral nutrition (Sharma and Dubey, 2005).

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 leads to plant death, at higher concentration (Marin, 1993). The main arsenic component in plants with poor growth or which have died was found to be arsenate (Mattusch et al., 2000). Phyto-toxic symptoms from arsenic to *Typha latifolia* were observed already at concentrations exceeding 300 mg kg⁻¹ in sediment, and 400 $\mu\text{g l}^{-1}$ in the water (Dushenko et al., 1995). Phytotoxicity study of Co in some plants has recently shown the adverse effect on shoot growth and biomass (Li et al., 2009). In addition to biomass, excess of Co restricted the concentration of Fe, chlorophyll, protein and catalase activity in leaves. Further, high level of Co also affected the translocation of P, S, Mn, Zn and Cu from roots to tops of plants.

Excess of Ni^{2+} in soil causes various physiological alterations and diverse toxicity symptoms such as chlorosis and necrosis in different plant species (Zornoza et al., 1999; Pandey and Sharma, 2002; Rahman et al., 2005). High uptake of Ni^{2+} induced a decline in water content of plants. Accumulation of excessive manganese (Mn) in leaves causes a reduction of photosynthetic rate (Kitao et al., 1997a, b). Necrotic brown spotting on leaves, petioles and stems is a common symptom of Mn toxicity (Wu, 1994).

The expression of iron toxicity symptoms in leaf tissues occurs only under flooded conditions, which involves the microbial reduction of insoluble Fe^{3+} to soluble Fe^{2+} (Becker and Asch, 2005). The appearance of iron toxicity in plants is related to high Fe^{2+} uptake by roots and its transportation to leaves and via transpiration stream. The Fe^{2+} excess causes free radical production that impairs cellular structure irreversibly and damages membranes, DNA and proteins (Arora et al., 2002; de Dorlodot et al., 2005).

1.3.5.3.2. *Microorganisms*

Toxic compounds in soils are often modified by microbes (Van Zwieten et al., 2003), but many such toxins also may hinder growth of soil microbes and impair their ability to promote plant growth. Additionally, fungi associated with roots have the potential to either increase or ameliorate the uptake of inorganic contaminants by plants. Consequently, mycorrhizal fungi are crucial in maintaining diverse populations of indigenous vegetation and act as a barrier to the uptake of toxic heavy metals by plants (Leyval et al., 1997). Sharples et al., (2000) presented evidence that the ericoid mycorrhizal fungus *Hymenoscyphus ericae* acts as a filter to maintain low arsenic uptake rates by roots of the plant *Calluna vulgaris* when growing in arsenic contaminated soil. In a study of evolved arsenate resistance in cultivars of the grass *Holcus lanatus*, Gonzalez-Chavez et al., (2002) found that colonization by the arbuscular-mycorrhizal fungus *Glomus* suppressed high affinity arsenate and phosphate transport into the roots. Conversely, mycorrhizal association with the fern *Pteris vittata* has been reported to stimulate arsenic accumulation by the host (Liu et al., 2005).

The mechanism of accumulation is poorly understood, but is mediated by rhizosphere microorganisms (Walter and Wenzel, 2002; Liu et al., 2005). Those microorganisms living in symbiotic association with plant roots in soils with long-term arsenic contamination. Microorganisms play an important role for arsenic and 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 metals and arsenic. There are four mechanisms involved with the removal; i.e. adsorption to the cell surfaces, complexation, 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 arsenic and heavy metal removal.
- Complexation: microorganisms can produce organic acids (e.g., citric acid), which may chelate toxic metals and arsenic, 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 arsenic and metal precipitation by producing hydrogen sulphide, which precipitate arsenic as their sulphides (e.g. As_2S_3). Sulphate reducing bacteria (SRB) transform SO_4^{2-} to H_2S , which promotes the extra-cellular precipitation of arsenic and metals from solution.
- Volatilization: metals like arsenic, mercury and some metals are transformed to volatile species as a result of microbial action. For example, microbially mediated methylation converts inorganic arsenic As(V) and As(III) to volatile and toxic species arsine (AsH_3), MMAA to monomethylarsine [MMA, $\text{AsH}_2(\text{CH}_3)$], DMAA to dimethylarsine [DMA, $\text{AsH}(\text{CH}_3)_2$] and TMAO to trimethylarsine [TMA, $\text{As}(\text{CH}_3)_3$] (Cullen and Reimer, 1989).

1.3.5.3.2.1. *Toxicity of heavy metals on microorganisms*

Species of microorganisms (e.g. Berdicevsky et al., 1993), strains of the same species (e.g. Romandini et al., 1992) and also activities of the same microbial species (e.g. Balsalobre et al., 1993; Torslov, 1993) can all show considerable differences in their sensitivity to metal toxicity.

Heavy metals are well known to be toxic to most organisms when present in excessive concentrations. In principle there are only two factors which may contribute to the

discrepancies between studies: (1) factors which modify the toxicity of the metals and (2) differences in sensitivity of the microorganism(s) or microbial process(es) (Giller et al., 1998). At high concentrations of heavy metals in soil ecotoxicological studies result in a decrease in the amount of microbial biomass, and a change in community structure (Maliszewska et al., 1985; Ohya et al., 1985; Naidu and Reddy, 1988; Aoyama et al., 1993; Frostegaard et al., 1993, 1996; Leita et al., 1995; Kozdroj, 1995; Speir et al., 1995; Kandeler et al., 1996; Knight et al., 1997).

Microorganisms differ in their sensitivity to metal toxicity and sufficient metal exposure will result in immediate death of cells due to disruption of essential functions, and to more gradual changes in population sizes due to changes in viability or competitive ability (Giller et al., 1998)

Evidence from the field suggests that under long-term metal stress there is a change in the genetic structure of the soil microbial community, without necessarily being an increase in metal tolerance. A decrease in the total soil microbial biomass under chronic metal stress has been observed in many field studies, but is likely to be preceded by changes in community structure (Giller et al., 1998). A decreased size of the microbial biomass can probably at least partially be explained by physiological causes such as a decrease in the microbial substrate utilization efficiency and an increased maintenance energy requirement. A decrease in the number of substrates which can be utilized and thus a reduction in the efficient exploitation of all ecological niches may also explain the decrease in the size of the biomass (Reber, 1992; Burkhardt et al., 1993).

1.3.5.3.3. Sulphide toxicity to plants

If organic matter accumulates and decomposes under anoxic conditions, phytotoxins are released into the soil. In healthy sites, reeds are able to oxygenate the rhizosphere by convective flow through rhizomes (Armstrong et al., 1996b), which may hence decrease concentration of sulphide in the rhizosphere.

Although sulphide may act as an inhibitor of N-uptake (Chambers et al., 1998; Mendelssohn and McKee, 1988), root absorption of both N and P did not seem to be hindered at die-back sites.

Sulphide may act as major phytotoxin, especially when environmental conditions such as waterlogged soil and high temperature affect gas diffusivity in roots, eventually enhancing the entrance of phytotoxins into the plant. High sulphide concentration may lead to toxic effects to aquatic plants, such as root decay (root blackening and increased flaccidity of the roots) and mortality (Armstrong et al., 1996b; Smolder and Roelofs, 1996a), reduced growth (Koch and Mendelssohn, 1989; Koch et al., 1990; Van der Welle et al., 2006) or even mortality (Lamers et al., 1998; Smolders et al., 1995a).

Both sulphide and organic acids induce the formation of abnormal anatomical features such as callus blocking aerenchyma channels, lignification and suberification of the surface layer of the root cells (Armstrong et al., 1996; Armstrong and Armstrong, 1999). On the other hand, callus blockage can also be induced by insect damage (Armstrong et al., 1996). It is known that sulphide is an inhibitor of aerobic respiration and nutrient uptake (Allan and Hollis, 1972; Mendelssohn and McKee, 1988). However, sulphide usually accumulates under anoxic conditions in brackish wetlands because of high sulphate concentration in the water (Armstrong et al., 1996).

Sulphide concentrations in sediment pore-water >1 mM have been found to induce stunted growth adventitious roots, lateral roots and buds, as well as callus formation in root and rhizomes, besides blockages in the vascular system (Armstrong et al., 1996; Armstrong et al., 1996; Armstrong et al., 1996). Additionally, Fürtig et al., (1996) found that energy metabolism in *Phragmites australis* is negatively affected even at sulphide concentration in pore-water as low as 1 mM.

Goodman et al., 1995, found negative effects of sulphide on sea-grass photosynthesis and increased mortality during die-back event have also been related to sulphide exposure (Carlson et al., 1994, 2002; Holmer et al., 2001). Intrusion of sulphide is considered to be the main cause for rapid die back event of *Thalassia testudinum* in Florida Bay (Borum et al., 2005).

Van der Welle, (2007) investigated the responses of the freshwater wetland species *J. effusus* L. and *Caltha palustris* to iron supply in sulphidic environments. *J. effusus* showed a double advantage under sulphide-rich condition: it does not suffer from sulphide toxicity since it can oxidize potentially harmful reduced compounds in its rhizosphere.

Sulphide toxicity, however, can be mitigated by the formation of highly insoluble metal sulphides like iron sulphides (FeS, FeS₂ or pyrite) or metal sulphide complexes (Huerta- Diaz et al., 1998; Smolders and Roelofs, 1995b; Wang and Chapman, 1999), thereby reducing both sulphide and metal toxicity. In areas where iron-rich groundwater is discharged, free S²⁻ concentration are usually low, as a result of iron sulphide precipitation.

1.3.5.3.4. Sulphide toxicity to microorganisms

The toxicity of sulphide in anaerobic reactors has been well studied. Koster et al., (1986) reported that a free sulphide of 250 mg S l⁻¹ caused 50 % inhibitions of methanogenesis in UASB granules. In a lactate-fed serum vial test, McCartney and Oleszkiewicz, (1993) observed a 50 % inhibition of the methanogenic activity at 100 mg l⁻¹ free sulphide. In an acetate-fed UASB reactor, a free sulphide of 184 mg l⁻¹ was also found to cause a 50 % inhibition of methanogenesis at neutral pH (Visser et al., 1996).

1.3.6. Application of the technology

There are an expanding number of application areas for constructed wetlands technology. During the early years (1985) of the development of the technology, virtually all emphasis was on the treatment of domestic and municipal wastewater. Later the emphasis was on domestic wastewater, agriculture wastewater and mine drainage water (Mandi et al., 1998; Gearheart, 1992; Knight et al., 2000). In recent years there has been a branching to include a very broad spectrum of wastewater, including industrial and storm-waters. Increasing attention is now also being paid to using constructed wetlands to treat leachate, contaminated groundwater and industrial effluents.

There are several roles for constructed wetlands in the treatment of domestic and municipal wastewaters. They can be positioned at any of several locations along the water quality improvement path. Constructed wetland technology is generally applied in two general themes for domestic and municipal wastewaters: for accomplishing secondary treatment and for accomplishing advanced treatment.

Constructed wetland treatment systems can provide secondary treatment of acid mine drainage/heavy metals containing industrial wastewater after mechanical pre-treatment consisting of a combination of screen, grit and grease chambers, sedimentation, septic and Imhoff tanks.

1.4. Aim of the work

The objective of this work was to improve the basic knowledge about acid mine drainage treatment (sulphate transformation processes which in turn affect the metal removal process) in constructed wetlands. Therefore, experiments for investigating the sulphate and heavy metals removal in laboratory based/model constructed wetlands have been performed. The specific objectives of this work were to focus and assess following aspects:

- To investigate different processes in model wetlands and to draw out a comparison in terms of efficiency among various type of model wetlands.
- To intensify the treatment processes with stimulation of the dissimilatory sulfate reduction in an autotrophic way by the use of hydrogen gas (Phytotechnicum).
- To estimation of the efficiency of different model wetland in terms of metal and sulphur removal.
- To compare efficiency of different plants in model wetlands.
- To characterize physico-chemical conditions in the model wetlands in both space and time.
- To understand the key factors influencing the efficiency of sulphate and metal removal in constructed wetlands.

2. Materials and Methods

Constructed wetland used in this study was called laminar stream subsurface horizontal flow system. From the name, the flow path through the operational systems was horizontal along the wetland bed. The laminar stream horizontal flow constructed wetland systems represented a more realistic and near to practice design. Both macro and micro gradients prevailed in this system. During the passage in the system, wastewater contaminants came in contact with a network of aerobic, anoxic and anaerobic zones (macro- and micro-gradients) in the gravel media where big variety of suspended and in biofilm fixed microorganisms and plant roots were grown.

2.1. Experimental sites

The experiments were done at two places:

- (1) Green House (UFZ, Leipzig)
- (2) Phytotechnicum (UFZ, Leipzig)

The work is done in two batches by using two different plant species namely *Juncus effuses* and *Phragmites australis* in four different types of experimental wetland systems in green house namely Unplanted Horizontal Sub-surface flow CW (Unplanted HSSF CW), Planted Horizontal Sub-Surface Flow CW (HSSF CW), Planted Horizontal Surface Flow CW (HSF CW) and Horizontal Hydroponic Root mat Filter CW (HHRMF) and two hydroponic systems with hydrogen gas as electron donor in phytotechnicum. This work is intended to study and compare the removal capacity of sulphate and heavy metals by the two different plants and to compare the removal capacity in different types of wetland.

2.2. Synthetic wastewater and its constituents

Model constructed wetlands were fed with tap water, trace mineral (TSM 3, 1 ml l⁻¹) and nutrient salt (Hakaphos, 0.1 g l⁻¹) for 3 months (November 2011 to January 2012) prior to starting of the experiments from February 2012. No addition of any organic carbon sources and heavy metals were made in the model wastewater feeding during this particular time period.

The synthetic AMD was prepared in tap water and modified according to Bissinger et al., (2001) with a mean pH of 2.65. The artificial AMD that was prepared and used in this investigation contained Fe, Mg, Ca, Al metals along with high amount of SO₄²⁻. The inflow concentrations of the used ingredients were (in mg l⁻¹): 650, 72.5, 19.5, 59.8, 28 of SO₄, Fe(3), Al, Ca and Mg respectively. In general, an inflow rate of 6 l/day was adjusted for all systems, which corresponds a hydraulic retention time of 5 days. In all cases, a trace mineral solution (Kusch, 1991) was added to the artificial wastewater (1 ml l⁻¹) containing (in g l⁻¹): EDTA-Na 0.1, FeSO₄.7H₂O 0.1, MnCl₂.4H₂O 0.1, CoCl₂.5H₂O 0.17, CaCl₂.6H₂O 0.1, ZnCl₂ 0.1, CuCl₂.5H₂O 0.02, NiCl₂.6H₂O 0.03, H₃BO₃ 0.01, Na₂MoO₄.2H₂O 0.01, H₂SeO₃ 0.001, HCl (3 mL).

2.2.1. Preparation of artificial AMD

The required amount of chemicals were weighed in the laboratory and is taken to the green house and phytotechnicum. In green house a container of capacity of 120L is filled by tap water and then the pH is adjusted to 2.65 using HCL and then other chemicals were added and mixed

by using air compressor. In phytotechnicum the same process is used to prepare the artificial AMD but instead of 120L container a 60L container is used as there are only two wetlands used for the experiment.

2.3. Treatment of synthetic acid mine drainage in the laboratory-scale horizontal flow constructed wetlands with *Juncus effuses* in green house (without external electron donor)

2.3.1. Experimental design

Four laboratory-scale horizontal subsurface flow model wetlands (Fig. 2.1) Unplanted Horizontal Sub-surface flow CW (Unplanted HSSF CW), Planted Horizontal Sub-Surface Flow CW (HSSF CW), Planted Horizontal Surface Flow CW (HSF CW) and Horizontal Hydroponic Root mat Filter CW (HHRMF) were established to investigate the fate of sulphate and heavy metals in the rhizosphere of wetland vegetation. The schematic diagram of a laboratory-scale subsurface horizontal flow wetland is shown in Figure 2.1

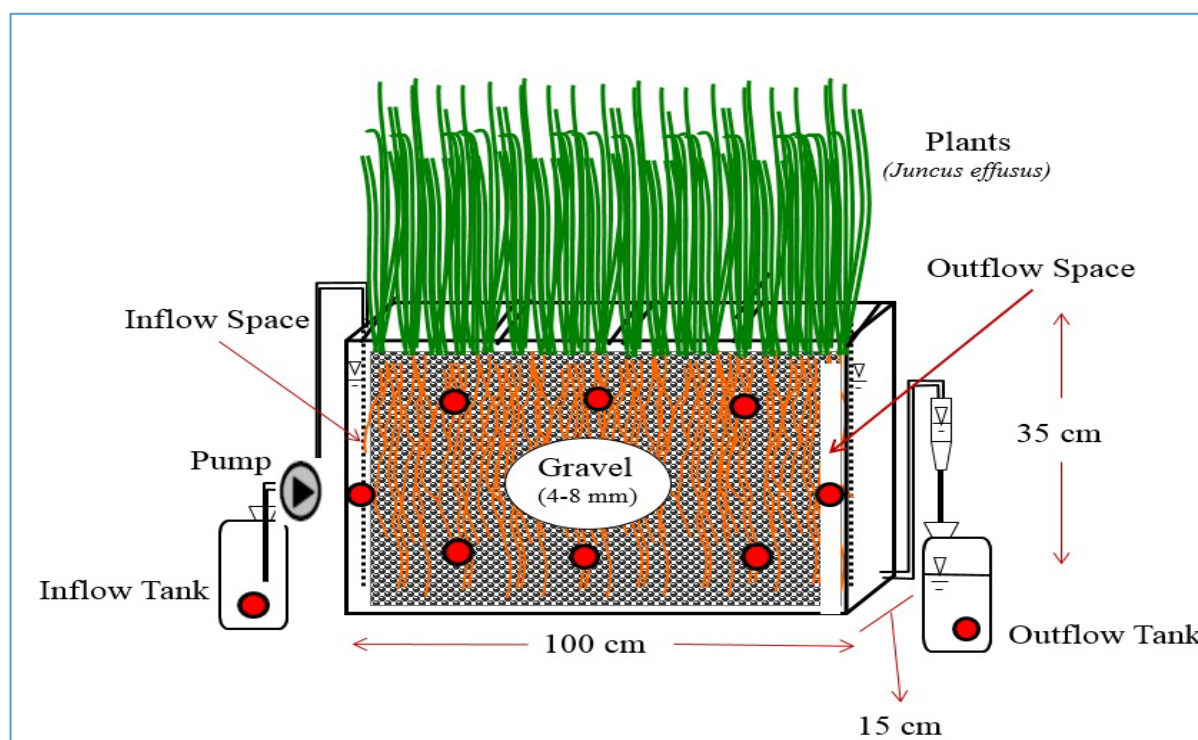


Figure 2. 1- Schematic diagram and layout (plan view) of horizontal subsurface flow laboratory-scale constructed wetland (Modified from Rahman, 2008).

The wetlands consisted of metallic containers of 100 cm in length, 15 cm in width and 35 cm in height. The unplanted gravel system and sub-surface flow system were uniformly filled with 65 kg gravel (with a diameter of 2–6 mm, a density of 1.665 g cm^{-3}) up to a height of 30 cm and a free pore volume of 25 l whereas surface flow system was filled with 60 kg gravels of the same diameter as the other two up to a height of 27 cm. The water level was adjusted to 5 cm below the surface in case of the unplanted gravel system and sub-surface flow system and 5 cm above the gravel for surface flow system. Sieves of perforated stainless steel were placed

3 cm in front of the inflow and outflow of the gravel bed. This free liquid volume should ensure an equal distribution of the inflow and a laminar liquid flow through the gravel bed. Model wastewater was pumped continuously from the storage tank to the inlet of each wetland by means of a well-calibrated peristaltic pump which ensured the same flow rate for all the experimental wetlands. The inflow rate was adjusted to an average value of 6 l d^{-1} ($\sim 4.2 \text{ ml min}^{-1}$), which provided a hydraulic retention time of 5 days. The soil matrix used in the wetlands was washed gravel in a range between 2-8 mm in diameter. No plants were grown on unplanted HSSF CW which was constructed in parallel representing as control wetland and the same model wastewater were fed into it. This control wetland unplanted HSSF CW provided a baseline to compare plant performances in the treatment wetlands HSSF CW, HSF CW and HHRMF.

Each laboratory-scale subsurface horizontal wetland was fed separately from the separate storage tank (50 l capacity). The storage tank was used for storing the synthetic wastewater to be treated in the wetlands. The tank had to be re-filled before being empty to ensure uninterrupted flow of wastewater into the wetland beds.

2.3.2. Plant biomass

Before starting the experiment, wetlands (HSSF CW, HSF CW and HHRMF) were planted uniformly with *J. effusus* in November 2011 with a mean shoot density of approximately 700, 780 and 733 shoots m^{-2} , respectively. This species of macrophytes was selected presumably on the basis of their common occurrence, rapid growth into their biomass (plant biomass production), rooting depth, tolerance to high loads of wastewater and abundance in natural wetlands. During the acclimatization period of approximately 3 months, plants were fed only with tap water and fertilizer (NPK Wasserlösliches Nährsalz, Hakaphos, concentration of 1 g l^{-1}) as plant nutrient source.

By August 2011, the plant shoots were well established and covered the entire surface of the model wetlands. Feeding with artificial AMD started from February 2012.

Number of green shoots were counted at least ones in every month throughout the whole operation time and also immediately after the termination of the experiment.

2.3.3. Experimental conditions

Experiment was conducted for a period of 10 months. This experiment had a sufficient duration to guarantee a representative number of samples taken from each experimental wetland. Artificial wastewater was freshly prepared in every 3 days to prevent microbial degradation during storage and operation. Synthetic AMD was prepared in a 120 L tank and an air compressor was used to mix the ingredients of the AMD (Table 2.1, 2.2 and 2.3) and then it was distributed evenly into each inflow tank.

Table 2. 1- Chemical composition of the artificial AMD

<u>Components</u>	<u>Amount</u>
H ₂ SO ₄ (0.5M)	1ml/L
Al ₂ (SO ₄) ₃ (18H ₂ O)	60mg/L
Fe ₂ (SO ₄) ₃	65mg/L
MgSO ₄ *7H ₂ O	72mg/L
CaSO ₄ *2H ₂ O	64mg/L

Ho(HoCl ₃)	1µg/L
Gd(GdCl ₃)	1µg/L
Ce(CeCl ₃)	1µg/L
TMS	1ml/L
TSS	1ml/L

Table 2. 2- Chemical composition of the trace mineral solution (TMS)

<u>Components</u>	<u>The original solution</u>	<u>Final concentration in the</u> <u>AMD</u>
	<u>mmol/L</u>	<u>mg/L</u>
Na ₂ WO ₄	0.00788	0.0026
Na ₂ MoO ₄	0.04001	0.0097
KBr	0.07991	0.0095
KI	0.03982	0.0066
Co(NO ₃)*6H ₂ O	0.04020	0.0117
CuSO ₄ *5H ₂ O	0.04005	0.0100
NiSO ₄ *6H ₂ O	0.04020	0.0113
VSO ₄	0.00603	0.0012
H ₂ SeO ₃	0.06017	0.0078
H ₃ BO ₃	4.011	0.248
MnSO ₄ *H ₂ O	50.8	4.2932
ZnSO ₄ *7H ₂ O	13.761	3.9568

Table 2. 3- Chemical composition of the trace salt solution (TSS)

<u>Components</u>	<u>The original solution</u>	<u>Final concentration in the</u> <u>AMD</u>
	<u>mmol/L</u>	<u>mg/L</u>
NaCl	15.2	15.2
(NH ₄) ₂ SO ₄	10.8	10.8
KH ₂ PO ₄	6.8	6.8
KNO ₃	2.00	2.00
K ₂ SO ₄	0.04	0.04

Model wetlands were placed in a greenhouse (Fig.2.2) with 16-h day length and operating under defined environmental conditions with a temperature of 22 ± 2 °C simulating an average summer day in moderate climates (Wiessner et al., 2005a). The operational periods lasted from September 2011 to December 2012.

The key concept of how a demonstration horizontal subsurface flow wetland for sulphate and heavy metal removal might work under carbon deficient and anoxic environmental condition was carried out and studied in all four model wetlands unplanted HSSF CW, HSSF CW, HSF CW and HHRMF in this experiment. The artificial AMD that was prepared and used in this investigation contained Fe, Mg, Ca, Al metals along with high amount of SO₄²⁻. The inflow concentrations of the used ingredients were (in mgL⁻¹): 650, 72.5, 19.5, 59.8, 28 of SO₄²⁻, Fe(3), Al, Ca and Mg respectively. No additional organic carbon sources were added to the

synthetic AMD. The only carbon source was the organic carbon diffused from the roots of the plants. This amount of diffused organic carbon was enough to ensure a persistent anaerobic condition in the wetland beds and allowed the activity of anaerobic microorganisms. For the experiment tap water was used instead of deionized water.



Figure 2. 2- Laboratory scale experimental set up in green house planted with *Juncus effuses*

2.4. Treatment of synthetic acid mine drainage in the laboratory-scale horizontal flow constructed wetlands with *Phragmites australis* in green house (without electron donor)

2.4.1. Experimental design

Four laboratory-scale horizontal subsurface flow model wetlands (Fig. 2.2) Unplanted Horizontal Sub-surface flow CW (Unplanted HSSF CW), Planted Horizontal Sub-Surface CW (HSSF CW), Planted Horizontal Surface Flow CW (HSF CW) and Horizontal Hydroponic Root mat Filter CW (HHRMF) were established to investigate the fate of sulphate and heavy metals in the rhizosphere of wetland vegetation.

The wetlands consisted of metallic containers of 100 cm in length, 15 cm in width and 35 cm in height. The unplanted gravel system and sub-surface flow system were uniformly filled with 65 kg gravel (with a diameter of 2–6 mm, a density of 1.665 g cm^{-3}) up to a height of 30 cm and a free pore volume of 25 l whereas surface flow system was filled with 60 kg gravels of the same diameter as the other two up to a height of 27 cm. The water level was adjusted to 5 cm below the surface in case of the unplanted gravel system and sub-surface flow system and 5 cm above the gravel for surface flow system. Sieves of perforated stainless steel were placed 3 cm in front of the inflow and outflow of the gravel bed. This free liquid volume should ensure an equal distribution of the inflow and a laminar liquid flow through the gravel bed. Model wastewater was pumped continuously from the storage tank to the inlet of each wetland by means of a well-calibrated peristaltic pump which ensured the same flow rate for all the

experimental wetlands. The inflow rate was adjusted to an average value of 7 l d^{-1} ($\sim 4.9 \text{ ml min}^{-1}$), which provided a hydraulic retention time of 4.2 days. The soil matrix used in the wetlands was washed gravel in a range between 2-8 mm in diameter. No plants were grown on Wetland 1 (W1) which was constructed in parallel representing as control wetland and the same model wastewater were fed into it. This control wetland unplanted HSSF CW provided a baseline to compare plant performances in the treatment wetlands HSSF CW, HSF CW and HHRMF gives the main constructive details of the treatment units. Each laboratory-scale subsurface horizontal wetland was fed separately from the separate storage tank (50 l capacity). The storage tank was used for storing the synthetic wastewater to be treated in the wetlands. The tank had to be re-filled before being empty to ensure uninterrupted flow of wastewater into the wetland beds.

2.4.2. Plant biomass

Before starting the experiment, wetlands (HSSF CW, HSF CW and HHRMF) were planted uniformly with *P. australis* in December 2013 with a mean shoot density of approximately 400, 433 and 420 shoots m^{-2} , respectively. This species of macrophytes was selected presumably on the basis of their common occurrence, rapid growth into their biomass (plant biomass production), rooting depth, tolerance to high loads of wastewater and abundance in natural wetlands. During the acclimatization period of approximately 3 months, plants were fed only with tap water and fertilizer (NPK Wasserlösliches Nährsalz, Hakaphos, concentration of 1 g l^{-1}) as plant nutrient source.

By March 2014, the plant shoots were well established and covered the entire surface of the model wetlands. Feeding with artificial AMD started from March 2014. Number of green shoots were counted at least ones in every month throughout the whole operation time and also immediately after the termination of the experiment.

2.4.3. Experimental conditions

Model wetlands were placed in a greenhouse (Fig. 2.3) with 16-h day length and operating under defined environmental conditions with a temperature of $22 \pm 2 \text{ }^{\circ}\text{C}$ simulating an average summer day in moderate climates (Wiessner et al., 2005a). The operational periods lasted from March 2014 to December 2014.

The key concept of how a demonstration horizontal subsurface flow wetland for sulphate and heavy metal removal might work under carbon deficient and anoxic environmental condition was carried out and studied in all four model wetlands un Planted HSSF CW, HSSF CW, HSF CW and HHRMF in this experiment. The artificial AMD that was prepared and used in this investigation contained Fe, Mg, Ca, Al metals along with high amount of SO_4^{2-} . The inflow concentrations of the used ingredients were (in mg l^{-1}): 650, 72.5, 19.5, 59.8, 28 of SO_4^{2-} , Fe(3), Al, Ca and Mg respectively. No additional organic carbon sources were added to the synthetic AMD. The only carbon source was the organic carbon diffused from the roots of the plants. This amount of diffused organic carbon was enough to ensure a persistent anaerobic condition in the wetland beds and allowed the activity of anaerobic microorganisms. For the experiment tap water was used instead of deionized water.



Figure 2. 3- Laboratory scale experimental set up in green house planted with *Phragmites australis*

2.5. Treatment of synthetic acid mine drainage in the laboratory-scale horizontal flow constructed wetlands with *Juncus effuses* Phytotechnicum (with H₂ as electron donor)

2.5.1. Experimental design

Two planted laboratory-scale hydroponic model wetlands were established to investigate the fate of sulphate and heavy metals in the rhizosphere of wetland vegetation, W1 without H₂ as electron donor and W2 with H₂ as electron donor. The schematic diagram of a laboratory-scale subsurface horizontal flow wetland is shown in Fig. 2.1.

The wetlands consisted of plastic containers of 100 cm in length, 15 cm in width and 35 cm in height. W2 is fed continuously with hydrogen gas through selective permeable silicone tubes placed at the bottom of the container (Fig. 2.5). The water level was adjusted to 5 cm below the surface in both the systems. Sieves of perforated plastic were placed 3 cm in front of the inflow and outflow of the wetland. This free liquid volume should ensure an equal distribution of the inflow and a laminar liquid flow through the wetland. Model wastewater was pumped continuously from the storage tank to the inlet of each wetland by means of a well-calibrated peristaltic pump which ensured the same flow rate for all the experimental wetlands. The inflow rate was adjusted to an average value of 6 l d⁻¹ (~4.2 ml min⁻¹), which provided a hydraulic retention time of 5 days. Both the wetlands were planted with almost equal amount of plants and the roots of the plants serve as the matrix in the wetlands.

No H₂ gas was fed into Wetland 1 (W1) which was constructed in parallel representing as control wetland and the same model wastewater were fed into it as of W2 which was continuously fed with H₂ at 1.8 bar pressure. Wetland W1 provided a baseline to compare plant performances in the treatment wetlands. Each laboratory-scale subsurface horizontal wetland

was fed separately from the separate storage tank (35 l capacity). The storage tank was used for storing the synthetic wastewater to be treated in the wetlands. The tank had to be re-filled before being empty to ensure uninterrupted flow of wastewater into the wetland beds.

2.5.2. Plant biomass

Before starting the experiment, wetlands (W1 and W2) were planted uniformly with *J. effusus* in January 2013 with a mean shoot density of approximately 705 and 715 shoots m^{-2} , respectively. This species of macrophytes was selected presumably on the basis of their common occurrence, rapid growth into their biomass (plant biomass production), rooting depth, tolerance to high loads of wastewater and abundance in natural wetlands. During the acclimatization period of approximately 3 months, plants were fed only with tap water and fertilizer (NPK Wasserlösliches Nährsalz, Hakaphos, concentration of 1 g l^{-1}) as plant nutrient source.

By March 2013, the plant shoots were well established and covered the entire surface of the model wetlands. Feeding with artificial AMD started from April 2013. Number of green shoots were counted at least ones in every month throughout the whole operation time and also immediately after the termination of the experiment.

2.5.3. Experimental conditions

Experiment was conducted for a period of 8 months. This experiment had a sufficient duration to guarantee a representative number of samples taken from each experimental wetland. Artificial wastewater was freshly prepared in every 3 days to prevent microbial degradation during storage and operation. Synthetic AMD was prepared in a 120 L tank and an air compressor was used to mix the ingredients of the AMD and then it was distributed evenly into each inflow tank (Table 2.1, 2.2 and 2.3).

Model wetlands were placed in a phytotechnicum (Fig. 2.4) with 16-h day length and operating under defined environmental conditions with a temperature of $22 \pm 2\text{ }^{\circ}\text{C}$ simulating an average summer day in moderate climates (Wiessner et al., 2005a). The operational periods lasted from April 2012 to November 2012.

The key concept of how a demonstration horizontal flow wetland for sulphate and heavy metal removal might work under carbon deficient and anoxic environmental condition (both in presence and absence of H_2 as electron donor) was carried out and studied in the two model wetlands W1 and W2, in this experiment. The artificial AMD that was prepared and used in this investigation contained Fe, Mg, Ca, Al metals along with high amount of SO_4^{2-} . The inflow concentrations of the used ingredients were (in mg l^{-1}): 650, 72.5, 19.5, 59.8, 28 of SO_4^{2-} , Fe(3), Al, Ca and Mg respectively. No additional organic carbon sources were added to the synthetic AMD. The only carbon source was the organic carbon diffused from the roots of the plants. This amount of diffused organic carbon was enough to ensure a persistent anaerobic condition in the wetland beds and allowed the activity of anaerobic microorganisms. For the experiment tap water was used instead of deionized water.



Figure 2. 4- Laboratory scale experimental set up in Phytotechnicum planted with *Juncus effuses*

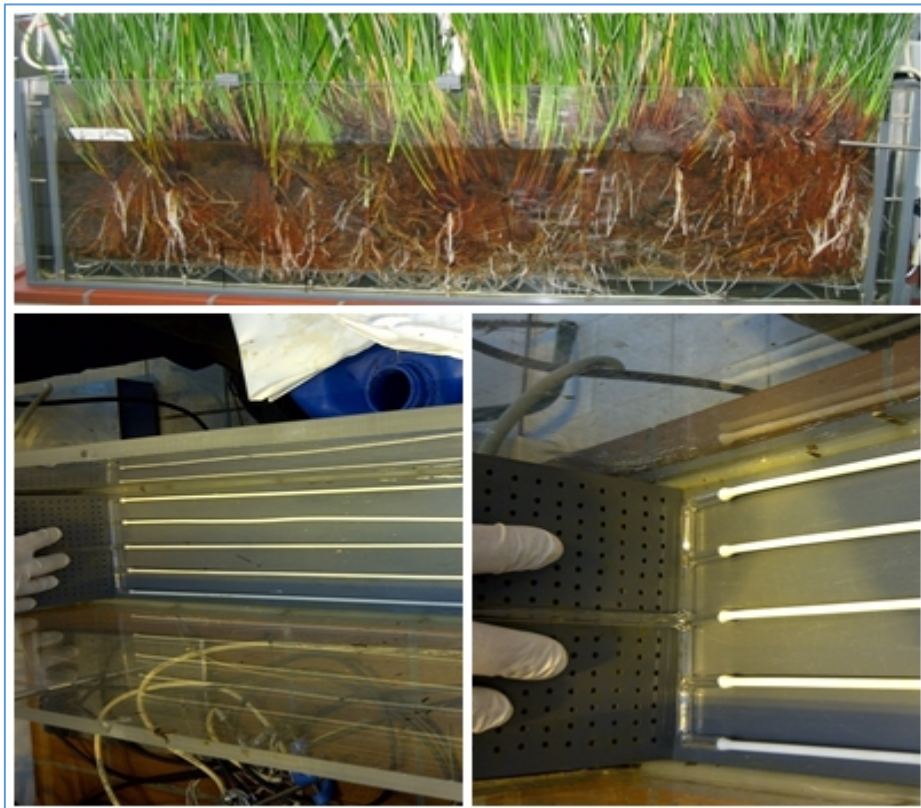


Figure 2. 5- Laboratory scale experimental set up in Phytotechnicum planted with *Juncus effuses* (1. Plant root mat, 2 & 3. Laying of hydrogen tubes at the bottom of the W2

2.6. Treatment of synthetic acid mine drainage in the laboratory-scale horizontal flow constructed wetlands with *Phragmites australis* Phytotechnicum (with H₂ as electron donor)

2.6.1. Experimental design

Two planted laboratory-scale hydroponic model wetlands were established to investigate the fate of sulphate and heavy metals in the rhizosphere of wetland vegetation, W1 without H₂ as electron donor and W2 with H₂ as electron donor.

The schematic diagram of a laboratory-scale subsurface horizontal flow wetland is shown in Fig. 2.1. The wetlands consisted of plastic containers of 100 cm in length, 15 cm in width and 35 cm in height. W2 is fed continuously with hydrogen gas through selective permeable silicone tubes placed at the bottom of the container (Fig.2.5). The water level was adjusted to 5 cm below the surface in both the systems. Sieves of perforated plastic were placed 3 cm in front of the inflow and outflow of the wetland. This free liquid volume should ensure an equal distribution of the inflow and a laminar liquid flow through the wetland. Model wastewater was pumped continuously from the storage tank to the inlet of each wetland by means of a well-calibrated peristaltic pump which ensured the same flow rate for all the experimental wetlands. The inflow rate was adjusted to an average value of 7 l d⁻¹ (~4.9 ml min⁻¹), which provided a hydraulic retention time of 4.2 days. Both the wetlands were planted with almost equal amount of plants and the roots of the plants serve as the matrix in the wetlands.

No H₂ gas was fed into Wetland 1 (W1) which was constructed in parallel representing as control wetland and the same model wastewater were fed into it as of W2 which was continuously fed with H₂ at 1.8 bar pressure. Wetland W1 provided a baseline to compare plant performances in the treatment wetlands. Each laboratory-scale subsurface horizontal wetland was fed separately from the separate storage tank (35 l capacity). The storage tank was used for storing the synthetic wastewater to be treated in the wetlands. The tank had to be re-filled before being empty to ensure uninterrupted flow of wastewater into the wetland beds.

2.6.2. Plant biomass

Before starting the experiment, wetlands (W1 and W2) were planted uniformly with *Phragmites australis* in December 2013 with a mean shoot density of approximately 420 and 427 shoots m⁻², respectively. This species of macrophytes was selected presumably on the basis of their common occurrence, rapid growth into their biomass (plant biomass production), rooting depth, tolerance to high loads of wastewater and abundance in natural wetlands. During the acclimatization period of approximately 3 months, plants were fed only with tap water and fertilizer (NPK Wasserlösliches Nährsalz, Hakaphos, concentration of 1 g l⁻¹) as plant nutrient source.

2.6.3. Experimental conditions

Experiment was conducted for a period of 8 months. This experiment had a sufficient duration to guarantee a representative number of samples taken from each experimental wetland. Artificial wastewater was freshly prepared in every 3 days to prevent microbial degradation during storage and operation. Synthetic AMD was prepared in a 120 L tank and an air compressor was used to mix the ingredients of the AMD and then it was distributed evenly into each inflow tank (Table 2.1, 2.2 and 2.3).

Model wetlands were placed in a phytotechnicum (Fig. 2.6) with 16-h day length and operating under defined environmental conditions with a temperature of 22 ± 2 °C simulating an average summer day in moderate climates (Wiessner et al., 2005a). The operational periods lasted from March 2014 to August 2014.

The key concept of how a demonstration horizontal subsurface flow wetland for sulphate and heavy metal removal might work under carbon deficient and anoxic environmental condition (both in presence and absence of H_2 as electron donor) was carried out and studied in the two model wetlands W1 and W2, in this experiment. The artificial AMD that was prepared and used in this investigation contained Fe, Mg, Ca, Al metals along with high amount of SO_4^{2-} . The inflow concentrations of the used ingredients were (in $mg\ l^{-1}$): 650, 72.5, 19.5, 59.8, 28 of SO_4^{2-} , Fe(3), Al, Ca and Mg respectively. No additional organic carbon sources were added to the synthetic AMD. The only carbon source was the organic carbon diffused from the roots of the plants. This amount of diffused organic carbon was enough to ensure a persistent anaerobic condition in the wetland beds and allowed the activity of anaerobic microorganisms. For the experiment tap water was used instead of deionized water.



Figure 2. 6- Laboratory scale experimental set up in Phytotechnicum planted with *Phragmites australis*

2.7. Maintenance

Prior to start of each experimental phase and several times during each phase operation, all the model wetland units were checked on regular basis and well-maintained to ensure the same initial and running conditions. Hence the systems were inspected on, at least, a weekly basis

concerning the overall functioning. Major attention was given to the inlet and outlet flow pipes, tubes and structures, which were checked twice a week after re-filling the inflow storage tank and being made empty of the three outflow tanks, as obstruction/clogging of the pipes due to inner-surface biofilm formation from continuous organic carbon loading in the influent and effluent could occur. A general cleaning of all the inter-connected pipes and tubes was usually undertaken twice a month.

2.8. Sampling

Pore water samples were collected on a monthly basis at seven consecutive locations. The location of sampling points are Inflow tank, 0 cm, 25 cm, 50 cm, 75 cm, 100 cm (Fig 2.1) and outflow tank along the flow path of the wetland. At location 25 cm, 50 cm, 75 cm of sampling points, pore water samples are collected from two depths i.e. 15 and 30 cm. Pore water samples were pumped out using long needle attached to pumps at a flow rate of 1L/3 h. Sampling locations at 0 and 100cm represent the inflow and outflow of the wetlands, respectively. Sample preservations (unless analyzed immediately) were made according to the standard preservation techniques suggested by various analytical methods for the compounds to be analyzed.



Figure 2. 7- Measurement of oxygen, temperature and pH while sampling

2.9. Analytical methods and calculations

All the collected samples were analyzed with the methods as described in the following sections. Parameters of all physical, chemical and biological activities which were analyzed during different experimental phases both in PFR and in horizontal subsurface flow model wetland operation are listed below along with brief analytical techniques and calculation procedure.

2.9.1. Sulphate

Sulfate was measured by a photometric method measuring the turbidity of barium sulfate (detection limit of 2.0 mg/L) at a wavelength of 880 nm. The photometric methods were measured by CADAS 100, LPG 210 spectrophotometer from DR LANGE.

2.9.2. Sulphide

Sulfide was analyzed with a photometric quick test (Hach Lange GmbH: LCW 053; detection limit of 0.15 mg/L) at a wavelength of 665nm. The photometric methods were measured by CADAS 100, LPG 210 spectrophotometer from DR LANGE.

2.9.3. Elemental sulphur

Elemental sulphur (S^0) was also determined according Rethmeier et al., 1997 by extracting samples with chloroform and the subsequent detection by HPLC (Beckman, USA) using a Li Chrospher 100, RP 18 column (5 μ m, Merck, Germany) and equipped with a UVdetector at 263 nm. The detection limit for elemental sulphur was about 0.064 mg l⁻¹.

2.9.4. Sulphite and thiosulphate

Concentrations of sulphite and thiosulphate were analyzed after derivatisation with monobromobimane and determined by HPLC (Beckman, USA) using fluorescence detector RF 551 (Shimadzu, Japan) and columns Li-Chrospher 60, RP Select B (250-4) according to Rethmeier et al. (1997).

2.9.5. Metals

Metals present in the artificial AMD are Al, Ca, Fe, Mg, and are measured by 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 0.005 mg/l of Fe, 0.02 mg/l of Al, 0.0005 mg/l of Ca and Mg. Variations of data from triplicate analysis were within ± 5 % of the average for all elements.

In ICP-AES, a sample solution is introduced into the core of Inductively Coupled argon plasma (ICP), which generates temperature of approximately 8000 degree Celsius. At this temperature all the elements become thermally excited and emit light at their characteristic wavelengths. The light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its constituent's wavelength. Within the spectrometer, this diffracted light is then collected by wavelength and amplified to yield an intensity measurement that can be converted to an elemental concentration by comparison with calibration standards.

2.9.6. Redox potential (Eh) and pH

In laboratory-scale horizontal subsurface flow wetland redox potential was measured in-situ by direct pumping out the pore water from the sampling point of the respective wetland with

the same flow rate as like the main inflow and recorded the data every 5 minutes. A SenTix ORP electrode connected to a multiline P4 (WTW, Germany) was used in this case. To prevent air contact, the electrode was placed in a small flow through cuvette. The inlet of the cuvette was connected with a long robust injection needle which was inserted into the sampling point of the horizontal flow model constructed wetlands. The outlet of the cuvette was connected to a pump to suck water samples through the cuvette and thus the pore water came in contact with the measuring electrode. Sets of readings from each wetlands were recorded. Another flow through cuvette with pH electrode was connected in series with redox measuring cuvette in order to obtain both the pH and rH values from the same sampling position at the same time.

The proper functioning of the electrodes were tested and calibrated regularly with WTW solution for redox potential (Pt/Ag/AgCl in 1 M KCl, +220 mV/25 °C) and for the pH using standard pH buffer (pH 4.01 and pH 7.00) solutions. Redox potential (rH) values were converted to the potential relative to the normal hydrogen reference electrode (*Eh*) by taking the sample temperature into account.

2.9.7. Dissolved oxygen and temperature

Dissolved oxygen (DO) was measured with a portable DO meter with automatic temperature compensation. Concentrations were measured in flow through mode using an optical oxygen trace sensor system (sensor FTC-TOS7 and instrument FIBOX-3-trace, PreSens, Regensburg, Germany). A separate temperature probe and DO probe were immersed into the sampling position and pore water was pumped out from each sampling position of the model wetlands through the DO probe into a flow cell cuvette which was connected to a lasersensitive optode and automatically calculated dissolved oxygen and temperature online into a computer software package supplied by the manufacturer.

Pore water pumping rate was adjusted to the same rate of the main inflow rate (4.1 ml min^{-1}) so as to achieve a more real in-situ sample. The probe was calibrated against saturated water of known temperature and adjusted for atmospheric pressure according to the manufacturer's instructions and was rinsed with deionized water between each reading.

Daily average temperature of the greenhouse was also recorded with a standard laboratory alcohol-filled thermometer.

2.9.8. Evapo-transpiration and water balance

Initial and final conditions (weight and volume) of inflow feeding water and outflow water (duration of 3-4 days) were measured in order to calculate the evapotranspiration of the system. It was controlled by balancing the inflow and the outflow amounts of water. The total water loss was divided by the time and the number of plants in the reactor to calculate the theoretical specific transpiration rate per plants.

Considering water loss due to evapotranspiration (EvT), area specific mass loading rates were also calculated in order to achieve actual rate of inflow mass loading and thereby subsequent removal rate produced by the horizontal subsurface-flow model wetland systems.

2.9.9. Shoot density

The numbers of the shoots were obtained periodically, at approximately 30-days intervals throughout the experimental period by counting the number of total green shoots and divided by the area to calculate the density of the plants.

2.9.10. Specific removal rate

The specific removal rates of the wetland systems were calculated as the difference between the specific inflow and outflow loading rates ($\text{g m}^{-2}\text{d}^{-1}$). Specific (inflow/outflow) loading rate = [concentration (mg l^{-1}) x flow rate (L d^{-1})]/area (m^2).

2.9.11. Removal efficiency analysis

Performance index of the constructed wetland systems for metals and other contaminant removal was calculated by comparing the inflow and outflow loading rates, which was termed as removal efficiency and expressed in percentage (%). The removal efficiency was calculated using the following equation:

$$\% \text{ Efficiency} = ((\text{Inflow loading rates} - \text{Outflow loading rates}) / \text{Inflow loading rates}) * 100$$

3. Results and Discussion

3.1. Results with *Phragmites australis* (from green house, without external electron donor)

3.1.1. Dynamics of sulphur removal

Dynamics of sulphate-sulphur removal and formation of sulphide within the wetland systems have a major influence on metal removal (Rahman, 2008). Detailed data on loads (Load/Area) of sulphur compounds (SO_4^{2-} -S mean values) and standard deviations (SD) in the four CWs are presented in Table 3.1.1 and 3.1.2, respectively.

Under C-deficient high redox and high oxic condition (Fig 3.1.1), marginal decrease in SO_4^{2-} -S load in the outflow of Unplanted HSSF CW was observed. The mean inflow SO_4^{2-} -S load was 3.2 g/m².d which corresponds to 0.06 g/l (in all four wetlands). Prevailing oxic condition and lack of electron donor inhibited microbial sulphate reduction and likely became concentrated with the formation of a S-pool in unplanted CW.

On the other hand despite the fact that the other three planted wetlands were also not provided with any external electron donor, there was marked decrease in the SO_4^{2-} -S load in the outflow was seen. The mean inflow SO_4^{2-} -S load was 3.2 g/m².d (in all four wetlands) (Fig 3.1.1) and the overall mean outflow load (g/m².d) was 0.45372, 1.0, and 0.8632 which resulted an overall mean SO_4^{2-} -S removal of 85.93%, 68.75% and 72.81% in corresponding HSSF CW, HSF CW and HHRMF respectively.

Though in planted CWs no external carbon sources were added which could have been a limiting factor for sulphate reduction (Stein et al., 2007) but the organic carbon from the roots of the plants (rhizodeposition products) were enough to stimulate rhizosphere microbial growth and activity (Munch et al., 2005; Nikolausz et al., 2008; Van de Moortel et al., 2010) for dissimilatory sulphate reduction and maintain a low redox conditions in the planted wetlands, which is not the case in unplanted wetland. The released carbon compounds could act as electron donors to be oxidized during sulphate reduction, thus leading to an enhanced sulphide production.

In Fig. 3.1.1 it is seen that the effluent load increase as the experiment progressed. This can be explained with the decline in the health conditions of the plants. The overall growth rate of the plants declined by 22%, 31% and 28% in HSSF CW, HSF CW and HHRMF CW respectively shown in Fig. 3.1.8 which resulted in the decrease in evapotranspiration rate (Fig. 3.1.9) in all planted wetlands. This decline in the number of green shoots in CWs resulted in the less influx of the electron donor in the form of rhizodeposits which resulted in the increase in effluent load.

From the Fig 3.1.2, it is seen that the removal rate varied slightly in between the two sampling depths (15 cm and 30 cm depth from the surface of the wetland). In all the planted wetlands, it is observed that at 30 cm sampling depth, the removal rates were higher as compared to 15 cm depth. This can be attributed to more anoxic and redox conditions at deeper layer along with more root cover which facilitates greater dissimilatory sulphate reductions.

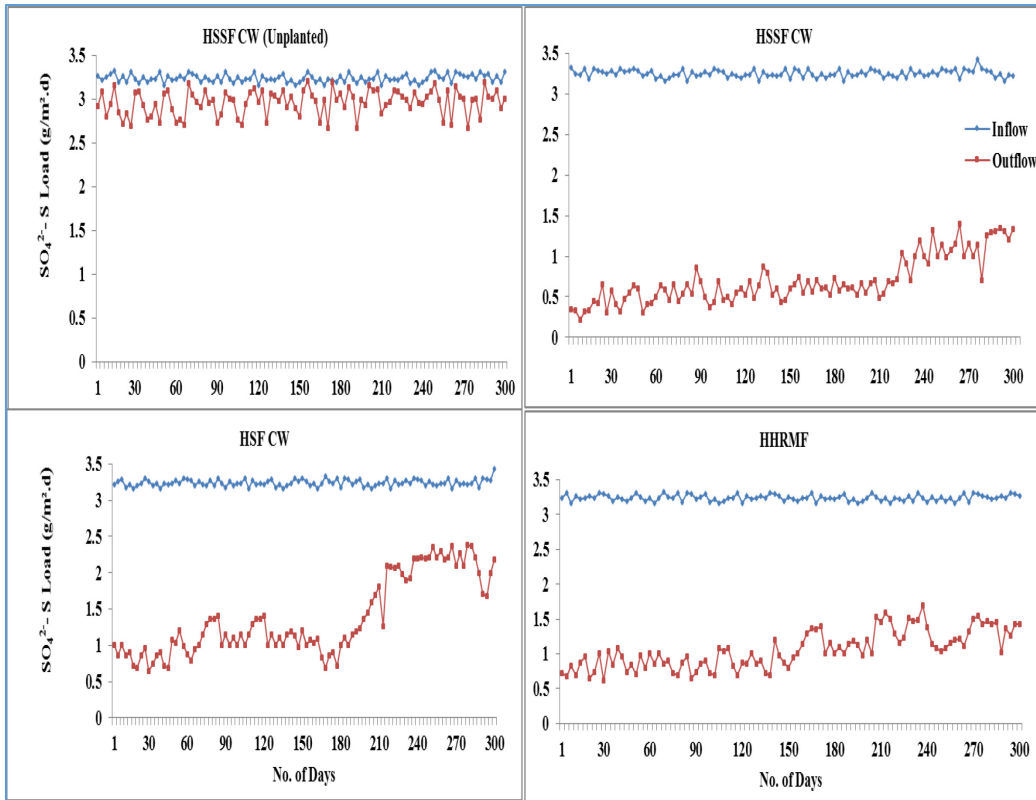


Figure 3.1. 1- Mean $\text{SO}_4^{2-}\text{-S}$ inflow and outflow load of wetlands.

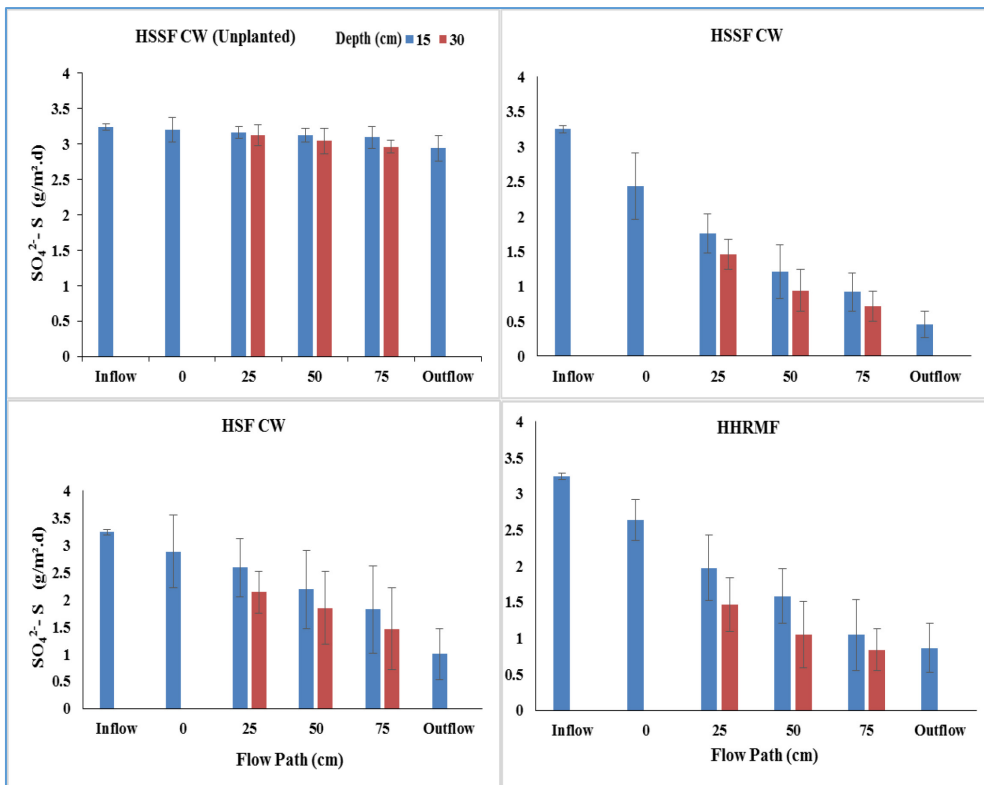


Figure 3.1. 2- Dynamics of mean $\text{SO}_4^{2-}\text{-S}$ load in the pore water along the flow path of the wetlands.

As the final product of dissimilatory sulfate reduction, sulfide was produced in all planted CWs. Strict and persistent reducing conditions resulted in anaerobic microbial sulphate reduction and subsequent increase in sulphide production (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman

et al., 2011) in all planted CWs. In this experiment the mean sulphide concentration measured in the outflow of HSSF CW, HSF CW and HHRMF were 23.2, 17.62 and 20.20 mg S²⁻ l⁻¹ which corresponds to 0.06, 0.036 and 0.045 g/m².d of S²⁻ respectively, Fig 3.1.3. In unplanted CW, since there was no external carbon source or plants to provide rhizodeposits, so sulphate reduction failed to occur and no S²⁻ was detected.

Sulfide generated from sulfate reduction can precipitate with metals to form metal sulfides, volatilizes (hydrogen sulfide emission), form organic sulfur compounds and undergo reoxidation in wetland sediments (Vymazal and Kröpfelová, 2008b). Since metals (e.g. Fe²⁺, Al³⁺) were present in the artificial wastewater feeding the systems, metal sulfides could be formed in the wetland models. The appearance of black precipitates showed that such precipitations occurred in the planted systems.

Sulfide concentrations exceeded 25 mg/L (0.07g/m².d) in planted CWs and were generally much higher from 25 cm to 75 cm along the flow path of the planted CWs. This is in agreement with the redox potential that is much lower at distances 25 cm and 75 cm than at distance 0 cm to 25 cm from inflow.

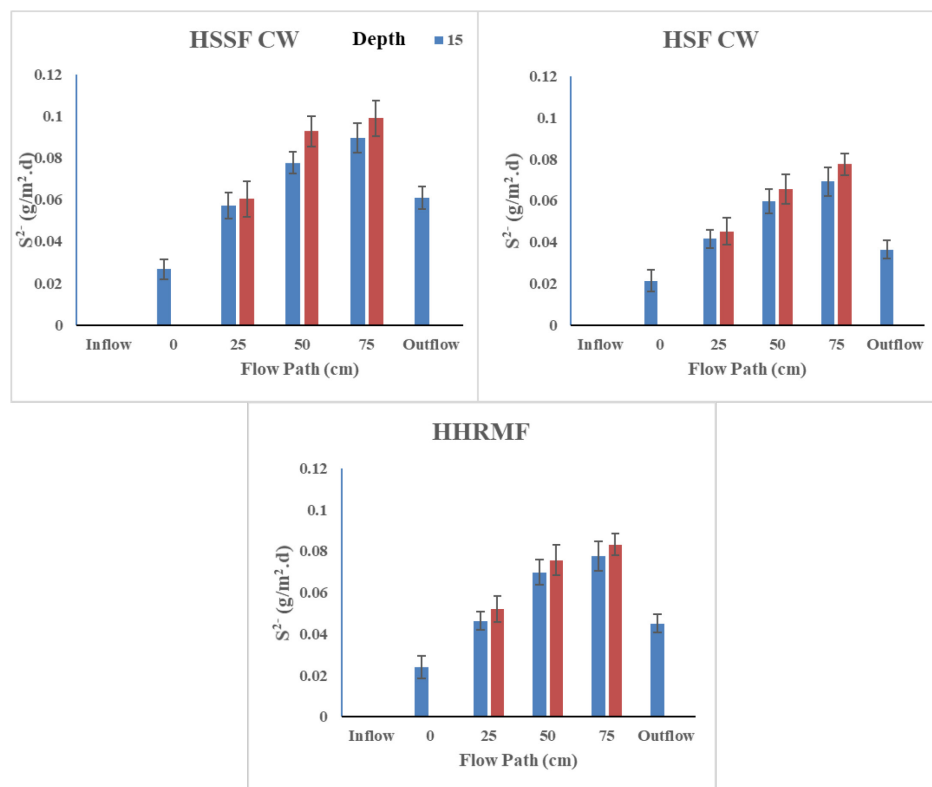


Figure 3.1. 3- Mean sulphide production (load/area) along the flow path of the wetlands.

It was evident that sulphate removal rate was higher in planted systems as compared to unplanted wetland (no sulphide was measured), due to the greater reducing conditions in the system with plants. Under this reducing conditions and presence of electron donor (rhizodeposits in case of planted wetlands) for the sulphate reducing bacteria resulted in better sulphate removal and in turn efficient metal removal (discussed in section 3.1.1).

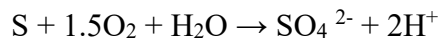
But plants exhibited toxic effects (decreasing water loss in terms of plant transpiration and less green shoots) probably due to sulphide toxicity along with high metal load (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011). As the experiment progressed the number of

healthy shoots decreased and the decrease in healthy shoots resulted in the less influx of rhizospheric carbon which in turn affects the sulphate removal. This is the major reason for the increase in the effluent load towards the end of the experimental period, Fig 3.1.1. The data for this experiment are shown in Fig. 3.1.8. Plants physiological inhibition of several helophytes was shown for S^{2-} concentrations of approximately 10 to 50 $mg\ l^{-1}$ (Armstrong et al., 1996; Chambers et al., 1998; Fürtig et al., 1996). The measured S^{2-} concentrations in this experiment were $>6\ mg\ l^{-1}$ in the outflow and $> 17\ mg\ l^{-1}$ inside the planted wetlands.

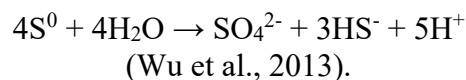
Further proof of dissimilatory sulphate reduction is provided by the detection of elemental sulphur in the planted wetlands as certain bacteria use elemental oxygen to carry out such oxidization of hydrogen sulfide to produce elemental sulfur. The concentration of elemental sulphur detected was 18.65, 11.48 and 15.34 $mg\ S^0\ l^{-1}$ which corresponds to 0.27, 0.46 and 0.37 $g/m^2.d$ (Fig 3.1.4) in HSSF CW, HSF CW and HHRMF CW respectively. In unplanted CW, since there was no source of external carbon source or plants to provide rhizodeposits, so sulphate reduction failed to occur and no S^0 was detected.

In CWs elemental sulfur can be formed via both chemical and biological sulfide oxidation ($2HS^- + O_2 \rightarrow 2S + 2OH^-$) (Chen et al., 2016; Wu et al., 2011). Elemental sulfur formed from bacterial sulfide oxidation can be deposited intracellularly or extracellularly. In many circumstances, white precipitates from elemental sulfur deposits have been observed in the outflows of treatment wetlands (Kadlec and Wallace, 2008). It is important to note that oxidation of sulphides produces oxidized sulphur species (i.e. S^0 , SO_4^{2-}) and may release associated metals or metalloids to the water column (Simpson et al. 1998). This may be the case of slightly elevated concentration of SO_4^{2-} -S in the outflow tank as compared to the outflow.

In this study, elemental sulfur concentration were high between 25 cm to 75 cm of the flow path in the planted wetlands. In HSSF CW and HHRMF the concentration of S^0 were between 20 to 25 $mg\ l^{-1}$ (0.02 to 0.05 $g/m^2.d$) whereas in HSF CW the concentration of S^0 was between 25 to 30 $mg\ l^{-1}$ (0.05 to 0.08 $g/m^2.d$). HSF CW has free surface of water on the top which is in contact with the oxygen as a result of this some amount of sulphide in the water can be oxidized to elemental sulphur. It is also seen that the S^0 production is slightly more at 15 cm depth as compared to 30 cm depth. This can be explained as the availability of oxygen at 15 cm depth is more as oxygen can diffuse to 15 cm depth as compared to 30 cm depth and also the root density is maximum at 15 cm depth in these wetlands so the oxygen transported from the roots to the CWs at this depth is also more. Oxygen released from plant roots has been reported to play an important role in oxidative processes in wetlands (Stottmeister et al., 2003; Wiessner et al., 2010). Oxidation of elemental sulfur can result in the elevation of sulfate in planted CWs:



In the wetlands, bacterial sulfur disproportionation could also contribute to the depletion of elemental sulfur, leading to the formation of sulfate and sulfide:



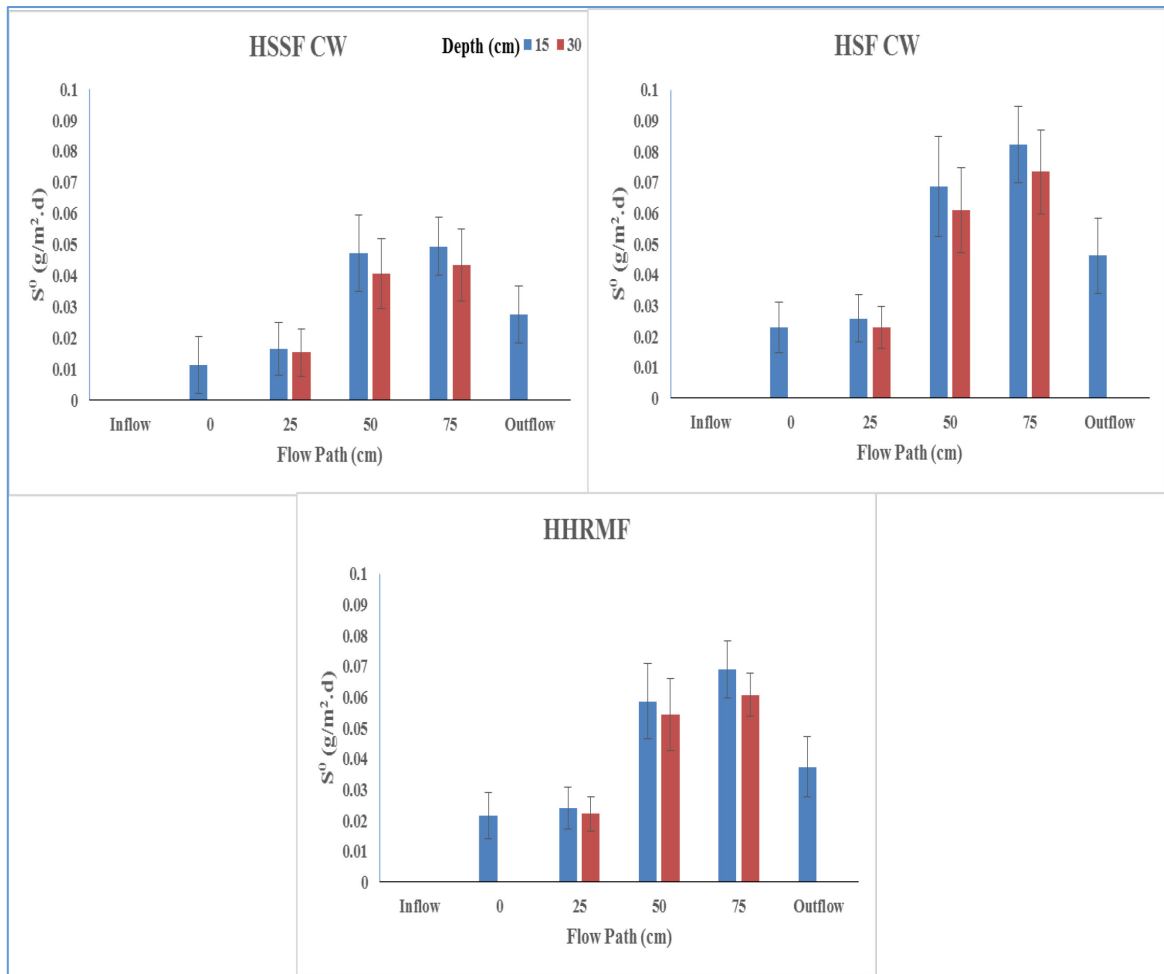


Figure 3.1. 4- Mean elemental sulphur production (load/area) along the flow path of the wetlands.

Overall, higher amounts of elemental sulfur in planted CWs were in good agreement with prior studies which showed that under more anaerobic conditions, more elemental sulfur was produced during sulfide oxidation (Celis-García et al., 2008; van den Ende et al., 1997).

Significant differences in the internal sulfur cycling and overall sulfur removal was noted among the model CWs. The synthetic wastewater contained sulfur mainly in the form of sulfate. The analyzed intermediately oxidized sulfur species (elemental sulfur, sulfite and thiosulfate) were detected inside all the planted systems. Since the influent did not contain any of the latter species, and as they can only be the product of abiotic or biotic oxidation of sulfide, it was concluded that DSR and sulfide oxidation processes occurred simultaneously.

Sulphur depositions (e.g. metal sulphide precipitations, elemental sulphur deposits) and the emission of hydrogen sulphide can result in total sulphur loss (Kadlec and Wallace, 2008). It has been documented that CWs could harbor an important inorganic and organic sulphur pool (Spratt and Morgan, 1990; Stottmeister et al., 2003; Wiessner et al., 2010).

The TS removal was highest in HSSF CW of about 80% followed by HHRMF, HSF CW and Unplanted HSSF CW about 66%, 61% and 8% (Table 3.1.1) respectively. Since in the effluent HSSF CW sulphate and other oxidized form of sulphur were found in less amount than the other CWs, hence sulfur transformations were more intensified in HSSF CW.

Table 3.1. 1- Sulphur mass balance in the planted and unplanted wetlands and mean removal of total sulphur (TS) in pore volume of planted and unplanted wetlands.

Wetlands	Influent (g/m ² .d)	Effluent (g/m ² .d)						Mean Removal TS*
	Total Sulphur	S ⁰	S ²⁻	SO ₃ ²⁻	S ₂ O ₃ ²⁻	SO ₄ ²⁻	Total Sulphur	
HSSF CW	3.2 ±0.06	0.0272 ±0.0121	0.0607 ±0.021	0.011 ±0.0016	0.08 ±0.019	0.453 ±0.2	0.6319 ±0.2537	80%
HSF CW	3.2 ±0.06	0.04568 ±0.023	0.0363 ±0.016	0.026 ±0.0019	0.132 ±0.0138	1.026 ±0.44	1.2659 ±0.4947	61%
HHRMF CW	3.2 ±0.06	0.03749 ±0.021	0.0448 ±0.018	0.020 ±0.0018	0.104 ±0.0142	0.863 ±0.39	1.0692 ±0.445	66%
Unplanted HSSF CW	3.2 ±0.06	Not Detected	Not Detected	Not Detected	Not Detected	2.94 ±0.19	2.94 ±0.25	8%

TS* is calculated as the sum of the 5 analyzed inorganic sulfur species.

3.1.2. Dynamics of iron removal

The dynamics of total iron (Fe²⁺ and Fe³⁺) with the values of mean inflow and outflow loads (Load/Area) and the removal efficiency are represented in Fig. 3.1.5 and Table 3.1.2. During the whole ten months of operation, a continuous supply of synthetic wastewater provided a mean inflow load of 0.116 g/m².d (2.18 mg/l) of Fe in all four reactors.

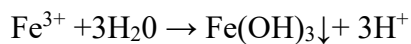
Under C-deficient anaerobic condition in the experiment, a highly efficient total Fe removal was observed in all three planted experimental wetlands. The mean outflow load was 0.0018, 0.00154 and 0.00161 g/m².d (Fig. 3.1.5 and Table 3.1.2) in HSSF CW, HSF CW and HHRMF respectively and the mean removal efficiency attained as 98%, 98.5% and 98.7% in HSSF CW, HSF CW and HHRMF respectively.

Table 3.1. 2- Iron mass balance in the planted and unplanted wetlands and mean removal of total iron (Tot. Fe) in pore volume of planted and unplanted wetlands.

Wetlands	Influent (g/m ² .d)		Effluent (g/m ² .d)			Mean Removal Tot. Fe
	Fe ³⁺	Fe ²⁺	Fe ³⁺	Fe ²⁺	Total Fe (g/m ² .d)	
HSSF CW	0.114 ±0.001	0.002 ±0.0002	0.0008 ±0.00003	0.00103 ±0.00103	0.0018 ±0.00106	98%
HSF CW	0.114 ±0.001	0.002 ±0.0002	0.00096 ±0.00005	0.00058 ±0.0003	0.00154 ±0.00035	98.5%
HHRMF CW	0.114 ±0.001	0.002 ±0.0002	0.00091 ±0.00006	0.00075 ±0.00004	0.00161 ±0.0001	98.7%
Unplanted CW	0.114 ±0.001	0.002 ±0.0002	0.110 ±0.036	0.0 ±0.0	0.121 ±0.036	9.7%

In the artificial AMD the iron was present in the form of ferric sulphate and the detection of ferrous iron was negligible. But as soon as the waste water enters the planted system, it is seen that the load of Fe(III) decreased and the load of Fe(II) increases (Fig. 3.1.5). Removal of Fe(III) under this condition is perhaps best explained by two methods i.e.

- (i) The effect of pH- The ferric form of iron remains soluble in water at acidic pH (2.5 and 3.5) but as soon as the pH increases (Fig. 3.1.6) it precipitates out in the form of hydroxides. This may be a reason for the decrease of ferric form of iron within the wetlands as the pH increased from 3.5 at inflow to > 4 at 25 cm length from the inflow, in all three planted wetlands. The Fe(III) reacts with water to form insoluble hydroxide (Kosolapov et al., 2004).



Initially most of the Fe(III) may have been removed as hydroxides due to the rise in pH. But at the same time there was an increase in the load of ferrous iron was seen and this phenomenon can be explained as microbial reduction of ferric form to ferrous form.

- (ii) The microbial reduction- Some of the Fe(III) may have been reduced to Fe(II) form, Fig 3.1.6. According to Kosolapov et al a wide range of anaerobic bacteria and archae bacteria are able to conserve energy through the reduction of Fe(III) to Fe(II). These Fe(II) form again can be removed by the formation of iron sulphides. Iron sulfides is formed as a result of microbial sulfate reduction (Rabenhorst et al., 1992). Sulfate-reducing bacteria (SRB) mediate dissolved sulfate reduction to hydrogen sulfide and metals are immobilized by the resulting sulfide. Iron sulphides formed in CWs may be iron mono sulfides (e.g. FeS) (Ahern et al. 2004, Morse and Rickard 2004) which is highly reactive and readily oxidizes on exposure to air, and the more stable forms of sulfur such as pyrite (FeS₂). Presence of black colour precipitates on the roots of the plants confirms the formation of FeS₂.

In the unplanted wetland, there is a marginal decline in the iron concentration even though there is no strong evidence of dissimilatory sulphate reduction. The Inflow load of Fe was 0.116 g/m².d and the outflow load was 0.107 g/m².d. There is a decrease of 4% in total iron concentration from inflow to outflow. The wetland was found to be oxic and there is a possibility of formation of oxyhydroxide. But for formation of iron oxyhydroxide, apart from oxygen the Fe should be present in ferrous form, since the iron used in this experiment is in ferric form (and concentration of ferrous form in inflow was also very low around 0.0375mg/l) so removal of iron in this oxyhydroxide form is likely less. Moreover, persistent oxic condition and lack of sufficient electron donor inhibited dissimilatory sulphate-reduction despite the relatively higher sulphate-sulphur concentrations in this wetland. So Under C-deficient aerobic condition and non-availability of ferrous form of Fe, the removal process can be best explained by the process of adsorption by the gravels of the wetland.

From the Fig. 3.1.6 it was observed that two-third of the total iron (most of them in the form of Fe(III)) was removed between 0 to 25 cm from the inflow. This is due to the fact that the pH was increased from 3.5 to 4.16. Sulfate reduction consumes acidity and raises water pH (Hedin and Nairn, 1992). This increase in pH from acidic pH facilitated the precipitation of Fe(III) hydroxide. The Fe(II) precipitates as mono sulfides (e.g. FeS) or pyrite. Soil sediments

examinations could not be performed due to constrain in time so the results provided are based on the examinations of pore water volume.

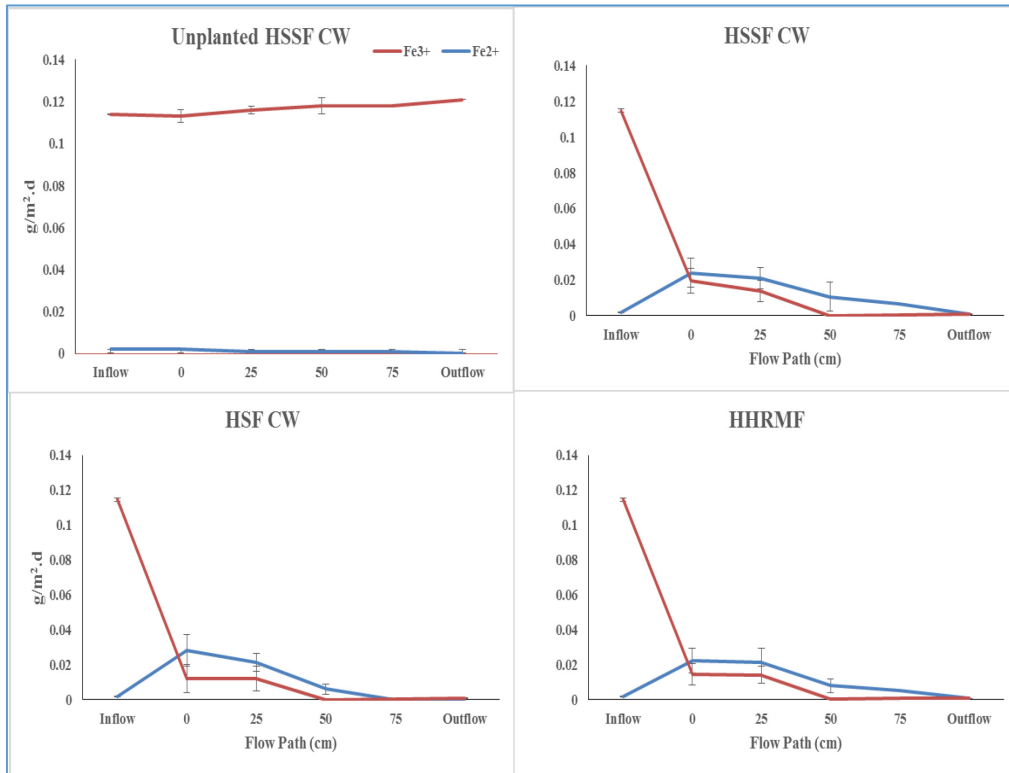


Figure 3.1. 5- Dynamics of mean Fe^{2+} and Fe^{3+} load in the pore water along the flow path of the wetlands.

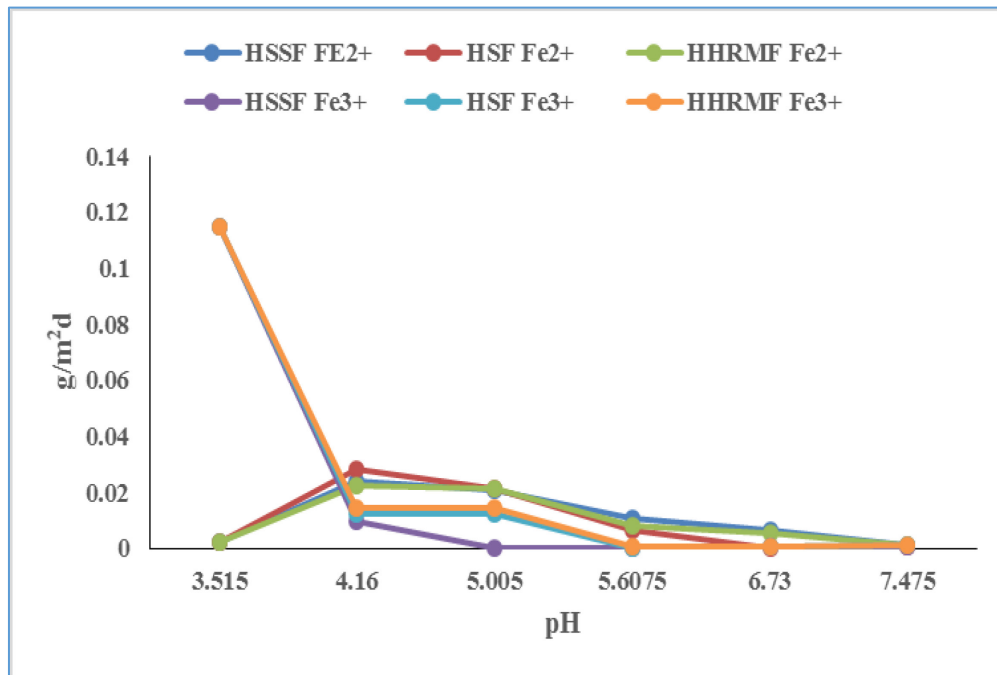


Figure 3.1. 6- Behavior of Fe^{2+} and Fe^{3+} with respect to the changes in pH in the planted wetlands.

According to Eger, 1994, one mole of sulfate would be reduced for each mole of divalent metal (Fe^{2+}) precipitated. The removal of each mole of aluminium and ferric iron (trivalent metal) requires 1.5 moles of sulfate reduction.

Table 3.1.3 shows the amount of sulphate used up for the removal of Tot Fe in the planted wetlands.

Table 3.1. 3- Total sulphate required for removal of total iron in the planted wetlands

Contaminants	Contaminants Removed (moles/m ² .d)			SO ₄ ²⁻ used up for removal of Fe ²⁺ and Fe ³⁺ (moles/m ² .d)		
	HSSF CW	HSF CW	HHRMF CW	HSSF CW	HSF CW	HHRMF CW
SO ₄ ²⁻	0.156	0.135	0.1331			
Fe ²⁺	0.000017	0.00003	0.000022	0.000017	0.00003	0.000022
Fe ³⁺	0.00202	0.002	0.002	0.00303	0.003	0.003
Tot. Fe	0.002037	0.00203	0.002022	0.0030347	0.00303	0.003022

3.1.3. Dynamics of aluminium removal

The dynamics of total aluminium with the values of mean inflow and outflow loads (Load/Area) and the removal efficiency are represented in Fig. 3.1.7. During the whole ten months of operation, a continuous supply of synthetic wastewater provided a mean inflow load of 0.2816 g/m².d (5.3 mg/l) Al in all four reactors.

Al³⁺ hydrolyzed in the water (which is known to occur by coordinating 6 water molecules to form an aquometal ion, Al(H₂O)₆(3+)) and then chemically precipitated as Al(OH)₃. It has been reported that the pH of minimum solubility for Al(OH)₃ falls in the range of 6.1– 6.3. (Snoeyink et al., 2003). Other studies have revealed that Al(OH)₃ has a minimum solubility in the pH range of 6 –7 (Kadlec and Knight, 1996; Metcalf & Eddy, 2003; Zouboulis and Traskas, 2005; Hu et al., 2006).

Under C-deficient anaerobic condition in the experiment, a highly efficient Al removal was observed in all three planted experimental wetlands. . The mean outflow load is 0.02, 0.05 and 0.03 g/m².d (Fig. 3.1.7) in HSSF CW, HSF CW and HHRMF respectively and the resulting outflow load showed nearly 10-fold declination in total Al and the mean removal efficiency attained as 93%, 82% and 89% in HSSF CW, HSF CW and HHRMF respectively. Removal of Al under this condition is perhaps best explained by formation of aluminium sulphate because in presence of dissolved sulfate Al may also precipitate as hydroxysulphate wetland (Kadlec and Wallace, 2009). Due to rise in pH (Fig. 3.1.12) in all three planted wetland Al precipitates as hydroxides (Eger, 1994) (because of the small oxmic microgradient present at the root zone) which is retained by the wetland.

In the unplanted wetland there is a marginal decline in the Al concentration in the outflow even though there is no strong evidence of dissimilatory sulphate reduction. The Inflow load of Al was 0.28 g/m².d and the outflow load was 0.25 g/m².d. There is a decrease of 12% in Al concentration from inflow to outflow. The wetland was found to be oxic and there is a

possibility of formation of oxyhydroxide. But for formation of aluminium oxyhydroxide, apart from oxygen the pH should be in neutral to alkali form, since the pH in unplanted wetland was in acidic form Al should occur as Al^{3+} form in aqueous form so removal of Al in this oxyhydroxide form is likely less. Moreover, persistent oxic condition and lack of sufficient electron donor (rhizospheric carbon) inhibited dissimilatory sulphate-reduction despite the relatively higher sulphate-sulphur concentrations in this wetland. So Under C-deficient aerobic condition and acidic pH of Al the removal process can be best explained by the process of adsorption by the gravels of the wetland.

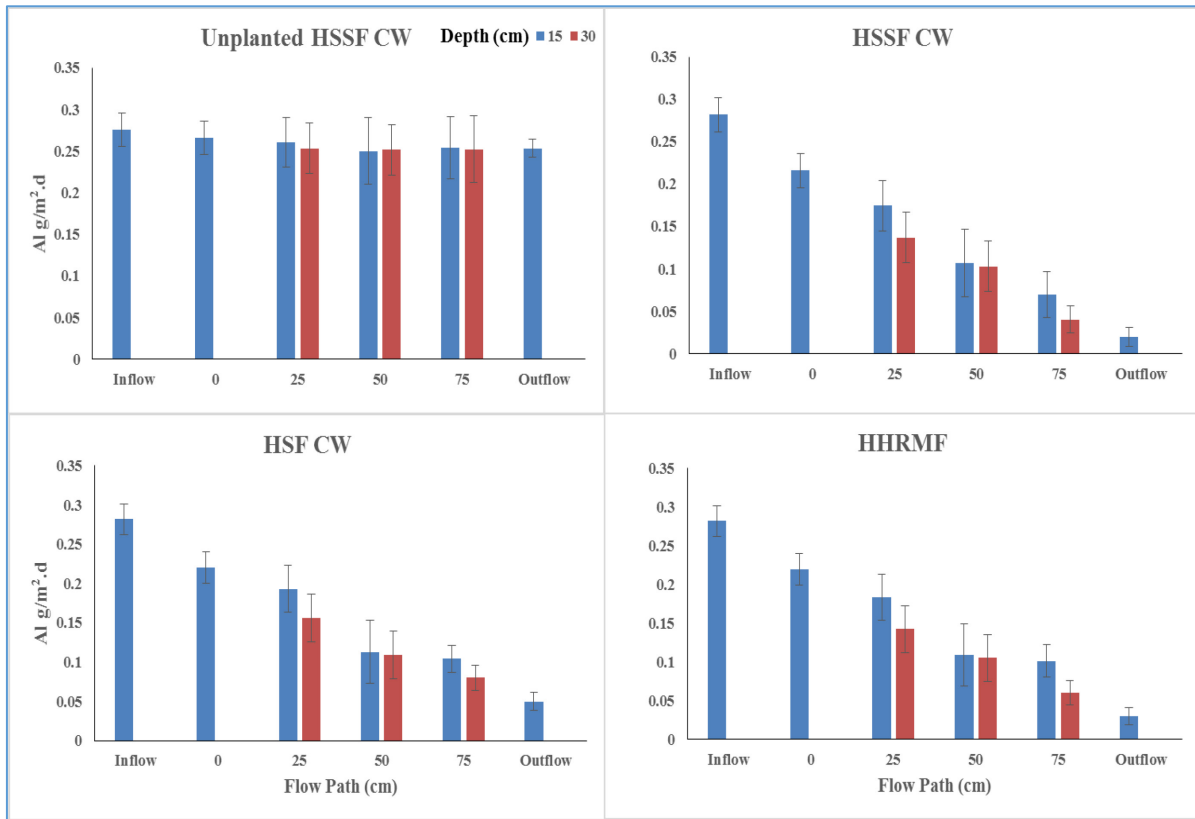


Figure 3.1.7- Dynamics of mean Al load in the pore water along the flow path of the wetlands.

3.1.4. Further parameters (shoot density, EvT, Eh and pH)

3.1.4.1. Growth of plant biomass (shoot density) and water loss (EvT)

Water loss from the wetlands as evapo-transpiration (EvT) was taken into consideration for the calculation of removal rates, which is the mass removal loading rates. This was particularly important to distinguish between planted and unplanted wetlands as because the rate of water loss due to the presence of plant biomass in planted wetlands hugely differed with the wetlands without plants (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011).

The growth status of the plant biomass in terms of total number of green and healthy shoots and therefore the variation in water loss via EvT is shown in Fig. 3.1.8. By the end of experiment, corresponding to 10 months of operation, there was decrease in overall shoot density. There was 22%, 31%, and 28% decrease in shoot density from an initial 3540 m⁻²,

3706 m⁻² and 3886 m⁻² to 2746 m⁻², 2560 m⁻² and 2800 m⁻² in HSSF CW, HSF CW and HHRMF CW respectively.

In the start the plants were able to take the stress and were healthy but as the experiment continued further, plants were stressed by the low pH and perhaps damaged by high sulphide formation. Due to strict anaerobic conditions, rapid sulphide formation within the anaerobic root zone might have caused mortality of wetland plants decreasing their growth rate (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011).

It appeared very important to notify that number of green shoots in the segment between 50 cm to 100 cm from inflow exhibited severe stress and shoot density decreased rapidly from an initial 3481 m⁻², 3640 m⁻² and 3792 m⁻² to only 2533m⁻², 2346 m⁻² and 2693 m⁻² respectively in HSSF CW, HSF CW and HHRMF CW at the end of experiment. Plant shoot density in this segment of wetlands declined rapidly due to the prolonged exposure to high loads of metals along with high rate of sulphide production and less nutrients being available to the plants in the latter half of the wetland.

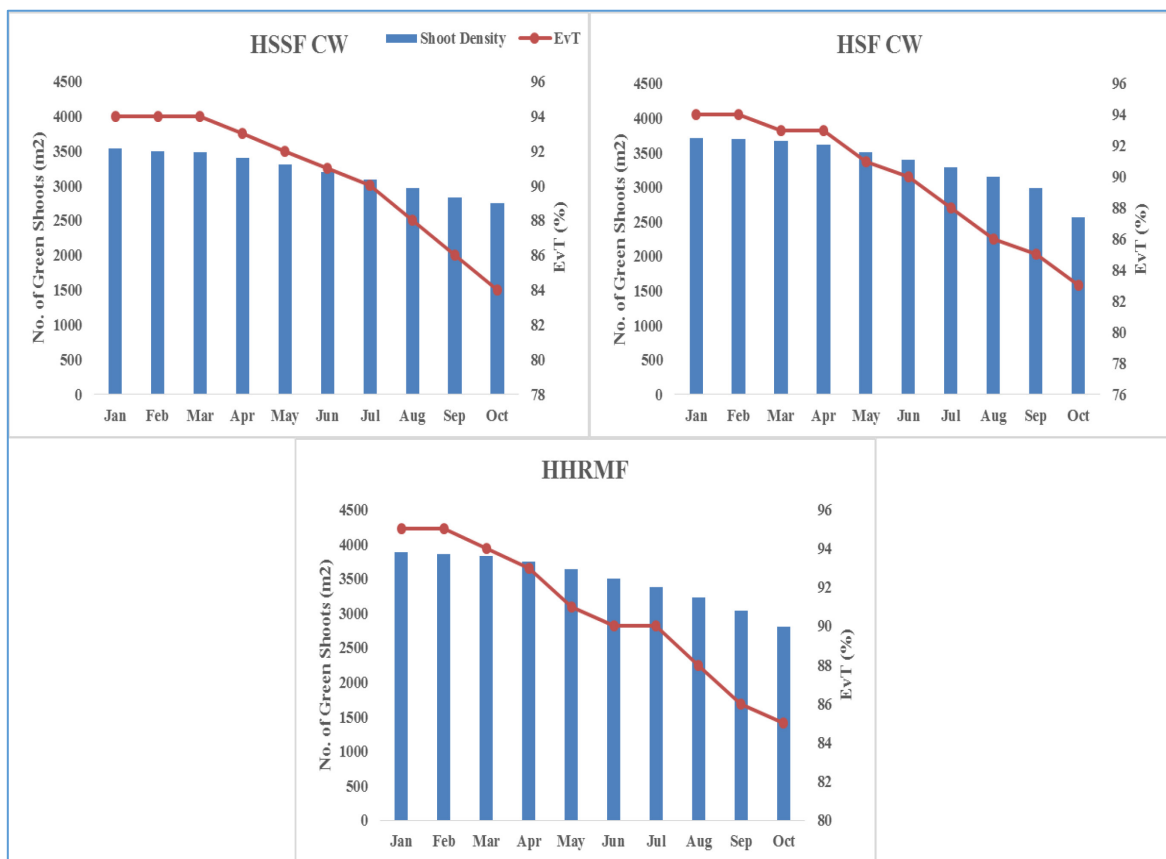


Figure 3.1. 8- Correlation between water loss by plant transpiration (EvT) and green shoot density during whole operation period in the planted wetlands.

In general, higher plant shoot density resulted in high water loss via plant transpirations but as the experiment progressed the number of healthy green shoots started to decrease and hence a decreasing tendency of water loss after the first four month of the experiment.

Temperature also plays a very important role for the water loss in CWs. There should be a direct relationship between the mean monthly temperature and the water loss, but as the shoot

density started to decrease the water loss also decreased irrespective of the mean monthly temperature.

In unplanted HSSF CW (control) no obvious changes or drastic fluctuations in EvT (between 5% to 8% EvT rate) (Fig. 3.1.9) was seen, so it suggests only evaporation from the unplanted HSSF CW bed was the only way for water loss due to the absence of plants.

The effect of the plants on the removal efficiency of heavy metals and sulphate was studied in planted HSSF CW, HSF CW and HHRMF CW as compared to unplanted HSSF CW (control) and in all cases. It was observed that planted wetlands considerably enhanced the retention of heavy metals than the unplanted HSSF CW. Likewise the presence of plants substantially improved sulphate retention within the wetland beds because plant roots serve as carriers for attached microbial growth, transfer oxygen and release exudates into the root zone, it leads to an efficient contaminant removal than in constructed wetlands without plants (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011).

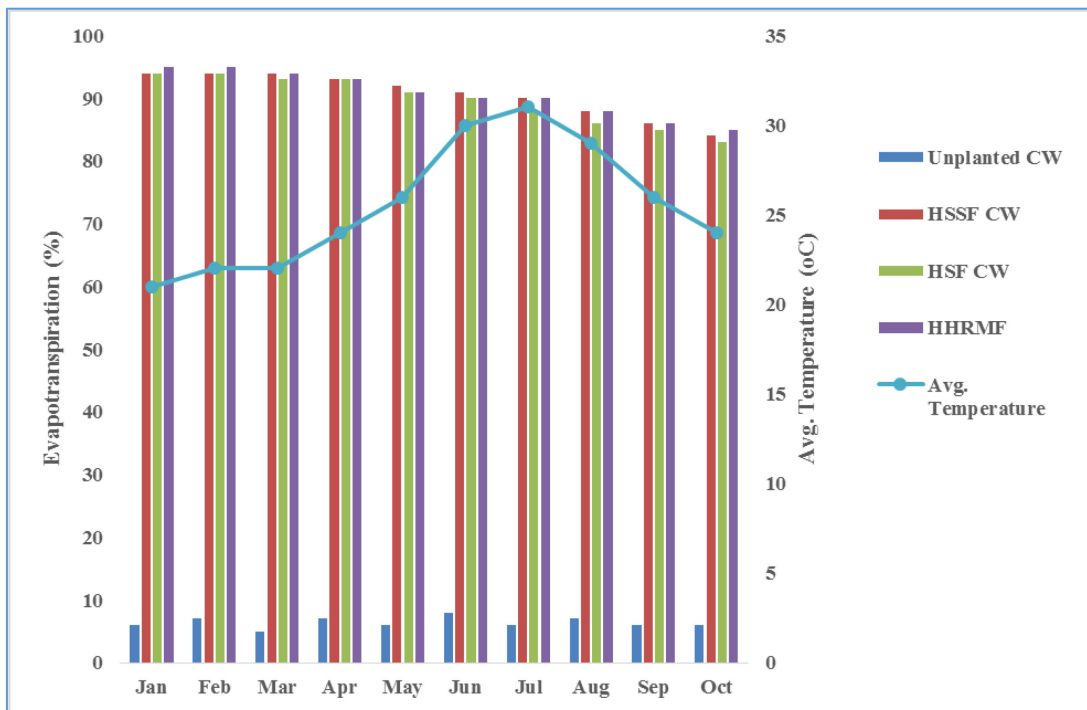


Figure 3.1. 9- Correlation between evapotranspiration (EvT) and temperature fluctuations during whole operation period in the wetlands.

3.1.4.2. *Redox potential (Eh), Dissolved oxygen (O₂) and pH*

In fact, the removal mechanisms of metals and sulphate are dependent on the pH and redox potential of the wetlands. The redox potential (Eh) shows the oxidation-reduction conditions and is related significantly to pollutant removal processes in CWs (Vymazal and Kröpfelová, 2008b; Wiessner et al., 2005a).

In this study, mean inflow oxygen concentration value was always very high around 8 mg/l during the entire experimental period in all the wetlands, whose results are presented in Fig. 3.1.10, varied dependent on system, distance from inflow, and depth. There was a marked differences in values between the unplanted and planted wetlands. The unplanted wetland was

always very oxic in nature whereas the planted wetlands were anoxic in nature. The highest concentration of DO was seen in the inflow region of all planted wetlands but the inside of system was strictly anaerobic. Of all the planted wetlands the HSSF CW was the most anoxic (8.126 mg/l at inflow 0.3432 mg/l at outflow) and the HSF CW to be more oxic (9.26 mg/l at inflow and 0.63 mg/l at outflow). The DO measured in HHRM was 8.13 mg/l at inflow and 0.416 mg/l at outflow. This trend was as well reflected on the redox potential levels in the different systems (Fig. 3.1.11). Low redox potential along with anaerobic conditions in the planted wetlands helped in carrying out dissimilatory sulphate reductions as presence of oxygen in the reactors hinders the process of dissimilatory sulphate reduction as it is toxic to sulphate reducing bacteria as well as it does not helps in attending the right redox potential needed for the process (Marschall et al., 1992).

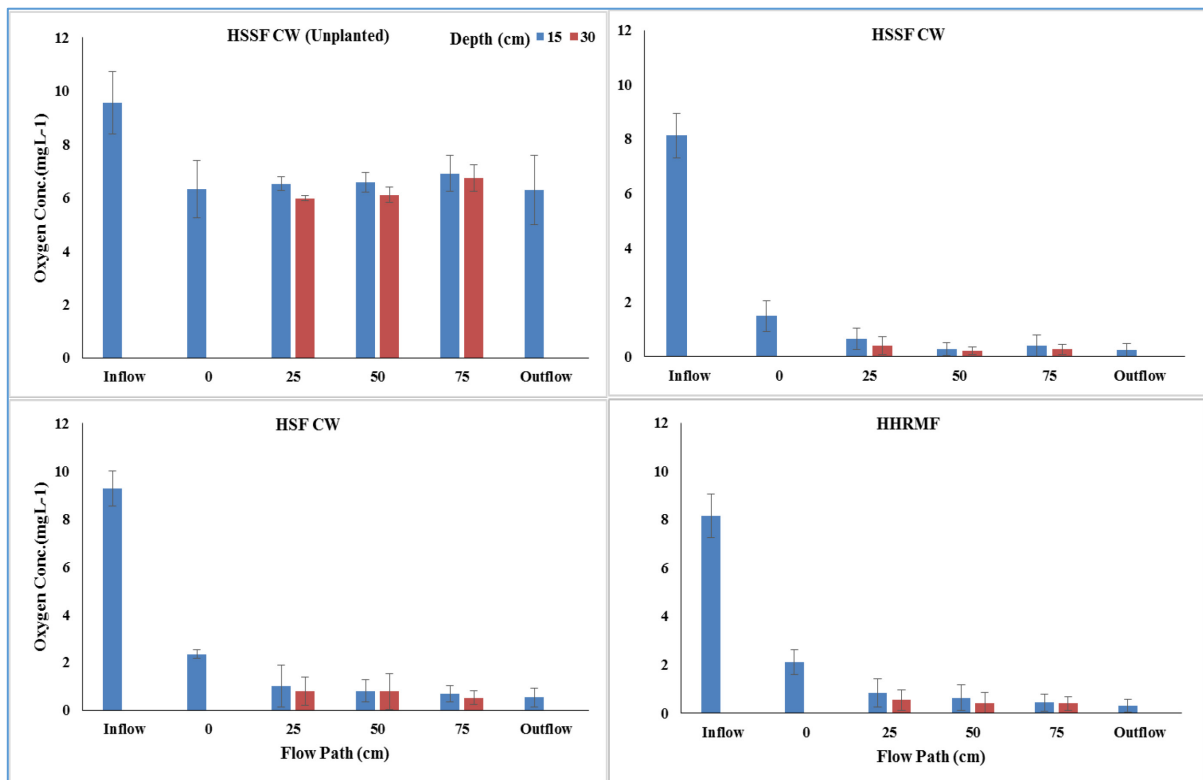


Figure 3.1. 10- Dynamics of mean dissolved oxygen concentrations in the pore water at different depths along the flow path of wetlands.

In the study, values of DO in most of the sampling points in planted CWs were below 0.5 mg/L. The results were corroborated with traditional observation that HSSF CWs are mostly anaerobic systems where DO concentrations are very low or undetectable (Garcia et al., 2010; Vymazal and Kröpfelová, 2008a).

Redox potential, whose results are presented in Fig. 3.1.11, varied dependent on system, distance from inflow, and depth. There was a statistically significant difference in redox values between different wetland types. The unplanted HSSF CW was characterized by stable and very high redox conditions between ~ 527 mV (Inflow) ~ 493 mV (75 cm, 30 cm depth). The most negative redox values were observed in the planted HSSF CW between ~ 519 mV (Inflow) and ~ -162 mV (75 cm, 30 cm depth) followed by HHRMF CW ~ 517 mV (Inflow) and ~ -153 mV (75 cm, 30 cm depth) and HSF CW ~ 510 mV (Inflow) and ~ -137 mV (75 cm, 30 cm depth) and these decreased statistically significantly with distance from inflow. Redox values

in the model CWs were found in the range of dissimilatory sulfate reduction ($E_h < -100$ mV) (Reddy and D'angelo, 1994).

Redox values in the HSF CW are higher among the planted wetlands as this system has a free water surface above the gravel. Though, the HHRMF CW was thought to have higher redox potential among the planted CWs but it was not the case as it did not have any free water surface because it was a root mat filter CW not a free floating root mat CW. While some amount of oxygen entered the systems with the inflow, plant roots remained main oxygen source along the flow path. Root exudates (Brix, 1997) and oxygen release by *P. australis* which can range from 0.02 to 12 g m⁻² d⁻¹ (Armstrong & Armstrong, 1990; Brix, 1990; Gries et al., 1990) both enhance microbial activity substantially. Therefore, low redox is product of the increased microbial turnover in the planted systems. Consistent low redox potential values were maintained in all planted CWs throughout the experiments which ensures the activity of anaerobic sulphate reducers.

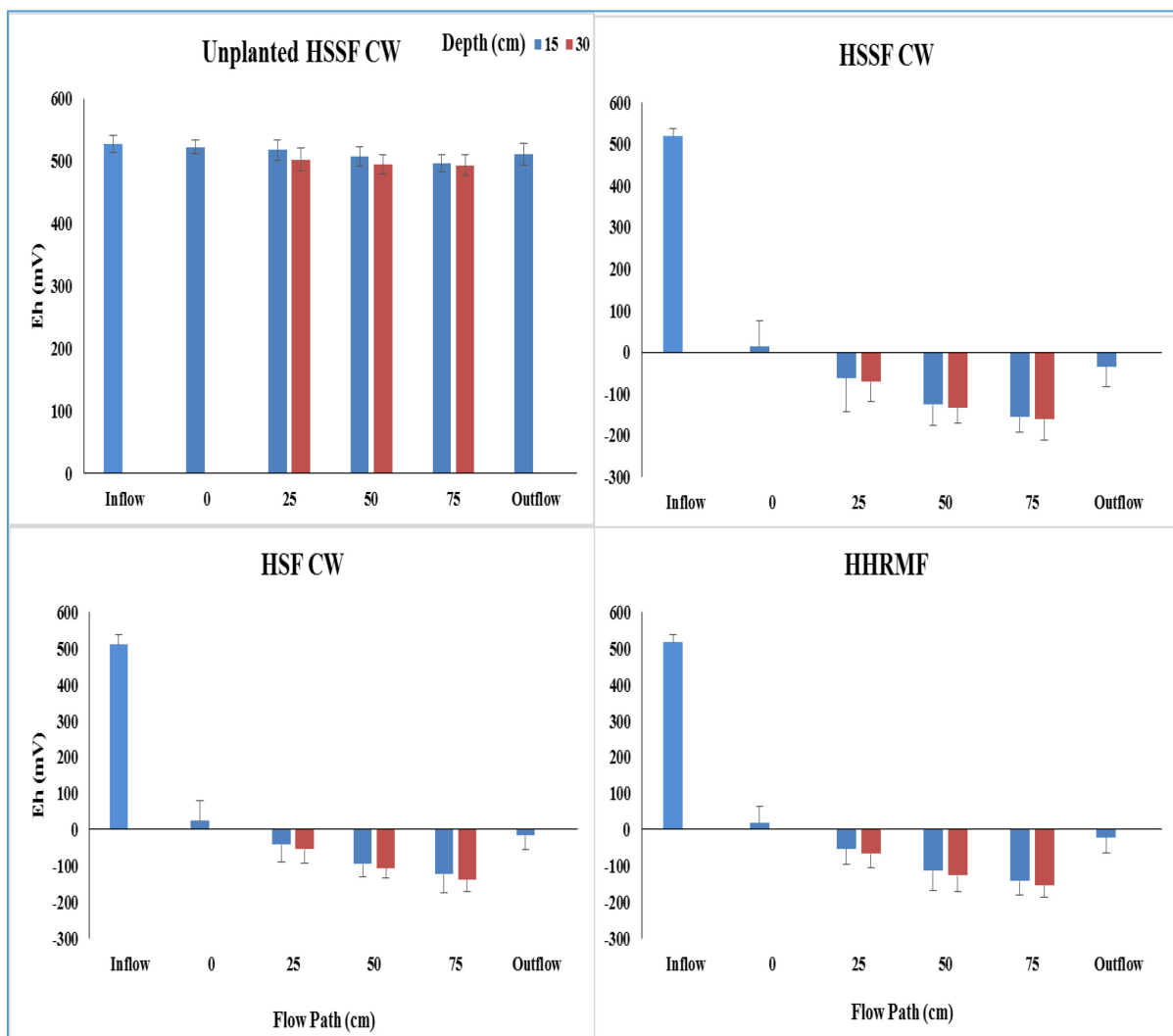


Figure 3.1. 11- Dynamic mean redox conditions in the pore water at different depths along the flow path of wetlands.

Fig. 3.1.12 shows the interrelationship between redox potential and DSR in this experimental study. In the inflow the redox potential tends to be very high in all the three planted wetlands with no sulphide being detected. As we move from inflow towards outflow the values for redox potential tends to be in negative. The most negative values was measured at 75 cm length from

the inflow. As the redox potential values becomes more and more negative we see an increasing trend in the sulphide production. Very low amount of sulphide was detected at 0 cm of the wetlands and then it increases towards the outflow. Sulphide production was highest at 75 cm length of the CWs where the redox values were also the lowest. This result is in agreement with the fact that DSR occurs at negative redox potential. The intensity of DSR increased from 25 cm to 75 cm length of the wetlands as the pH between 25 cm and 75 cm is within the range of 4.5 to 6.5 which is the ideal pH for maximum activity of the bacteria carrying out DSR (Jong and Parry, 2003).

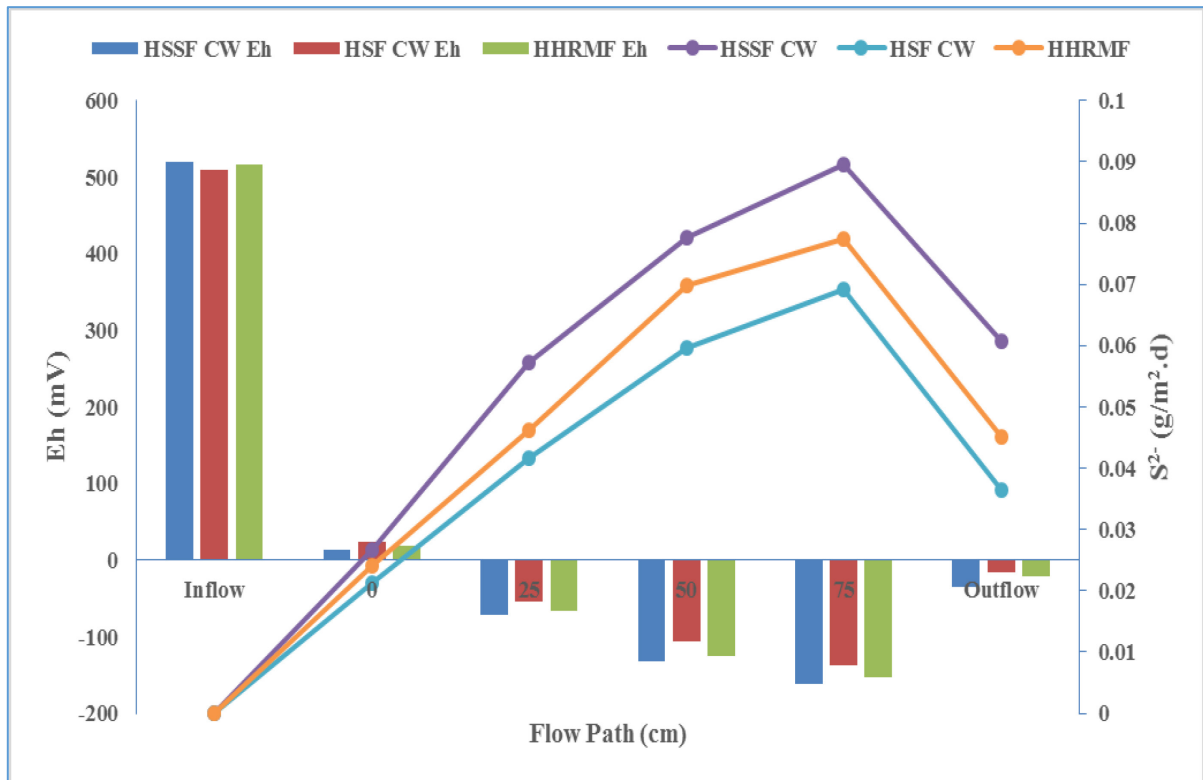


Figure 3.1. 12- Correlation between redox potential and DSR leading to sulphide production along the flow path in the planted wetlands.

pH is an important factor influencing microbial processes and is associated with nutrient and metal removal in CWs (Reddy and D'angelo, 1994). The pH behavior in the experimental model wetlands is shown in Fig. 3.1.13. In this study, mean inflow pH value was always in the range of 3.5 to 3.6 during the entire experimental period in all the wetlands. pH varied dependent on system, distance from inflow and depth.

There was a marked differences in pH values between the unplanted and planted wetlands. The highest pH was seen in the outflow of all the wetlands. The outflow pH value is highest in HSSF CW at 7.8 followed by HHRMF CW at 7.6 and HSF CW at 7.3. The change in the pH value from acidic to alkalinity is due to the dissimilatory sulphate reduction by anaerobic sulphate reducers. The increase in the pH furthers helps in the metal (Fe and Al) precipitations. The rhizospheric carbon through decomposition produces organic acid metabolites which could have decreased the pH in the planted wetlands but the pH was well buffered by the sulphate reducers (Tiedje et al., 1984; Küsel and Alewell, 2004). The pH value of unplanted HSSF CW was found to be relatively unchanged (Inflow pH value 3.70 and Outflow pH value 3.72) in the system with respect to inflow pH throughout the experiment.

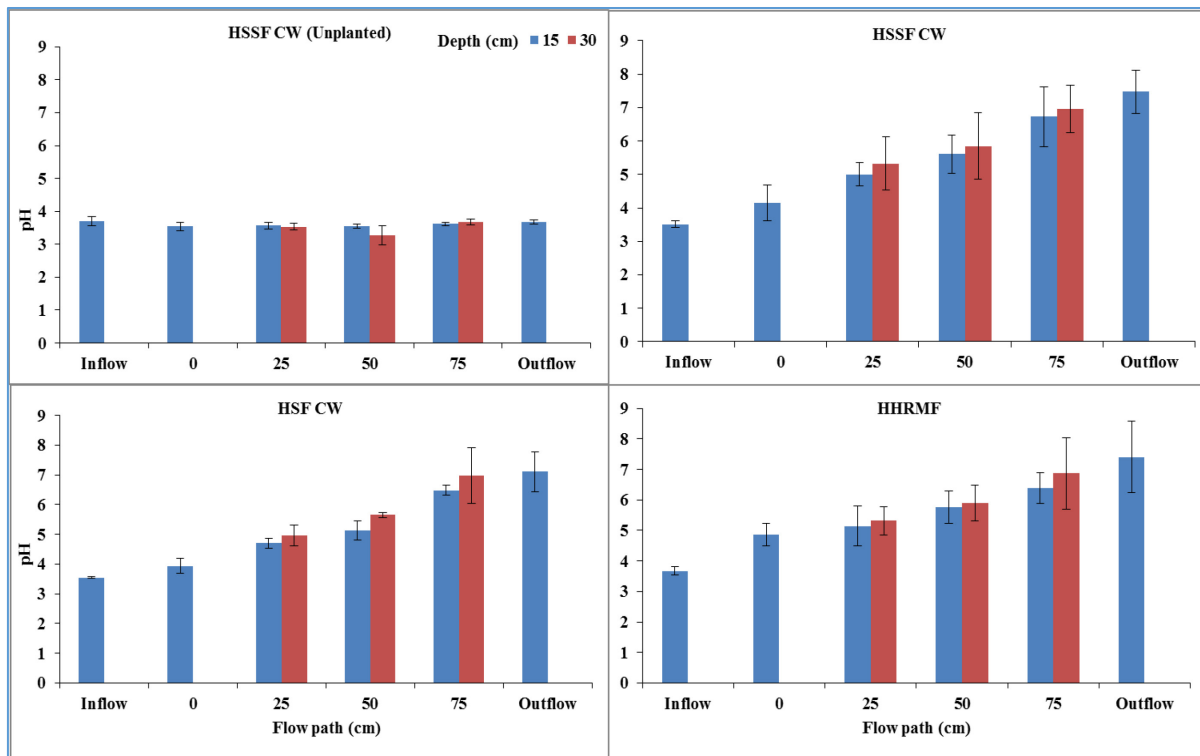


Figure 3.1. 13- Dynamics of mean pH at different depths along the flow path of wetlands.

The increase in pH in the planted CWs can be directly related to the constant removal in the SO_4^{2-} -S load from the waste water in planted wetlands whereas in unplanted CW was no decrease in the sulphur load hence there was no change in pH detected. In the (Fig. 3.1.14) it is seen that as the sulphate is being removed from the artificial AMD the pH also increases simultaneously (Jong and Parry, 2003) which studies is in agreement with all previous research. In the unplanted wetland it is seen that no DSR took place as a result there was no change in the pH values from inflow to outflow.

Metals like Al are mostly pH dependent for their removal as they do not form stable sulphides in water (Jong and Parry) and are more likely removed as precipitation of hydroxide (Eger, 1994). Studies have revealed that $\text{Al}(\text{OH})_3$ has a minimum solubility in the pH range of 6 –7 (Kadlec and Knight, 1996; Metcalf & Eddy, 2003; Zouboulis and Traskas, 2005; Hu et al., 2006).

In this study (Fig. 3.1.15) it is seen that Al is removed at a steady and slow rate from 0 to 25 cm in the planted wetlands and then from 25 cm to outflow there was a rapid removal of Al from the wastewater. The pH at 25 cm was 4.5 but after 25 cm the pH range was between 5.5 to 7. According to Kadlec and Knight, 1996 aluminium hydroxide has the minimum solubility within this range of pH so the precipitation of aluminium hydroxide occurred more vigorously. A mean removal efficiency of > 89 was seen in all the three planted wetlands which is agreement with the studies conducted by Christensen et al. In unplanted wetland the pH was unchanged throughout the flow path of the wetland which is around 3, so almost no removal in Al was seen in this wetland.

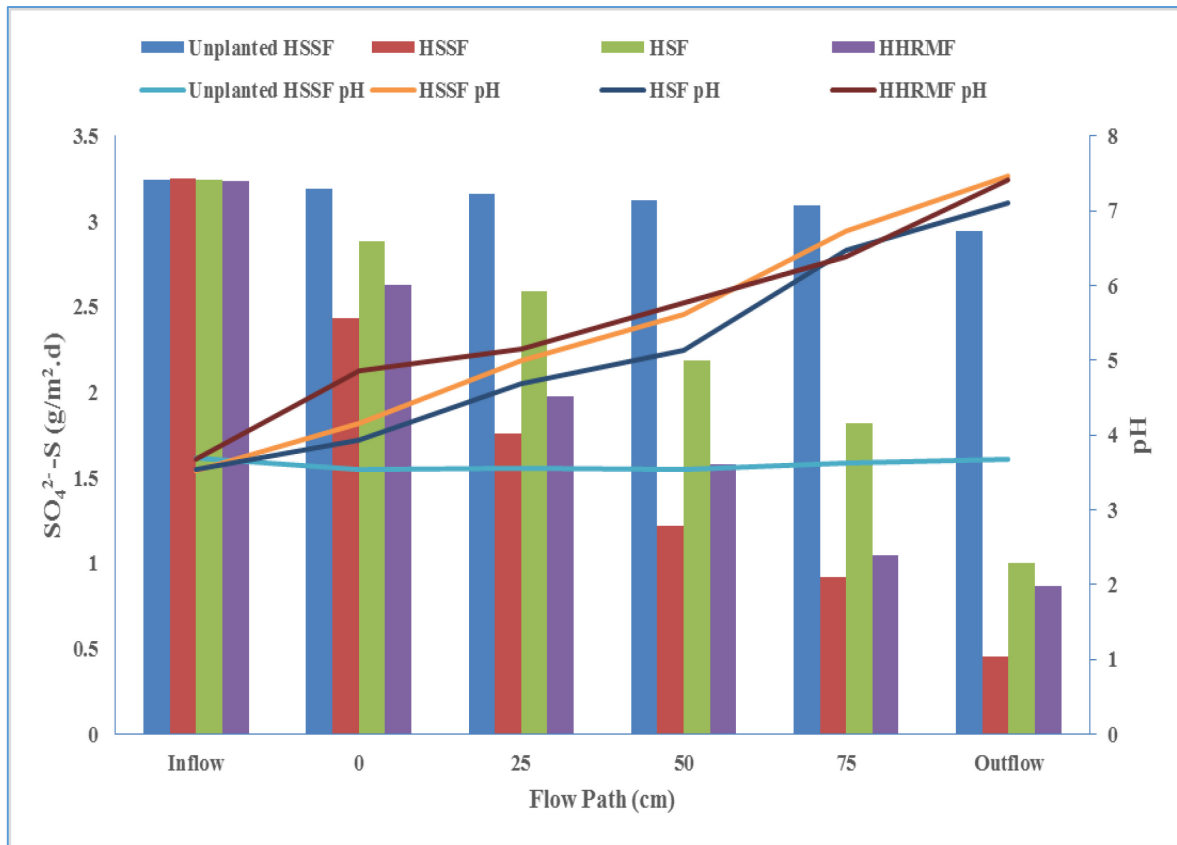


Figure 3.1. 14- Correlation between sulphate removal and pH dynamics along the flow path of in unplanted and planted wetlands.

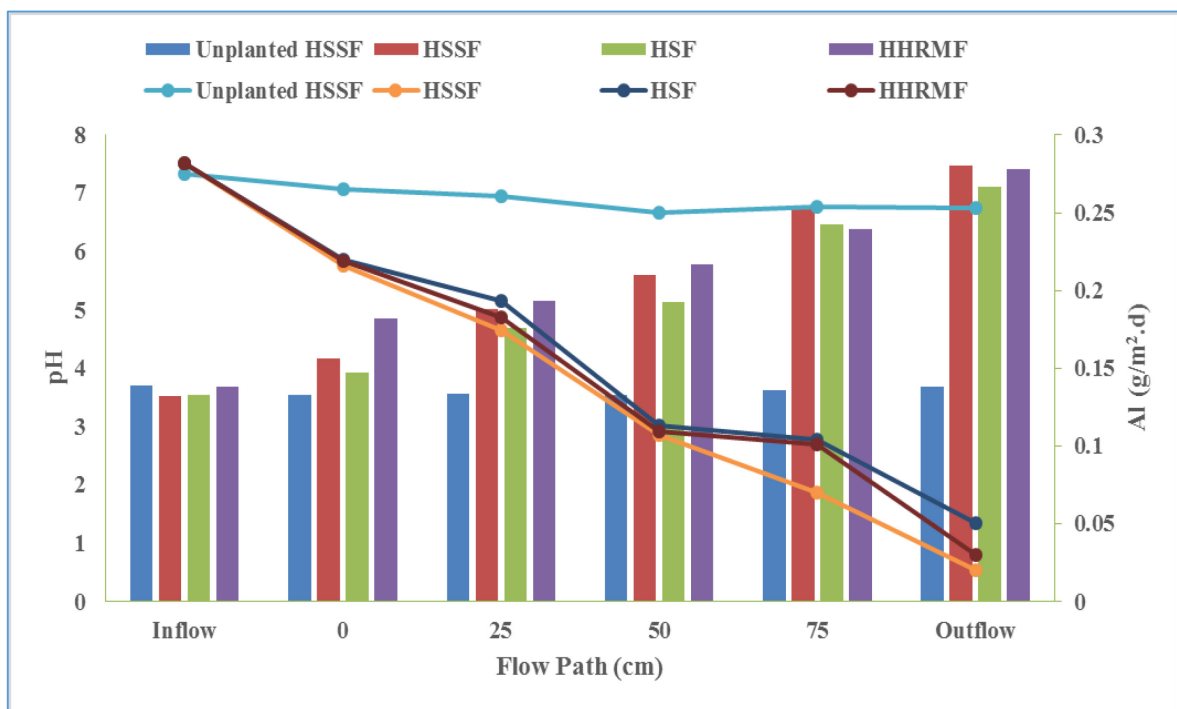


Figure 3.1. 15- Correlation between pH and Al removal along the flow path of unplanted and planted wetlands.

3.2. Results with *Phragmites australis* (from Phytotechnicum, with H₂ as external electron donor)

In phytotechnicum the two wetlands chosen were HHRMF CW as they are easy to maintain. In CW1 no external electron donor is provided only the rhizospheric carbon act as electron donor whereas in CW2 in addition to rhizospheric carbon, hydrogen as external electron donor is supplied. Hydrogen is an attractive electron donor for sulfate reduction because its free energy of sulfate reduction is more favorable (Warounsak and Ajit, 2007). Sulfate reducing bacteria are generally more efficient in hydrogen utilization than methanogenic bacteria (Davidova and Stams, 1996); therefore, using hydrogen as an electron donor has an advantage over using organic compounds. The equation below shows how hydrogen can act as an electron donor.



3.2.1. Dynamics of sulphur removal

The dynamics of sulphate-sulphur removal and formation of sulphide within the wetland systems have a major influence on metal removal. Though both the systems maintained strict anaerobic conditions but CW2 was found to be more oxygen deficient than CW1. Detailed data on loads (Load/Area) of sulphur compounds (mean values) and standard deviations (SD) in both the CWs are presented in Table 3.2.1 and 3.2.2, respectively.

Despite the fact that CW1 was not provided with any external carbon source or any other form of electron donor there was marked decrease in the SO₄²⁻-S load in the outflow. The mean inflow SO₄²⁻-S load was 3.2 g/m².d which corresponds to 0.06g/l (Fig. 3.2.2) and the mean outflow load was 1.046 g/m².d which resulted a mean removal of 67.5%.

Though in both the CWs no external carbon sources were added which could have been a limiting factor for sulphate reduction (Stein et al., 2007) but the organic carbon from the roots of the plants (rhizodeposition products) were enough to stimulate rhizosphere microbial growth and activity (Munch et al., 2005; Nikolausz et al., 2008; Van de Moortel et al., 2010) for dissimilatory sulphate reduction and maintain a low redox conditions in the planted wetlands, which is not the case in unplanted wetland. The released carbon compounds could act as electron donors to be oxidized during sulphate reduction, thus leading to an enhanced sulphide production. In CW2, hydrogen as external electron donor is supplied to the wetland. The mean load of SO₄²⁻-S in the inflow was 3.2 g/m².d (0.06g/l) and the mean load of SO₄²⁻-S in outflow was 0.5 g/m².d which resulted in a mean removal of 84.3%. It shows a higher mean removal as compared to CW1 due to the intensification of sulphate reduction process enhanced by the supply of hydrogen as external electron donor. The organic carbon substrate was supplied from the roots as the as rhizodeposits.

In Fig. 3.2.1 it is seen that the effluent load increased as the experiment progressed. This can be explained with the decline in the health conditions of the plants. The overall growth rate of the plants declined by 28% and 29% in CW1 and CW2 respectively shown in Fig.3.2.8. This decline in the number of green shoots in CWs resulted in the less influx of the electron donor in form of rhizodeposits which resulted in the increase in effluent load.

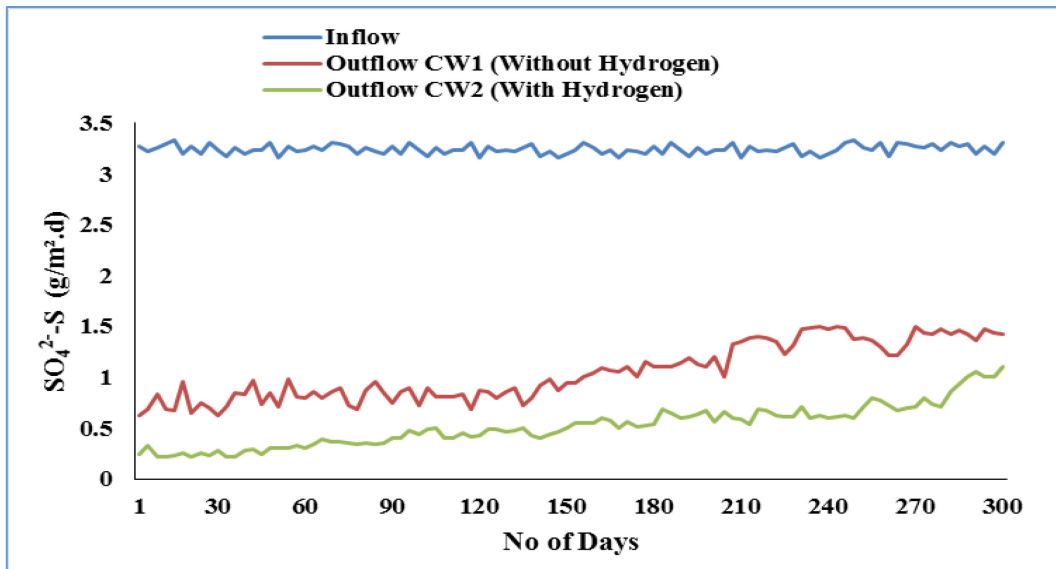


Figure 3.2. 1- Mean $\text{SO}_4^{2-}\text{-S}$ inflow and outflow load of the wetlands.

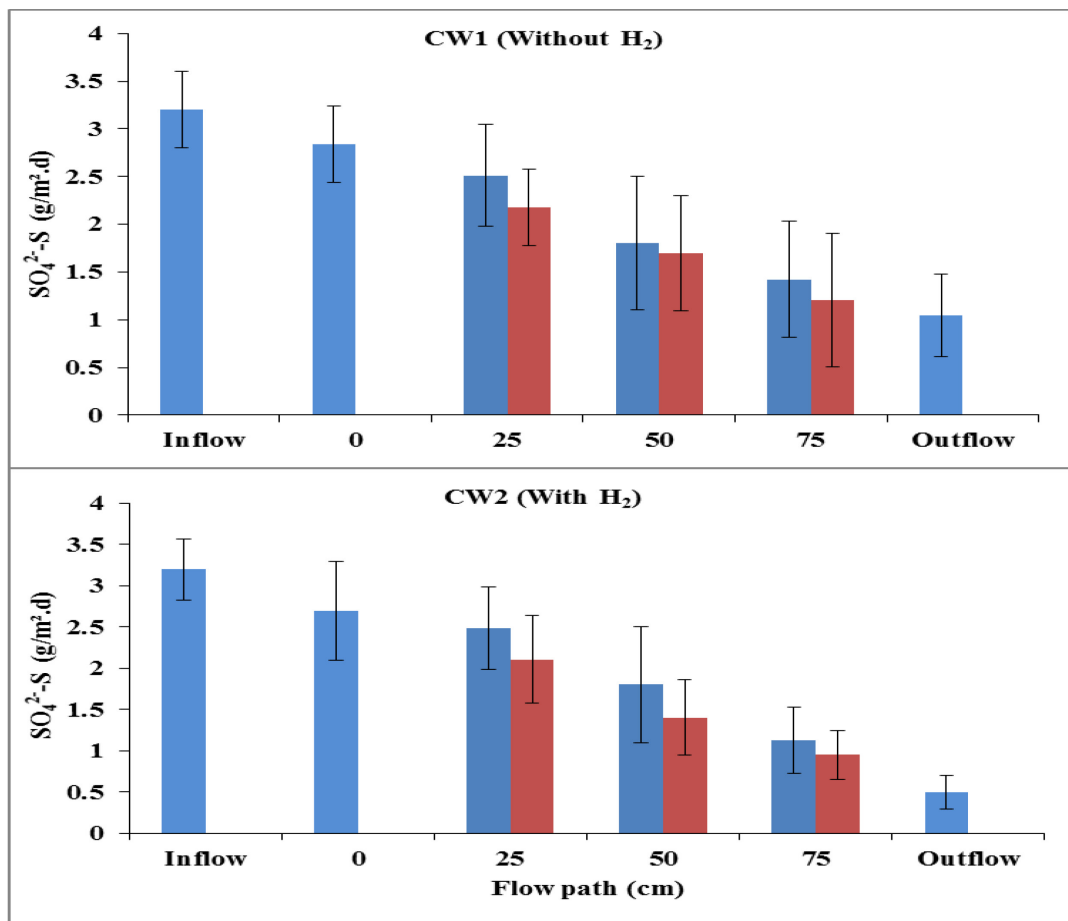


Figure 3.2. 2- Dynamics of mean $\text{SO}_4^{2-}\text{-S}$ load in the pore water along the flow path of the wetlands.

From the Fig 3.2.2, it is seen that the removal rate varied slightly in between the two sampling depths (15 cm and 30 cm depth from the surface of the wetland). It is seen that $\text{SO}_4^{2-}\text{-S}$ (Fig. 3.2.1) removal is higher at 30 cm depth of the wetlands than the 15 cm depth. This can be

attributed to more anoxic and reducing conditions (Fig. 3.2.9 and 3.2.10) at deeper layer along with more root cover which facilitates greater dissimilatory sulphate reductions.

As the final product of dissimilatory sulfate reduction, sulfide was produced in both the CWs. Strict and persistent reducing conditions resulted in anaerobic microbial sulphate reduction and subsequent increase in sulphide production (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011). In this experiment sulphide found in the outflow of planted wetlands with mean concentrations of 11.41 and 16.3 mg S l⁻¹ which corresponds to 0.043 and 0.087 g/m².d in CW1 and CW2 respectively (Fig. 3.2.3).

Even though both wetlands maintained strict reducing and anaerobic conditions but higher amount of sulphide production was seen in CW2 than CW1 owing to the supply of external electron donor which shows comparatively very high reducing activities in CW2. It was evident that sulphate removal rate was higher in CW2 than in CW1, due to the greater reducing conditions in CW2 (H₂ as electron donor) than CW1 without organic carbon as external electron donor. This high sulphate removal also attributed to the higher metal removal in CW2 than the CW1 (discussed in section 3.2.1) under reducing conditions and presence of H₂ as electron donor for the respiration of sulphate reducers.

Sulfide generated from sulfate reduction can precipitate with metals to form metal sulfides, volatilizes (hydrogen sulfide emission), form organic sulfur compounds and undergo reoxidation in wetland sediments (Vymazal and Kröpfelová, 2008b). Since metals (e.g. Fe²⁺, Al³⁺) were present in the artificial wastewater feeding the systems, metal sulfides could be formed in the wetland models. The appearance of black precipitates showed that such precipitations occurred in the planted systems.

Sulfide concentrations exceeded 30 mg/L (0.1g/m².d) in both the CWs and were generally much higher from 25 cm to 75 cm along the flow path of the planted CWs. This is in agreement with the redox potential that is much lower at distances 25 cm and 75 cm than at distance 0 cm to 25 cm from inflow.

It was evident that sulphate removal rate was higher in CW2 as compared to CW1, due to the greater reducing conditions in CW1 due to the use of H₂ as electron donor. Under this reducing conditions the sulphate reducing bacteria resulted in better sulphate removal and in turn efficient metal removal.

But plants exhibited toxic effects (decreasing water loss in terms of plant transpiration and less green shoots) probably due to sulphide toxicity along with high metal load (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011). As the experiment progressed the number of healthy shoots decreased and the decrease in healthy shoots resulted in the less influx of rhizospheric carbon which in turn affects the sulphate removal. This is the major reason for the increase in the effluent load towards the end of the experimental period. The data for this experiment are shown in Fig.3.2.8. Plants physiological inhibition of several helophytes was shown for S²⁻ concentrations of approximately 10 to 50 mg l⁻¹ (Armstrong et al., 1996; Chambers et al., 1998; Fürtig et al., 1996). The measured S²⁻ concentrations in this study were >10 mg l⁻¹ in the outflow and > 25 mg l⁻¹ inside the wetlands.

Further proof of dissimilatory sulphate reduction is provided by the detection of elemental sulphur in both the wetlands as certain bacteria use elemental oxygen to carry out such

oxidization of hydrogen sulfide to produce elemental sulfur. The concentration of elemental sulphur detected in out flow were 4.7 and 3.9 mg l^{-1} in which corresponds to 0.025 and 0.02 g/m 2 .d in CW1 and CW2 respectively (Fig. 3.2.4).

In CWs elemental sulfur can be formed via both chemical and biological sulfide oxidation ($2HS^- + O_2 \rightarrow 2S + 2OH^-$) (Chen et al., 2016; Wu et al., 2011). Elemental sulfur formed from bacterial sulfide oxidation can be deposited intracellularly or extracellularly. In many circumstances, white precipitates from elemental sulfur deposits have been observed in the outflows of treatment wetlands (Kadlec and Wallace, 2008). It is important to note that oxidation of sulphides produces oxidized sulphur species (i.e. S^0 , SO_4^{2-}) and may release associated metals or metalloids to the water column (Simpson et al., 1998). This may be the case of slightly elevated concentration of SO_4^{2-} in the outflow tank than at the outflow.

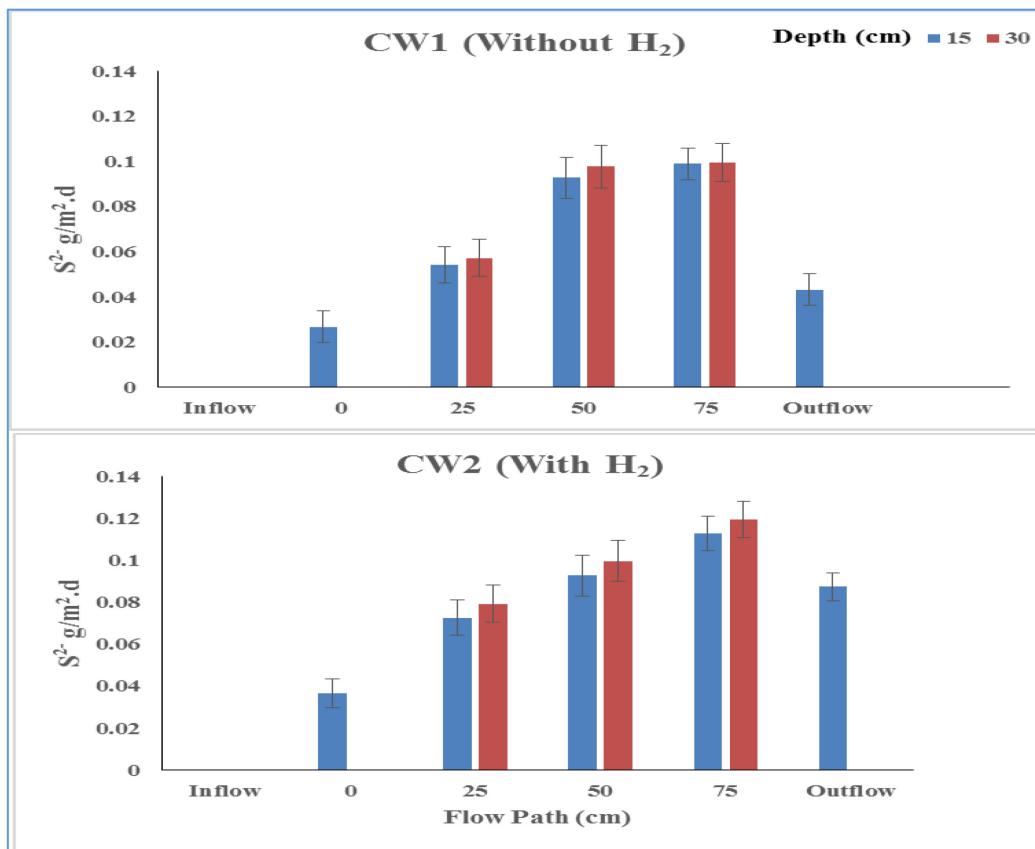


Figure 3.2. 3- Mean sulphide production (load/area) along the flow path of the wetlands.

In this study, elemental sulfur concentration were high between 25 cm to 75 cm of the flow path in the planted wetlands. In CW1 and CW2 the concentration of S^0 were between 20 to 25 mg l^{-1} (0.02 to 0.08 g/m 2 .d) Both the CWs were hydroponic wetlands as a result of this some amount of sulphide in the water can be oxidized to elemental sulphur. It is also seen that the S^0 production is slightly more at 15 cm depth as compared to 30 cm depth. This can be explained as the availability of oxygen at 15 cm depth is more as oxygen can diffuse to 15 cm depth as compared to 30 cm depth and also the root density is maximum at 15 cm depth in these wetlands so the oxygen transported from the roots to the CWs at this depth is also more.

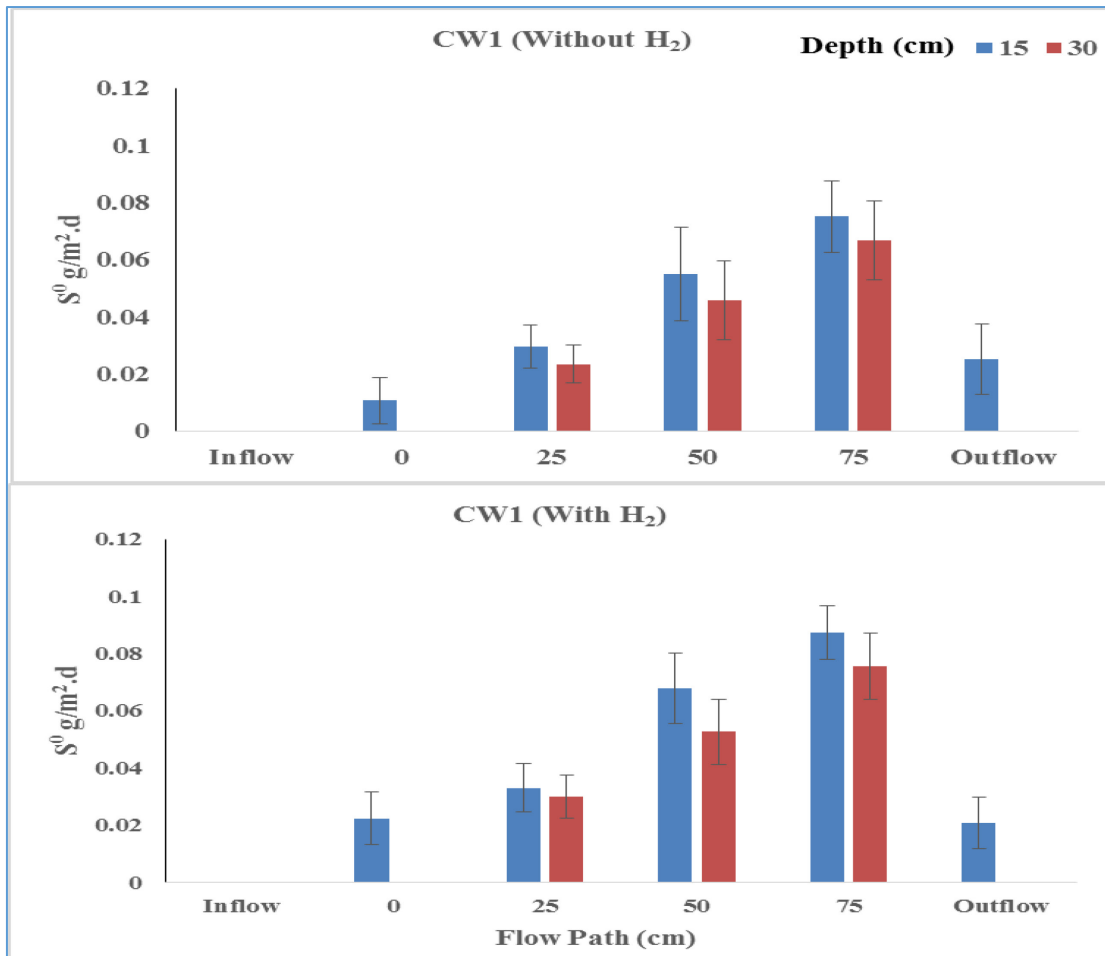
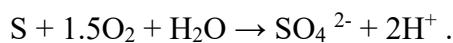


Figure 3.2. 4- Mean elemental sulphur production (load/area) along the flow path of the wetlands.

Oxygen released from plant roots has been reported to play an important role in oxidative processes in wetlands (Stottmeister et al., 2003; Wiessner et al., 2010). Oxidation of elemental sulfur can result in the elevation of sulfate in planted CWs:



In the wetlands, bacterial sulfur disproportionation could also contribute to the depletion of elemental sulfur, leading to the formation of sulfate and sulfide:



Overall, higher amounts of elemental sulfur in CW2 were in good agreement with prior studies which showed that under more anaerobic conditions, more elemental sulfur was produced during sulfide oxidation (Celis-García et al., 2008; van den Ende et al., 1997).

Significant differences in the internal sulfur cycling and overall sulfur removal was noted among the model CWs. The synthetic wastewater contained sulfur mainly in the form of sulfate. The analyzed intermediately oxidized sulfur species (elemental sulfur, sulfite and thiosulfate) were detected inside all the systems. Since the influent did not contain any of the latter species, and as they can only be the product of abiotic or biotic oxidation of sulfide, it was concluded that DSR and sulfide oxidation processes occurred simultaneously.

Sulphur depositions (e.g. metal sulphide precipitations, elemental sulphur deposits) and the emission of hydrogen sulphide can result in total sulphur loss (Kadlec and Wallace, 2008). It has been documented that CWs could harbor an important inorganic and organic sulphur pool (Spratt and Morgan, 1990; Stottmeister et al., 2003; Wiessner et al., 2010).

The TS removal was highest in CW2 of about 73% followed by CW1 about 61% (Table 3.2.1) respectively. Since in the effluent CW2 sulphate and other oxidized form of sulphur were found in less amount as compared to CW1, hence sulfur transformations were more intensified in CW2.

Table 3.2. 1- Sulphur mass balance in the wetlands and mean removal of total sulphur (TS) in pore volume of the wetlands.

Wetlands	Influent	Effluent						Mean Removal
		S ⁰	S ²⁻	SO ₃ ²⁻	S ₂ O ₃ ²⁻	SO ₄ ²⁻	Total Sulphur	
CW1 (Without H ₂)	3.2 ±.07	0.025 ±0.023	0.043 ±0.016	0.015 ±0.0014	0.109 ±0.0174	1.046 ±0.43	1.238 ±0.4878	61%
CW2 (With H ₂)	3.2 ±.07	0.020 ±0.021	0.087 ±0.018	0.0203 ±0.0017	0.206 ±0.0142	0.518 ±0.47	0.8513 ±0.5249	73%

TS* is calculated as the sum of the 5 analyzed inorganic sulfur species.

3.2.2. Dynamics of iron removal

The dynamics of total iron (Fe²⁺ and Fe³⁺) with the values of mean inflow and outflow loads (Load/Area) and the removal efficiency are represented in Fig. 3.2.5 and Table 3.2.2. During the whole ten months of operation, a continuous supply of synthetic wastewater provided a mean inflow load of 0.116 g/m².d (2.18mg/l) in both the reactors.

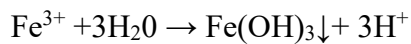
Under C-deficient anaerobic condition in the experiment, a highly efficient Fe removal was observed in both the experimental wetlands. The mean outflow load was 0.001911 and .001059 g/m².d CW1 and CW2 respectively and the mean removal efficiency attained was 98.5% in CW1 and 99.2 in CW2.

Table 3.2. 2- Iron mass balance in the wetlands and mean removal of total iron (tot. Fe) in pore volume of wetlands.

Wetlands	Influent		Effluent		Total Fe	Mean Removal
	Fe ³⁺	Fe ²⁺	Fe ³⁺	Fe ²⁺		
CW1	0.114 ±0.001	0.002 ±0.0002	0.000407 ±.00001	0.000752 ±0.0003	0.001911 ±0.00031	98.5%
CW2	0.114 ±0.001	0.002 ±0.0002	0.000307 ±0.0001	0.000687 ±0.0003	0.001059 ±0.0004	99.2%

In the artificial AMD the iron was present in the form of ferric sulphate and the detection of ferrous iron was negligible. But as soon as the waste water enters the planted system, it is seen that the load of Fe(III) decreased and the load of Fe(II) increases (Fig.3.2.5). Removal of Fe(III) under this condition is perhaps best explained by two methods i.e.

- (i) The effect of pH- The ferric form of iron remains soluble in water at acidic pH (3 and 3.5) but as soon as the pH increases (3.2.6) it precipitates out in the form of hydroxides. This may be a reason for the decrease of ferric form of iron within the wetlands as the pH increased from 3.5 at inflow to > 4 at 25 cm length from the inflow, in all three planted wetlands. The Fe(III) reacts with water to form insoluble hydroxide (Kosolapov et al., 2004).



Initially most of the Fe(III) may have been removed as hydroxides due to the rise in pH. But at the same time there was an increase in the load of ferrous iron was seen and this phenomenon can be explained as microbial reduction of ferric form to ferrous form.

- (ii) The microbial reduction- Some of the Fe(III) may have been reduced to Fe(II) form. According to Kosolapov et al a wide range of anaerobic bacteria and archae bacteria are able to conserve energy through the reduction of Fe(III) to Fe(II). These Fe(II) form again can be removed by the formation of iron sulphides. Iron sulfides is formed as a result of microbial sulfate reduction (Rabenhorst et al., 1992). Sulfate-reducing bacteria (SRB) mediate dissolved sulfate reduction to hydrogen sulfide and metals are immobilized by the resulting sulfide. Iron sulphides formed in CWs may be iron mono sulfides (e.g. FeS) (Ahern et al. 2004, Morse and Rickard 2004) which is highly reactive and readily oxidizes on exposure to air, and the more stable forms of sulfur such as pyrite (FeS₂). Presence of black colour precipitates on the roots of the plants confirms the formation of FeS₂.

From the Fig. 3.2.6 it was observed that two-third of the total iron (most of them in the form of Fe(III)) was removed between 0 to 25 cm from the inflow. This is due to the fact that the pH was increased from 3.5 to 4.3. Sulfate reduction consumes acidity and raises water pH (Hedin and Nairn, 1992). This rise in pH from acidic pH facilitated the precipitation of Fe(III) as hydroxide. The Fe(II) precipitates as mono sulfides (e.g. FeS) or pyrite. Soil sediments examinations could not be performed due to constrain in time so the results provided are based on the examinations of pore water volume.

According to Eger, 1994, one mole of sulfate would be reduced for each mole of divalent metal (Fe²⁺) precipitated. The removal of each mole of aluminium and ferric iron (trivalent metal) requires 1.5 moles of sulfate reduction.

Table 3.2.3 shows the amount of sulphate used up for the removal of Tot Fe in the planted wetlands.

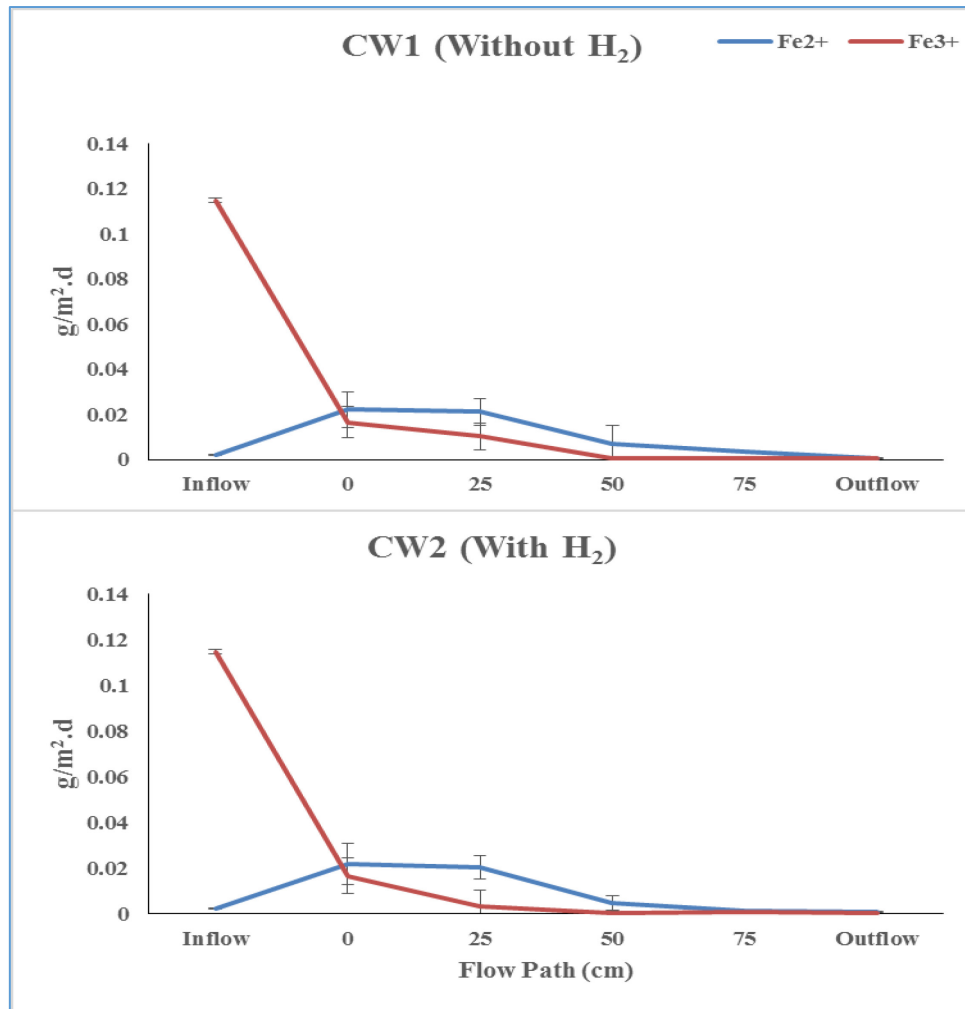


Figure 3.2. 5- Dynamics of mean Fe²⁺ and Fe³⁺ load in the pore water along the flow path of the wetland.

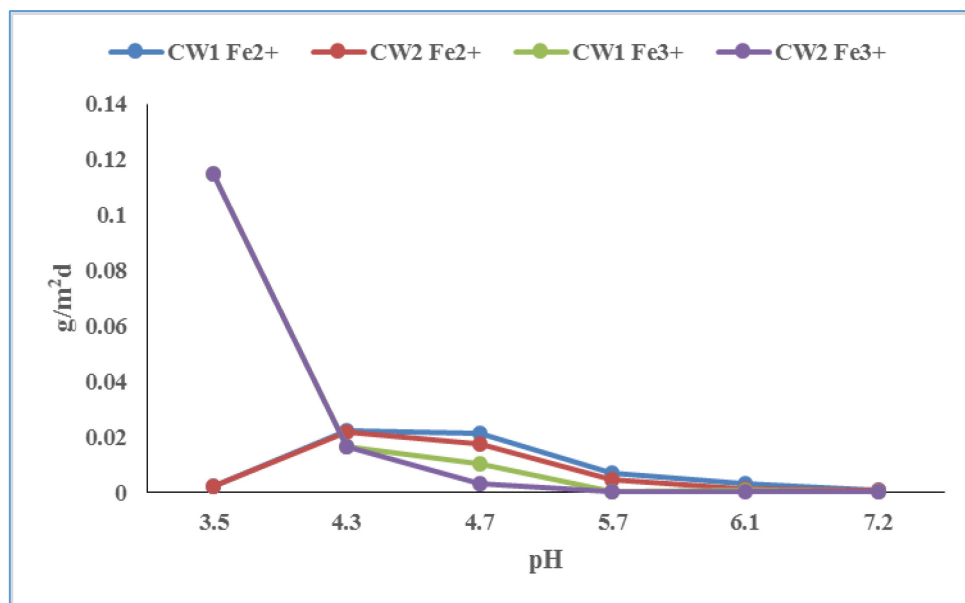


Figure 3.2. 6- Behavior of Fe²⁺ and Fe³⁺ with respect to the changes in pH in the planted wetlands.

Table 3.2. 3- Total sulphate required for removal of total iron in the wetlands

Contaminants	Contaminants Removed (moles/m ² d)		SO ₄ ²⁻ used up for removal of Fe ²⁺ and Fe ³⁺ (moles/m ² d)	
	CW1	CW2	CW1	CW2
So ₄ ²⁻	0.1346	0.1676		
Fe ²⁺	0.000022	0.000023	0.000022	0.000023
Fe ³⁺	0.0020	0.00204	0.003	0.00306
Tot. Fe	0.002022	0.002063	0.003022	0.003083

3.2.3. Dynamics of aluminium removal

The dynamics of total aluminium with the values of mean inflow and outflow loads (Load/Area) and the removal efficiency are represented in Fig. 3.2.7. During the whole ten months of operation, a continuous supply of synthetic wastewater provided a mean inflow load of 0.28 g/m².d (5.3 mg/l) Al in both the reactors.

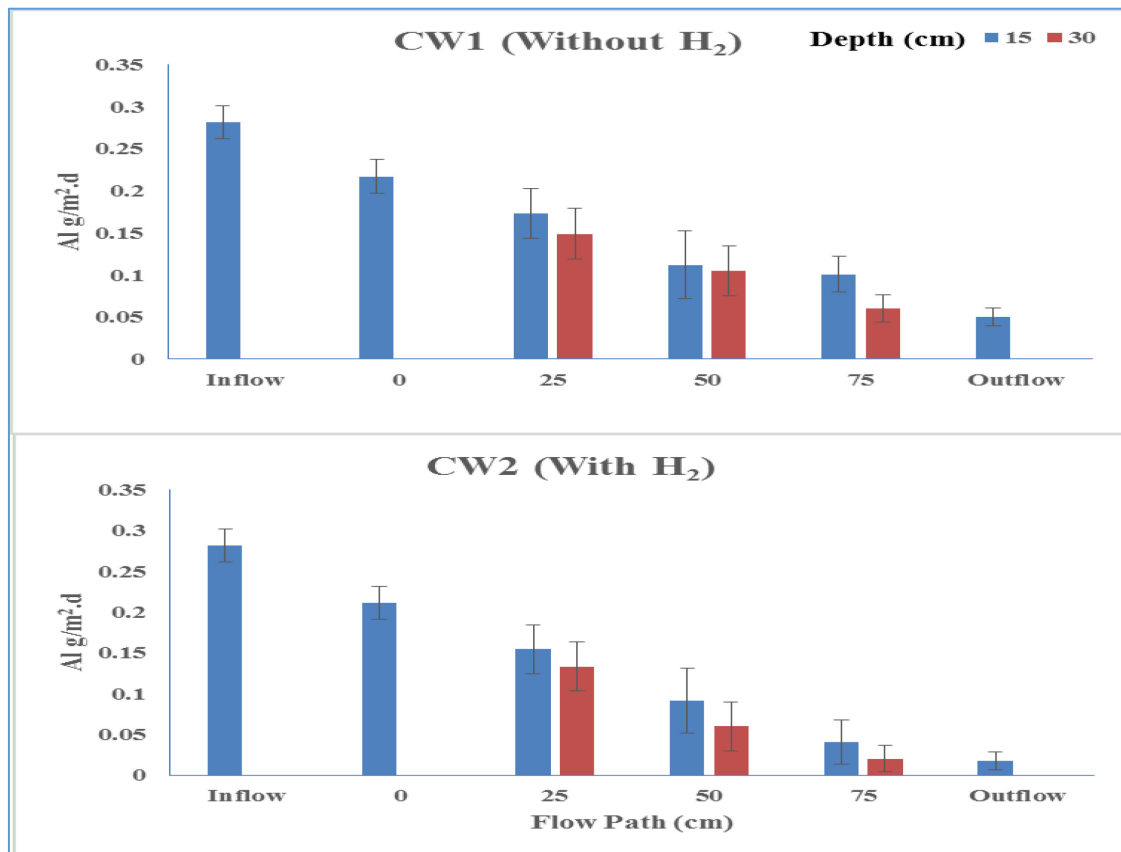


Figure 3.2. 7- Dynamics of mean Al load in the pore water along the flow path of the wetlands.

Al³⁺ hydrolyzed in the water (which is known to occur by coordinating 6 water molecules to form an aquometal ion, Al(H₂O)₆(3⁺)) and then chemically precipitated as Al(OH)₃. It has been reported that the pH of minimum solubility for Al(OH)₃ falls in the range of 6.1 – 6.3.

(Snoeyink et al., 2003) Other studies have revealed that $\text{Al}(\text{OH})_3$ has a minimum solubility in the pH range of 6 – 7. (Kadlec and Knight, 1996; Metcalf & Eddy, 2003; Zouboulis and Traskas, 2005; Hu et al., 2006)

Under C-deficient anaerobic condition in the experiment, a highly efficient Al removal was observed in both the experimental wetlands. The mean outflow load is 0.05 and 0.017 $\text{g}/\text{m}^2\cdot\text{d}$ in CW1 and CW2 respectively and the resulting outflow load showed nearly 10-fold declination in total Al and the mean removal efficiency attained as 82% and 94% in HSSF CW1 and CW2 respectively (Fig. 3.2.7). Removal of Al under this condition is perhaps best explained by formation of aluminium sulphate because in presence of dissolved sulfate Al may also precipitate as hydroxysulphate wetland (Kadlec and Wallace, 2009). Due to rise in pH (Fig. 3.2.11) in both the wetlands Al precipitates as hydroxides (Eger, 1994) (because of the small oxic microgradient present at the root zone) which is retained by the wetland. In CW2 hydrogen as electron donor helped in the stimulation of dissimilatory sulphate reduction which resulted in 5.72% more removal of Al.

3.2.4. Further parameters (shoot density, EvT, Eh, DO and pH)

3.2.4.1. *Growth of plant biomass (shoot density) and water loss (EvT)*

Water loss from the wetlands as evapo-transpiration (EvT) was taken into consideration for the calculation of removal rates, which is the mass removal loading rates. This was particularly important to distinguish between planted and unplanted wetlands as because the rate of water loss due to the presence of plant biomass in planted wetlands hugely differed with the wetlands without plants (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011).

The growth status of the plant biomass in terms of total number of green and healthy shoots and therefore the variation in water loss via EvT is shown in Fig. 3.2.8. . By the end of experiment, corresponding to 10 months of operation, there was decrease in overall shoot density. There was 28% and 29% decrease in shoot density from an initial 3960 m^{-2} and 4080 m^{-2} to 2886 m^{-2} and 2926 m^{-2} in CW1 and CW2 respectively.

In the start the plants were able to take the stress and were healthy but as the experiment continued further, plants were stressed by the low pH and perhaps damaged by high sulphide formation. Due to strict anaerobic conditions, rapid sulphide formation within the anaerobic root zone might have caused mortality of wetland plants decreasing their growth rate (Rahman 2008).

It appeared very important to notify that number of green shoots in the segment from 50 cm to 100 cm from inflow exhibited severe stress and shoot density decreased rapidly from an initial 3200 m^{-2} and 3360 m^{-2} to only 2380 m^{-2} and 2286 m^{-2} respectively in CW1 and CW2 at the end of experiment. Plant shoot density in this segment of wetlands declined rapidly due to the prolonged exposure to high load of metals along with high rate of sulphide production and less nutrients being available to the plants to the latter half of the wetland.

In general, higher plant shoot density resulted in high water loss via plant transpirations but as the experiment progressed the number of healthy green shoots started to decrease and hence a decreasing tendency of water loss after the first four month of the experiment. Temperature also plays a very important role for the water loss in CWs. There should be a direct relationship between the mean monthly temperature and the water loss, but as the shoot density started to decrease the water loss also decreased irrespective of the mean monthly temperature.

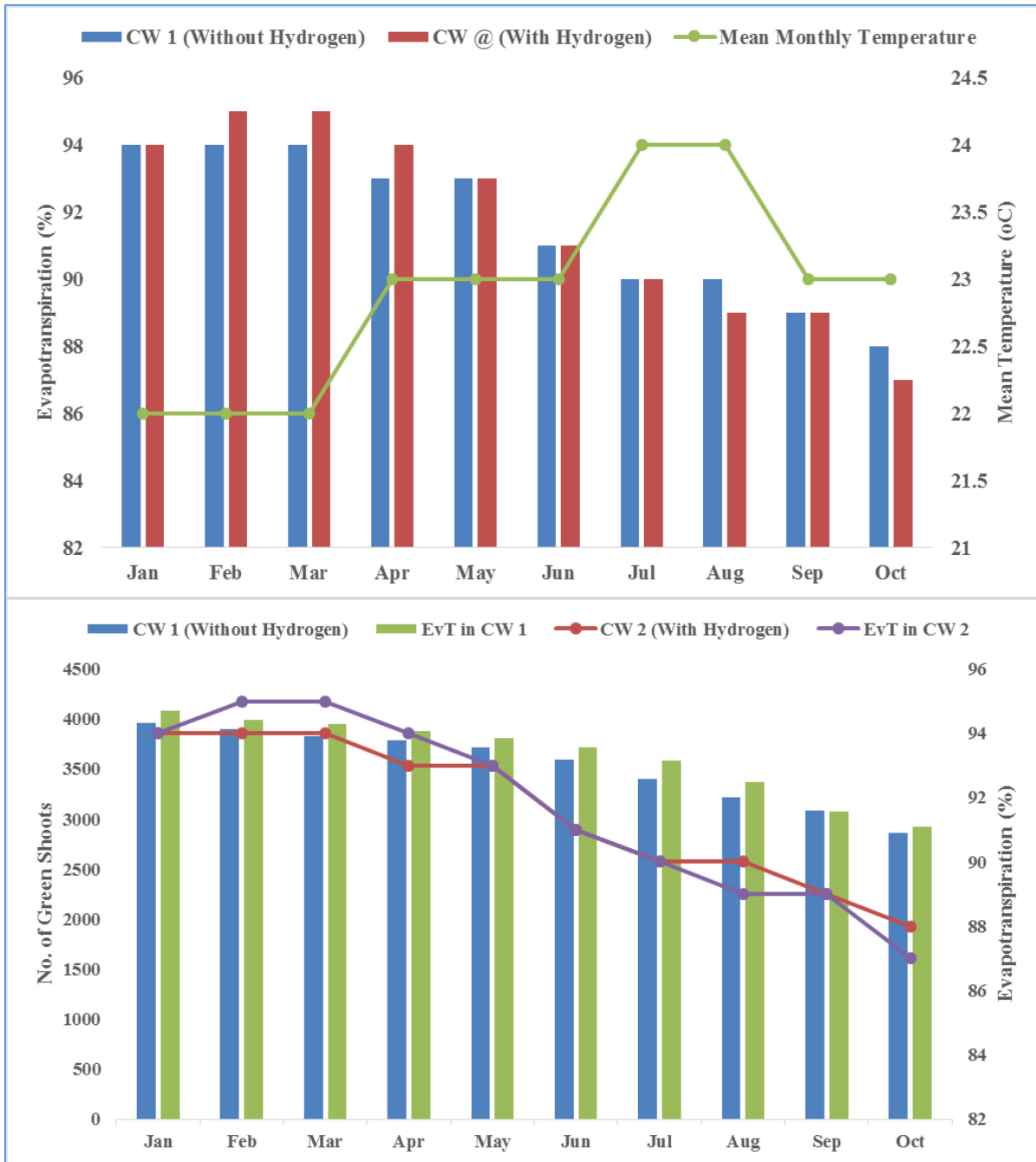


Figure 3.2. 8- (Top) Correlation between evapotranspiration (EvT) and temperature fluctuation during whole operation period in the wetlands. (Bottom) Correlation between water loss by plant transpiration (EvT) and green shoot density during whole operation period in the planted wetlands.

Temperature also plays a very important role for the water loss in CWs. Unlike the green house, here in phytotechnicum there is not much fluctuation in temperature as the temperature is maintained between 23°C to 25°C. The mean monthly temperature almost remained constant with very minor fluctuation during different months (Fig. 3.2.8).

The effect of the plants on the removal efficiency of heavy metals and sulphate was studied in both the wetlands, CW1 (without H₂ as electron donor) and CW2 (with H₂ as electron donor). It was observed that CW2 considerably enhanced the retention of heavy metals than CW2. Likewise, presence of H₂ substantially improved sulphate retention within the wetland beds.

3.2.4.2. *Redox potential (Eh), Dissolved Oxygen (O₂) and pH*

In fact, the removal mechanisms of metals and sulphate are dependent on the pH and redox potential of the environment. The redox potential (Eh) shows the oxidation-reduction conditions and is related significantly to pollutant removal processes in CWs (Vymazal and Kröpfelová, 2008b; Wiessner et al., 2005a).

In this study, mean inflow oxygen concentration value was always very high around 8 mg/l during the entire experimental period in all the wetlands, whose results are presented in Fig. 3.2.9, varied dependent on system, distance from inflow, and depth. The inside of the wetland wetlands were anoxic. The highest concentration was seen in the inflow region of both the wetlands but the inside of system was strictly anaerobic. CW2 (mean O₂ concentration 8.87 mg/l at inflow, 0.21 mg/l at outflow) was found to be more anoxic than CW1 (mean O₂ concentration 8.96 mg/l at inflow, 0.4325 mg/l at outflow). This trend was as well reflected on the redox potential levels in the different systems (Fig. 3.2.10). Low redox potential along with anaerobic conditions in the planted wetlands helped in carrying out dissimilatory sulphate reductions as presence of oxygen in the reactors hinders the process of dissimilatory sulphate reduction as it is toxic to sulphate reducing bacteria as well as it does not helps in attending the right redox potential needed for the process (Marschall et al., 1992).

In the study, values of DO in most of the sampling points in planted C were below 0.5 mg/l. The results were corroborated with traditional observation that HSSF CWs are mostly anaerobic systems where DO concentrations are very low or undetectable (Garcia et al., 2010; Vymazal and Kröpfelová, 2008a).

Redox potential, whose results are presented in Fig. 3.2.10, varied dependent on system, distance from inflow, and depth. There was a statistically significant difference in redox values between the two wetlands. The most negative redox values were observed in CW2 between ~-497 mV (Inflow) and ~ -178 mV (75 cm from inflow, 30 cm depth), whereas in CW1 the maximum and minimum redox values are ~424 mV (Inflow) and ~ -157 mV (75 cm from inflow, 30 cm depth) respectively. In CW2 more redox conditions prevailed than in CW1 this is due to the supply of H₂ as electron donor which ensure a greater reducing environment in CW2. The redox values decreased significantly with distance from inflow (Fig. 3.2.10) in both the wetlands. While some amount of oxygen entered the systems with the inflow, plant roots remained main oxygen source along the flow path. Root exudates (Brix, 1997) and oxygen released by *P. australis* which range from 0.02 to 12 g m⁻² d⁻¹ (Armstrong & Armstrong, 1990; Brix, 1990; Gries et al., 1990) enhance microbial activity substantially. Consistent low redox potential values were maintained in both the CWs throughout the experiments which ensures the activity of anaerobic sulphate reducers. Redox values in the model CWs were found in the range of dissimilatory sulfate reduction (Eh < -100 mV) (Reddy and D'angelo, 1994).

Fig.3.2.11 shows the interrelationship between redox potential and DSR in this experimental study. In the inflow the redox potential tends to be very high in both the wetlands with no sulphide being detected. As we move from inflow towards outflow the values for redox potential tends to be in negative. The most negative values were measured at 75 cm length from the inflow. As the redox potential values becomes more and more negative, we see an increasing trend in the sulphide production. Very low amount of sulphide was detected at 0 cm of the wetlands and then it increases towards the outflow. Sulphide production was highest at 75 cm length of the CWs where the redox values were also the lowest. This result is in agreement with the fact that DSR occurs at negative redox potential. The intensity of DSR

increased from 25 cm to 75 cm length of the wetlands as the pH between 25 cm and 75 cm is within the range of 4.5 to 6.5 which is the ideal pH for maximum activity of the bacteria carrying out DSR (Jong and Parry, 2003).

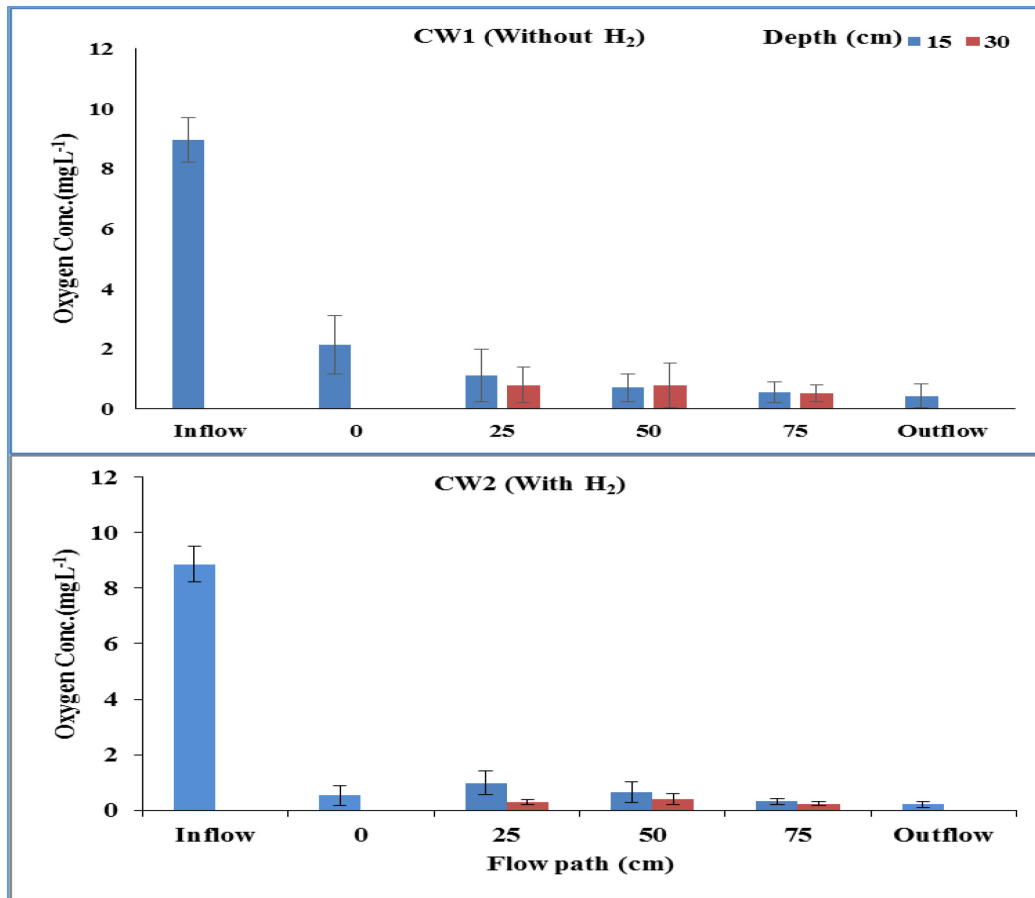


Figure 3.2. 9- Dynamics of mean dissolved oxygen concentrations in the pore water at different depths along the flow path of wetlands.

pH is an important environmental factor influencing microbial processes and is associated with nutrient removal in CWs (Reddy and D'angelo, 1994). The pH behavior in the experimental model wetlands is shown in Figure 3.2.12. In this study, mean inflow pH value was always in the range of 3.5 to 3.6 during the entire experimental period in all the wetlands. In both the wetlands pH varied dependent on system, distance from inflow, and depth. The highest pH was seen in the outflow of all the wetlands. The outflow pH value were 7.34 and 7 in CW2 and CW1 respectively (Fig. 3.2.12). The change in the pH value from acidic to alkalinity is due to the dissimilatory sulphate reduction by anaerobic sulphate reducers. The increase in the pH furthers helps in the metal precipitations. The rhizospheric carbon through decomposition produces organic acid metabolites which could have decreased the pH in the planted wetlands but the pH were well buffered by the sulphate reducers (Tiedje et al., 1984; Küsel and Alewell, 2004).

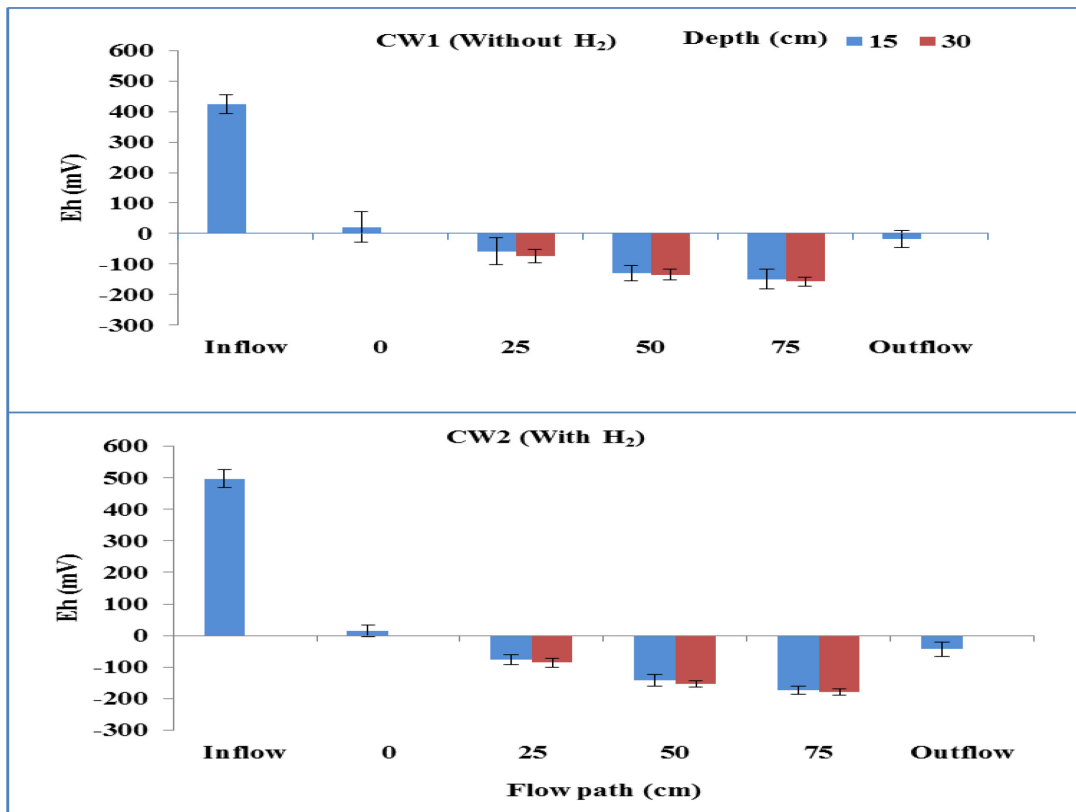


Figure 3.2. 10- Dynamic mean redox conditions in the pore water at different depths along the flow path of wetlands.

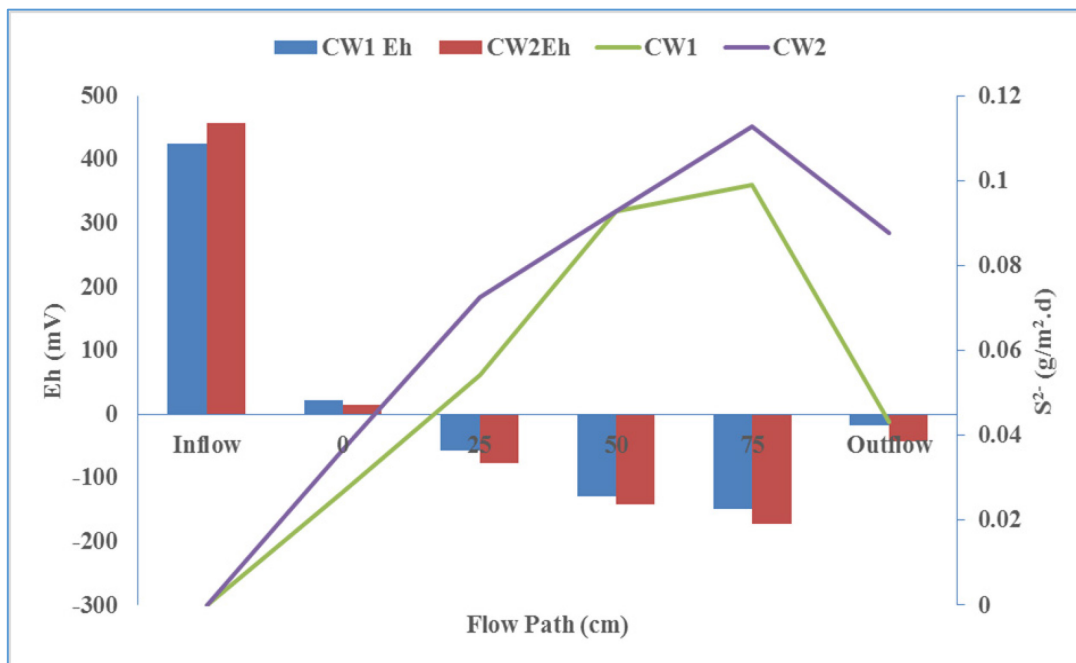


Figure 3.2. 11- Correlation between redox potential and DSR leading to sulphide production along the flow path of the wetlands.

The increase in pH in the planted CWs can be directly related to the constant removal in the SO_4^{2-} -S load from the waste water in both the wetlands. In the (Fig. 3.2.13) it is seen that as the sulphate is being removed from the artificial AMD the pH also increases simultaneously (Jong and Parry, 2003) which is in agreement with all previous research. The intensity of

sulphate removal is slightly high in CW2 in comparison to CW1 this is due to the intensification of DSR in CW2 due to the availability of H₂ as external electron donor.

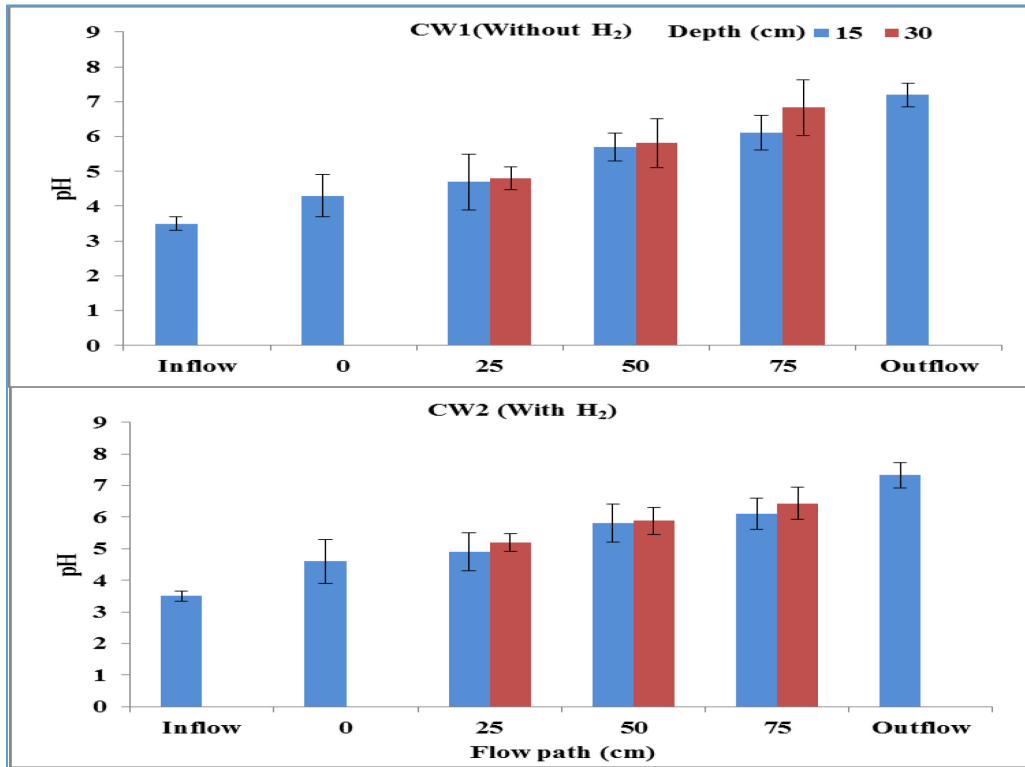


Figure 3.2. 12- Dynamics of mean pH at different depths along the flow path of wetlands.

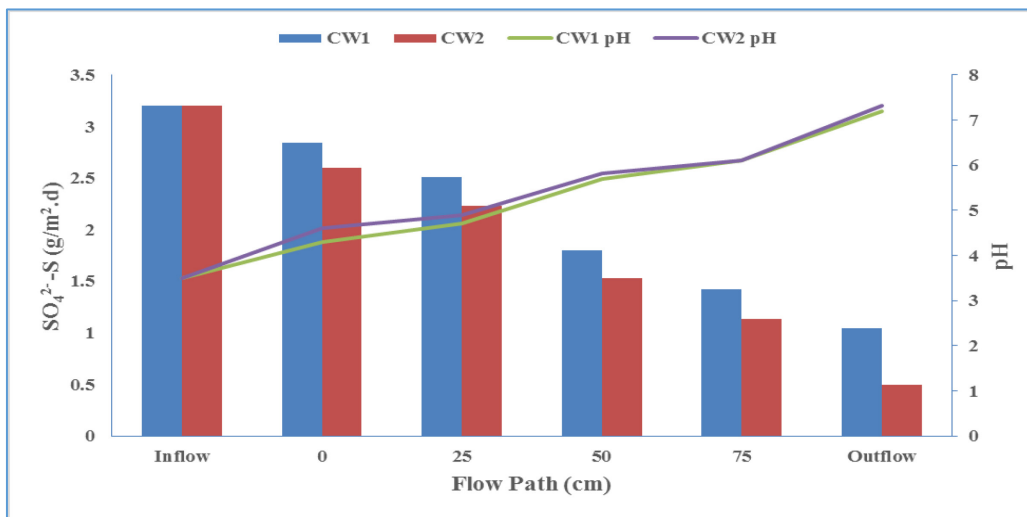


Figure 3.2. 13- Correlation between sulphate removal and pH dynamics along the flow path of wetlands.

Metals like Al are mostly pH dependent for their removal as they do not form stable sulphides in water (Jong and Parry) and are more likely removed as precipitation of hydroxide (Eger, 1994). Studies have revealed that Al(OH)₃ has a minimum solubility in the pH range of 6 –7 (Kadlec and Knight, 1996; Metcalf & Eddy, 2003; Zouboulis and Traskas, 2005; Hu et al., 2006).

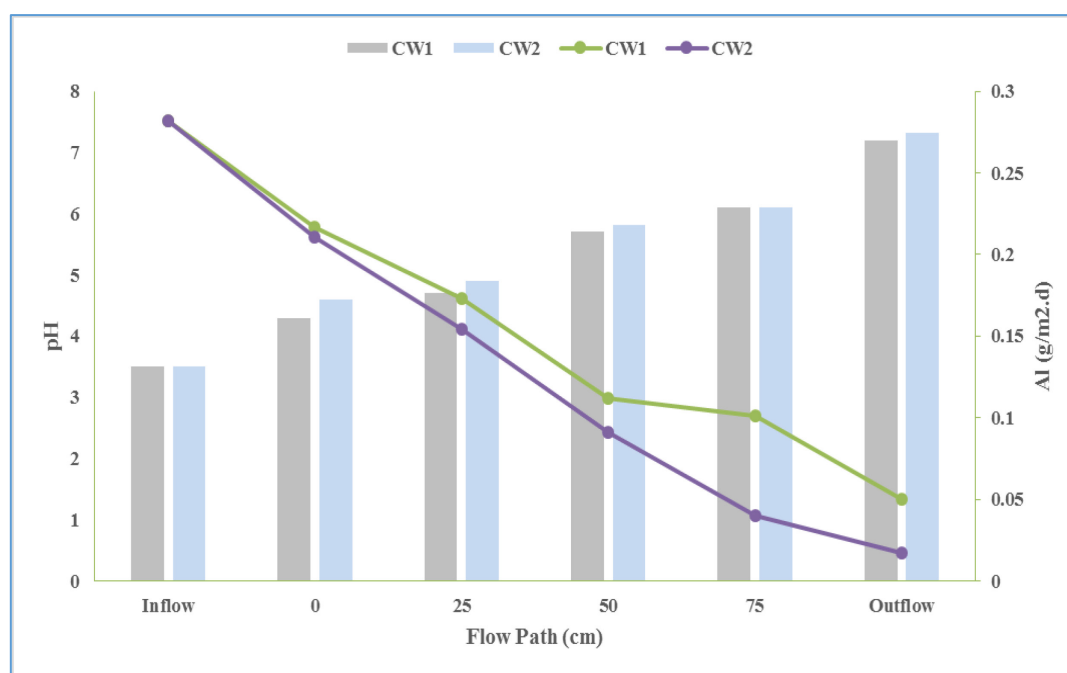


Figure 3.2. 14- Correlation between pH and Al removal along the flow path in the wetlands.

In this study (Fig. 3.2.14) it is seen that Al is removed at a steady and slow rate from 0 to 25 cm in both the wetlands and then from 25 cm to outflow there was a rapid removal of Al from the wastewater. The pH at 25 cm was 4.5 but after 25 cm the pH range was between 5.5 to 7. According to Kadlec and Knight, 1996 aluminium hydroxide has the minimum solubility within this range of pH so the precipitation of aluminium hydroxide occurred more vigorously. A mean removal efficiency of > 89 was seen in all the three planted wetlands which is agreement with the studies conducted by Christensen et al. The removal rate of Al is more in CW2 as the pH of the pore water in CW2 is slightly high than that of CW1.

3.3. Results with *Juncus effusus* (from green house, without external electron donor)

3.3.1. Dynamics of sulphur removal

Dynamics of sulphate-sulphur removal and formation of sulphide within the wetland systems have a major influence on metal removal.

Under C-deficient high redox and high oxidic condition (Fig. 3.3.1) in Unplanted HSSF CW, no significant removal in the SO_4^{2-} -S load was observed. The mean inflow SO_4^{2-} -S load was 3.2 g/m².d (0.06 g/l) (Fig 3.3.1) and the mean SO_4^{2-} -S load in the outflow was 3 g/m².d (0.056g/l) and this accounts for the 10% of the removal efficiency. Prevailing oxidic condition, high redox potential and lack of electron donor inhibited microbial sulphate reduction and likely became concentrated with the formation of a S-pool in unplanted WC.

The main aim of the experiment was to rely on the rhizospheric carbon as electron so no external carbon sources were added to the artificial AMD. There was a marginal decrease in the SO_4^{2-} -S load in the outflow of the planted CWs. The mean inflow SO_4^{2-} -S load was 3.2 g/m².d (in all planted wetlands) (Fig. 3.3.1) and the overall mean outflow load (g/m².d) was 2.8, 2.9 and 2.88. There was no significant removal of SO_4^{2-} -S load seen in this experiment. The overall mean removal efficiency stands at 18.2%, 17.14% and 17.7% in corresponding HSSF CW, HSF CW and HHRMF respectively. Most of the removal was attained in the first eight months of the experiment as compared to the last eight months of the experiment.

Furthermore, the limited removal of the SO_4^{2-} -S load cannot completely be explained with reference to dissimilatory sulphate reduction, as in the last eight months, the non-detection of sulfide in the systems is consistent with the high oxidic conditions and very high redox potential. DSR is expected to take place at a redox potential below -100 mV (Faulwetter et al. 2009).

It can also be inferred that there was not much influx of rhizodeposits in the CWs in the last eight months of the experiment as there was a constant decrease in the number of healthy green shoots of *J. effuses* owing to the very low pH of the artificial AMD and high load of contaminants. As compared with *P. Australis* the influx of rhizodeposits was less in *J. effuses* (Zhai et al., 2013).

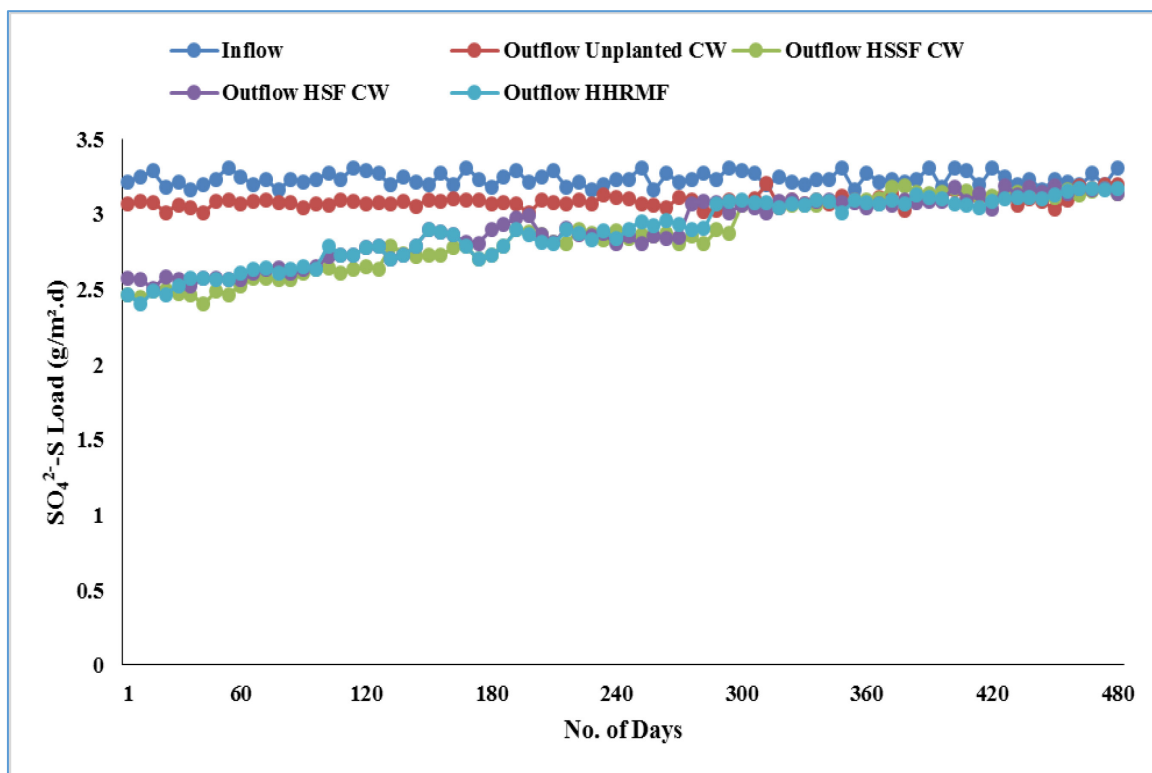


Figure 3.3. 1- Mean SO_4^{2-} -S inflow and outflow load of the wetlands.

The limited removal of the SO_4^{2-} -S load in the first few months of the experiment can be due to intake by plants, adsorption to the gravel matrix and marginally due to DSR due to the presence of multi gradient redox process (both micro- and macro) in constructed wetland conditions (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011, Bezbaruah and Zhang, 2004; Wiessner et al., 2005b). This however does not exclude the possibility that some DSR occurred in anoxic niches of the *J. effusus* systems, and that subsequent reoxidation of

sulfide to sulfate took place (Wiessner et al. 2008b). In addition, in the presence of metals, sulfide can form a precipitate and be immobilized inside the CWs. Though sulphide was not detected in the last eight months of the experiment but in the initial months of the experiment there was occasional detection of sulphide in low concentration whereas elemental sulphur remained undetected.

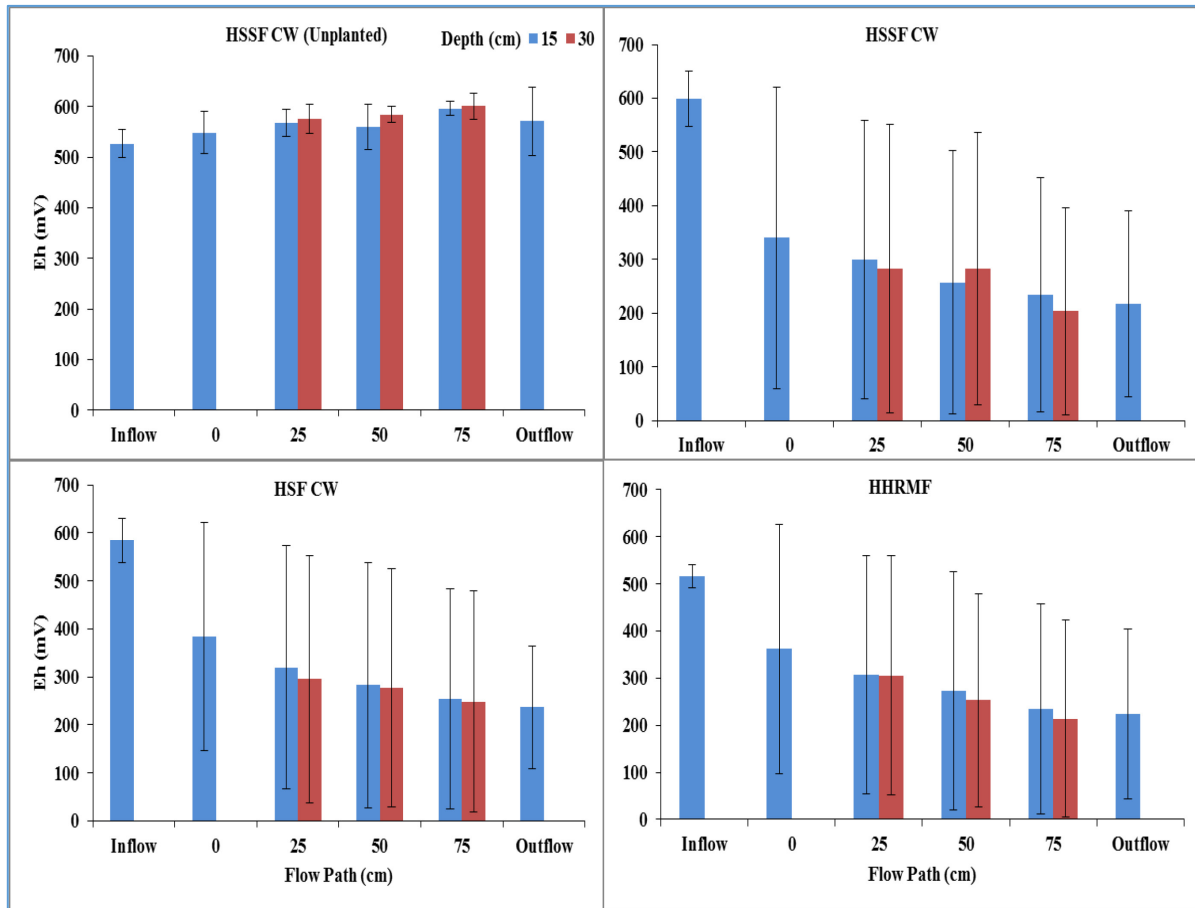


Figure 3.3. 2- Dynamic mean redox conditions in the pore water at different depths along the flow path of wetlands.

The overall redox potential in all planted wetlands were very high for dissimilatory sulphate reduction. Redox potential, whose results are presented in Fig. 3.3.2 The unplanted HSSF CW was characterized by stable and very high redox conditions between ~527 mV (Inflow) ~493 mV (75 cm, 30 cm depth). The least redox values recorded in the planted HSSF CW between 8 mV (75 cm, 30 cm depth) followed by HHRMF CW ~ 20 mV (75 cm, 30 cm depth) and HSF CW ~ 14 mV (75 cm, 30 cm depth). These redox values were measured in the initial few months of the experiment. In the later part of the experimental phase the redox inside the wetlands were greater than 100mV. Redox values in the HSF CW are more positive among the planted wetlands as this system has a free water surface above the gravel. Though, the HHRMF CW was thought to have higher redox potential among the planted CWs but it was not the case as it did not have any free water surface because it was a root mat filter CW not a free-floating root mat CW.

As the experiment proceeded there was a decrease in healthy shoots (3.3.7 and 3.3.8) which resulted in increase in redox potential values. In this experiment consistent low redox potential

values were not maintained in all planted CWs throughout the experiments which affected the activity of anaerobic sulphate reducers and in turn the efficiency of sulphate and metal removal.

In this study, mean inflow oxygen concentration value was always very high around 8 mg/l during the entire experimental period in all the wetlands, whose results are presented in Fig. 3.3.3, varied dependent on system, distance from inflow, and depth

The unplanted wetland was always very oxic in nature whereas the planted wetlands were slightly anoxic in nature. The highest concentration was seen in the inflow region of all planted wetlands but the inside of system it was less aerobic. During the initial part of the experiment the planted wetlands were less aerobic when compared with the latter half of the experimental phase. Of all the planted wetlands the HSSF CW was the most anoxic (2.91 mg/l at outflow) and the HSF CW to be more oxic (3.26 mg/l at outflow). This high DO level is reflected in the positive redox values (Fig. 3.3.2)

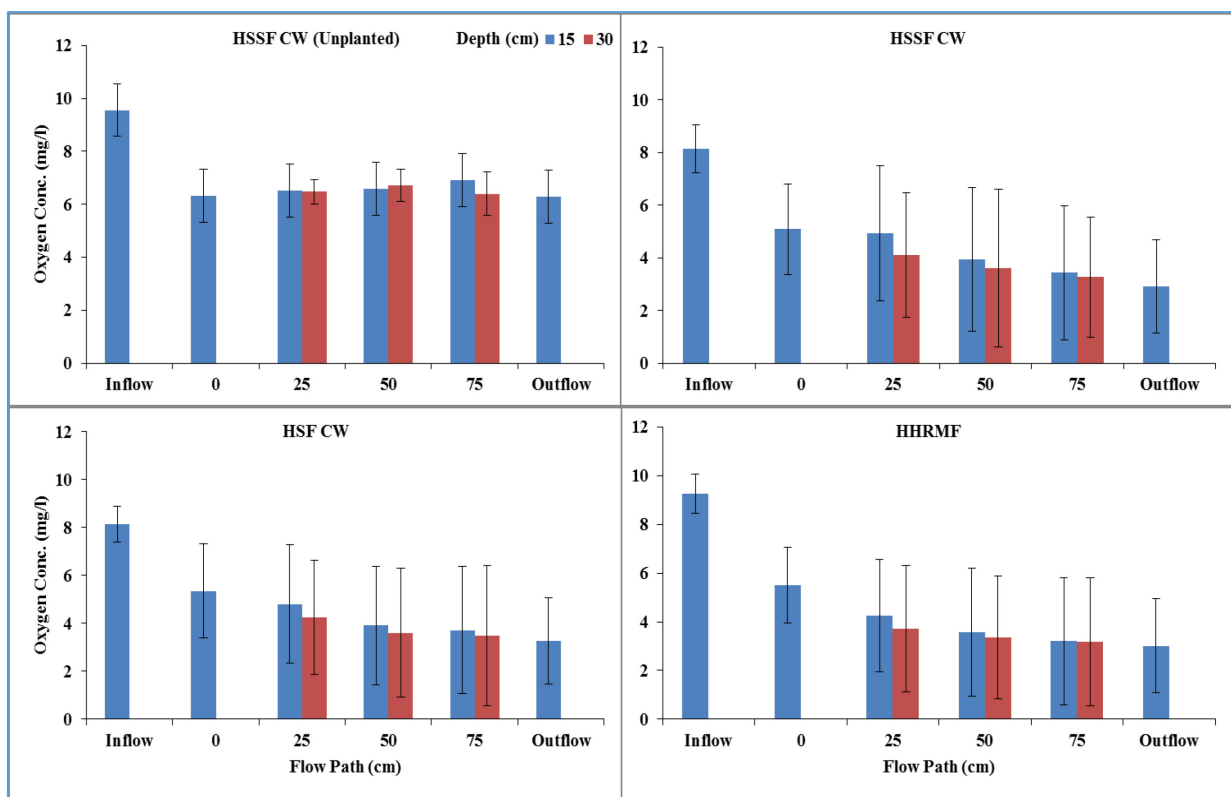


Figure 3.3. 3- Dynamics of mean dissolved oxygen concentrations in the pore water at different depths along the flow path of wetlands.

3.3.2. Dynamics of iron removal

The values of mean inflow and outflow concentrations and the removal efficiency are represented in Figure 3.3.4. During the whole 16 months of operation, a continuous supply of synthetic wastewater provided a mean inflow load of 0.1166 g/m².d (2.18mg/l) of Fe in all four reactors.

In the unplanted wetland there was an overall removal of 6% of Fe (outflow load 0.0264 g/m².d). The wetland was found to be oxic and there is a possibility of formation of oxyhydroxide. But for formation of iron oxyhydroxide, apart from oxygen the Fe should be present in ferrous form, since the iron used in this experiment is in ferric form so removal of

iron in this oxyhydroxide form is likely less. Moreover, persistent oxic condition and lack of sufficient electron donor inhibited dissimilatory sulphate-reduction despite the relatively higher sulphate-sulphur concentrations in this wetland. So, Under C-deficient aerobic condition and non-availability of ferrous form of Fe the removal process can be best explained by the process of adsorption by the gravel matrix of the wetland.

The mean outflow loads ($\text{g}/\text{m}^2\cdot\text{d}$) are 0.093, 0.095 and 0.094 which makes the overall mean removal efficiency in all three planted wetlands as 20%, 18% and 19% in HSSF CW, HSF CW and HHRMF respectively. Most of the removal was attained in the first eight months of the experiment as compared to the last eight months of the experiment. As the pH in the in the outflow was around 4.5 in the first eight months of the study, some of the Fe^{3+} ions may have precipitated as hydroxides as the solubility of Fe^{3+} ions decrease with the rise in pH (Kosolapov et al., 2004).

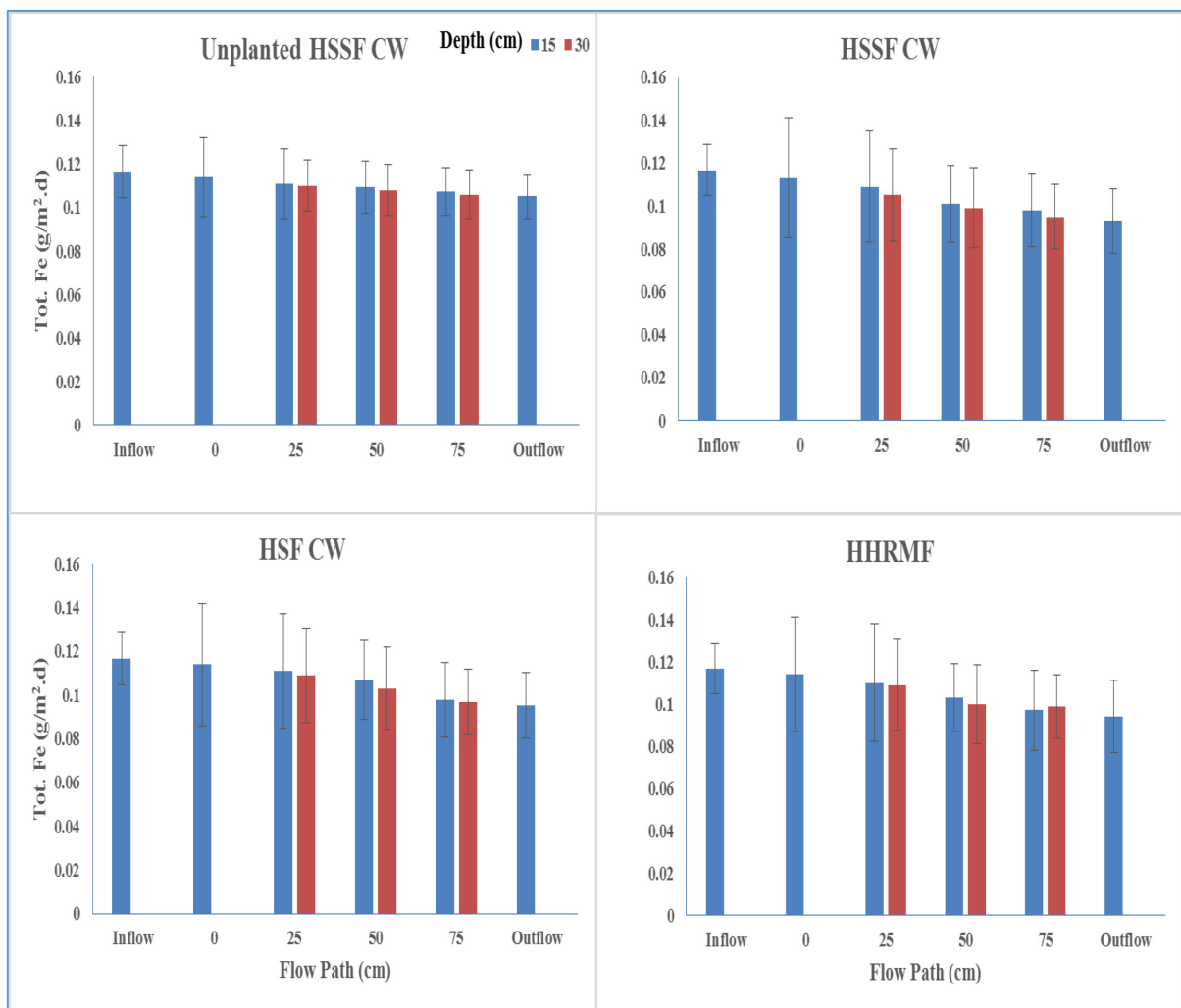


Figure 3.3. 4- Dynamics of mean total Fe load in the pore water along the flow path of the wetlands.

In this study, mean inflow pH value was always in the range of 2.5 (Fig. 3.3.5) during the entire experimental period in all the wetlands. There was not much differences in pH values between the unplanted and planted wetlands. The highest pH was seen in the outflow of all the planted wetlands in the first phase of the experiment. The outflow pH value was around 4.5. The slight rise in the pH value is due to the removal of limited amount of sulphate. But towards the second part of the experimental phase the pH of outflow was ~ 3 in all planted wetlands. The decrease

in the number of healthy shoots reduced the sulphate removal process resulting in the fall of pH in the planted wetlands (Tiedje et al., 1984; Küsel and Alewell, 2004). The pH value of outflow in unplanted HSSF CW was found to be relatively unchanged (Inflow pH value 3.70 and Outflow pH value 3.72) with respect to inflow pH throughout the experiment.

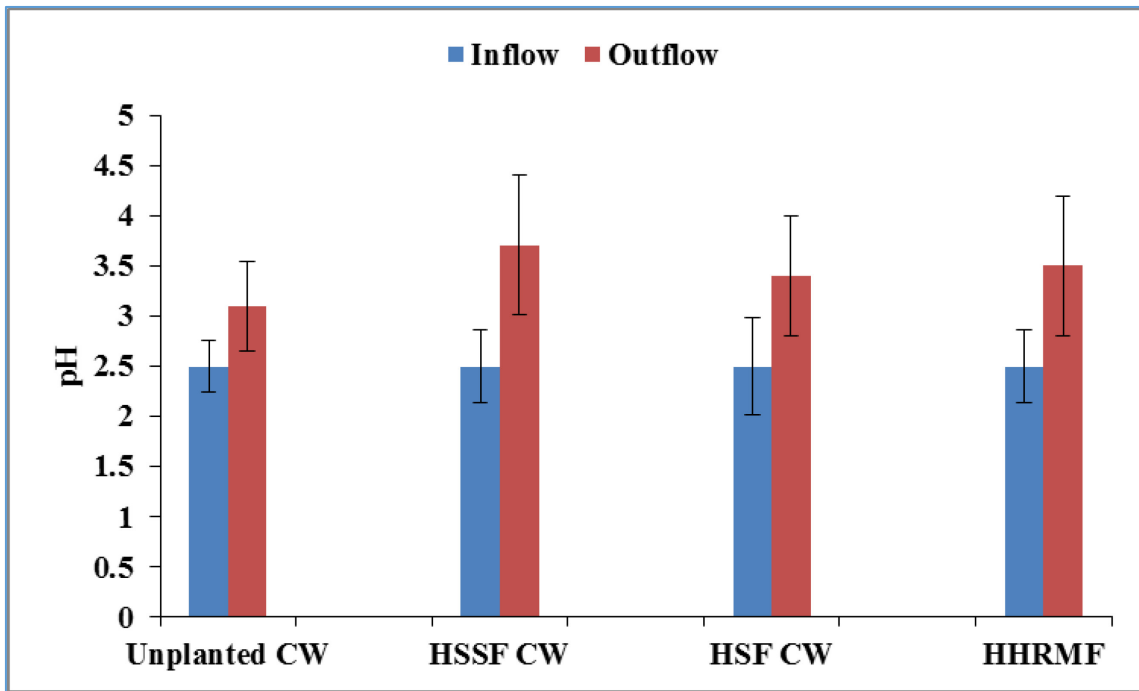


Figure 3.3. 5- Dynamics of mean pH at different depths along the flow path of wetlands.

3.3.3. Dynamics of aluminium removal

The values of mean inflow and outflow load and the removal efficiency are represented in Fig. 3.3.6. During the whole 16 months of operation, a continuous supply of synthetic wastewater provided with a mean inflow load of 0.28 g/m².d (5.3 mg/l) of Al in all four reactors.

In the unplanted wetland there is a decrease of 10% (outflow load 0.015 g/m².d) in Al concentration from inflow to outflow. The wetland was found to be oxic and there is a possibility of formation of oxyhydroxide. But for formation of aluminium oxyhydroxide, apart from oxygen the pH should be in neutral to alkali, since the pH in unplanted wetland was in acidic range Al should occur as Al³⁺ form in aqueous form so removal of Al in this oxyhydroxide form is likely less. Moreover, persistent oxic condition and lack of sufficient electron donor inhibited dissimilatory sulphate-reduction despite the relatively higher sulphate-sulphur concentrations in this wetland. So, Under C-deficient aerobic condition and acidic pH of Al the removal process can be best explained by the process of adsorption by the gravels of the wetland.

The mean outflow loads (g/m².d) are 0.0217, 0.232 and 0.233 which makes the overall mean removal efficiency in all three planted wetlands are 17%, 14% and 16% in HSSF CW, HSF CW and HHRMF respectively. Most of the removal was attained in the first eight months of the experiment as compared to the last eight months of the experiment. In Fig. 3.3.5 it is seen that the overall mean pH was around 4.5. According to Kadlec and Knight, 1996, the minimum solubility of aluminium hydroxide falls within the range of 5.5 to 7, the removal of aluminium by hydroxide precipitate can be ruled out. So, the marginal removal of Al under this condition is perhaps best explained as adsorption by the gravel matrix of the wetland.

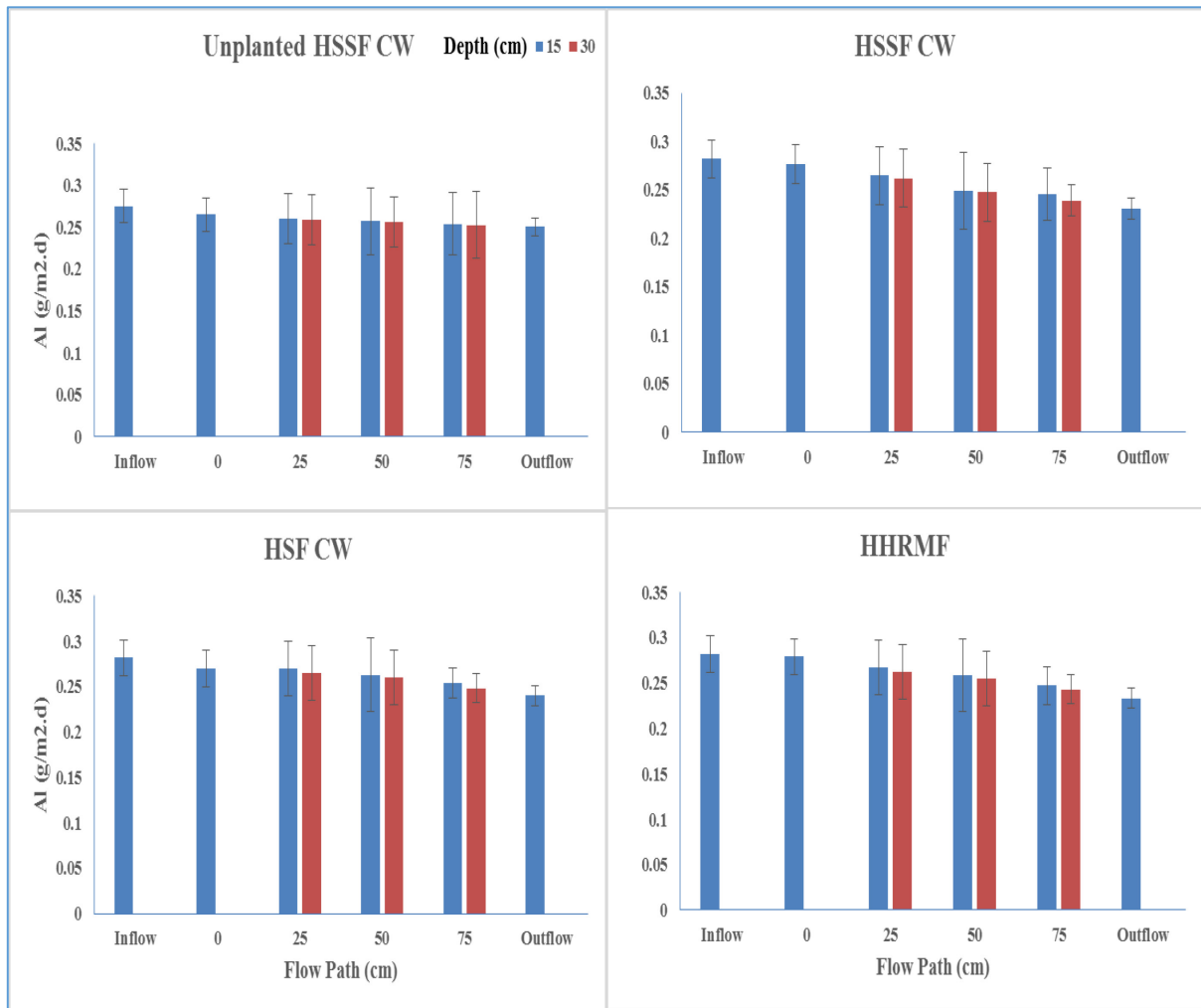


Figure 3.3. 6- Dynamics of mean Al load in the pore water along the flow path of the wetlands.

3.3.4. Growth of plant biomass (shoot density) and water loss (EvT)

Water loss from the wetlands as evapo-transpiration (EvT) was taken into consideration for the calculation of removal rates, which is the mass removal loading rates. This was particularly important to distinguish between planted and unplanted wetlands as because the rate of water loss due to the presence of plant biomass in planted wetlands hugely differed with the wetlands without plants (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011).

The growth status of the plant biomass in terms of total number of green and healthy shoots and therefore the variation in water loss via EvT is shown in Fig. 3.3.7. By the end of experiment, corresponding to 16 months of operation, there was constant decrease in overall green shoot density and by the end of the experiment there were no green shoots available in any of the planted wetlands which explains the decrease in efficiency in terms of sulphate and metals removal.

From the start of the experiment the plants were under stress and the shoots started to turn yellow and as the experiment continued further, plants were more stressed by the low pH and perhaps damaged by high loading rate of metals.

In general, higher plant shoot density contributed to an increment of water loss via plant transpirations. But since there was a decrease in the number of green shoots hence a decreasing tendency of water loss was seen throughout the experiment. Irrespective of the temperature there was a decreasing trend seen in the evapotranspiration rate in all planted wetlands (Fig. 3.3.8).

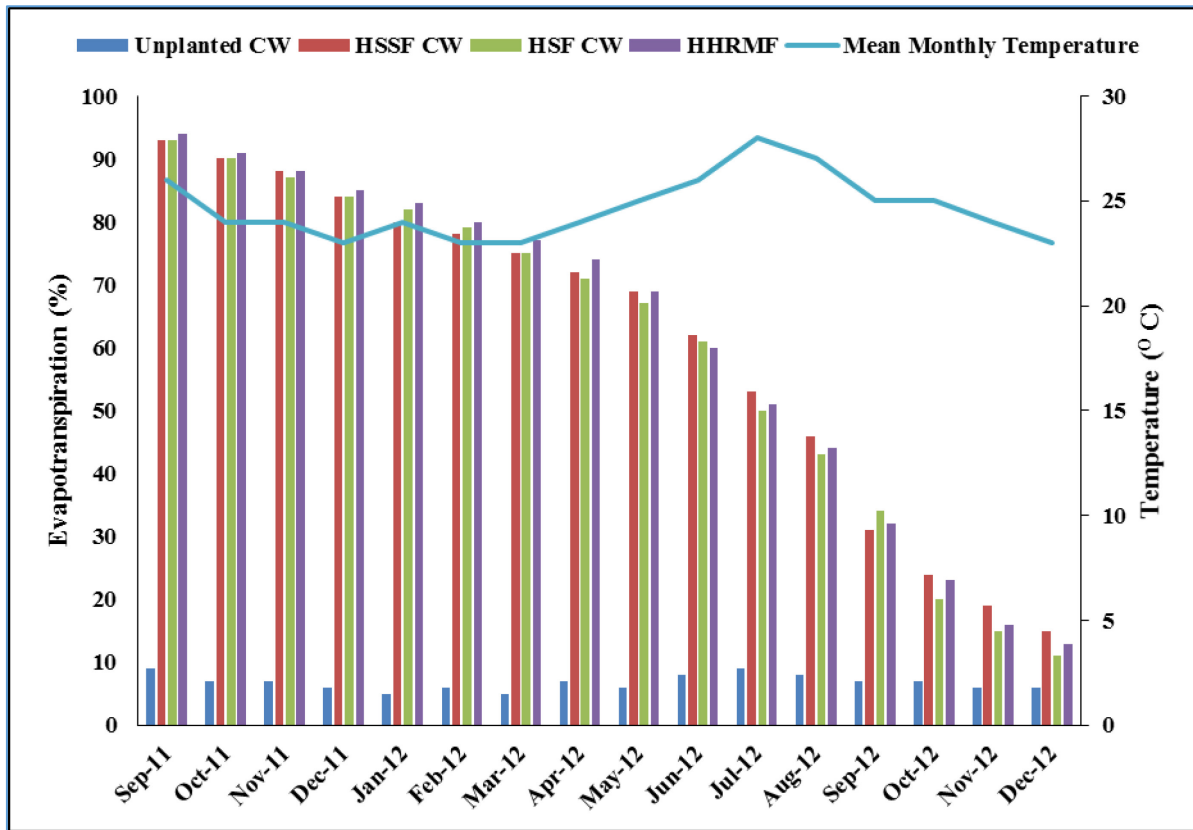


Figure 3.3. 7- Correlation between evapotranspiration (EvT) and temperature fluctuation during whole operation period in the wetlands.

With no obvious changes or drastic fluctuations (between 4% to 9% EVT rate) (Fig. 3.3.7) in unplanted HSSF CW (control), where evaporation from the surface of the wetland bed to the atmosphere was the only way for water loss due to the absence of plants and subsequent plant transpirations.

The effect of the plants on the removal efficiency of heavy metals and sulphate was studied in all experimental phases of planted HSSF CW, HSF CW and HHRMF CW as compared to unplanted HSSF CW (control) and in all cases, it was observed that in planted wetlands retention of metals was marginally higher than that of the unplanted HSSF CW. Likewise the presence of plants also marginally improved sulphate retention within the wetland beds because plant roots serve as carriers for attached microbial growth and release organic exudates into the root zone which leads to contaminant removal in planted CWs than in CWs without plants (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011).

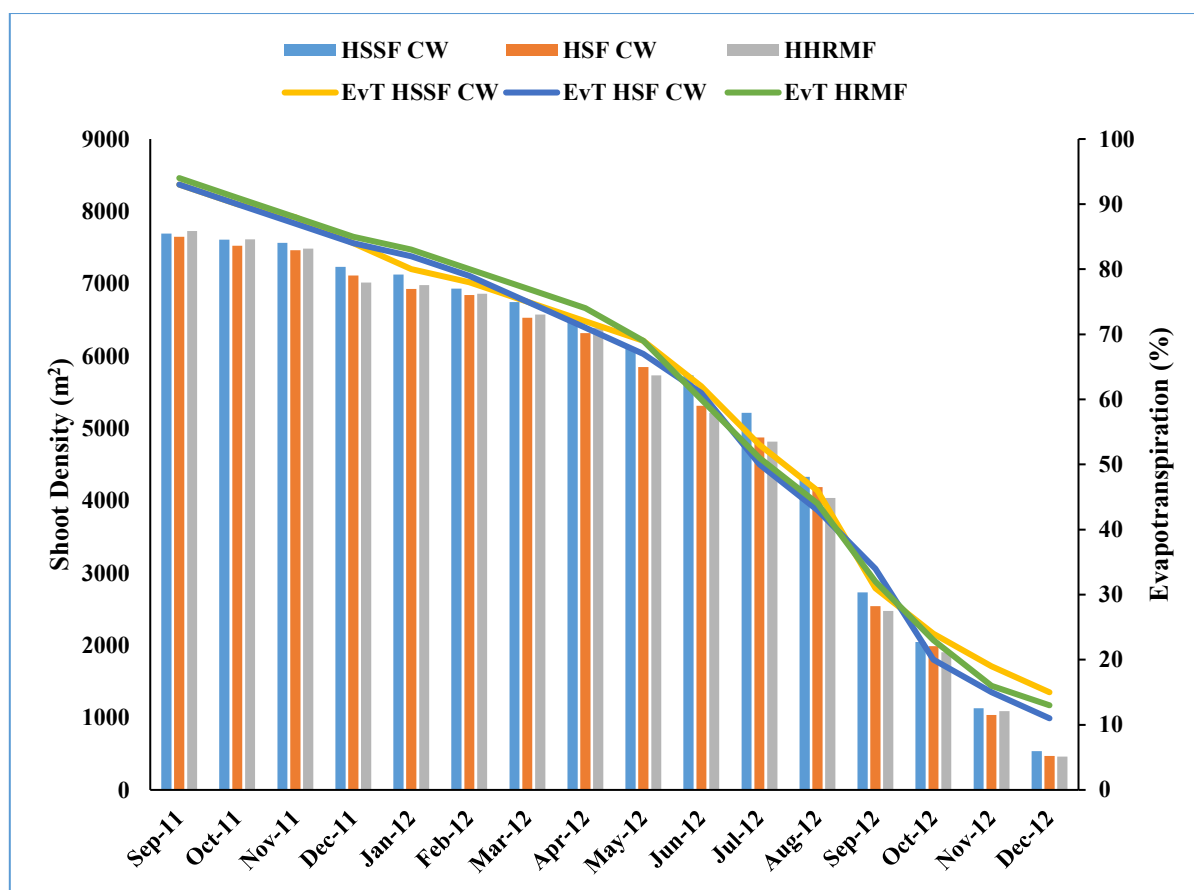


Figure 3.3. 8- Correlation between water loss by plant transpiration (EvT) and green shoot density during whole operation period in the planted wetlands.

3.4. Results with *Juncus effuses* (from Phytotechnicum, with H₂ as external electron donor)

3.4.1. Dynamics of sulphur removal

In this experiment H₂ gas was provided as external electron donor in CW2 and CW1 was kept as control without any H₂ gas. No organic source of carbon is provided to both the wetlands, the only organic carbon source was the rhizodeposits from the root zone.

The dynamics of sulphate-sulphur removal and formation of sulphide within the wetland systems have a major influence on metal removal.

In the experiment it is seen that there was a marginal decrease in the SO₄²⁻-S load in the outflow than the inflow. The mean inflow SO₄²⁻-S load was 3.2 g/m².d (0.06g/l) (Fig. 3.4.1) and the overall mean outflow load (g/m².d) was 2.8 and 2.6. Though there was no significant removal of sulphate seen in this experiment owing to the bad health of the plants in the later part of the experiment but the overall mean removal efficiency stands at 20% and 23% in CW1 and CW2 respectively. Most of the removal was attained in the first eight months of the experiment as compared to the last eight months of the experimental phase.

In the first eight months of the experiment the removal efficiency was 19% and 25% but in the later part of the experiment the efficiency fell to 4% and 9% respectively in CW1 and CW2. It

can be stated that the deterioration of the health of plants resulted in the less efficient sulphate removal in the CWs. As the plant suffered from the high acidic conditions, the less carbon from the rhizodeposits were available from the plants which failed to maintain the reducing conditions needed for dissimilatory sulphate reduction.

Furthermore, the limited removal of the SO_4^{2-} -S load cannot completely be explained with reference to dissimilatory sulphate reduction, as in the last ten months, the non-detection of sulfide in the systems is consistent with the high oxic conditions and very high redox potential.

It can also be inferred that there was not much influx of rhizodeposits in the CWs in the last eight months of the experiment as there was a constant decrease in the number of healthy green shoots of *J. effuses* owing to the very low pH of the artificial AMD and high load of contaminants. As compared with *P. Australis* the influx of rhizodeposits was less in *J. effuses* (Zhai et al., 2013).

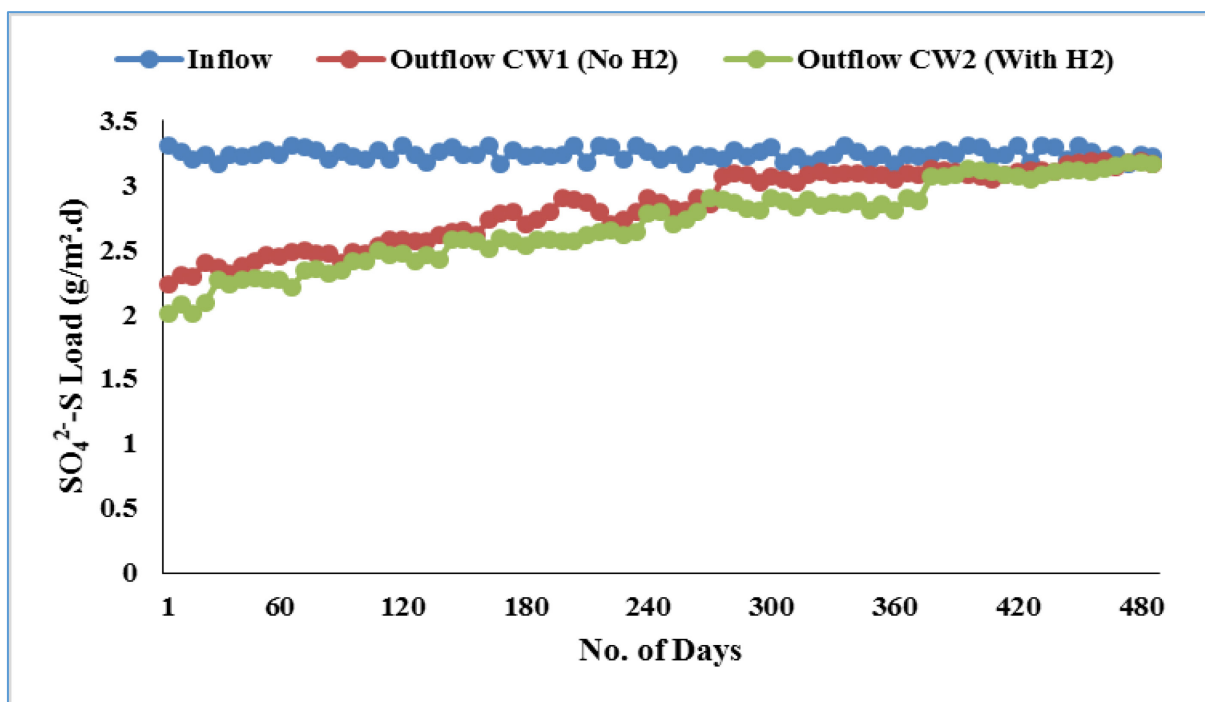


Figure 3.4. 1- Mean SO_4^{2-} -S inflow and outflow load of wetlands.

The limited removal of the SO_4^{2-} -S load in the first few months of the experiment can be due to intake by plants, adsorption to the gravel matrix and DSR due to the presence of multi gradient redox process (both micro- and macro) in constructed wetland conditions (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011, Bezbaruah and Zhang, 2004; Wiessner et al., 2005b). This however does not exclude the possibility that some DSR occurred in anoxic niches of the *J. effusus* systems, and that subsequent reoxidation of sulfide to sulfate took place (Wiessner et al. 2008b). In addition, in the presence of metals, sulfide can form a precipitate and be immobilized inside the CWs. Though sulphide was not detected in the last ten months of the experiment but in the initial months of the experiment there was occasional detection of sulphide in low concentration whereas elemental sulphur remained undetected.

In fact, the removal mechanisms of metals and sulphate are dependent on the pH and redox potential of the environment. The overall redox potential in all planted wetlands were very high for dissimilatory sulphate reduction.

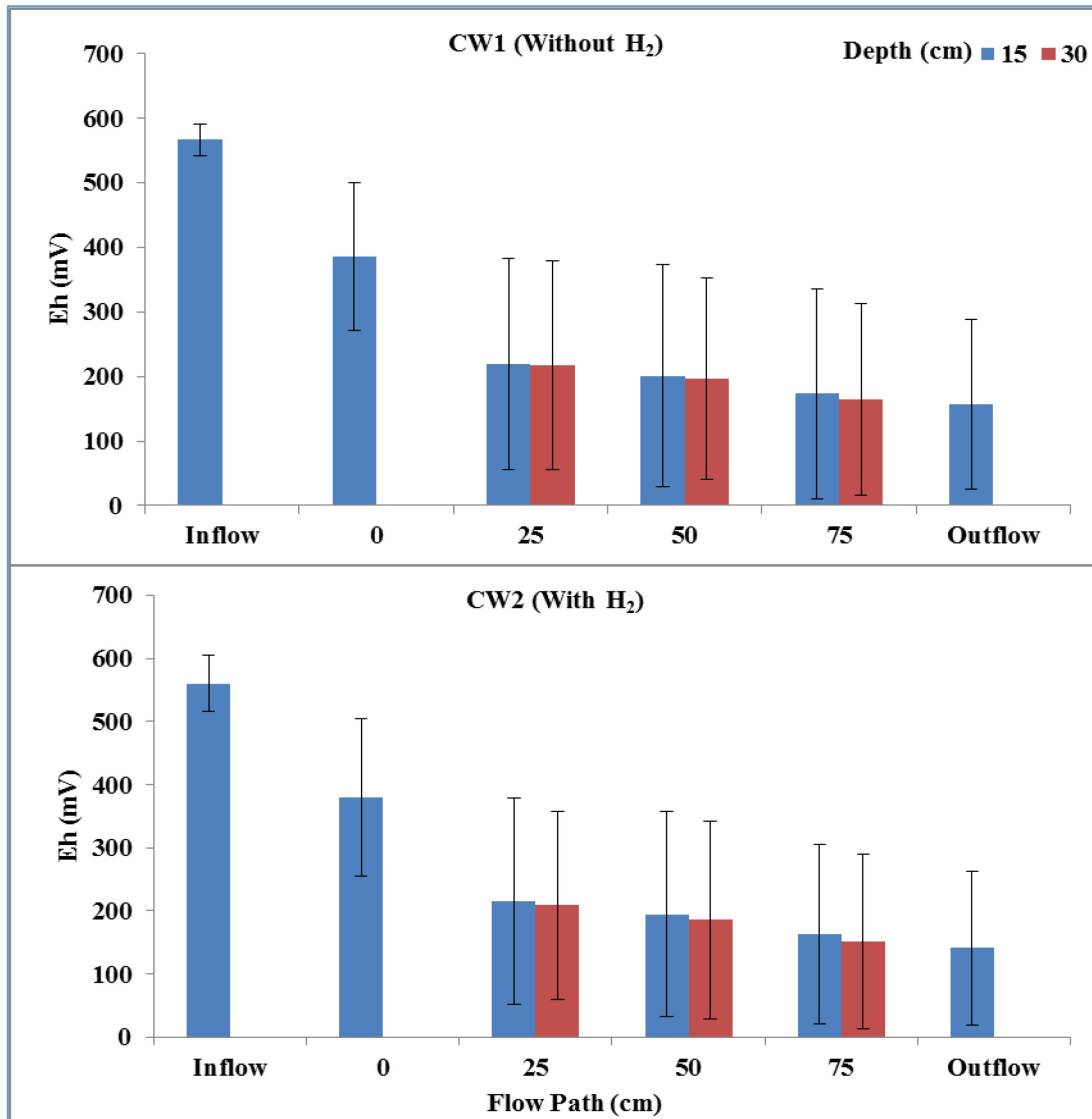


Figure 3.4. 2- Dynamic mean redox conditions in the pore water at different depths along the flow path of wetlands.

The value for the redox potential are presented in Fig. 3.4.2. The least redox values recorded in the CW2 ~ 5 mV (75 cm, 30 cm depth) and in CW1 between ~ 12 mV (75 cm, 30 cm depth) in the initial phase of the experiment. As the experiment proceeded and the decrease in healthy shoots resulted in increase in redox potential values. In this experiment consistent low redox potential values were not maintained in both the CWs throughout the experiments which affected the activity of anaerobic sulphate reducers and in turn the efficiency of sulphate and metal removal process.

In this study, mean inflow oxygen concentration value was always very high around 8 mg/l during the entire experimental period in both the wetlands, whose results are presented in Fig. 3.4.3, varied dependent on system, distance from inflow and depth.

Both the wetlands were always oxic in nature. The highest concentration was seen in the inflow of both the wetlands but the inside of system it was less aerobic as compared to inflow. During the initial part of the experiment the planted wetlands were less aerobic when compared with the latter half of the experimental phase. Of all the planted wetlands the CW2 was the most anoxic (.5 mg/l at 75cm, 30 cm depth) and CW1 to be more oxic (1 mg/l at 75cm, 30 cm depth). This high DO level is reflected in the positive redox values (Fig. 3.4.2).

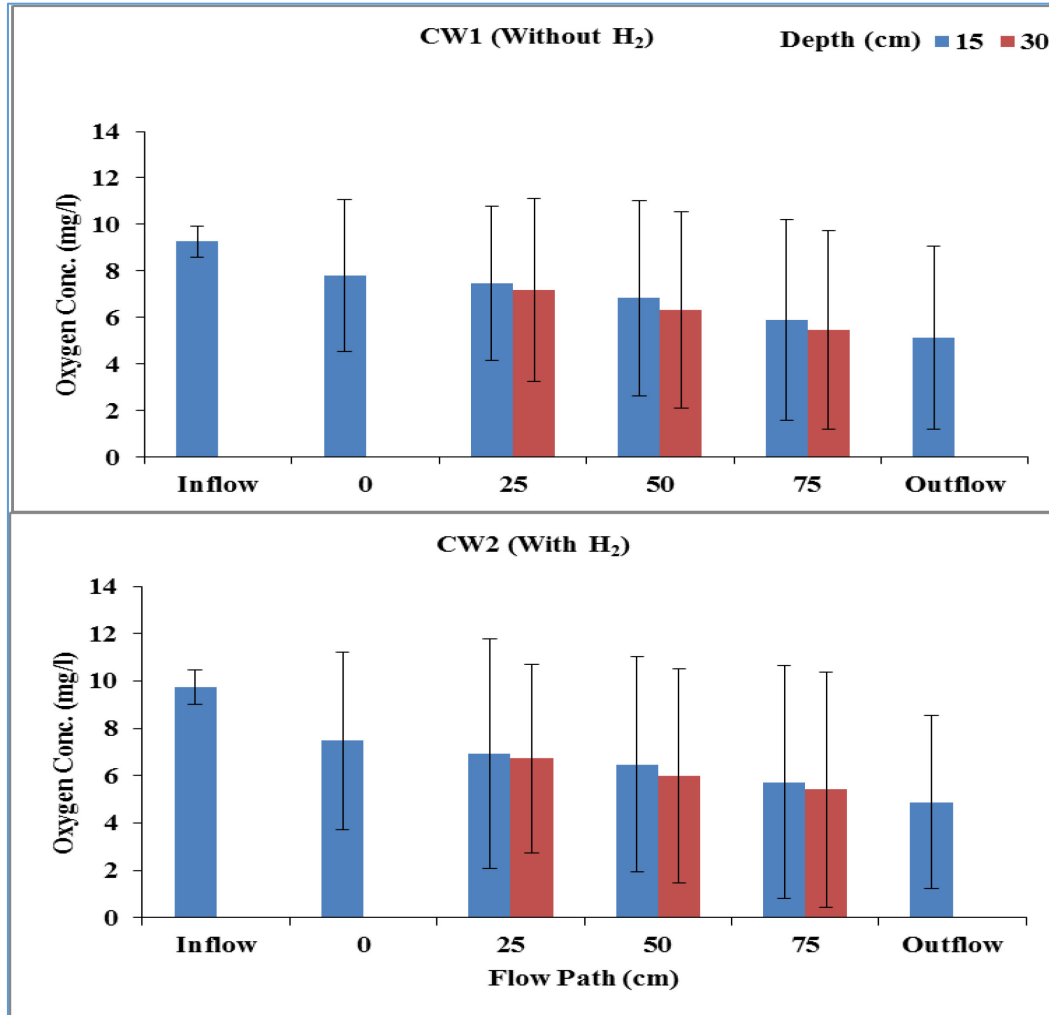


Figure 3.4. 3- Dynamics of mean dissolved oxygen concentrations in the pore water at different depths along the flow path of wetlands.

3.4.2. Dynamics of iron removal

The values of mean inflow and outflow loads and the removal efficiency are represented in Fig. 3.4.4. During the whole 16 months of operation, a continuous supply of synthetic wastewater provided a mean inflow load of 0.1166 g/m².d (2.18 mg/l) of Fe in all four reactors.

The mean outflow load were 0.096 and 0.093 g/m².d which makes the overall mean removal efficiency as 18% and 20% in CW1 and CW2 respectively. Most of the removal was attained in the first eight months of the experiment as compared to the last experimental phase. As the pH in the in the outflow was around 4.5 in the first eight months of the study, some of the Fe³⁺ ions may have precipitated as hydroxides as the solubility of Fe³⁺ ions decrease with the rise in pH (Kosolapov et al., 2004).

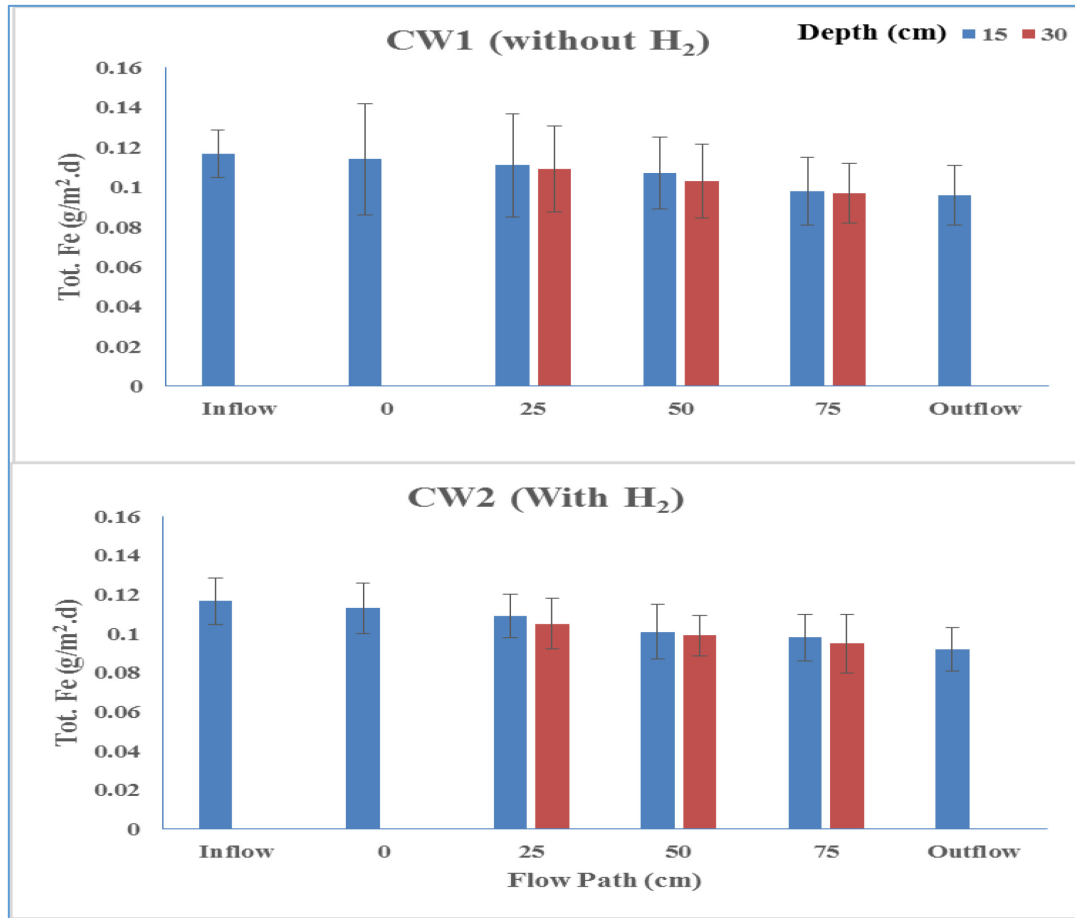


Figure 3.4. 4- Dynamics of mean Fe load in the pore water along the flow path of the wetlands.

In this study, mean inflow pH value was always in the range of 2.5 (Fig. 3.4.5) during the entire experimental period in both the wetlands. There was not much differences in pH values between the wetlands. The highest pH was seen in the outflow of both the wetlands in the

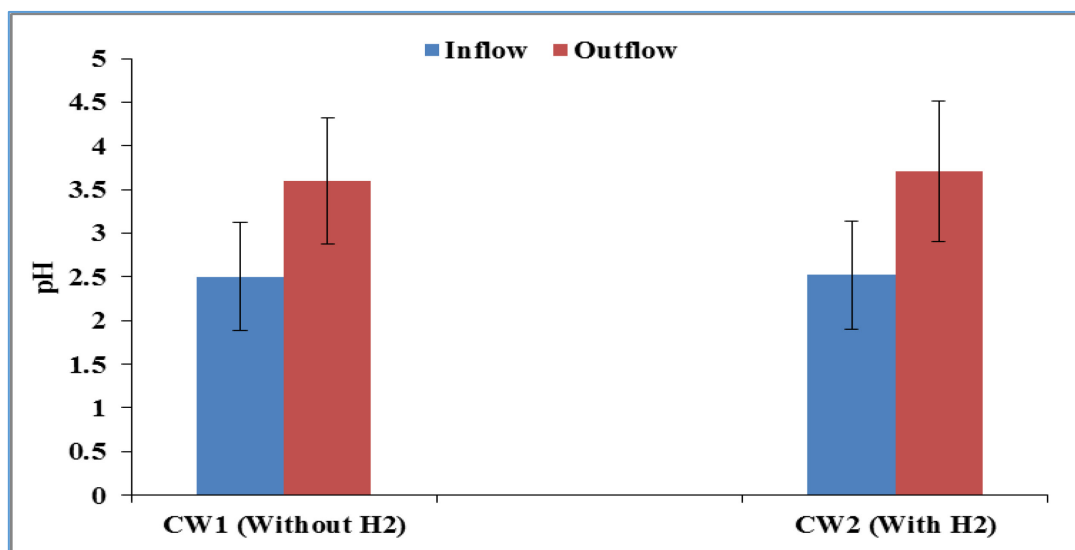


Figure 3.4. 5- Dynamics of mean pH at different depths along the flow path of wetlands.

first phase of the experiment. The outflow pH value was around 4.5. The rise in the pH value is due to the removal of limited amount of sulphate. But towards the second part of the experimental phase the pH of outflow was ~ 3 in all planted wetlands. The decrease in the number of healthy shoots reduced the sulphate removal process resulting in the fall of pH in the planted wetlands (Tiedje et al., 1984; Küsel and Alewell, 2004).

3.4.3. Dynamics of aluminium removal

The dynamics of total aluminium with the values of mean inflow and outflow loads and the removal efficiency are represented in Fig. 3.4.6. During the whole 16 months of operation, a continuous supply of synthetic wastewater provided a mean inflow load of 0.28 g/m².d (5.3 mg/l) of Al in both the wetlands.

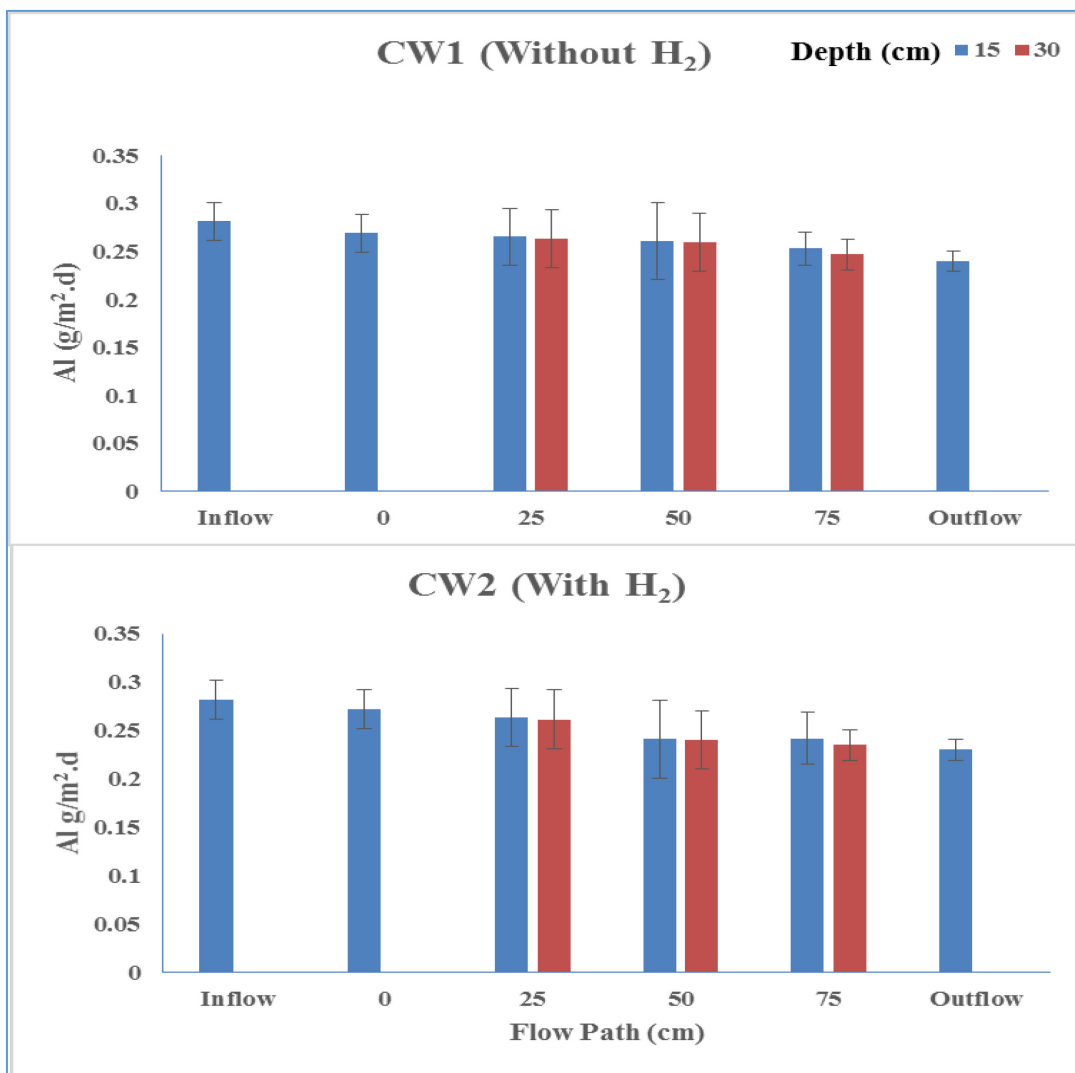


Figure 3.4. 6- Dynamics of mean Al load in the pore water along the flow path of the wetlands.

The mean outflow loads are 0.024 and 0.023 g/m².d which makes the overall mean removal efficiency in all three planted wetlands are 14% and 17% in CW1 and CW2 respectively. Most of the removal was attained in the first eight months of the experiment as compared to the last phase of the experiment.

The wetland was found to be oxic and there is a possibility of formation of oxyhydroxide. But for formation of aluminium oxyhydroxide, apart from oxygen the pH should be in neutral to alkali, since the pH in unplanted wetland was in acidic range Al should occur as Al^{3+} form in aqueous form so removal of Al in this oxyhydroxide form is likely less.

In Fig.3.4.5 it is seen that the overall mean pH was around 4.5. According to Kadlec and Knight, 1996, the minimum solubility of aluminium hydroxide falls within the range of 5.5 to 7, the removal of aluminium by hydroxide precipitate can be ruled out. So the marginal removal of Al under this condition is perhaps best explained as adsorption by the gravel matrix of the wetland.

3.4.4. Growth of plant biomass (shoot density) and water loss (EvT)

Water loss from the wetlands as evapo-transpiration (EvT) was taken into consideration for the calculation of removal rates, which is the mass removal loading rates.

The growth status of the plant biomass in terms of total number of green and healthy shoots and therefore the variation in water loss via EvT is shown in Fig.3.4.7. By the end of experiment, corresponding to 16 months of operation, there was constant decrease in overall green shoot density and by the end of the experiment there were no green shoots available in any of the planted wetlands which explains the decrease in efficiency in terms of sulphate and metals removal.

From the start of the experiment the plants were under stress and the shoots started to turn yellow and as the experiment continued further, plants were more stressed by the low pH and perhaps damaged by high loading rates of metals. In general, higher plant shoot density contributed to an increment of water loss via plant transpirations. But since there was a decrease in the number of green shoots hence a decreasing tendency of water loss was seen throughout the experiment. Irrespective of the temperature there was a decreasing trend seen in the evapotranspiration rate in all planted wetlands (Fig. 3.4.8).

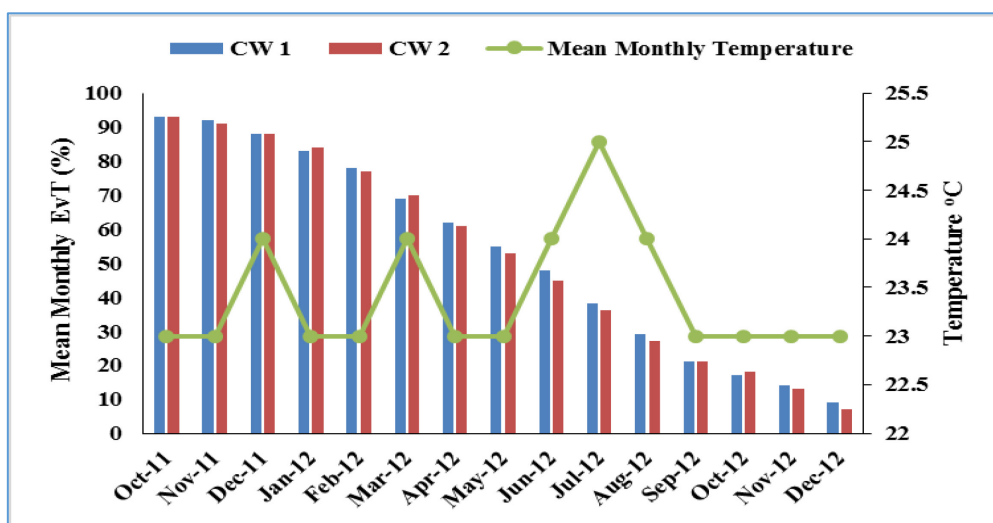


Figure 3.4. 7- Correlation between evapotranspiration (EvT) and temperature fluctuation during whole operation period in the wetlands.

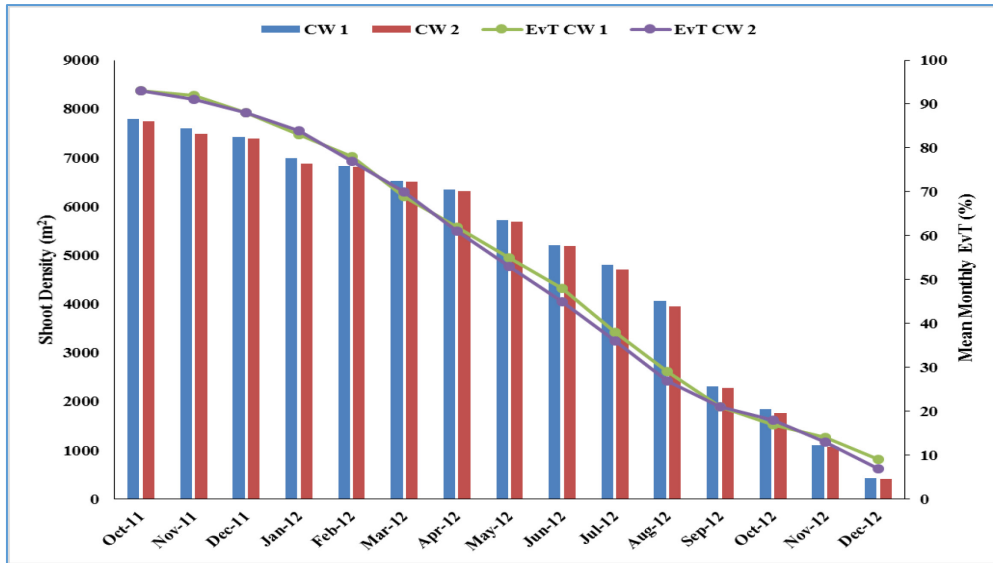


Figure 3.4. 8- Correlation between water loss by plant transpiration (EVT) and green shoot density during whole operation period in the planted wetlands.

3.5. Outcomes and general remarks

Based on the results of this study the important observations and interpretations can be summarized as follows:

- In greenhouse, without external electron donor, the above data suggest that HSSF CW has the highest efficiency in the removal of total sulphur (80%) followed by HHRMF (66%) and HSF CW (61%) in the experiment with *P. australis* but in the experiment with *J. Effusus*, HSSF CW shows an efficiency of 18% followed by HHRMF and HSF CW at 17 each. The sulfate removal rates were 3.5 to 4 times higher in the *P. australis* as compared to the *J. effuses*. DSR was estimated to be the main process of sulfate removal and was proved by the detected sulfide in the *P. australis* CWs.
- In case of Fe removal, a high mean removal efficiency of >98% is obtained in all the three wetlands planted with *P. australis* which is in accordance with previous research (Luca et al., 2011 and Xu et al., 2009) where more than 50% of total Fe is removed at one-third length of the wetlands. In case of Al a mean removal efficiency of >89% is seen in all the three wetlands planted with *P. australis* which is in accordance with previous research (Wood and McAtamney, 1996) whereas in the experiment with *J. effuses* the efficiency for Fe removal is around 4% and for Al removal is 17%.
- Since acid mine drainage is characterized by very low pH and high concentration of heavy metals and sulphate, choice of plants plays a major role in the treatment. In the experiment with *J. effuses*, even under controlled environment, it is seen that the plants could not withstand the harsh conditions and started to suffer immediately as the AMD is fed into the wetlands due to which the performances of the wetlands were very poor. But on the other hand, in the experiment with *P. australis* shows a much-improved performances in the planted wetlands. *P. australis* proved to be more robust than *J. effuses* as *P. australis* could withstand the acidic conditions till the end of the experimental period whereas *J. effuses* started to die few days after the start of the experiment.
- The results in this study indicated that there were significant differences between the unplanted and planted experimental wetlands in terms of removal rates. In planted CWs the sulphate removal efficiency is >60% whereas in unplanted CW the sulphate removal efficiency is ~8%.
- The simultaneous changes in redox potential proved to be one of the important factors for the sulphate and Fe removal efficiency in constructed wetlands. In the planted wetlands (*P. Australis*) throughout the experiment a negative redox potential is maintained inside the wetlands. Redox potential was in turn the main indicator for which sulfur transformations occur in a CW. At low redox potential, the available sulphate was utilized as a terminal electron acceptor for the oxidation of organic matter (rhizodeposits). Where as in unplanted wetland the redox potential was always in the positive scale which shows that no sulphate reduction took place. The results highlighted the importance of plant and their biochemical and physical processes for the removal process of the contaminants despite the adverse effect of metals, pH and sulphide toxicity on the plants.

- pH plays an important factor in the removal of metals like Fe and Al. When the pH raises from acidic towards neutral metals like Fe and Al precipitates as hydroxides and oxyhydroxides. The rise in pH is associated with removal of sulphate from the water. Due to high intensity of DSR in the planted wetlands the sulphate was removed from the artificial AMD which resulted in the increase in pH towards neutral range which in turn helped in the precipitation of Al and Fe.
- It is very necessary to optimize the design of CWs by selecting a more suitable species of helophytes for an intended treatment objective. For example, application of *J. effusus* CWs can be advantageous when the wastewaters intended for treatment are not low in organic carbon and/or when the removal of carbon is intended, since *J. effusus* releases considerable oxygen (Wiessner et al. 2002b) that can stimulate the oxidation of organic matter and at the same time does not release large amount of organic carbon in form of rhizodeposits that may pose extra treatment requirement.
- On the other hand, *P. australis* can be a suitable helophyte for treatment of contaminated waters when organic carbon is limited for e.g. to promote sulfate reduction; or for the treatment of acid mine drainage. This would provide economic savings as well as environmental benefits since the current practice in these cases is to add external organic carbon to stimulate the required processes (Henze 1991).
- The overall experimental results suggested the practicability of applying horizontal subsurface-flow wetlands planted with *P. australis* in pilot-scale basis for a long-term investigation and afterwards full-scale operation units to treat acid mine drainage prior to disposal to the nearby water body (rivers, lakes etc.)
- In phytotechnicum, with H₂ as electron donor, the mean removal efficiency of sulphate in CW1 (without H₂ as electron donor) was 61% whereas the mean removal efficiency of CW2 (with H₂ as electron donor) was 73%. With hydrogen as electron donor the performance efficiency was increased by almost 12% for sulphate removal and almost 6% increase in Al removal efficiency. The removal efficiency for total Fe for both the wetlands is > 98%.
- From this experiment it is seen that hydrogen has proved to be an efficient, cost effective and clean (can be produced by electrolysis of water by using green energy) electron donor, further research-based investigation must be performed into using hydrogen in pilot- scale plants in future.
- Metals tends to be retained in wetland vegetation and sediments, this waste may be localized in the environment but this sediment deposits may have adverse effects on wetland vegetation and its efficiency (Rahman, K.Z, 2008, Rahman et al., 2008, Rahman et al., 2011). So, for wetland systems to function as efficient and effective manner, a practical and viable waste disposal mechanism for the heavy metal contaminated biomass and sediments must be developed.
- Further studies should focus on understanding i) the effects and mechanisms of sulphur transformation processes on the removal efficiencies in constructed wetlands, ii) microbial pathways for various chemical transformation processes, iii) effect of metals and sulphur toxicity on plants and microbes.

- The above experimental data suggests that constructed wetlands can be effective in removing sulphate and Fe from Acid Mine Drainage under carbon limited and anoxic conditions (as discussed in details in chapter 3) before their disposal into the water bodies. These results encourage the construction of a pilot-scale wetland coupled with H₂ as electron donor to further investigate the effectiveness of constructed wetlands (in large scale) for the treatment of wastewater from mine drainage.
- In general, we can conclude that the design parameters in terms of flow direction/path/type, aeration scheme and choice of plants affect directly the sulfur cycle processes in the system. Therefore, of all the parameters that influence DSR (e.g. availability of electron donors/acceptors, temperature, etc.), should be taken into consideration as they are the most influencing factor needed for setting up CWs.

4. Conclusions

Cost effectiveness and energy efficient technologies for the treatment of wastewater are the need of the hour in developing as well as in industrialized countries. Among all the treatment processes, the anaerobic treatment process technology for wastewater stands out in terms of its capability of high energy saving potential. In general, constructed wetlands are increasingly used, especially for the treatment of AMD as they have the advantages of using the nature-based process or nature's energy to perform various physical, chemical and biological processes.

The performances of wetlands are determined by various factors/processes occurring in the general environment and as well as in the wetlands which in turn influence the final outcome/efficiency of the system. Of the several processes occurring in the wetlands, the oxidation and reduction process occurring at the root zone in the system is the key factor for the removal process of the contaminants like sulphur and Fe. The other factors which play decisive role for the contaminant removal are pH, temperature, choice of plants, loading rate of wastewater and retention time. For efficient removal of contaminants and smooth functioning of the wetland systems, a deep knowledge of the interactions between various key elements needs to be analyzed and understood in all possible ways.

It was shown that the planted horizontal subsurface-flow constructed wetland (with *Phragmites*) had the highest treatment performance for sulphate and heavy metals removal than the unplanted horizontal subsurface-flow constructed wetland which has the lowest efficiency, under C-deficient (absence of external electron donor) and this illustrates the importance of the presence of plants in the treatment system as process like dissimilatory sulphate reduction and co-precipitation can occur simultaneously in the root zone of treatment wetlands due to simultaneous change in redox (aerobic-anaerobic) conditions.

Furthermore, it is seen from the experiment that the choice of proper plants for the treatment process in constructed wetlands is as important as any other factor. *Phragmites australis* proved to be more robust and effective in treating AMD as it can withstand the extreme low pH as unlike *Juncus effuses* which are very sensitive to low pH which in turn decreases the efficiency of the system.

Moreover, the choice and effect of external electron donor on the final outcome/efficiency of the treatment process is immense. Organic electron donor like methanol and propionic acid has high impact on the treatment process with increased efficiency but their further metabolites could result in tertiary contaminants. But, H₂ gas on other hand acts as much cleaner, efficient and cost-effective electron donor in terms of its production (Khotari et al., 2008)

Further, it has been a huge challenge dealing with the toxic waste sediments and bio solids (dead plants from the wetlands) especially generated after the treatment of AMD. So proper approach and technologies should be researched and implemented for proper post treatment waste management for the betterment of the environment.

In future better understanding and knowledge of these above-mentioned aspects would help us to widen the treatment process of constructed wetlands and provide better working knowledge for the construction of highly modified constructed wetlands which can prove to be much more efficient in comparison to various other available technologies.

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