Effect of depth and plants on pollutant removal in Horizontal Subsurface flow constructed wetlands and their application in Ethiopia

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Kurzzusammenfassung

Die vorliegende Arbeit behandelt die Schadstoffeliminierung, das Nitrifikationspotenzial und das hydrodynamische Verhalten horizontal durchflossener Bodenfilter (*subsurface flow-constructed wetlands*, HSSFCW) in Abhängigkeit von ihrer Beettiefe und Bepflanzung. Die betreffenden Untersuchungen erfolgten in Langenreichenbach (Deutschland) von September 2010 bis September 2012 und in Arba Minch (Äthiopien) von Juli 2012 bis März 2013. An beiden Standorten wurden unbepflanzte und bepflanzte (*planted*, p) Bodenfilter mit Beettiefen von 25 cm (H25) und 50 cm (H50) im Pilotmaßstab bei hydraulischen Aufenthaltszeiten (*hydraulic resicence time*, HRT) von 6 Tagen untersucht. In Arba Minch erfolgten zudem durch Einstellung erhöhter flächenbezogener Belastungsraten (*hydraulic loading rate*, HLR) Untersuchungen bei HRT von 1,5 Tagen (H25) und 3 Tagen (H50). Während in Arba Minch nur die Influenten und Effluenten untersucht wurden, erfolgten in LRB zusätzlich Analysen von Lösungen verschiedener Probenahmepunkte in den Bodenfiltern selber.

Tracer-Studien in LRB belegten einen höheren hydraulischen Wirkungsgrad von Bodenfiltern mit einer Tiefe von nur 25 cm. In Filtern beider Beettiefen zeigte Pflanzenbewuchs diesbezüglich nur geringe Effekte.

In LRB waren bei gleichen HRT die flächenbezogenen Eliminierungsraten in Bodenfiltern mit Beettiefen von 50 cm für BSB_5, TOC, TN und *E. coli* signifikant höher als die in Bodenfiltern mit 25 cm Tiefe. Für NH_4^+-N zeigten sich hier keine Unterschiede. Die volumenbezogenen Eliminierungsraten der Bodenfilter H25p und H50p für TN und NH_4^+-N waren durch signifikante Unterschiede gekennzeichnet. Hingegen wurden die BSB_5, TOC, TN, NH_4^+-N und *E. coli* nicht durch die Beettiefe beeinflusst. Der Effekt der Bepflanzung auf die Eliminierung von TN und NH_4^+-N war im Gegensatz zu der von $CBSB_5$, TOC und *E. coli* sowohl bei Volumen- als auch Flächenbezug signifikant.

Bei gleicher HLR und damit unterschiedlichen HRT differierten in Arba Minch die flächenbezogenen Eliminierungsraten für TSS, BSB_5, CSB, TKN, PO_4^{3-}-P und NH_4^+-N in Bodenfiltern mit 25 cm und 50 cm Bodentiefe nicht signifikant voneinander. Hinsichtlich der volumenbezogenen Raten zeigte sich für TKN eine signifikant höhere Eliminierung in den H25 Becken. Die Bepflanzung wirkte sich hier sowohl auf die flächen- als auch volumenbezogenen Eliminierungsraten von TKN, NH_4^+-N und PO_4^{3-} - P positiv aus.

Unter Verwendung des P-k-C* Modells war die Konstante k_A, bei 20°C für TN und NH_4^+-N in der Anlage Arba Minch erhöht und der BSB_5 in beiden unbepflanzten Becken auf einem ähnlichen Niveau.

Abstract

This study investigated the effect of depth and plants on pollutant removal in Horizontal Subsurface Flow Constructed Wetlands (HSSFCW). The hydrodynamic behaviour and nitrification potential of the wetlands were also assessed. In order to study the effect of depth and plants on HSSFCW, two independent studies were conducted. The first was from September 2010 to September 2012 at Langenreichenbach (LRB), Germany and the second was from July 2012 to March 2013 in Arba Minch, Ethiopia. At both sites four pilot-scale beds (planted and unplanted) with a water depth of 25 cm and 50 cm were constructed. The systems at LRB were operated at the same hydraulic residence time (HRT) and samples were collected from the influent, effluent and from internal points along the wetland length. At Arba Minch, only inlet and outlet monitoring was conducted at the same HRT and at the same hydraulic loading rate (HLR).

Tracer studies in LRB demonstrated that the 25 cm deep systems had higher hydraulic efficiency than the 50 cm deep beds and there was little difference in hydraulic efficiency between planted and unplanted beds at the same depth.

At the same HRT (LRB), the areal mass removal rate for $CBOD_5$, TOC, TN and *E.coli* of 50 cm deep beds were significantly greater than 25 cm deep beds while the NH_4^+-N was the same. The volumetric mass removal rate was significantly different for TN and NH_4^+-N between H25p and H50p but the same for $CBOD_5$, TOC, TN, NH_4^+-N and *E.coli* with respect to depth. The areal and volumetric mass removal rate of the $CBOD_5$, TOC and *E.coli* were not significantly different but TN and NH_4^+-N of planted beds were significantly greater than the unplanted beds.

At the same HLR (Arba Minch), the areal mass removal rate of TSS, $CBOD_5$, COD, TKN, PO_4^{3-}-P and NH_4^+-N were not significantly different for 25 cm and 50 cm deep wetlands. The volumetric mass removal rate of TKN was significantly different but TSS, $CBOD_5$, COD and NH_4^+-N were not significantly different with respect to the 25 cm and 50 cm deep beds. The areal and volumetric mass removal rate of TSS, COD and $CBOD_5$ were not significantly different between planted and unplanted beds. However, areal and volumetric rates for planted beds were significantly greater than the unplanted beds for TKN, NH_4^+-N and PO_4^{3-}-P. In conclusion, 25 cm deep beds are more advantageous in performance than 50 cm deep beds at the same hydraulic loading rates.

Based on the P-k-C* model, the $k_{A,}$ at 20^0C of $CBOD_5$ for LRB and Arba Minch were the same for unplanted wetlands at the same HRT and the k_A of TN and NH_4^+-N of the Arba Minch was higher than that of LRB.

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List of Abbreviations

symbol	definition
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
BOD	Biochemical oxygen Demand
$CBOD_5$	Carbonaceous biochemical oxygen demand
COD	Chemical oxygen Demand
CSTR	Continuous stirred tank reactor
DO	Dissolved oxygen
DTD	Detention time distribution
ET	Evapotranspiration
H25	25 cm deep un planted bed
H25p	25 cm deep planted bed
H50	50 cm deep un planted bed
H50p	50 cm deep planted bed
HLR	Hydraulic loading rate
HSSFCW	Horizontal subsurface flow constructed wetlands
HSSF	Horizontal subsurface flow
KVD	Rate coefficients (K) value distribution
LRB	Langenreichenbach
LMMRM	Linear mixed model repeated measure analysis
nHRT	Nominal hydraulic retention time
NTIS (N)	Number of tanks in series
NH_4^+-N	Ammonia nitrogen
NO_3^--N	Nitrate nitrogen
NO_2^--N	Nitrite nitrogen
ORP	Oxidation reduction potential
PO_4^{3-}-P	Phosphate phosphorus
RTD	Retention time distribution
SSF	Subsurface flow
TW	Treatment wetland
TN	Total nitrogen
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
TSS	Total suspended solids

List of Symbols

symbol	definition
A	area
C	Effluent concentration
C^*	Background concentration
C_i	Inlet concentration
e_v	Hydraulic efficiency
E_h	Oxidation reduction potential at standard values
h	depth
k_A	Areal rate coefficient
k_v	Volumetric rate coefficient
p	Apparent tanks-in- series number (p-value)
q	Areal hydraulics loading rate (mm/d)
Q	Flow rate
Q_i	Inflow rate
Q_o	Outflow rate
t	time
T	Temperature
τ	Hydraulic retention time (hydraulic detention time)
τ_i	Hydraulic retention time of a single hypothetical tank, TIS
τ_n	Nominal detention time (V/Q)
Γ	Gamma function
ε	Porosity of gravel bed
Θ	Dimensionless time
θ	Temperature factor
σ^2	variance
σ_θ^2	the normalized variance
t_d	normalized delay time
λ_t	normalized detention time
λ_p	normalized peak time

Publications:

- Kinfe Kassa, Tom Headley, Jaime Nivala, Katy Bernhard Manfred van Afferden, Matthias Barjenbruch, Roland Müller (2013) Comparison of the nitrification potential of passive and aerated horizontal subsurface flow constructed wetlands Wetpol 2013, 5th international Symposium, Nantes, France.

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1 Introduction

1.1 Background of the research

Wastewater treatment technologies are based on the combination of physical, chemical and biological mechanisms in treating wastewater and they can be classified into natural systems (wetlands and waste stabilization ponds) and conventional systems (trickling filters, activated sludge, etc) (Pescod 1992). Conventional systems are characterized by higher rate of biological process (aerobic) at the expense of intensive energy consumption and high operation and skilled manpower requirement. While natural systems use aquatic plants and organisms at low capital cost and less sophisticated operation and maintenance (Crites and Tchobanoglous 1998).

Constructed wetlands are designed to utilize naturally occurring processes associated with vegetation, soils, and their associated assemblages to assist in treating wastewater (Kadlec and Knight 1996, U.S.EPA 2000). The relatively slow rate of operation and land intensive nature of constructed wetlands in comparison to high energy intensive conventional wastewater treatment systems is a disadvantage. However, constructed wetlands are designed to utilize natural processes and limited fossil fuel input that do not disturb the environment and require less trained manpower for their operation in comparison to other technologies (Kadlec and Wallace 2009). Constructed wetlands are used to treat many wastewater effluents coming from different sources including: municipal, industrial wastewaters, storm water and agricultural effluents (Vymazal 2011).

There are two types of constructed wetlands according to the hydraulic characteristics of the system: surface flow wetland and subsurface flow wetlands. Subsurface flow constructed wetlands are divided into two subcategories: vertical and horizontal subsurface flow constructed wetlands. Overall, subsurface flow treatments are more effective than surface flow wetlands at removing pollutants at high application rates. However, surface flooding and media clogging can result in reduced efficiency of subsurface systems (Shutes 2001, Wallace and Knight 2006).

HSSFCW have historically been constructed with depths of 50 cm or more (U.S.EPA 2000, Brix and Arias 2005, DWA 2006). One factor which encourages designers to build HSSFCWs deeper is to reduce the surface area, based on the assumption that treatment performance is primarily determined by the hydraulic detention time (HRT) (Reed and Brown 1995, Reed et al. 1995). If this assumption is true, then two HSSFCW of different depths but operated with the same HRT should give the same pollutant removal performance. However, this assumption has been called into question based on available evidence from monitoring and research which suggests treatment performance is more strongly governed by area-related factors (U.S.EPA 2000). For example, the amount of plants and associated roots in a HSSFCW is more dependent on the wetland area than its subsurface volume.

Another factor that is often considered is the rooting depth of the plants. Based on the assumption that the plants play an important role in treatment, the philosophy is based on the fact that the depth of a HSSFCW should ideally be limited to the rooting depth of the plants used. A commonly cited study for this is Gersberg et al. (1986) which predicted that the penetration of the plant root depth was 30–76 cm depending on the species and as a result these findings are commonly cited in guidelines for designs. However, there is some danger in extrapolating rooting depths of plants growing in natural wetlands with relatively low nutrient concentrations and organic loads while being rooted in soft and sandy sediments. Rooting depths measured in such conditions are often greater than those observed for plants growing in gravel media treating wastewater. The shear strength of gravel can provide a resistance to root development while the high oxygen demand of the wastewater provides a further impediment to root development. Consequently, studies indicate that plant roots penetrate into considerably shallower (mostly <30 cm) gravel-bed systems (Tanner 2001). The majority of plant roots occurs within the top 20 cm of the gravel media where their contribution towards treatment is minimal below that (Headley et al. 2003, Headley et al. 2005). Kadlec & Wallace (2009) explained the rooting depths (20-30 cm) do not differ much among species in nutrient rich waters. Assuming plant roots play an important role in treatment processes, and then it would be reasonable to assume that the wetted media depth in HSSFCW should be limited to match the rooting depth in order to avoid water short-circuiting beneath the root zone and potentially receiving less treatment. Based on such assumptions, it can also be hypothesized that treatment performance in HSSFCW cannot strictly be considered as an areal phenomenon, since a lower rate of pollutant removal might be expected for deeper beds where a significant fraction of the water bypasses the root zone.

Research over recent years has raised questions about the optimal depth for horizontal subsurface flow constructed wetlands (HSSFCWs), with shallower beds which force the water to flow through the root zone seemingly more efficient than deeper ones, especially with regard to aerobic processes. Garcia et al. (2003) and Garcia et al. (2004) reported the removal of pollutants in HSSFCW increased with decreasing depth and attributed these effects to differences in the chemical environment (high oxidation reduction potential) which is responsible for increasing nitrification. The authors conducted the experiment with HSSFCW systems with 27 cm deep planted beds. HSSFCW systems that showed the best nitrification performance were shallow gravel horizontal flow cells because their flow is restricted to the most effective portions of the plant root zone in the top of 15-30 cm (Tanner 2001). Oxygen is supplied to the nitrifiers from a combination of the oxygen release from roots and rhizomes and oxygen diffusion and convection directly from the atmosphere (Tanner 2001). Brix (1994b) summarized the calculated oxygen release of *Phragmites australis* roots 0.02-12 g m^{-2} day^{-1}, the wide range of the values ascribed partly by the different experimental methods and season of measurement. With regard to oxygen and distribution of degrading bacteria (Gagnon et al. 2007) showed that aerobic or facultative bacteria were present in greater numbers within planted beds rather than unplanted beds, particularly on root surfaces. The attached biofilm suggest root oxygen release and low activity of these bacteria in interstitial water. These findings suggest why shallow planted constructed wetland beds may be more efficient than deeper ones and unplanted ones.

It is hypothesized that limiting the depth of HSSFCW to the active rooting depth of the plants (ca. 25 cm) may improve treatment by forcing the wastewater to pass directly through the root zone, thereby enhancing plant-related effects such as root oxygen leakage. This depth designs were chosen based on the results of Garcia et al. (2005).Therefore in this thesis, the removal efficiency of shallow (25 cm wetted depth planted and unplanted) and deeper (50 cm wetted depth planted and unplanted) constructed wetland beds are compared for domestic wastewater treatment. The research was conducted partly at a pilot plant in Langenreichanbach, Germany under a temperate climate, and partly in Arba Minch, Ethiopia under semi arid climate conditions. The efficiency of the wetlands during the first two years of operation was studied and the performance evaluation of four wetlands was investigated from September 2010 to September 2012 in Langenreichenbach, Germany. The hydraulic characteristics of the wetlands were investigated using tracer studies. In addition, the nitrification potential in the wetlands were also analysed at LRB site in Germany. The effect of depth and presence of plants on treatment performance was also studied in the semi arid environment of Arba Minch, Ethiopia. The objectives of the study were to investigate the effect of depth and plants on the performance of the pilot scale HSSFCW in treating municipal wastewater under both temperate continental and semi-arid climates.

1.2 Problem statement

The previous introduction has identified that there is a knowledge gap in regards to the effective of depth for treatment performance in horizontal subsurface flow constructed wetlands. There is a need to identify which wetland depth is the most effective and to understand the role of plants in wastewater treatment. Thus, a set of HSSFCW were established in Germany and Ethiopia with wetted depths of 25 and 50 cm, including both planted and unplanted versions, in order to answer the following aims and objectives. The overall aims of the project were:

1. to investigate if there is a difference in hydraulic characteristic between 25 and 50 cm deep beds.
2. to determine if there is a difference in the pollutant removal rates in 25 cm and 50 cm deep horizontal flow systems with the same nominal detention time,
3. to determine if wetland plants have an effect on water quality parameters and pollutant removal rates,
4. to examine the spatial variation in nitrification potential within planted constructed wetlands with a depth of 25 and 50 cm compared with an aerated system,
5. to determine if there is a difference in the pollutant removal rates in 25 and 50 cm deep HSSF wetland at the same hydraulic loading rate. and
6. to determine if there is climatic effect in pollutant removal rates.

1.3 Thesis outline

The thesis presents a summary of the literature in regard to the current body of knowledge on constructed wetlands, followed by field work based on laboratory experiments and results from shallow and deep constructed wetlands in Germany and Ethiopia. Finally, the findings are summarized and presented along with general conclusions.

Chapter 1 presents the introduction, objective and outline of the thesis. Chapter 2 presents an overview of the current relevant literature on the type of constructed wetland under discussion, their removal mechanisms, and finally their potential advantage for developing country applications. Chapter 3 provides the experimental work on the effect of depth and plants in pollutant removal in HSSFCW treatment wetlands studied at Langenreichenbach in Germany from September 2010 to 2012. The results for hydraulics characterization, assessing performance of the wetlands, and measuring nitrification potential experiments and results are also outlined in Chapter 3.

Chapter 4 presents experimental work about the performance removal efficiency of 25 and 50 cm deep planted and unplanted HSSCWs in Arba Minch, Ethiopia. Finally, in Chapter 5 synthesis, conclusion and recommendations are provided.

2. Literature review: Constructed wetlands for the treatment of municipal wastewater

Overview

This section discusses the current knowledge of wetlands, with an emphasis on HSSFCW with respect to the type, history, hydraulics and pollutant removal mechanisms and the relevance of this technology to developing countries. This chapter is arranged in the following subsections and order. Section 2.1 discusses the historical development of the wetlands, Section 2.2 the types of constructed wetlands, Section 2.3 HSSF constructed wetlands, and Section 2.4 components of constructed wetland and 2.5 pollutant removal mechanisms, Section 2.6 wetland hydrology and hydraulics, Section 2.7 tracer studies, Section 2.8 treatment wetland models and kinetics and Section 2.9 Constructed wetlands for developing countries and finally Section 2.10 summarizes the important conclusions from the chapter.

2.1 Historical development of treatment wetlands

Natural wetlands are usually found between water bodies and terrestrial areas. These systems naturally screen and collect pollutants such as silt and nutrients as they migrate towards water bodies. They include a range of environments from those that are rarely or never flooded to the areas that are often inundated. The Ramsar Convention, 1971, Article 1.1, although not scientifically precise, states that "wetlands are areas of marsh fen, peat land or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water to a depth not exceeding six meters" (Secretariat 2006).

Natural wetlands were historically used as wastewater discharge sites (Kadlec and Knight 1996). Natural wetlands are still used for wastewater treatment under controlled conditions and the use of constructed wetlands has increased in the past five decades (Vymazal and Kröpfelová 2008). The purposeful construction and study of wetlands to treat wastewater was started at the Max Plank Institute in 1952 by Seidel (Vymazal 2011). Research in this area has accelerated since 1985 because of the simplicity of the systems in regard to mechanical operation, biological complexity and high level of treatment. The other attractive advantage for developing countries is that construction may be completed using local materials and labour.

The first full size horizontal subsurface flow artificial wetland was constructed in 1974 in Germany and the first full scale vertical flow constructed wetland was used in the Netherlands in 1975 but vertical flow wetlands dated back to the time of Seidel (Kadlec and Knight 1996).

2.2 Types of constructed wetlands

Constructed (artificial, manmade or engineered) wetlands are manmade systems designed primarily to replicate the treatment that has been observed to occur when polluted water enters a natural wetland (Kadlec and Wallace 2009). Constructed wetlands are classified according to water flow characteristics in the system and by the types of macrophytes that grow in the wetland: surface flow systems, subsurface flow systems, hybrid systems and zero discharge systems (Vymazal and Kröpfelová 2008). Surface flow systems further subdivided based on the type of macrophytes that grow on them as free floating macrophytes (e.g. duck weed and water hyacinth), submerged macrophytes, free floating leaved macrophytes and floating mat macrophytes whereas subsurface flow wetlands may be subdivided into horizontal and vertical flow (downflow, upflow or tidal) wetlands based on the hydrological mode of flow (IWA, 2000). The type of constructed wetland is shown in Figure 2.1. An extensive classification of wetlands was recently published by Fonder and Headley (2013).

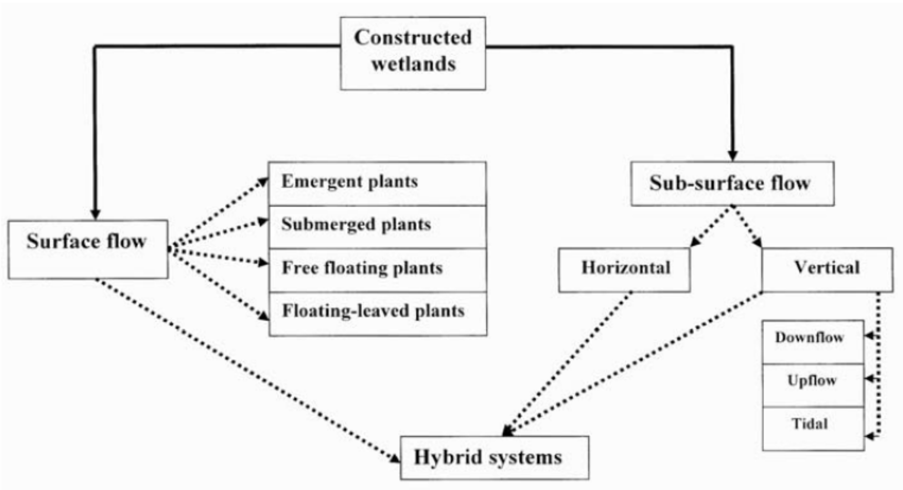


Figure 2.1: Types of constructed wetland taken from (Vymazal and Kröpfelová 2008)

In order to exploit the advantage of the different systems, combined or hybrid systems, surface and subsurface flow constructed wetland systems can be arranged in different configurations to complement each other and obtain improved performance (Vymazal 2011). The most common configuration to date has been a vertical flow stage followed by horizontal subsurface flow wetland cells, the vertical systems remove organics and TSS and provide nitrifying conditions while horizontal systems denitrify and further remove organics and TSS (Kadlec and Wallace 2009). Example of hybrid systems are listed by Vymazal and Kröpfelová (2008). The surface and subsurface flow constructed wetlands are shown in Figure 2.2.

In France a wetland was developed called French system which has a two stage vertical bed designed with a criteria of 1.2-1.5 m^2/PE in the first stage (larger in size) and 0.8 m^2/PE for the second stage, with gravel and sand, respectively (Molle et al. 2005, Troesch and Esser 2012). The main difference of these from the previous is that no pre-treatment (like septic tank) is required therefore sludge treatment and primary treatment is done simultaneously in the first bed and the second vertical beds work like the normal vertical bed (Vymazal and Kröpfelová 2008). It is claimed to have high removal of organic matter and ammonia.

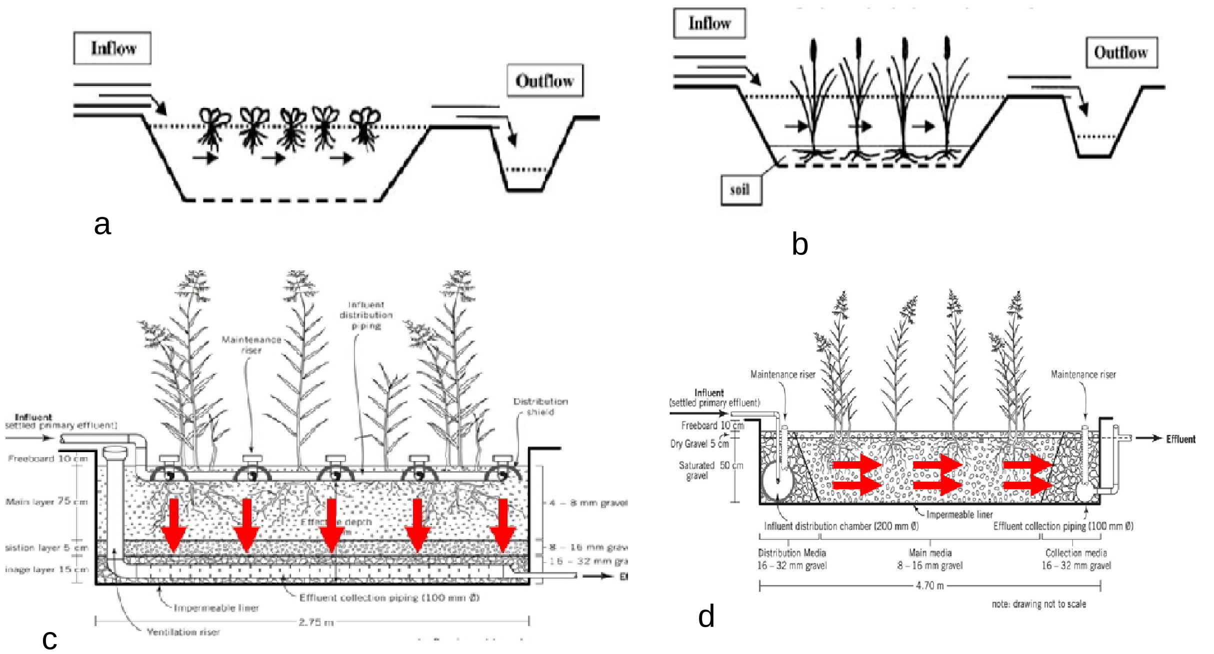


Figure 2.2: Types of constructed wetlands. Free flow constructed wetlands (a &b); vertical (c) and horizontal (d) subsurface flow constructed wetlands. Figure a and b was taken from (Vymazal 2007) and c and d was taken with some modification from (Nivala 2012).

A wetland system different from these is based on a willow tree as sewage discharge is called zero discharge system (Brix and Gregersen 2002). The system losses the water by evapotranspiration (no effluent) and the nutrients absorbed by fast growing willow for biomass production (Brix and Arias 2005). The zero discharge system is good for areas which do not have space to discharge the treated effluent.

Based on our objective, the discussions in the coming sections are focused on the horizontal subsurface flow constructed wetlands.

2.3 Horizontal subsurface flow wetlands

Subsurface flow constructed wetlands are reliable and cost effective treatment methods which can be used for domestic, municipal, and industrial wastewaters. Their application ranges from family homes, schools, industry and municipalities and their main significant advantages of the systems include the lack of odour, mosquitoes and other insect vectors, and minimal risk of public exposure and contact with the water in the system (U.S.EPA 1993). Subsurface flow constructed wetlands have been used for domestic wastewater treatment after primary settling with good success in meeting secondary effluent standards (TSS; $BOD_5 \leq 30$ mg/L) (Vymazal and Kröpfelová 2008). HSSFCW are effective in removing TSS, organic matter, microbial pollution and heavy metals. However, the removal of organic matter is limited by the shortage of oxygen (Vymazal 2011).

HSSCWs have a porous substrate which is saturated with water except for the uppermost layer, whereas in vertical flow constructed wetlands the bed is intermittently saturated and typically free-draining. In horizontal flow constructed wetlands, water flows slowly through a porous medium under the surface of the bed in a more or less horizontal path and reaches the outlet zone and is collected by an outlet device (Figure 2.2d). In the meantime, the wastewater will come into contact with aerobic, anoxic and anaerobic zones; the plant roots and rhizomes have been reported to leak oxygen into the substrate (Brix 1987).

Sizing of HSSFCW is done in the range from “rule of thumb” to the complex dynamic models (Vymazal and Kröpfelová 2008). But the most acceptable model between these ranges in the design of HSSFCW is first order black-box approach, which is particularly suitable for sizing systems (Garcia et al. 2010). The output from the model, areal rate coefficient (k_A), is used to size wetlands (Vymazal and Kröpfelová 2008). However, areal rate coefficient is not constant and variable as many authors provide different values from place to place (Rousseau et al. 2004), so for design purposes it is advisable to calculate at the same conditions when a wetlands is constructed. Besides, BOD_5, nitrogen, phosphorous and pathogen have different rate coefficient which means different area of wetlands, so the choice depends on the objective of the project (target parameter) (Kadlec and Knight 1996). Nitrogen requires larger area than organic carbon. The P-k-C* model is one of the state of the art black box model with modified parameters to take care of pollutant behaviour in the wastewater including degradation and hydraulic behaviour (Kadlec and Wallace 2009). P-k-C* model equation is discussed in section 2.8.

The second method is the use of rule of thumb to design area of wetland which is initially based on the hydraulic or organic loading rates (Vymazal and Kröpfelová

2008). For instance, for municipal sewage 5 m^2PE^{-1} is used (Vymazal 2011). Typical design guidelines based on the rule of thumb and effluent quality requirement for horizontal and vertical beds are shown in Table 2.1 and 2.2 for Germany and USA, respectively. Rule of thumb values are conservative and claims quality effluent but at the expense of construction cost (Rousseau et al. 2004). Besides, the rule of thumb is developed for certain region, they may not be used directly for the tropical weather condition. Therefore black box models is relatively more flexible as it allows adjustment in the design for discharge and temperature conditions of the wetland (U.S.EPA 1993). Therefore, rate coefficient is worth calculating.

Table 2.1: Vegetated soil filter design values from German standard (DWA 2006)

	unit	Vertical beds	Horizontal beds
Area required	m^2/PE	≥ 4	≥ 5
Minimum area	m^2	≥ 16	≥ 20
COD load	g COD/(m^2.d)	≤ 20	≤ 16
Hydraulic load	mm/d	≤ 80	≤ 40
Depth of the filter material	cm	≥ 50	≥ 50

Table 2.2: Guidelines of design criteria for HSSFCW (U.S.EPA 2000)

parameter	Areal loading rate g/(m^2d)	Required effluent (mg/L)
BOD_5	6	30
	1.6	20
TSS	20	30
TKN	Use another treatment process in conjunction to HSSFCW	

As part of sizing of HSSFCW, depth is an important parameter due to its influence in the construction cost and performance of the systems. HSSFCW have historically been constructed with depths of 50 cm or more. Although some field experiments showed better performance for shallower beds (George et al. 1994, García et al. 2004), virtually all HSSF wetlands have been designed with beds depths of 30-60 cm (Kadlec and Wallace 2009). According to Kadlec (2009) bed depth in HSSFCW is usually on the order of 60 cm. In the U.S., most bed depths are approximately 0.6 meters (U.S.EPA 1993). In the German standard, Table 2.1, the required media depth is 0.5 m or more (DWA 2006). One factor which encourages designers to build HSSFCWs deeper is to reduce the surface area, based on the assumption that treatment performance is primarily determined by HRT (Reed and Brown 1995, Reed et al. 1995). Another factor that is often considered, and somewhat misleading, is the rooting depth of the plants. A commonly cited study is Gresberg et al. (1986) which predicted plant depth goes as much as 76 cm in soil. However, the majority of plant roots remain in the top 20 cm of the bed (Headley et al. 2005). Deeper wetland beds can provide certain benefits which may include providing additional storage for pollutants via increased retention time and reducing the risk of freezing (Kadlec and Wallace 2009).

2.4 Components of Horizontal Subsurface Flow Wetlands

Overview

In this subtopic the bed media, liners and treatment wetland macrophytes used in subsurface flow constructed wetland are discussed.

2.4.1 Bed media

In subsurface constructed wetlands, media also called substrate or aggregate perform the function of rooting material for macrophytes, surface for microbial biofilm growth, screen organic and inorganic suspended matter, distribute inflow and collect outflow water (U.S.EPA 2000). Keeping the water level below the surface of the bed also reduces the risk of human contact with pathogens, and reduces the opportunities for breeding vector organisms such as mosquitoes. Media may include mineral aggregates (ranges from sand to gravel), soils, manmade aggregate and industrial by-products. Some materials have desirable properties of high concentrations of Ca and Mg carbonates as they provide adsorption sites for phosphorous (Kadlec and Wallace 2009). According to Kadlec and Wallace (2009) potentially active industrial by products include blast furnace slag, crushed rock, fly ash, crushed concrete, burnt oil shale, iron ochre and wood chips. The media used in subsurface flow constructed wetlands is usually gravel ranging in sizes from 3 to 32 mm. In the inlet zone the gravel may be as large as 50 mm (Crites and Tchobanoglous 1998). The larger media at the inflow and outflow areas is used to distribute the water evenly and to reduce the risk of clogging.

The microorganisms responsible for degradation of pollutants are located at the surface of the media and the smaller sized media has a larger surface area than coarser media (Vymazal and Kröpfelová 2008). Media selection depends on the requirement of adequate hydraulic conductivity to reduce the risk of clogging and experience showed that soil and sand are very susceptible to clogging (U.S.EPA 2000). Well graded media (containing all gravel sizes in the selected range) is better than poorly graded media as it offers greater pore space and provides good removal of particulate matter. In countries, where constructed wetlands are widely applied, there is a difference in the choice of filling material for the horizontal wetlands even within the same country, for instance U.S. America (Vymazal and Kröpfelová 2008). This depends on the availability of the construction material nearby because the cost and handling of gravel is very high and is in the range of 40 - 55 percent of the total construction cost (U.S.EPA 2000). The difference of choice among the countries for filling materials depends on their experience and conditions (Vymazal and Kröpfelová 2008). Table 2.3 shows an example of filter media used in different countries.

Table 2.3: Example of HSSF wetland bed materials

Source	Media size, mm	k_f	d60/d10	Main material	Country
DWA (2006)	≥ 0.2 to ≤ 0.4	10^{-3} - 10^{-4}	<5	Sand	Germany
(U.S.EPA 2000)	20-30			gravel	USA
Kadlec and Wallace (2009)	3-8	2 X 10^{-3} - 10^{-4}		gravel	Austria
Brix and Arias (2005)			<4	d10 (0.3-2 mm), d60 (0.5-8mm)	Denmark

The HSSFCW bed cannot maintain its conductivity due to deposition of solid in the wetland (Vymazal and Kröpfelová 2008). Clogging of HSSFCW occurs especially with in the first ¼ to 1/3 of the beds from the inlet zone, and thus reduce hydraulic conductivity and results in surface flooding or ponding (U.S.EPA 1993). Mechanisms responsible for clogging are deposition of inert suspended matter, accumulation of refractory organic material, chemical precipitation, biofilm formation on the media and pore volume occupation by the plant roots on the upper surface of the bed (Kadlec and Wallace 2009).

2.4.2 Impermeable lining

Natural and artificial liners are usually used in horizontal subsurface constructed wetlands to prevent the seepage of pollutants to groundwater. The most popular liners used in constructed wetlands include poly vinyl chloride (PVC), low and high density polyethylene (LLDPE, HDPE), reinforced plastics and clay (U.S.EPA 2000).

Although PVC is cheaper, easier to work with and resistant to puncture, it has the least resistance to UV light if it is not covered. LLDPE and HDPE are more resistant to UV light but is more expensive (Kadlec and Wallace 2009). Reinforced plastics have a woven net of nylon or polypropylene embedded in order to offer additional strength but they are also expensive. Clay with a permeability 10^{-8} m/s or less is an economical alternative and sustainable if it is found close to the site (Cooper 1990, U.S.EPA 2000). The installed compacted thickness is usually on order of 30 cm (Kadlec and Wallace 2009).

2.4.3 Macrophytes

Macrophytes used in horizontal subsurface flow constructed wetlands have the ability to grow in high nutrient, high organic load and high sediment carrying waters. The root of the macrophytes can tolerate certain level of anoxic (Vymazal and Kröpfelová 2008). There are thousands of plant species which can grow in wetlands but the four most frequently used are: cattails (*Thypha Sp*.), Common reed (*Phragmatis australis*), bulrushes (*Schoenplectus sp*.) reed canary grass (*Phalaris arundinacea)* are typical species used in subsurface flow constructed wetlands (Kadlec and Wallace 2009). However, many locally available emergent species could be used for horizontal flow constructed wetlands (Vymazal and Kröpfelová 2008).

There are many general functions of vegetation in constructed wetlands. The most important physical functions of wetland macrophytes in treating wastewater are shading, blocking wind, transpiration, flow resistance, particulate trapping, and provision of surface area for attached microorganisms and litter supply (Tanner 2001). The other functions of macrophytes is the metabolism which includes nutrient uptake, creation of organic matter, oxygen supply (Brix 1994a, Kadlec and Wallace 2009). Plants facilitate microbial activity in wetlands by providing sites for attached growth, as well as carbon and oxygen in the root system (Brix 1994b).

Although with most constructed wetland for domestic wastewater treatment, microbial and physical transformations are more important than the plant uptake of pollutants. However, during the initial plant growth phase, direct plant immobilization is an important mechanism especially for some pollutants (IWA 2000). Plants uptake nutrients, metals and some pollutants from wastewater in HSSFCW with their root during active plant growth (U.S.EPA 2000). Net annual estimates of uptake for phosphorus and nitrogen ranges for phosphorus, 1.8 to 18 g P/(m^2.y) and for nitrogen, 12 to 120 g N/(m^2.y) by emergent wetland plant (Reddy et al. 1989). To maximize nutrient removal, harvesting should be done before the start of senescence. Plants uptake of nutrients and metals and harvesting do not play significant role in wastewater treatment when compared with a HSSFCW load with primary treated domestic wastewater (U.S.EPA 2000, Vymazal and Kröpfelová 2008).

Wetland plants also introduce organic carbon into the bed as roots exudates. Decomposing wetland plants and plant excudates are potential sources of biodegradable organic carbon for denitrification but are also sources of ammonia from organic nitrogen (U.S.EPA 2000). Plant root exudates of organic carbon and nitrogen are the largest at the beginning of the senescence. Some of the plant exudates are anti microbial, inhibitory to other plant growth or some support organisms growth (Vymazal and Kröpfelová 2008).

Decomposition of the plant material can contribute additional organic material and nitrogen to the wastewater (U.S.EPA 2000). The plant material on the surface of the bed may also be washed into the bed overtime. Organic matter provides sites for material exchange and microbial attachment, and is a source of carbon, the energy source that drives some of the important biological reactions in wetlands. The above ground parts of macrophytes may contribute some BOD during senescence but they do not interact with the wastewater directly when they are alive (Kadlec and Knight 1996).

All vascular plant roots require gaseous exchange mechanisms for the supply of oxygen and removal of carbon dioxide resulted from metabolic processes. One adaptation to flooding is the development of aerenchymous (internal aearation system) plant tissues that transport gases to and from the roots through the vascular tissues of the plant from above the water where it is in contact with the atmosphere (Tanner et al. 1995). This provides an aerated root zone thereby lowering the plant`s reliance on external oxygen diffusion through water and soil (Armstrong 1978). Plant root also affects hydraulic conductivity. Plant roots remain in the top part of HSSFCW because of the availability of nutrients and water which this resulted in reducing the hydraulic conductivity at the top and favouring short cutting (more flow through the media without root) at the bottom (Fisher 1990, DeShon et al. 1995, Sanford et al. 1995, Tanner and Sukias 1995).

Organic compounds are degraded aerobically as well as anaerobically by bacteria attached to plant roots, rhizomes and media surfaces. The oxygen required for aerobic degradation is supplied directly from the atmosphere by diffusion or oxygen leakage from the macrophyte roots and rhizomes in the rhizosphere; the amount of oxygen released by plant roots is in the range of 0.02-12g/m^{2}d by different research (Armstrong et al. 1990, Brix 1994b).

2.5 Pollutant removal mechanisms in HSSFCW

Overview

HSSFCW have fixed biofilm reactors in which pollutants removal is achieved by the interaction physical, chemical and biochemical processes between the pollutant and the biofilm (Garcia 2010). The main pollutants of concern from municipal wastes include total suspended solids, organic carbon (biochemical oxygen demand and total organic carbon) nitrogen, phosphorus, and pathogens. Their occurrence and removal mechanisms are discussed in this section.

2.5.1 Total suspended solids

Total suspended solids are solid materials, including organic and inorganic that are suspended in wastewater. They are derived from wastewater and are a by-product of decomposing wetland vegetation. The primary mechanisms of total suspended solids removal are flocculation, filtration, settling and larger particles have a priority in removal (U.S.EPA 2000). In HSSFCW total suspended solids settles into micro pockets or it is filtered out by flow restriction (Kadlec 2000). In general, because of the nature of suspended matter and the physical mechanism of removal, TSS is removed faster than the other pollutants. The total suspended solids occur within the first part of the bed (Bavor et al. 1989, Nguyen 2001, Vymazal 2003). Larger biodegradable materials filtered in the wetland can be a source of BOD as they degrade and re-enter the water column (U.S.EPA 2000). They may also be degraded into smaller pieces.

2.5.2 Organic matter

Wastewater, irrespective of its source, contains a wide variety of organic compounds and they can be measured as total organic carbon (TOC), biochemical oxygen demand (BOD) and chemical oxygen demand (COD). HSSFCW receive organic matter from the external source or influent wastewater and from the primary production in the wetland itself C* (internal loading or background concentration). C* has a significant effect when the wetland is planned to treat wastewater having low concentration of organic matter (Garcia et al. 2010). Besides physico-chemical removal, the removal pathways of organic carbon in subsurface wetland is aerobic, facultative, anaerobic and obligate anaerobic and these processes occur in different zones of the wetland (Kadlec and Wallace 2009). Heterotrophic aerobic removal, denitrification, anaerobic degradation followed by fermentation in anaerobic zones is the main removal mechanisms for organic matter removal presented in this section.

Organic compounds which are degraded by microorganisms exist as settleable, suspended solid and soluble forms. In subsurface flow constructed wetlands particulate BOD removal from wastewater occurs rapidly and physically by entrapment by the plant roots and media and through settling. The trapped material and soluble organic material are decomposed primarily by attached and suspended microroganisms which are essentially a fixed biofilm (Garcia et al. 2010). Soluble organic pollutants are decomposed aerobically as well as anaerobically by microorganisms attached on the media surface and plant roots and rhizomes (Gagnon et al. 2007). Atmospheric oxygen diffusion or leakage from the macrophyte is the main contributor for the aerobic degradation (Vymazal and Kröpfelová 2008).

Wetlands are ideal environments for chemical transformations because of the range of oxidation states that naturally occur in wetland soils (Armstrong 1978). Table 2.4 shows the order of major electronic acceptors in the degradation of organic matter in wetlands.

Aerobic degradation of soluble organic matter (e.g. carbohdrates) by aerobic heterotrophic bacteria and autotrophic bacteria is shown in Equation 2.1.

$$(CH_2O) + O_2 \longrightarrow CO_2 + H_2O \qquad \text{Eq 2.1}$$

The autotrophic bacteria degrade organic compounds in the presence of nitrate or nitrite under anaerobic conditions and it is called dentrificatin. Glucose in eq 2.2 is used to represent organic compounds.

Table 2.4: The order of electronic acceptor on the degradation of organic matter with their reduction potential and type of respiration, (Vymazal and Kröpfelová 2008)

Electron acceptor	(E_h) Redox potential (mV)	Type of respiration
$O_2 \longrightarrow H_2O$	+400 to +700	Aerobic
$NO_3^- \longrightarrow N_2$	+220 to +250	Denitrification
$Mn \longrightarrow Mn^{2+}$	+200	Manganese Reduction
$Fe^{3+} \longrightarrow Fe^{2+}$	+100 to +120	Ferric Reduction
$SO_4^{2-} \longrightarrow S^{2-}$	-100 to -200	Sulfate Reduction
$CO_2 \longrightarrow CH_4$	-200 to -300	Methanogenesis

$$C_6H_{12}O_6 + 4NO_3^- \longrightarrow 6CO_2 + 6\ H_2O + 2N_2 + 4e^- \qquad \text{Eq 2.2}$$

In horizontal subsurface flow constructed wetlands there is an insufficient supply of oxygen to the bacterial community so a multi-step anaerobic degradation process prevails (Cooper et al. 1996). The first step in anaerobic degradation is production of fatty acids (acetic acids, lactic acids, butyric acids) Reddy and Graetz (1988) followed by fermentation of the fatty acids to alcohols and carbon dioxide and hydrogen, shown Eq 2.3-2.5. Fermentation occurs in absence of electron acceptors (Bitton 2005).

$$C_6H_{12}O_6 \longrightarrow 3CH_3COOH \text{ (acetic acid) } + H_2 \qquad \text{Eq 2.3}$$

$$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH \text{ (lactic acid)} \qquad \text{Eq 2.4}$$

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH \text{ (ethanol) } + 2CO_2 \qquad \text{Eq 2.5}$$

In the second step, iron and sulfate reducing and methane forming bacteria use the end product of fermentation. The electron acceptors are presented in Table 2.2.
Iron reduction occurs in anoxic or anaerobic zones, Eq.2.6:

$$CH_3COO^- + 8Fe^{3+} + 3H_2O \longrightarrow 8Fe^{2+} + CO_2 + HCO_3^- + 2H_2O + 8H^+ \qquad \text{Eq 2.6}$$

Sulfate reduction occurs in anaerobic zones by strict anaerobes (Bitton 2005) in Eq 2.7, 2.8:

$$2CH_3CHOHCOO^- + SO_4^{2-} + H^+ \longrightarrow 2CH_3COO^- + 2CO_2 + 2H_2O + HS^- \qquad \text{Eq 2.7}$$

$$CH_3COO^- + SO_4^{2-} + 2H^+ \longrightarrow 2CO_2 + 2H_2O + HS^- \qquad \text{Eq 2.8}$$

Methanogenesis occurs in anaerobic zone at pH range 6.5 to 7.5 (Bitton 2005) (Eq. 2.9 & 2.10)

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O \qquad \text{Eq 2.9}$$

$$CH_3COO^- + 4H_2^{\,-} \longrightarrow 2CH_4 + H_2O + OH^- \qquad \text{Eq 2.10}$$

Methane formers operate only in pH of 6.5 to 7.5 so over production of acid affects methane forming bacteria and results in production of odorous compounds in the wetlands (Vymazal et al. 1998a).

The wetland BOD removal is not improved at higher wetland temperature. Organic matter removal rates are not related to changes in water temperatures which implies that physicochemical and biological mechanisms are the principal removal mechanisms (McNevin et al. 2000). However, background BOD increases at high temperature and outlet BOD is generally higher in summer than in winter. There are typically gentle annual cycles in the effluent BOD from HSSF wetlands (Kadlec and Wallace 2009) as in Eq: 2.11.

$$C = C_{avg}(1 + A.\cos(\omega(t - t_{\max}))) \qquad \text{Eq 2.11}$$

Where,

A= trend fractional amplitude, dimensionless; C= concentration, mg/L; C_{avg}.= mean annual concentration, mg/L; t= year day, d; t_{max}= year day for maximum concentration, d; ω= annual period, 0.01721d^{-1}; mean fractional amplitude is 35% of the mean

2.5.3 Nitrogen

In wastewater, the forms of nitrogen of greatest interest are nitrate, nitrite, ammonia, and organic nitrogen. All these forms of nitrogen, as well as nitrogen gas (N_2), are biochemically inter-convertible and are components of nitrogen cycle. In wastewater, nitrogen exists in the form of inorganic, organic and/or soluble and particulate forms.

Organic nitrogen is defined as organically bound nitrogen in the tri-negative oxidation state includes natural materials such as proteins, and peptides, nucleic acids and urea, and numerous synthetic organic materials. Analytically, organic nitrogen and ammonia can be determined together and referred as “Kjeldahl nitrogen”, a term that reflects the technique used in its determination (APHA et al. 1999). In wastewater ammonia is produced largely by deamination of organic nitrogen-containing compounds and by hydrolysis of urea in wastewater.

The most common removal or transformation pathways of nitrogen are chemical and physical transformation processes. Chemical transformations include: ammonification (minerlaization), nitrification, denitrification, assimilation and decomposition where the physical processes include: plant translocation, ammonia volatilization, filtration, sorption onto substrates and sedimentation (IWA 2000, Vymazal and Kröpfelová 2008). There is no simple model to express the inflow and outflow changes of nitrogen. According to Spieles and Mitsch (2000), nitrification and dentrification mechanisms are the major nitrogen removal processes in constructed wetlands. The removal of nitrogen in constructed wetlands ranges from 25 to 85% (Lee et al. 2009).

The nitrogen removal mechanisms and transformations in constructed wetlands are explained below.

Ammonification

Transformation of organic nitrogen into ammonium ion using enzymes by microorganisms is called Ammonification (Vymazal 2007). The ammonification rate depends on the temperature, pH, C/N ratio (Reddy and Patrick 1984); and Ammonificaiton proceeds more rapidly than nitrification (Kadlec and Knight 1996).

Ammonia volatilization

The escape of ammonia as gas from wastewater is called volatilization and the process is a physicochemical. Aqueous solution of ammonia is in equilibrium with ammonium ion and hydroxide ion as in Eq: 2.12. According to this equation, pH increase favours the reverse reaction to form ammonia gas and enhances evaporation with temperature. Ammonia removal process by volatilization is not high because the pH is not alkaline in wetlands (Cooper et al. 1996, Vymazal et al. 1998b).

$$NH_3\,(aq) + H_2O \rightleftharpoons NH_4^+\,(aq) + OH^-\,(aq) \qquad \text{Eq 2.12}$$

According to Reddy and Patrick (1984) volatilization of nitrogen in the form of ammonia is insignificant below pH value 7.5.

Nitrification

Nitrification is a biological oxidative process of ammonia (NH_4^+) conversion to nitrite (NO_2^-) which is then subsequently oxidized to nitrate (NO_3^-). Nitrification is an important removal mechanism used in a number of treatment processes to control ammonia (Vymazal 2007). Oxidation of ammonium to nitrate is carried out in two stages by chemolitotrophic bacteria which are widely distributed in soils, water and wastewater (Bitton 2005). For the two stages the chemical equations are shown by Eq 2.13 and E.q. 2.14. The oxidaiton of ammonium to nitrate is performed by ammonium oxidizing bacteria (e.g.nitrosomonas in most wastewater environment) and nitrite oxidation to nitrate is performed by a group of nitrite oxidizing bacteria (e.g nitrobacter) (Bitton 2005).

$$NH_4^+ + 1.5\ O_2 \longrightarrow NO_2^- + H_2O + 2H^+ \qquad \text{Eq 2.13}$$

$$NO_2^- + \tfrac{1}{2}\ O_2 \longrightarrow NO_3^- \qquad \text{Eq 2.14}$$

In the nitrification process, ammonia conversion to nitrite is slower than nitrite conversion to nitrate so therefore ammonia oxidation to nitrite is the rate determining step for the overall ammonia oxidation (Schmidt 1982). Although not significant, nitrification is also carried out by heterotrophic bacteria and Fungi (Verstraete and Alexander 1972, Falih and Wainwright 1995).

Nitrification is influenced by factors: pH, alkalinity, temperature, BOD_5/ TKN, microbial population, dissolved oxygen, availability of toxic chemicals and concentration of ammonia/ nitrite concentration (Tchobanoglous and Burton 1991). Influence of temperature, pH and dissolved oxygen further explained.

In bacterial mediated reactions, temperature is likely to play a larger role in nitrogen oxidation rates compared with other factors. Nitrification affected by temperature in all seasons and by other seasonal factors which linked to plant physiology related to root oxygen release (Garcia et al. 2010). According to Schmidt (1982), the optimum temperature range for nitrification is 20-40^{0}C and lower temperature decreases ammonia oxidation by bacteria (Abeliovich 1987). According to Bitton (2005) review, the optimum temperature for nitrifiers growth is 25-30^{0}C. The pH of the wastewater for nitrification is between 7.5 - 8.5 (U.S.EPA 1975). Alkalinity is consumed by nitrification but compensated by denitrification step (Bitton 2005)

Oxygen is one of the other important factors. Nitrification occurs as low as 0.05 mg/L dissolved oxygen but low oxygen is not favourable (Abeliovich 1987). According to studies, when the concentration of dissolved oxygen is less than 1-2 mg/L, nitrification is reduced substantially (Hammer and R.L. 1994, Lee et al. 1999). Diffusion from atmosphere and leakage from root of plants are the source of oxygen for the nitrification process in wetlands (Armstrong et al. 1990, Brix 1994a). Passive HSSFCW are predominantly anaerobic so they are not very effective in removing ammonia because the nitrification step is affected by low oxygen (Kadlec and Knight 1996). The limited oxygen availability is responsible for incomplete oxidation of ammonia to nitrate which is the main reason for poor ammonia removal (Vymazal et al. 1998a, Vymazal 2002).

Dentrification

The process of reduction of nitrate to gaseous nitrogen by facultative anaerobes is called denitrification. In this process, denitrifying bacteria (heterotrophic bacteria) reduce nitrate and nitrite (terminal electron acceptors) into the nitrogen molecule (N_2) using organic carbon as the electron donor under anoxic conditions (Koike and Hattori 1978). Many organisms are capable of denitrification, and E_h is required to be +350 to +100mV (Hauck 1984, Kadlec and Wallace 2009). The dentrification sequential steps are shown in Eq 2.15 .(Bitton 2005)

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \qquad \text{Eq 2.15}$$

The microorganisms involved in denitrification switch to oxygen as terminal electron acceptor when the environment changed to aerobic condition so denitrification should be conducted in anaerobic condition (Bitton 2005). The area with high concentration of nitrate promotes a more rigorous and robust population of denitrifiers (Sirivedhin and Gray 2006). Nitrate concentration, microbial flora, type and quality of organic carbon source, pH (6-8), plant species type, absence of oxygen, redox potential and moisture and presence of denitrifying bacteria are some of the factors influencing the rate of denitrification (Focht and Verstraete 1977, Garcia et al. 2010). However, the optimum values of these factors vary among different authors.

In wetlands, aerobic microorganisms closer to the root can conduct nitrification reactions whereas denitrification reactions only occur with microbes away in the anoxic bulk soil (Vymazal 2007, Lee et al. 2009). It is believed that there is an oxygen gradient established from the root surface to the bulk soil and water media because more oxygenated towards the root than in the bulk solution (Bezbaruah and Zhang 2004).

In the absence of organic matter as electron donor and dissolved oxygen, autotrophic ammonia oxidizers produce nitrogen gas (N_2) by using ammonium (NH_4^+) as electron donor and nitrite (NO_2^-) as electron acceptor (Bitton 2005).

Plant uptake

Plants utilize nitrogen generally in the form of ammonium and nitrate, ammonium is a more preferable. Plant uptake contributes to the seasonal dynamic of nitrate and ammonium removal and account for a significant part of the wetland nitrogen removal with rapid nitrogen uptake during the growth period (Vymazal 2007, Kadlec and Wallace 2009). Generally, the nitrogen uptake will be high in a newly built wetland. If the old plants are not removed, the nitrogen taken up by them will be returned back to the wetland. According to Lee et al.(2009) assimilation of nitrogen by plants and algae is typically 1-34%, in comparison to 60-95% by denitrification.

Ammonia adsorption

Another possibility of nitrogen removal is electrostatic attractions. Since ammonium ion is positively charged, it is readily adsorbed to sediment particles and litter; however, NO_3^- does not use this process because of their negative charge (Vymazal 2007).

Factors affecting nitrogen removal efficiency

Nitrogen is available at different oxidation states and is removed with different mechanisms therefore numerous environmental factors affect its removal. The major factors are temperature, detention time, vegetation type and distribution, climate and microbial communities. Generally, temperature is one of the main environmental factors that affect the rate of reaction and activity of microorganisms. Nitrification and denitrification conversion reactions are also impacted by temperature changes (Langergraber 2007). Most microbial communities increase nitrogen removal efficiency at temperature above 15^{o}C and the nitrification and denitrification activities decrease at water temperatures below 15^{o}C or above 30^{o}C (Kuschk et al. 2003). Bacteria are not adapted to low temperatures prevailing at 3.1^{0} C (Kern 2003).

Hydraulic residence time also play a major role in nitrogen removal. Total Kjeldahl nitrogen concentration decrease exponentially in treated effluent with the increased hydraulic detention time of wastewater (Huang et al. 2000). In most wetland systems, nitrogen removal requires longer detention time than BOD_5 (Kadlec 2009).

2.5.4 Phosphorous

Phosphorous exists in wastewater as polyphosphate and orthophosphate in soluble and particulate forms in the range of 10- 20 mg/L (Bitton 2005). Phosphorous removal in natural treatment systems occurs by plant uptake, adsorption, complexation and precipitation. Direct settling or sedimentation can account for the removal of any influent phosphorous associated with particulate matter. Sorption of orthophosphate on manganese and iron containing gravel is one potential form of removal (Kadlec and Knight 1996). In this case, the media has to be replaced or regenerated to provide consistent phosphate removal. Another removal mechanism of phosphorous is through plant uptake and this can be improved by harvesting. Harvesting the wetland plant increases the phosphate removal and removes the chance of returning the nutrient back into the wastewater (Vymazal and Kröpfelová 2008). With detention time of less than ten days, available data indicate that 30 to 50 percent phosphorus is removed in wetlands (WEF 2001). If the purpose of the construction of a treatment plant is to remove phosphate using HSSF wetland, the selected filter media should have an adsorption site for phosphate.

2.5.5 Pathogens

Pathogenic microorgansims found in domestic wastewater contain bacteria, viruses, protozoa or helminth eggs and enter the environment from the faeces of infected hosts and contaminate a new host when the host consumes infected water (WHO 2006a). The level of contamination of domestic wastewater with pathogenic microorganisms is related to the health of the local community or the existence of infected persons in the population (U.S.EPA 2002). Viruses (the smallest) are commonly associated with wastewater, since they multiply in the gut and are excreted in large numbers in faeces (Cisneros 2011). The second group are bacteria which are the most common and abundant pathogens in wastewater and ranges in size from 0.2 to 5 μm (Tchobanoglous and Burton 1991). When protozoa stay outside the infected host, they persist as dormant stages called cysts and oosysts and are most commonly transmitted to the healthy host by oral route (Bitton 2005). The last group are pluricellular organisms with sizes ranging from 1 mm to several meters. Helminths provoke heleminthasis, which causes different kinds of diseases characterized by undernourishment, anaemia, and stunted growth and in developing countries the number of people affected may be as high as 25-33% of the population (Cisneros 2011). Protozoan pathogens and helminth worms are particular importance for tropical and subtropical countries (Rivera et al. 1995). Irrigating with untreated wastewater can result to increased helminth infection (mainly *Ascaris lumbricoides*) viral and bacterial infections such as typhoid, cholera, *Helicobacter pylori*, norovirus in consumers (WHO 2006b).

The most commonly tested faecal bacteria indicators to know the contamination level of wastewater are total coliforms, faecal coliforms, *Eschericia coli* (*E. coli*), faecal streptococci and *Enterococci* (Dufour et al. 2012). The best indicators of health risk are *E. coli* and *Entercocci* (U.S.EPA 2013).

Physical, chemical and biological (including predation) pathways are the main removal mechanism of pathogens in natural treatment systems. Chemical and biological factors include oxidation, exposure to biocides from plant exertion, antimicrobial activity of root exudates, predation, activity of lytic bacteria or viruses, retention in biofilms and natural die-off (Gersberg et al. 1989, Vymazal and Kröpfelová 2008). Stevik et al. (2004) in their review pointed out that two mechanisms responsible for immobilization of pathogens in wastewater moving through a porous media are straining and adsorption; straining mechanism is the physical blocking of movement of bacteria. Helminth eggs found in wastewater are resistant to conventional disinfectants: chlorine, ozone and UV light (Cisneros 2011). Because of their size, filtration and adsorption is the main physical mechanism of removal. When wastewater is used for irrigation purposes, that water should have a concentration of less than ≤ 1 egg/L for irrigation water used for food to be eaten raw (WHO 2006b).

Pathogen removal is one of the most important purposes of constructed wetland. From the available data on wetland systems, a 2 to 3 log reduction in faecal coliforms can be expected with a 5 to 10 day detention time (WEF 2001). Removal of *Faecal coliform* depends on hydraulic retention time and filtration media grain size (García et al. 2003). Log removal increase with HRT increase until day 3 and also the inactivation of the FC changes from (0.1- 2.7 log) to (0.7-3.4 log) with (5-25 mm diameter gravel) and (2-13mm diameter) gravel, respectively. Tanner et al. (1998) also reported the increased removal of FC with increase of hydraulic residence time. In most cases, this reduction is not enough to meet discharge limit so therefore supplemental disinfection is required to meet discharge compliance.

2.6 Wetland Hydrology and hydraulics

Hydrology (rainfall, ground water characteristics, inflow and outflow and evapotranspiration) are most important variable in wetland design and in maintaining the wetland components to ensure they meet their performance requirements (Kadlec and Knight 1996). Wetland hydrology determines the length of time the water and pollutant spends in the wetland, and thus the opportunity for interactions between water borne substances and the wetland ecosystem. Natural wetlands are impacted by extremely variable flows (stochastic in character) and variations in input but manmade treatment wetlands have almost constant inflow (Kadlec and Wallace 2009); however, ET and precipitation affect the uniformity of flow in these systems.

In order to assess the performance of the treatment wetland, determination of the hydrological characteristics is necessary. A consideration of all terms for inflows and outflows gives a simple water balance or budget of the wetland. The water balance in the wetland can be written as Eq 2.16 (Kadlec and Knight 1996).

$$\frac{dV}{dt} = Q_i + Q_c + Q_{sm} - Q_o - Q_b - Q_{gw} + (p * A) - (A * ET) \qquad \text{Eq 2.16}$$

Where,

dV/dt =net volume change per day (m^3/day), V= water storage (volume) in wetland, m^3 , Q_i = daily flow to the system (m^3/day), Q_{gw}= infiltration rate to ground water (m/day), Q_o= daily outflow from system (m^3/day), Q_b= bank loss rate (m^3/day), Q_c= catchment runoff (m^3/day), Q_{sm}= snow melt rate, (m^3/day), P= daily precipitation rate (m/day), ET= evapotranspiration rate (m/day), A= total surface area of the wetland (m^2)

Treatment wetlands are normally isolated from ground water and most of the water would leave via stream flow in most cases. Therefore, Eq 2.16 can be simplified by neglecting terms which are insignificant to give Eq 2.17 and Eq 2.18 if the wetland is lined the ground water and bank loses can be neglected and snow melt ignored in some locations. With this adjustment, the calculation is reduced the data requirement. The volume of the wetland changes with season determines the time the pollutant stays in the wetland for further treatment or it may let the pollutant out before getting enough treatment. It is reasonable to assume that in most regions the fluctuation is most likely to be from ET and precipitation (Armstrong 1978).

$$\frac{dV}{dt} = Q_i - Q_o + A(p - ET) \qquad \text{Eq 2.17}$$

$$Q_i = Q_o + A(ET - p) \qquad \text{Eq 2.18}$$

Since ET is an important parameter and difficult to measure, the method is discussed here. ET is a combined process of evaporation from the water and soil surface and transpiration of water from emergent portion of the pants. Treatment wetlands condition the warm air by ET by loss of the latent heat of vaporization of water otherwise the prevailing temperature burns the vegetation (Kadlec and Knight 1996). The ET rate varies from plant to plant and place to place but it has to be considered in the design. ET shows a diurnal cycle and seasonal cycles because it is directed by solar radiation (IWA 2000).Thus, ET loss reduces the water volume in the wetland (increasing the concentration of contaminants) but not increasing the mass of contaminants being discharged in the effluent (Wallace and Knight 2006).

To estimate ET, there are several related measurements of wetland water losses. Three of the methods are the following: relating ET to class A pan evaporation, closed bottom lysimeters and ETo computation from metrological information for regional using of Penman-Montieth or Priestley-Taylor energy balance methods (Kadlec and Wallace 2009). ET can be calculated from ETo for a specific crop by multiplying with a crop coefficient (Eq 2.19).

$$ET=K_c*ET_o \qquad \text{Eq 2.19}$$

Where ET is expressed by Eq. 2.19 and K_c is the crop coefficient for the wetland plant and ET_0 evapotrasnpiration potential of the region where the wetland located.

For a sealed wetland a mass balance can be calculated with Eq 2.18 by measuring flow and precipitation (Headley et al. 2012). Or if planted and unplanted systems are available for the same sized wetlands constructed side by side, ET estimated by Q_{out} unplanted less Q_{out} planted.

In working with wetlands hydrological parameters are important and should be considered in reporting in order to obtain the actual mass removal.

2.7 Tracer Studies

Actual wetland flow hydraulics does not follow complete mixed reactor or ideal plug flow models. The deviation from plug flow and complete mix reactor models of existing constructed wetlands can be determined by conducting tracer tests. In this section, ideal flow reactors, non-ideal flow in reactors, tracer analysis, types of tracer in use, tracer response curves, tracer measurement interpretation and models of non ideal flow in reactors are explained. One of the important results of tracers test is the determination of the mean tracer retention time. The nominal retention time is the active water volume divided by average flow rate. A number of tracer studies that was conducted on constructed wetlands suggested that they equated to 4-6 equally sized complete mixed reactors (tanks in series) (Kadlec and Wallace 2009).

The plug flow reactor (PFR) and the continuously stirred tank reactor (CSTR) are the two ideal extreme reactor models in terms of the degree of mixing, from none to infinite. In plug flow reactors, there is no back mixing so the detention time of all flow fractions in the reactor equal to the theoretical detention time while in the CSTR there is back-mixing and different (varying) detention time. Since the hydrodynamic conditions in the constructed wetland tend to exist between these extremes, mixing rate can be mathematically approximated from equally sized CSTR and PFR tanks connected in series. As the number of CSTR increases it approaches the PFR (Kadlec and Wallace 2009).

The chemicals used for a tracer are inert and found at low concentrations in the environment. They include fluorescein, lithium chloride, potassium permanganate, rhodamine, sodium chloride and potassium bromide. The shape of the resulting outflow concentration versus time curve shows the degree of short circuiting or back mixing within the wetland and the actual detention time (Tchobanoglous et al. 2003). The effluent concentration of the wetland determined by collecting grab samples or by using automatic instruments samplers.

Pulse and step input methods are the most commonly used test methods. In the tests after adding known amount of tracer, the concentration of the tracer collected at the effluent end versus time and the shape of the curve indicates the character of the reactor. The data is then to plot a time versus effluent tracer concentration graph (Teefy 1996). This tracer information can be used as a diagnostic tool to ascertain flow characteristics of a particular reactor. In order to analyze the detention time distribution of the fluid in a reactor E(t) is developed.

The tracer response curve illustrates the entire range of detention times observed in the wetland which is called detention time distribution. The concentration versus time curve for PFR, CSTR and TIS are shown in Figure 2.3.

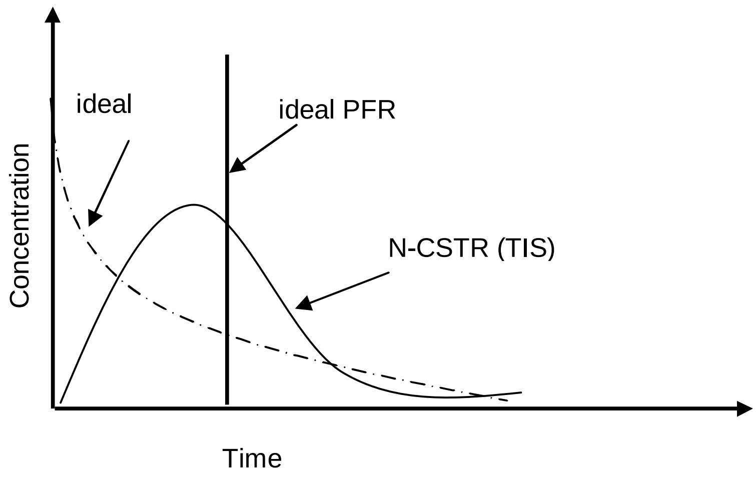


Figure 2. 3: Pulse input tracer curves for plug flow reactor (PFR), continuously stirred tank reactor (CSTR) and tank in series (TIS).

Eq 2.20- 2.27 are based on (Teefy 1996, Tchobanoglous et al. 2003). The mean and the variance are equal to the 1st and 2nd moments of the distribution (Tchobanoglous et al. 2003).The RTD function f(t) for an impulse tracer introduced into a steadily flowing system is

$$\tau = \int_0^\infty tf(t)dt \qquad \text{Eq 2.20}$$

$$f(t) = \frac{QC(t)}{\int_0^\infty QC(t)dt} = \frac{C(t)}{\int_0^\infty C(t)dt} \qquad \text{Eq 2.21}$$

Where C(t)= exit tracer concentration, g/m^3

Q= water flow rate, m^3/d

$$\tau = \frac{\int_0^\infty tC(t)dt}{\int_0^\infty C(t)dt} \approx \frac{\sum t_i c_i \Delta t}{\sum c_i \Delta t} \qquad \text{Eq 2.22}$$

Where, τ= mean detention time

t_i and C_i are the time and corresponding concentration at the ith measurements

Δt = the time increment between effluent tracer measurements.

The spread of the time distribution curve or its variance is calculated

$$\sigma^2 = \frac{\int_0^\infty t^2 c(t)dt}{\int_0^\infty c(t)dt} - \tau^2 \approx \frac{\sum t_i^2 C_i \Delta t}{\sum C_i \Delta t} - \tau^2 \qquad \text{Eq 2.23}$$

When the area under RTD curve for a slug input is normalized, the normalized curve is known as E curve. The peak concentration of E- curve is 1. The variance of E curve can also be normalized.

$$N = \frac{\tau_m^2}{\sigma^2}$$ Eq 2.24

$$\theta = \frac{t}{\tau}$$ Eq 2.25

$$\sigma_\theta^2 = \frac{\sigma^2}{\tau^2}$$ Eq 2.26

Normalized results help comparison with other similar works.

The mass of tracer recovered, M_t can also be estimated from pulse input tracer data according to Equation 2.27.

$$M_t = Q\sum_{i=1}^{i=n} C_i \Delta t = \frac{V_R}{\tau_m}\sum_{i=1}^{i=n} C_i \Delta t$$ Eq 2.27

Where, Q is the water flow rate

Tanks in series (TIS)

Tanks in series model (TIS) is explained here in its use for constructed wetland to calculate the experimental mean detention time (τ), variance (σ^2), normalized variance (σ_Θ^2).

The tracer results obtained are fitted and modelled using different techniques. The use of more accurate hydraulic models includes tanks-in-series and tanks-in-parallel, in combination with first-order kinetics, allows the observed variability to be taken into account. TIS model requires two parameters N (number of tanks) and τ (the mean tracer detention time). The TIS model is a gamma distribution of detention times as described in Eq 2.28 (Levenspiel 1972).

$$g(t) = \frac{N}{\tau\,\Gamma(N)}\left(\frac{Nt}{\tau}\right)^{N-1}\exp\left(-\frac{Nt}{\tau}\right)$$ Eq 2.28

Where,

Γ(N) = gamma function of N; N= number of tanks (shape parameter), unit less; t= actual time, d; τ = mean detention time, d

In the limit as N becomes very large, the gamma distribution becomes the plug flow (PF) distribution. In reality, this limitation is not observed in treatment wetlands. The TIS hydraulic model is flexible to describe both mixing and preferential flow paths for a wide range of hydraulic efficiencies.

For the TIS model

$$\sigma_\theta^2 = \frac{1}{N} \quad \text{Eq 2.29}$$

$$\frac{1}{N} = \frac{\tau - \tau_p}{\tau} \quad \text{Eq 2.30}$$

The relationship between τ_p and τ_n can be illustrated with the effective volume ratio, Equation 2.31 (Thackston et al. 1987). The effective volume is strongly influenced by the length-to-width ratio (Persson and Wittgren 2003).

$$\lambda = \frac{\tau_p}{\tau_n} = Hydraulic\ \ efficiency \quad \text{Eq 2.31}$$

Where, λ= wetland hydraulic efficiency, dimensionless
τ_p= peak value

If poor hydraulic conditions prevail, the HRT and effective volume can be reduced, resulting in lower removal efficiencies of the wetland system (Persson and Wittgren 2003)

$$e_v = \frac{V_{active}}{(LWh)_n} = \frac{\varepsilon\eta h}{h_n} = \frac{\tau}{\tau_n} \quad \text{Eq 2.32}$$

Where, e_v =wetland volumetric efficiency, dimensionless

V_{active}= active wetland volume, m^3; ε= fraction of volume occupied by water, dimensionless; η=gross areal efficiency, dimensionless; h= water depth, m; h_n= nominal water depth; $(LWh)_n$ = nominal wetland volume, m^3

Tracer study values are not an end by itself but it has to be applied into models to predict performance values different water quality parameters. The following section is about wetland models used in constructed wetlands.

2.8 Treatment wetland performance models and kinetics

Models are available for constructed wetlands to simulate the behaviour of wetland hydraulics and represent the treatment performance. In wetlands, black box and mechanistic models have been used (Garcia et al. 2010). A requirement for large numbers of parameters and a lack of in-depth knowledge limits the mechanistic models application as a design tools so black box approaches are practically used for sizing systems. The black box design models can be broadly grouped into areal loading models and volumetric models types. A design model developed by Kadlec and Knight (1996) is an example of an areal loading model. Wetland model developed by Reed and Brown (1995) and Crites and Tchobanoglous (1998) are examples of volumetric models.

Kadlec and Knight (1996) propose Eq 2.33 from mass balance to describe the removal of various constituents in wetlands:

$$\frac{C_0 - C*}{C_i - C*} = \exp(-\frac{k_A}{q}) \qquad \text{Eq 2.33}$$

Where, q= hydraulic loading rate (m/d)

- C_i and C_o=inflow and outflow concentration, respectively (kg/m^3)
- k_A = areal removal rate constant (m/d)
- C*= residual or background constituent concentration (kg/m^3)

This equation is particularly useful for estimating the area of wetland necessary to achieve a given removal. Rearranging Equation 2.33 gives

$$A = \frac{Q}{k_A} \ln \frac{C_0 - C*}{C_i - C*} \qquad \text{Eq 2.34}$$

Where, Q = flow rate through the wetland

Equation 2.34 has limitations because it does not consider water loss and gain, rate constants were developed with small wetlands and inclusion of C* results in excessive sizing. Constructed wetlands are designed to remove external loading but internal loading (C*) are come from the wetland components itself, such as wetland plants. Plants the largest source of background concentration because they release leachates and excudates (Garcia et al. 2010).

Reed et al.(1995) proposed Equation Eq 2.35 where k_V is based on volume.

$$\frac{C_o}{C_i} = \exp(-k_V t) \qquad \text{Eq 2.35}$$

Where k_V = rate constant at the wetland temperature T t= hydraulic detention time in the wetland

k_T are estimated from a standardized value at 20^0C using Equation 2.36

$$k_T = k_{20}\theta^{(T-20)} \qquad \text{Eq 2.36}$$

Where θ= modified Arrhenius temperature factor, dimensionless; T= water temperature, ^{0}C; k_T= Areal rate coefficient, k_v or volumetric rate coefficient, k_A at temperature T;
k_{20}= Areal rate coefficient, k_v or volumetric rate coefficient, k_A at 20^0C.

The required wetland area corresponding to the Equation 2.35 is given by Equation 2.37.

$$A = Q_A \frac{\ln(\frac{C_i}{C_0})}{k_T y n} \qquad \text{Eq 2.37}$$

Where, $$Q_A = \frac{Q_{in} + Q_{out}}{2} \qquad \text{Eq 2.38}$$

Where, y= average depth of flow in the wetland; n= porosity of the wetland

Crites and Tchobanoglous (1998) proposed Equation 2.37 like (Reed et al. 1995) with some modifications in the parameter estimates and formulations.

The tanks in series (TIS) model for continuous flow systems

However, the current black box wetland design models are based on steady state water flow conditions and first order decay of pollutants as opposed to the reality (Kadlec and Knight 1996). This has resulted in oversized and less efficient wetlands. Continuous flow system like wetlands are not spatially uniform, there are plants, microorganisms, short circuiting, and dead zones. So the hydraulic model must account for these effects through the use of the TIS model (Kadlec and Wallace 2009).

Water passes through N tanks in series, and loses a portion of the contaminant load in each. For the case of no water losses or gains, the steady flow contaminant mass balance for the j^{th} tank is

$$QC_{j-1}-QC_j=kA(C_j-C^*) \qquad \text{Eq 2.39}$$

Where C_j= concentration in and leaving tank j, g/m^3, A= wetland area (m^2)
For the entire sequence of tanks based on input and output, these mass balances combine to

$$\frac{C-C^*}{C_i-C^*}=(1+\frac{k_A\tau}{Nh})^{-N} \qquad \text{Eq 2.40}$$

Where

C= outlet concentration, g/m^3; C_i= inlet concentration, g/m^3; C*= background concentration, g/m^3; k_A = removal rate constant, m/d; N= number of tanks; τ = detention time, d; h=nominal wetland water depth, m

Rate constant, k_A and hydraulic parameter, N are the two reaction parameters. Longitudinal concentration profiles may also be derived from this model.

$$\frac{C-C^*}{C_i-C^*}=(1+\frac{k_A\tau y}{Nh})^{-N} \qquad \text{Eq 2.41}$$

y= fractional distance through the wetland.
This equation shows the longitudinal decreasing of concentration from the inlet to the outlet up to the value of C*.
N is obtained from tracer curves and it is a fitting parameter and does not represent the physical configuration of the wetland.

P-k-C* Model

Equation 2.40 and 2.41 are meant for single compound (e.g. glucose) and not for compounds of a complex mixture in nature like TSS, BOD, Total nitrogen etc. Due to their chemical nature, individual compounds in BOD for instance are composed of different degrees of biodegradability at the same conditions so they have different rate of removal. So In this case it is relevant to use a distribution of k values (KVD) than single k value (Kadlec and Wallace 2009). Equation 2.42 is the average k values at any time during the reduction process.

$$k=\frac{k_i}{(1+\beta t)^n} \qquad \text{Eq 2.42}$$

Where k=rate constant during weathering process

k_i= inlet rate constant; n= mixture of k values distribution, dimensionless; t= length of time the mixture has weathered in the wetland; β= mixture k values distribution weathering parameter, d^{-1}

The P-k-C* model is preferred and promoted by Kadlec and Wallace (2009) for determining removal rate coefficients and for sizing treatment wetlands from operational data. This model is a variant from equation 2.33 formulation of the first-order k-C* model (Kadlec and Knight 1996).

Physical factors like topography, geometry, vegetation distribution and density should be included in calculations since they affect hydraulic efficiency and non plug flow conditions. Weathering factor should be included for mixture parameters like BOD, COD, and TSS. The presence of degradable mixture or chemical will cause a reduction in the N value determined from an inert tracer experiment. The relaxed TIS concentration model is P-k-C* model (Kadlec and Wallace 2009), can therefore, and be defined as

$$\frac{C - C^*}{C_i - C^*} = \frac{1}{(1 + k_A / pq)^p} = \frac{1}{(1 + k_V \tau / p)^p} \qquad \text{Eq 2.43}$$

Where k_A = is the modified first order areal rate constant, m/d; k_V= modified first order volumetric rate constant, d^{-1}; P= apparent number of TIS, always P ≤ N

$$k_v = \frac{k_A}{\varepsilon h} \qquad \text{Eq 2.44}$$

Where, ε=wetland porosity, dimensionless; h= wetland water depth, m

Table 2.5: Background concentration of pollutants in HSSF treatment wetlands, C* (Kadlec and Wallace 2009)

C* values for	Approximate values, mg/l
BOD_5	5
Organic nitrogen	1.5
Total nitrogen	1
PO_4^{3-} , NO_3^-, NH_4^+	0
Faecal coliform, E. coli	0

In the earlier TIS model, the effects of hydraulic efficiency were described by the parameter N, the number of TIS. In the P-k-C* model, N has been replaced by P and combines the effects of hydraulic efficiency (N; DTD) and weathering pollutants (K-value distribution, effect of pollutant weathering) (Kadlec and Wallace 2009). Since the microbial ecology, the configuration, the hydraulic loading, climatic conditions, pollutant concentration affect the results; extrapolation of rate constants or model parameters is not a good practice for different situation when using black box approach models (Kadlec 2000).

The goal of the design calculation is the selection of wetland area that will provide the necessary treatment in all seasons of the year. So conducting a tracer and estimate parameter N and τ by data fitting then calculate k from P-k-C* model and based on the model estimate outlet concentration.

2.9 Constructed wetlands for developing countries

The United Nations, during the Millennium Summit in New York in 2000 and the World Summit on Sustainable Development in Johannesburg (WSSD) in 2002, developed a series of Millennium Development Goals (MDGs) aiming to achieve poverty eradication and sustainable development by rapidly increasing access to basic requirements such as clean water, energy, health care, food security and the protection of biodiversity. The specific target set for the provision of water supply and sanitation services is to halve the proportion of people without access to safe drinking water and adequate sanitation by 2015 (WHO 2006b).

Wastewater is increasingly used for agriculture in both developing and industrialized countries, with the principal driving forces being increasing water scarcity and stress, population increase and recognition of wastewater as a resource (WHO 2006b). It is estimated that within the next 50 years, more than 40% of the world´s population will live in countries facing water stress or water scarcity (Hinrichsen et al. 1998). Fresh water is already scarce in many parts of the world and population increase in water scarce regions will further increase its value. It is estimated that around 62 % of the 8 billion population will live in water stressed countries by 2025 (Arnell 1999). Growing competition between the agricultural and urban uses of high quality fresh water supplies, particularly in arid, semiarid and densely populated regions, will increase the pressure on this diminishing resource.

Agriculture is the largest consumer of freshwater in the world with it accounting for nearly 70% of all extractions of fresh water (Gleick 2000 , FAO 2002). As fresh water becomes increasingly scarce due to population growth, urbanization and climate change, the use of wastewater in agriculture will increase even more. At least 10% of the world`s population is thought to consume foods produced by irrigation with wastewater (Smit and Naser 1992). Wastewater is often a reliable year round source of water, and it is considered a method of combining water and nutrient recycling, thus, increasing household security though food production (WHO 2006b). The use of wastewater for crop irrigation as a source of fertilizer so often promoted in order to reduce the need for artificial fertilizer, hence it provides a form of nutrient recycling.

When introducing wastewater in agriculture to achieve the goal mentioned above, it should have low risk to the consumers and the farmers. The wastewater has to be treated to an appropriate level to reduce health risks. Decentralized wastewater management is being progressively considered because it is less resource intensive and more ecologically sustainable (Tchobanoglous et al. 2003). Constructed wetlands are one of the technologies preferable because of the low construction costs, low management requirements and complexity and low operation and maintenance cost requirement (Kivaisi 2001). In addition, they require less skilled manpower than more technical systems. If this technology is implemented then there may be significant health and environmental benefits. Wetlands are able to tolerate fluctuations in flow, facilitate water reuse and recycling, provide habitat for many wetland organisms and can be built to fit harmoniously into the landscape (U.S.EPA 1995).

In spite of their advantage and favourable weather for the technology in tropical countries the rate of adoption of constructed wetlands technology in developing countries is slow because of lack of awareness and local expertise (Kivaisi 2001). When planning and designing for the introduction of an environmental technology or transferring from industrialized world to developing countries, the socio-cultural dimension needs to be included in the sustainability assessment: the support from the public and local government is very important to the success of the project (Moller et al. 2012). Besides, it is not possible to import the technology designed for temperate climates directly to a tropical climate. The design has to be adapted to warm climates in order to improve performance, particularly higher mass loadings than lower mass loadings, shorter residence time than longer residence time to fit the situation (Garfi et al. 2012). The proper management and operation requires proper design considerations. However, there are some experiences from African countries including Egypt, Morocco, Tunisia, Kenya, South Africa, Uganda (Vymazal and Kröpfelová 2008). In Tanzania, pilot scale wetlands were studied to treat waste stabilization pond effluents (Mashauri et al. 2000, Senzia et al. 2003, Kaseva 2004). In Ethiopia, to the knowledge of the author, currently constructed wetland systems used in three sites that shows wetland is unknown to the public and government.

In summary, the various advantage of constructed wetland for wastewater treatment suggests that there may be opportunities for the technology to be implemented in the developing world in order to contribute in solving the worlds impending water shortages.

2.10 Summary of literature review

This chapter presented the historical development of constructed wetlands and the type of constructed wetland designs available which, this includes subsurface and surface flow constructed wetlands and finally hybrid constructed wetlands. The major information presented focused on the horizontal subsurface flow constructed wetland and its major components in the constructed wetland: liners, media, macrophyte and pollutant removal mechanisms. To study the hydraulics characteristics of a wetland the tracer study methods were presented and the selected models used in wetlands were discussed. Finally the application of wetlands in developing countries was discussed.

Although HSSF systems are good at removing organic matter (80-90%) and total nitrogen (25-50%), there are problems related to optimum dimensioning of the bed. One of the design parameter is the effective depth of wetlands used in treating wastewater with HSSFCW. Traditionally, wetlands are designed with 50 cm deep or more in order to save space and costs associated with construction. Recent studies have shown that shallow beds are more effective in removing pollutants than deeper beds. This needs to be studied further at different climatic conditions. Currently, there is very little practical information about the most effective depth of wetlands bed for pollutant. The next chapters attempt to answer research questions on the effect of depth and plant on performance of HSSCW.

A. Langenreichenbach Pilot Study, Leipzig, Germany

3 The effect of depth and plants on pollutant removal in HSSFCW

Overview

In this chapter, the experimental method and the results of the effect of depth and plants on HSSFCW at Langenreichenbach (LRB), Germany is presented. Three experiments were conducted at LRB namely: tracer studies, performance evaluation and nitrification potential measurement of the wetlands.

Section 3.1 presents the methods which includes the location, description of the site, description of the wetlands, procedures for tracer analysis, analytical methods of the physicochemical parameters, nitrification potential experimental procedures and the statistical methods used.

Section 3.2 presents the results and discussion of the tracer experiment and in this section the water balance of the wetlands and the analysis of the hydraulic characteristics of the wetlands are presented.

Section 3.3 presents the results and discussion of the treatment performance of the wetlands. The overall yearly and two yearly average performance is presented and compared among the four wetlands, then the effect of season on bimonthly average value comparison for total suspended solids, $CBOD_{5,}$ TOC, TN, ammonia nitrogen and *E. coli* rate coefficients are discussed. Finally, the internal performances of the wetlands are compared.

Section 3.4 discusses the results of the spatial variation in nitrification potential with inter and intra difference in a HSSFCW with an ex-situ method and infer nitrifying bacterial population distribution. Therefore, the rates of nitrification in planted, unplanted, aerated, none aerated, deep or shallow depth and inlet or outlet side of the wetland systems are reported.

3.1 Methods

3.1.1 The wetlands

The HSSFCW is located at the LRB Ecotechnology Facility (shown in Figure 3.1) located at LRB which is East of Leipzig, Germany (51.5°N, 12.9°E) (shown Figure 3. 2). The four HSSFCW with a surface area of 5.64 m^2 (1.2m X 4.7 m) and a wetted gravel depth of 50 cm and 25 cm are presented in Table 3.1. Each bed was lined with a polyethylene liner and covered with geotextile fabric, and was then filled with 8-16 mm alluvial gravel of 38 % porosity. The inlet and outlet of the wetlands was filled with coarse gravel 16-32 mm to minimize clogging. Figure 3.3 and 3.4 show the profile view of the wetlands and composition of the wetlands.

Figure 3.1: The Ecotechnology research facility at Langenreichenbach, Germany. The horizontal subsurface flow wetlands are located in the middle of right side of the picture. Photo credit Andr´e Künzelmann/ UFZ.

During monitoring time the air temperature ranged from -4.3 - 25^0 C. This thesis was based on four passive horizontal subsurface flow constructed wetland from the site which consists of 15 pilot scale subsurface flow wetlands (Figure 3.1 & Table 3.1).

Table 3.1: Details of the saturated horizontal subsurface flow constructed wetlands.

code	*P. australis*	Wetted Media Depth (m)	Hydraulic loading rate (HLR), (L/m^2d)	Main Media Type, gravel	Loading Interval (hour)	Surface Area (m^2)
H25	unplanted	0.25	18	8–16 mm	0.5	5.64
H25p	planted	0.25	18	8–16 mm	0.5	5.64
H50	unplanted	0.50	36	8–16 mm	0.5	5.64
H50p	planted	0.50	36	8–16 mm	0.5	5.64

The introduction of seedlings (5/m^2) was completed in September 2009 and was fertilized with nutrients until June 2010. The wetlands were dosed 2 litres and 4 litres for 25 cm and 50 cm deep beds, respectively every 30 minutes.

Figure 3.2: Map of Germany showing location of Leipzig

Sampling wells were installed at 12.5 - 25 - 50 - 75% internal points along the central axis of the four HSSFCW. In 50 cm deep wetlands, each internal sampling point consists of three lengths of 20 mm diameter PVC pipe shown in Fig 3.5, capped on the lower end with a perforated PVC tee; samples were collected from the middle pipe. In Figure 3.6 the 25 cm deep wetland, a sampling PVC well was placed at each of four points along the internal point of the wetland.

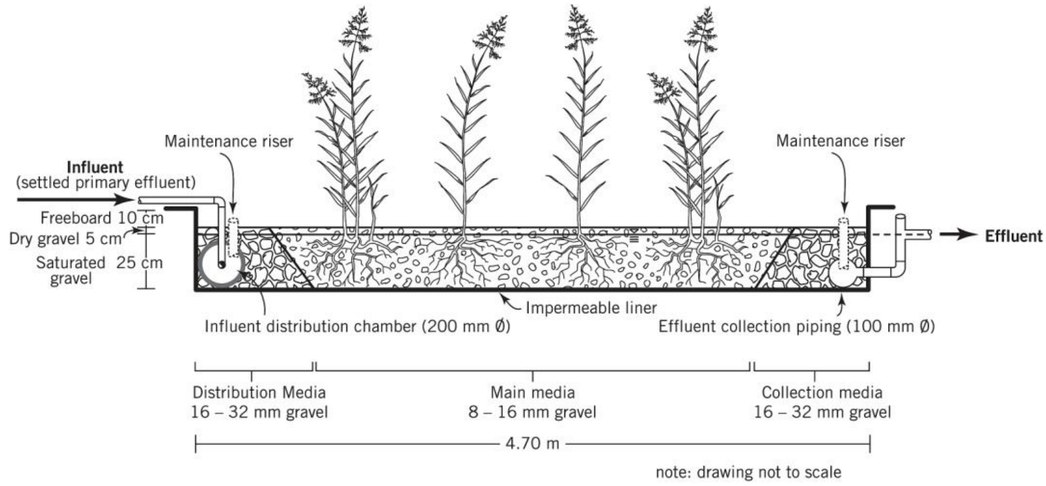


Figure 3.3: Horizontal subsurface flow wetlands scheme for the 25 cm deep beds.

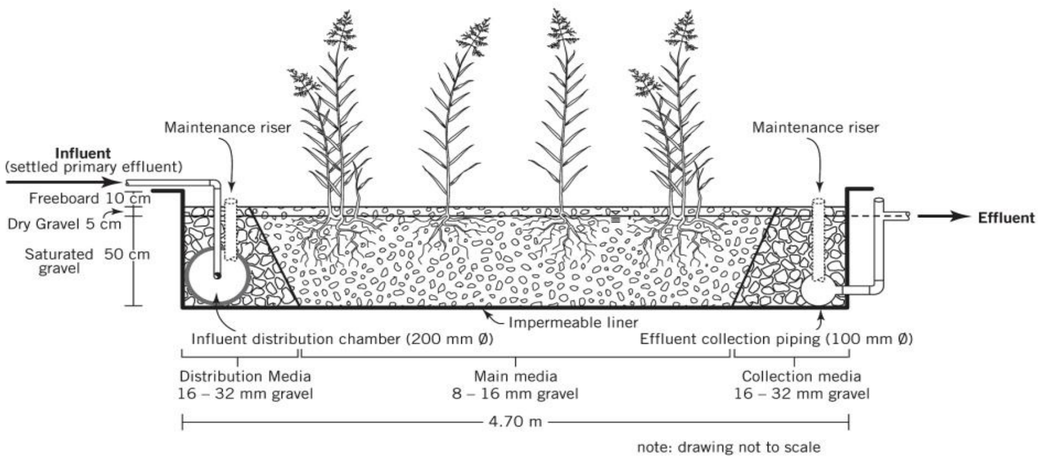


Figure 3.4: Horizontal subsurface flow wetlands scheme for the 50 cm deep beds

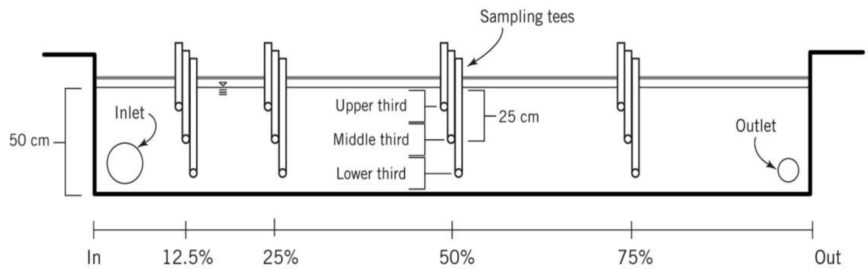


Figure 3.5: Cross sectional view of the intermediate sampling tee wells that enable sampling of the water column along the length of the 50 cm deep wetland. For this experiment the middle port was used for sampling.

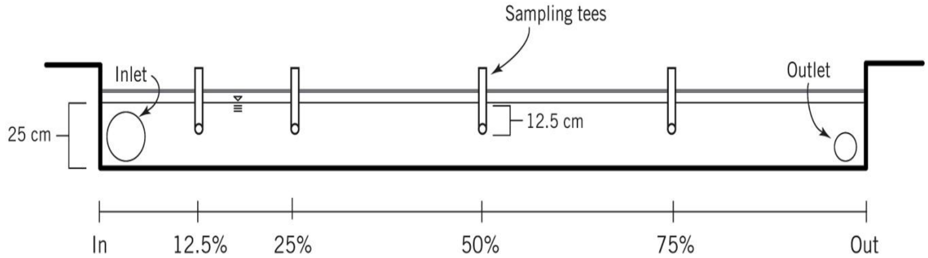


Figure 3.6: Cross sectional view of the intermediate sampling tee wells that enable sampling of the water column along the length of the wetland in 25 cm deep wetlands.

Figures from 3.3 to 3.6 were taken from Nivala (2012). The inlet/ outlet experiment was conducted in September 2010 – 2012. Internal samples were collected in September 2010- Sept 2011. The beds were dosed every 30 minutes with primary treated municipal effluent at an average hydraulic loading rate (HLR) of 18 and 36 mm/d for the 25 cm and 50 cm deep wetlands. Outflow from the wetlands were measured by a float valve connected to a computer with the capacity to record the number of fills events. Water samples were collected bi-weekly for the inlet outlets and every 3 weeks for the internal sampling wells.

Temperature, humidity, rainfall, wind speed and direction, air pressure and solar radiation of the LRB research facility was measured by an onsite weather station.

The H25p, H25, H50p and H50 wetlands water balance was mainly influenced by wastewater inflow and outflow. Rainfall was one of the contributors to the inflow and ET was responsible for the loss of water. Since the system was lined, it is presumed that the loss of water from the system was only by ET and the outflow. The ET rate of the planted beds was approximated from out flow of the unplanted bed minus the outflow of the planted beds with the same depth and surface area and inflow or the ET expressed as in Eq 3.1.

$$ET = Q_i + P - Q_o \qquad \text{Eq 3.1}$$

Where, Qi = wetland influent (mm/day); Q_o = wetland effluent (mm/day); ET=evapotranspiration (mm/day); P= Precipitation (mm/day)

3.1.2 Gravel Porosity analyses

The inlet and outlet of the wetlands were packed with 16-32 mm gravel and the main media was 8-16 mm gravel with a porosity of 38% with uniformity coefficient of 2. The gravel was selected in order to ensure clogging not to occur soon by taking different International experiences. The porosity of the 8-16 mm gravel was measured in 200 litre basin, placed on a levelled surface. The container was filled with measured volume of water to a mark then the water emptied and the container was filled with gravel in question to the mark and levelled on the top and was filled with measured amount of water to the level. The percentage of the volume of water added in the container with the gravel to the water added without gravel was calculated as percentage porosity of the gravel.

3.1.3 Water sampling and analysis

Water samples were collected in 500 mL containers, tightly closed and placed in an ice box for transport to the UFZ, Leipzig. Samples for *E. coli* were collected in 100 mL sterilized glass containers, tightly closed and transported in a separate ice box to the laboratory. Depth samples from the internal wells were collected using a peristaltic pump. The contents of each sample well were pumped out immediately prior to sample collection in order to ensure that samples represented fresh wastewater that had infiltrated into the well from the surrounding area at the intended depth. Water sample temperature was measured during sampling and also during measurement of pH, conductivity, DO and ORP.

Water samples for $CBOD_5$, TN, TOC, *E. coli*, TSS, NH_4^+, NO_3^-, and NO_2^- were conducted at the Environmental- and Biotechnology laboratory at the Helmholtz Environmental Research Centre (UFZ) in Leipzig, Germany.

The TOC was measured according to the European standard DIN EN 1484 by *Total Organic Carbon Analyzer TOC.*V_{CSN} from *Shimadzu*. The procedure is a two stage process that results in all carbon being combusted at 1000^0C to transform all carbon in to CO_2 and in a separate reactor samples are acidified to get CO_2 from inorganic carbon compounds. The carbon dioxide produced in the two steps is measured with infrared spectroscopy and the difference between total carbon and the inorganic carbon is the TOC.

TN was analysed according to the European standard DIN EN 12660, using the *Total Nitrogen measuring unit TNM-1 from Shimadzu*. The analytical procedure includes combustion of all the nitrogen in the sample at 700^0C to transform in to NO. The NO allowed reaction with ozone to give NO_2 which results in excitement and later emissions in the form of visible light for detection.

Conductivity, dissolved oxygen, temperature, and redox potential were measured onsite using a WTW multi 350i universal meter with the relevant electrodes. $CBOD_5$ was analyzed by WTW oxitop manometric respirometers that relate oxygen uptake to the change in pressure caused by oxygen consumption while maintaining a constant volume. As the micro-organisms respire they use oxygen converting the organic carbon in the solution to CO_2, which is absorbed to the potassium hydroxide (KOH) which is present in the form of a pellet in the lid of the bottle. This results in pressure drops in the system, which is directly proportional to BOD values, which are measured by a pressure sensor. The BOD values are stored in the sensor memory and can be called up on the large-format display. Before measurement, the sample BOD range was estimated for the selection of measurement range.

Total suspended solids were analyzed gravimetrically by filtering measured amounts of sample through a 1.5 μm pore diameter glass microfiber filter, 934-AH™ and then oven dried at 103^0C according to the standard methods.

E.coli was measured with the IDEXX™ method. Samples were serially diluted to appropriate dilution factor and mixed thoroughly with IDEXX nutrient. This mix was poured (air bubbles removed) in to an IDEXX envelope with many wells and sealed with a machine and incubated for 18 hours at 36^0C. The results evaluated by counting the number of glowing wells in UV light and comparing them with the reference provided from the manufacturer.

Ammonium, nitrate and nitrite measurements are described in the nitrification experiment section.

The pollutant mass balance for constructed wetlands was calculated Kadlec and Knight (1996) and Kadlec and Reddy (2001) as shown in Equation 3.2. In order to take care of the water loss and gains from planted beds, areal load removal rates calculated for each bed using Eq 3.2. Volumetric mass removal rate was also used by dividing eq 3.2 by the depth and porosity of the media. Percent concentration reduction was used only in few case in this document because it do not reflect the internal chemical dynamics of the wetland such as production and water loss and gains (Kadlec and Wallace 2009)..

$$\text{areal mass removal rate } \left(\frac{\mathrm{g}}{\mathrm{m^2 d}}\right) = \frac{C_i Q_i - C_o Q_o}{A} \qquad \text{Eq 3.2}$$

Where Q_i is the inflow rate (m^3/day), Qo is the average outflow rate (m^3/day), C_i and Co are the parameter concentrations in the influent and effluent, respectively (g/ m^3), they are the average of two months and A is the area of the wetland (m^2). The mass volume removal of the wetlands was calculated by dividing Equation 3.2 by the depth and porosity of the wetland.

As identified in Equation 2.18 and 2.19, the average wastewater flow accounts for the effects of water gains and losses (precipitation, ET and infiltration) that occur in a constructed wetland. Defining Q_{in} as the wetland influent flow rate and Q_o as the effluent flow rate, the average wastewater flow rate is expressed as Equation 3.3 (U.S.EPA 1995). Eq 3.3 was used in the calculation of the nominal hdraulic residence (nHRT) time during the internal concentration analysis to take care of water loss and gain (Headley et al. 2013).

$$Q_{ave} = \frac{Q_{in} + Q_o}{2} \qquad \text{Eq 3.3}$$

3.1.4 Tracer experiment

Tracer was conducted with potassium bromide and fluoresein dye simultaneously.
In order to determine the hydraulic characteristics between H25, H25p, H50 and H50p a tracer study using potassium bromide (KBr) were conducted in November 2012 and April 2013. Eight and fifteen grams of oven dried potassium bromide (KBr) was dissolved each in two litres of primary treated wastewater to apply in the 25 and 50 cm deep wetlands, respectively. The mixed solution was added to the inlet of the wetlands with a single-shot injection simultaneously when feeding the wetlands with the wastewater. The added KBr solution was stirred with a rod for about 3 minutes to create turbulence and avoid salt stratification or settling at the inlet. The KBr amount selected was based on the volume of the wetland and the maximum concentration it gives for analysis. At the same time a VWR-TP II automatic sampler started to collect samples from the effluent of the wetlands at 4 hours interval. After four days of sampling, the samples were filtered and stored at 4^0C until the tracer experiment was finished. The tracer sampling experiment was conducted for 10 days for 25 cm deep beds and 12 days for 50 cm deep bed to obtain a complete response of the tracer injection. The bromide concentration in each sample was measured using anionic ion chromatography according to DIN 38405 D19 with automatic IC machine DX500 from DIONEX at the UFZ, Leipzig.

7 ml and 14 ml of 10.2 mg/L fluoresein was applied for 25 and 50 cm deep wetlands respectively in the same manner like KBr and the fluoresein was measured with automatic Fluoresein sensing electrode at the outlet onsite.

The detention time distribution (DTD) of the wetlands were plotted using the technique from Headley and Kadlec (2007) and Kadlec and Wallace (2009). The raw data was plotted C(t) versus time as in Section 2.7. In order to compare systems analysed with different concentration and situations the raw DTD was normalized; C(t) to C'(t) = C(t)*Q/M and τ to $\Theta = t/\tau$ dimensionless forms. Dimensionless DTD function is a tracer mass fraction at a corresponding fraction of mean tracer detention time.

The tanks in series (TIS) model which enables gamma distribution was used to fit the curves. The curve fitting exercise (trial and error) was performed to obtain a model which best describes the tracer response curve for H25p, H25, H50p and H50 wetlands. The iteration exercise was conducted with SOLVER, Microsoft Excel™ to minimize the sum of squares errors (SSQE) between the TIS - DTD model gamma function and the observed normalized DTD. Values of the number of tanks in series and mean detention time were the variables entered in the distribution curve until the best fitting was obtained. The DTD curve for the TIS model can be represented by Eq 2.28 (Levenspiel 1972).

Hydraulic variables from the curve of each of the wetland were the average detention time, mode (the peak in the tracer concentration), variance and the time the tracer is first detected and the derivation of this hydraulic efficiency (degree of short circuiting and dead zones) and mass recovery were evaluated and discussed.

3.1.5 Calibration of the P-k-C* model

The P-k-C* model was applied to calculate rate coefficient (k) for water quality parameters for $CBOD_5$, TOC, TN, NH_4^+-N and *E.coli* for bimonthly average in and outflow concentrations. The P-k-C* model combines the first order degradation rate coefficient and the TIS hydraulic model. The P-value which includes KVD and DTD is less than N calculated from the tracer analysis and C* are assumed (Ci>C*) and taken from Kadlec and Wallace (2009) as shown in Table 2.5. k_A was calculated for each parameter by rearranging Eq 2.43 for the two year period.

In order to be able to compare rates of degradation between systems, it is common to calculate k_A at 20^0C as a standard condition.

Eq 2.43 and Eq 2.36 combined to give Eq 3.4 which resulted in two unknowns that are rate coefficient (k_A) and temperature factor (θ) at 20^0C.

$$C_{\mathrm{Calculated}} = C^* + \frac{C_i - C^*}{\left(1+\frac{k_{20}\theta^{(T-20)}}{Pq}\right)^P} \qquad \text{Eq 3.4}$$

$$\text{sum of square errors} = \text{minimum} = \sum_{i=1}^{n}(C_{\mathrm{measured}} - C_{\mathrm{Calculated}})^2 \qquad \text{Eq 3.5}$$

SOLVER tool from MS Excel™ 2007 was used to minimize the sum of squares errors (SSQE) between the measured and predicted concentration values of the effluent in Eq 3.5. The Excel sheet for evaluation of k_{20} and θ is shown in Appendix 2 Table 1.

3.1.6 Sampling for Nitrification potential measurement

Nitrification potential was conducted in the passive HSSFCW (H25, H25p, H50, and H50p) and aerated beds (HA, HAp) in February 2011 and May 2011. H25, H25p, H50 and H50p were explained in section 3.1.1. The planted *(Phragmites australis)* and unplanted aerated beds had a surface area of 5.64 m^2 and a depth of 100 cm and the main bed was filled with gravel in a size range from 8-16 mm. The acronym for the wetland was horizontal aerated and planted (HAp) and the horizontal aerated and unplanted labelled HA. The aerated beds were designed to receive an air at a flow rate of approximately 2.4 m^3/h, and received a flow of approximately 720 L/d.

Gravel and root samples for nitrification potential measurements were collected at a distance of 80 cm away from the inlet and outlet side and about 30-40 cm from the side of the wetlands between depths of 12.5 - 20 cm from all wetlands using cylindrical sampling core cutter. Similarly, root samples were collected for the planted beds from the same spot. The samples were kept in an ice box and transported to the laboratory. Temperature of the pore water was also measured to know the environment of the gravel and root sampled during collection.

3.1.7 Determination of nitrification potential

Nitrification potential was carried out as described by different authors (Belser and Meyer 1982, Kyambadde 2004). Nitrification potential solution was prepared by mixing 7.5 ml of 0.2 M KH_2PO_4, 17.5 ml of 0.2 M K_2HPO_4, and 75 ml of 25 mM $(NH_4)_2SO_4$ solution and diluted and pH adjusted to 7.2 and further diluted to 5 litres with distilled water.

Two hundred fifty grams of wet sediment sample of the beds were mixed with 150 ml nitrification potential solution. Care was taken not to remove the gravel and root biofilm while transferring and weighing. The gravel suspension in excess nitrification potential solution was incubated in partially screwed 500 ml bottles at 20^0C and was shaken on a rotary shaker at rate of 120 rpm in a horizontal position to optimize the transfer of oxygen from the air to the gravel and buffer solution.

Fifteen grams of carefully selected root hair (rhizomes were not included) of the 25 cm, 50 cm deep and aerated planted beds were mixed with 200 ml nitrification potential solution. Young roots of *P. Australis* release oxygen, whereas no release was detected from old roots and rhizomes (Brix and Schierup 1990) and therefore assumed that nitrifying bacteria accumulate around Selection of young roots because young roots rather than rhizomes. The plant root suspension was incubated in the same manner as the gravel incubation.

The production of nitrate and nitrite during 24 hours incubation was measured by periodic pipette withdrawal of aliquots at 0, 2, 4, 8 and 24 hours from the starting of the experiment. The pipette solution was first filtered through a 0.45 µm pore syringe filter to remove solid particles and nitrifying bacteria to stop further nitrification. The filtered sample was analyzed for nitrite, nitrate and ammonium and stored at -20^0 C if not analyzed on time. Samples collected in May 2011 were analyzed for ammonium, nitrate and nitrite concentrations colourimetrically according to *DIN 38 406 ES, DIN 38 405 D9 and DIN 38 405 D10 by EPOS Analyzer 5060 from Eppendorf*. Samples collected in February 2011 were analyzed by HACH Lange spectrophotometers using relevant reagents for ammonium, nitrate and nitrite ion. The results from ammonium analysis were used for the February data analysis.

Samples taken from each point were incubated in three separate bottles and the supernatant was analysed as noted in the previous paragraph for ammonium, nitrate and nitrite in the time given and the concentration obtained was averaged to use for the y axis of the curve. The gravel and the plant root after nitrification potential analysis were dried at 103^{0}C and weighed so that ammonia oxidation rates were normalized to gravel and root dry weight. The nitrification potential in mg-N/g-dw/hour was calculated as the slope of the linear regression of accumulated nitrogen mass divided by dry gravel or root weight against time. Nanogram, microgram and milligram were used depending on the value of the concentration Eq 3.6.

$$\textbf{Nitrification potential} = \frac{\textbf{ng N}}{\textbf{g dw.hour}} \qquad \text{Eq 3.6}$$

3.1.8 Statistical methods

Statistical procedure was carried out using SPSS® statistics version 16 software package (IBM Corporation, USA). Linear Mixed Model (LMM) with repeated measures methods was used to check the significance of performance with areal and volumetric mass removal rate of H25, H25p, H50 and H50p beds for TSS, $CBOD_5$, TN, NH_4^+-N and *E.coli* parameters. LMM with repeated measures was chosen rather than ANOVA because the data do not satisfy the requirement of independence which is the fundamental assumption of ANOVA. The significance were checked at α=0.05. The procedure followed in other works (Headley et al. 2013).

To determine if the rate of pollutant concentration change with nominal residence time (nHRT) across from inlet to outlet varied between the H25, H25p, H50 and H50p, the analysis of covariance (ANCOVA) procedure was conducted in excel sheet as described by (Zar 2010). The regression equations described the relationship between concentration and nHRT across the wetland bed. For pollutants that did not exhibit linear relationship with nHRT, the concentration data was log transformed. For ANCOVA, at first the slope of the concentration of the pollutant with nHRT was conducted between wetlands for significance if the slopes were the same then the y-Intercept (elevations) was checked. In this experiment, the elevations were the same in four of the wetlands because the same raw water was feed (same concentration) so they were no need to compare. The analysis was conducted in other works

(Headley et al. 2005, Headley et al. 2013). The parameters treated with ANCOVA were TOC, TN, NH_4-N and DO.

One way of ANOVA (post HOC test) was used in order to compare values of the ET difference between the wetlands and to determine the differences of the normalized (variance, detention time, delay time, and peak time) of the wetlands in hydraulic efficiency check using SPSS version 16.

3.2 Results and discussion of hydraulic characteristics of wetlands

Overview

The first part of this section is the inflow, outflow, ET data presentation and discussion and the second part is fitting the gamma distribution model to the actual tracer concentration data and calculating the hydraulic parameters for H25, H25p, H50, and H50p and discussing their implications.

3.2.1 Evapotranspiration rates

Based on the inflow and outflow of the wetlands, the bimonthly averages flows and the calculated ET are shown for Sept 2010 to August 2012 in Figure 3.7. Although the absolute ET values in Figure 3.7 looks the same, from the average values calculated the ET of the H25p was 23 % and H50p was 12 % of the influent wastewater, respectively. From these result, H25p had the highest ET when compared with H50p which may be ascribed to H25p plant roots were found distributed throughout the water zone contrary to the H50p where bottom (below 30 cm) water zones have relatively low root distribution. In support to these observations, the high ET in H25p was observed to the point that the wetland was not giving effluent in some summer days. However, when compared statistically using ANOVA the ET rate of the H25p and H50p have not significantly different from each other ($p=0.92$) at $\alpha = 0.05$. Both the H25p and H50p have almost the same cover of plants but the volume of water in the wetland were not the same.

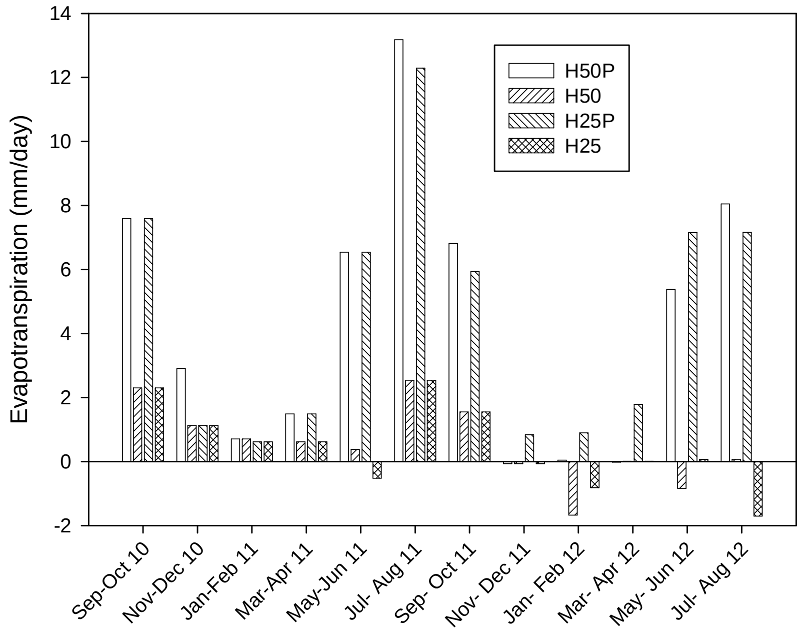


Figure 3.7: Averaged ET of H25p and H50p wetland at LRB experimental site collected from Sept 2010 to August 2012. The peaks were in summer and the highest values were from July to August 2011. The Negative values of Evaporation in January and February ascribed to the ice melting.

3.2.2 Hydraulic behaviour of HSSFCW with depth and plants

The hydraulic efficiency represents the ability of a wetland to distribute its flow uniformly throughout its volume, maximizing contact time of pollutant and optimizing degradation (Holland et al. 2004). Depth and presence of plants are some of the factors affecting hydraulic efficiency of wetlands. Hydraulic efficiency of wetlands is measured by way of tracer studies after reducing the DTD to a single number from the output of the data. Comparison of H25, H25p, H50 and H50p wetlands after conducting replicate tracer analysis four parameters are used to identify the effect of depth and plants. Therefore, the DTD curve and the hydraulic efficiency are discussed with the normalized variance (σ_θ^2), normalized delay time (t_d), normalized detention time (λ_t) and normalized peak time (λ_p) for H25p, H25, H50p and H50 beds. The Normalization procedure removes the effect of different working condition (such as different flow or mass of tracer added) by isolating the dispersive and mixing characteristics of the system and to make comparison possible (Holland et al. 2004). Ideally, normalization makes tracer curve area and centroid at one in normalized DTD (Holland et al. 2004).

Figures 3.8- 3.11 shows one of the normalized gamma distribution of detention times and the data plotted against the dimensionless detention time for H25p, H25, H50p and H50. The exit curves not follow plug flow or complete mix reactors but tank in series with typical bell shaped response (Kadlec and Wallace 2009). The normalized tracer curve of the 25 cm planted and unplanted beds was symmetric as shown in the Figure 3.8 and 3.9, however the tracer curves from the 50 cm deep planted and unplanted beds was bimodal with high peak closer to the origin than the 25 cm deep beds followed by a smaller peak not clearly seen but a slight rise as shown in Figure 3.10 and 3.11. This bimodal nature of DTD curves of the 50 cm deep indicates short circuiting or preferential flow path. Thus, in the 50 cm deep wetlands, tracer detention time is lower than the nominal detention time.

The tracer curve exit curves are presented in Table 3.2 for the demonstrated DTD in Figure 3.8-3.11. Since the tracer analysis was conducted more than once for each wetland, the average values of the hydraulic efficiency parameters values with standard deviations is shown in Figure 3.12 and the pair wise comparison in Table 3.3.

Four of the wetlands were working at 5.3 days nominal detention times and the calculated detention time of the wetlands obtained from the tracer curve was 5.1, 4.3, 3.4 and 3.4 days for H25p, H25, H50p and H50 beds, respectively. The tracer mass recovery varied from 47 to 80 %. The average tracer recoveries of H25p were lower than the other three beds. It may have been that in H25p, the two years of operation might have increased the root and rhizome structure in the available 25 cm depth impact the movement of the tracer of the bed.

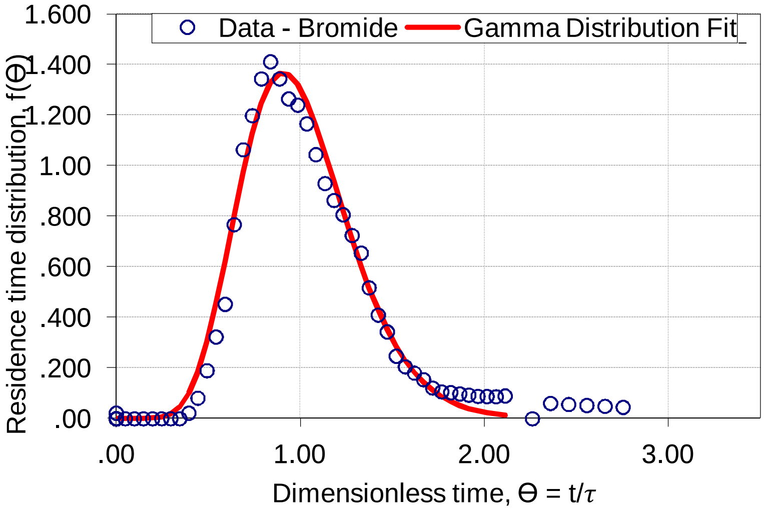


Figure 3.8: Fitting of the gamma distribution model to the experimental bromide tracer concentration (O) on H25p.

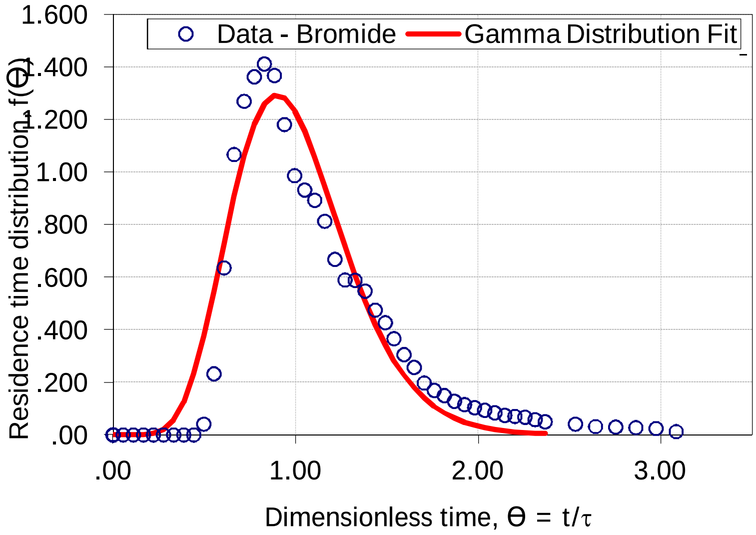


Figure 3.9: Fitting of the gamma distribution model to the experimental bromide tracer concentration (O) on H25.

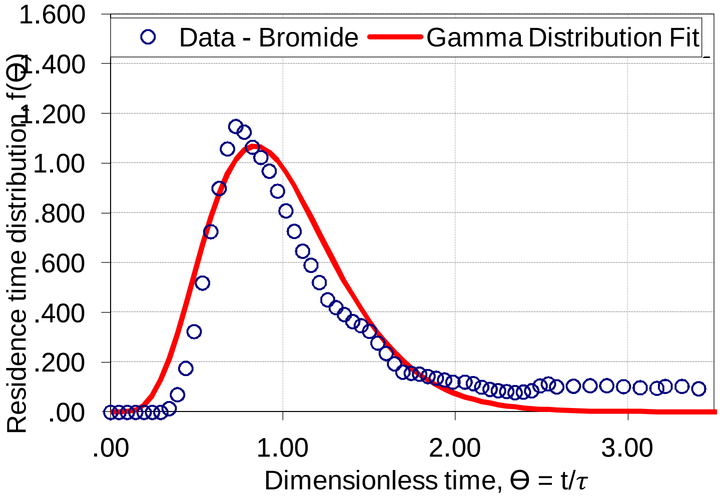


Figure 3.10: Fitting of the gamma distribution model to the experimental bromide tracer concentration (O) on H50p.

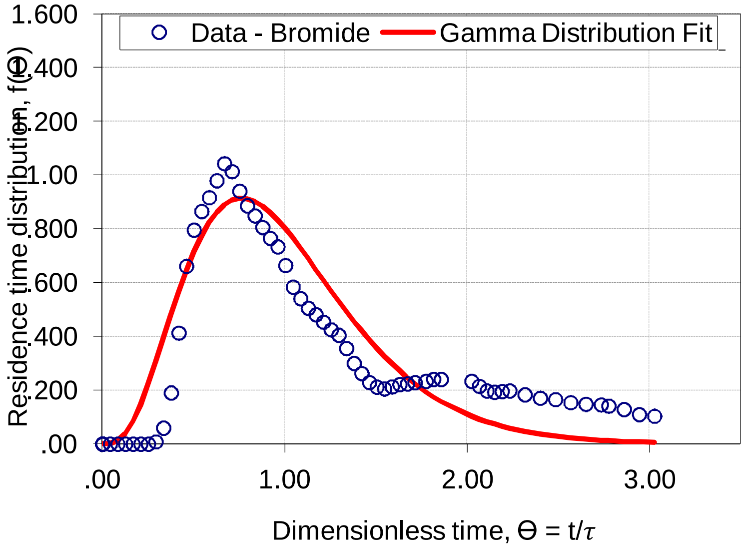


Figure 3.11: Fitting of the gamma distribution model to the experimental bromide tracer concentration (O) on H50.

Table 3.2: Results of hydraulic characteristics for 25 cm and 50 cm deep beds using bromide tracer experiments breakthrough curves. The data were obtained from best fit gamma distribution curve and raw data. $\tau = N\tau_i$; τ_n, = nominal residence time**.**

Parameters	H25p	H25	H50p	H50
Measured time	Apr. 2013		Nov. 2012	
τ_n, d	5.3	5.3	5.3	5.3
τ_i, d	0.5	0.5	0.6	0.9
τ, d	5.1	4.5	3.4	4.0
σ^2 (variance)	0.1	0.1	0.2	0.2
NTIS, N	10.8	9.6	6.2	4.2
Mass recovery, %	69	81	78	74
e_v, %	90	87	72	82
Delay time, d	2.0	2.3	1.2	1.2
Peak time, d	4.3	3.5	2.5	2.7

λ_p is the time recorded from the addition of the tracer to the observation of the maximum concentration of the tracer at the outlet of the wetland. The peak is also called the mode of the concentration distribution (Kadlec 1994). According to Persson et al (1999) λ_p is a hydraulic efficiency, incorporating the effects of mixing scale and short circuiting. The results of the parameter are shown in Figure 3.12(a) and the pair wise comparison in Table 3.3. 25 cm deep beds had shown the maximum peak value with H25p indicating more than the H25. When the statistical results are compared, the 25 cm deep beds were not significantly different at a $p = 0.135$, which implies there was no significance of plant effect on the hydraulic efficiency. This is supported with the results of 50 cm deep beds at a $p = 0.876$ in this case H50 and H50p had almost the same average values as shown in Figure 3.12a. The comparison of the depth effect on the hydraulic efficiency as in Table 3.3 shows that, 25 cm deep and 50 cm deep bed were statistically different whether they are planted or unplanted, which means 25 cm deep beds had shown a good hydraulic efficiency with respect to λp than the 50 cm deep beds.

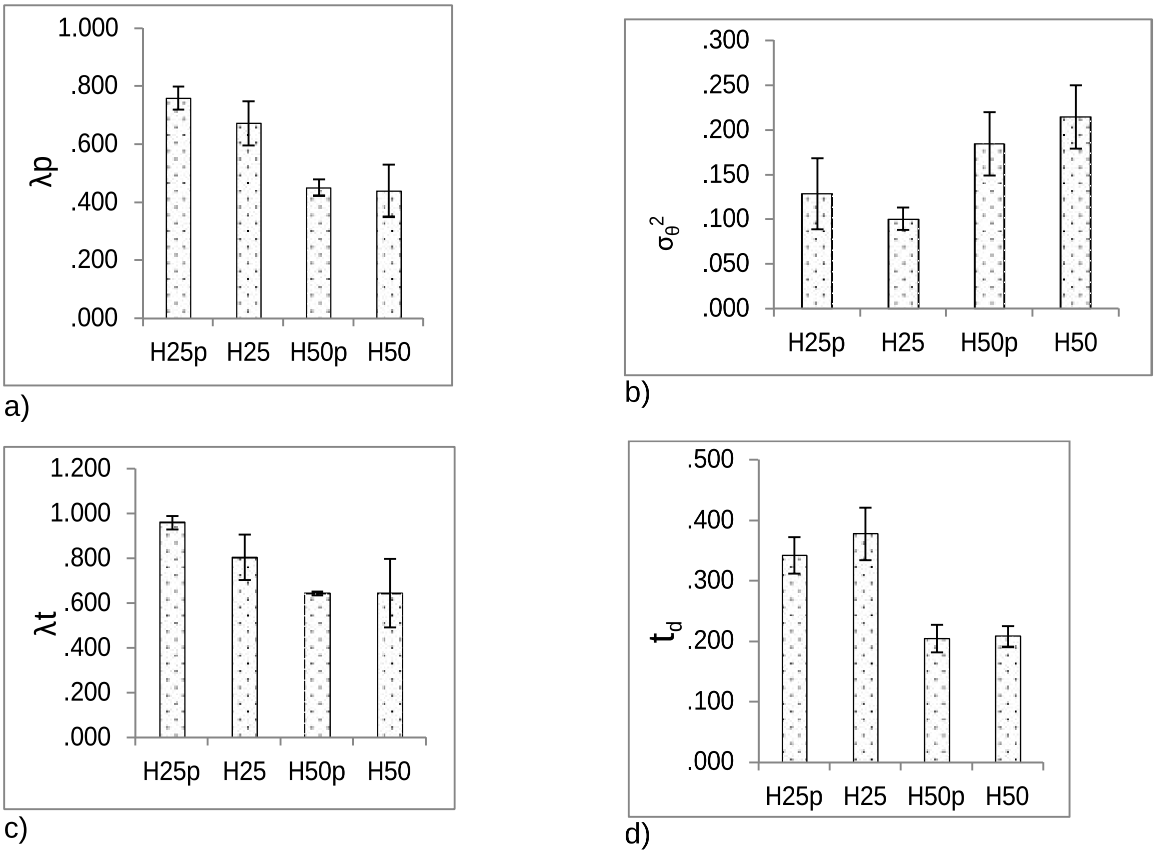


Figure 3.12: Statistics of the detention time distribution characteristics for H25p, H25, H50p and H50 beds. Comparisons were made for the four wetland beds (mean± st.dev.) for a) λp, b) σ_θ^2, c) λ_t and d) t_d.

Table 3.3: P value of the wetlands hydraulic behaviour compared pair wise at a significant value of α=0.05 with LSD.

Comparing wetlands		p value at α=0.05			
		λ_p	$\boldsymbol{\sigma_\theta^2}$	λ_t	t_d
H25p	H25	0.135	0.320	0.269	0.244
H25p	H50p	0.002	0.100	0.065	0.004
H25	H50	0.006	0.010	0.251	0.001
H50	H50p	0.876	0.380	0.924	0.879

The variance of the tracer response curve (DTD) used as a measurement of hydraulic efficiency by describing the scale of mixing in the wetland (Holland et al. 2004). Variance is the dispersing of the tracer pulse after travelling though the wetland (Kadlec 1994). In Figure 3.12 (b), the average $\boldsymbol{\sigma_\theta^2}$ of the 50 cm deep bed wetlands was almost twice as much as the 25 cm deep beds which means, there was relatively more mixing in the 50 cm deep beds than in the H25 beds. Comparison of the values in Table 3.3 indicates that there was no plant effect for

H25/H25p or H50/H50p significantly at p= 0.32 and 0.38, respectively. There was significant effect of depth on the variance for the unplanted beds; however, the H25p and H50p had variance not significantly different at a value of p=0.1. Since the data from the tracer analysis was fitted with tanks in series, that the number of tanks in series (NTIS) is the inverse of variance (Kadlec and Knight 1996, Kadlec and Wallace 2009). The NTIS for 25 cm deep beds had a value of about NTIS =10 and that of the 50 cm deep beds were around 5.

The NTIS which is a shape parameter of DTD shows the same hydraulic behaviour like variance to tell about the characteristics of the wetlands. The NTIS values calculated for the four wetlands is presented in decreasing order of H25p>H25>H50p> H50. 25 cm deep wetlands have higher NTIS values than the 50 cm deep wetlands, which means H25 and H25p were relatively closer to the plug flow (Mena et al. 2011). When NTIS has a value close to 1, it corresponds to a completely mixed system and a value of NTIS close to ∞ is a plug flow extreme. The plug flow is the optimum flow for the treatment processes theoretically and gives a high hydraulic efficiency (Persson et al. 1999). So from the results H25 and H25p had higher NTIS or lower mixing than H50/H50p which implies relatively higher hydraulic efficiency in terms of variance or NTIS.

The detention time found from the tracer studies was less than the nominal detention time. According to the U.S.EPA(2000) the mean actual detention time in many horizontal subsurface flow constructed wetlands has frequently been found to be 40-80 % less than the nominal HRT because of the loss of pore volume, dead volume or preferential flow. Researchers have found significant differences between actual and nominal detention time in HSSF wetland systems and attributed this to dead volume created by the root from plant growth (Breen and Chick 1995, DeShon et al. 1995, Tanner and Sukias 1995, Mandi et al. 1998). Which means not all of the volume of the wetland was involved in the flow path, as was assumed in the nominal detention time calculation (Kadlec and Wallace 2009). Dead zones of near zero flow velocity occurred in the inlet left and right and outlet left and right corners of the rectangular shaped wetland basin (Thackston et al. 1987). This reasoning might be an explanation of dead zones created to result in low residence time.

According to Thackston et al (1987) hydraulic efficiency (λ_t) as the ratio of the measured detention time to the theoretical detention time calculated for the wetland. In the Figure 3.12(c), the average hydraulic efficiency of the 25 cm deep beds were better than the 50 cm deep wetlands and the order was H25p>H25>H50>H50p. Although there was difference between the wetlands for λ_t, the values were not significantly different as shown in Table 3.3. Holland et al (2004) also reported that 16.6 and 39.8 cm deep wetlands had no significant difference for λ_t although the average λ_t of the shallow water level was higher than the deep water level. This indicates that most of the volume in the shallow bed was used as opposed to deeper beds. This behaviour enables shallow beds to use the properties offered by the presence of roots and the air water interface for improved performance.

Delay time is the minimum time that the tracer reaches the effluent. According to Holland et al (2004) the minimum delay time for the tracer to reach the effluent is a characteristic of the wetland DTD which identifies short circuiting. A wetland with small delay time of the length in situations like H25p, H25, H50p and H50 indicates short circuiting. As shown in Figure 3.12(d), the delay time of the 25 cm deep wetland was larger than the 50 cm deep bed wetlands. From Table 3.3 results, there was no effect of plant significantly for H25/H25p or H50/H50p. However there was significant effect of depth for normalized delay time whether they are planted or unplanted. This implies deeper beds display higher levels of encourage short circuiting than the shallow beds.

In summary, the hydraulic efficiency parameters responded uniquely, 25 cm deep bed was better than 50 cm bed with λ_t although there was no significant difference statistically. This result led to significant difference in delay time of the 50 cm deep wetland when compared from the 25 cm deep beds. This gave rise to the high spread of the DTD curve of the 50 cm beds which implies a decrease in hydraulic efficiency compared to 25 cm deep wetlands. This general explanation is given with respect to wetlands having 25 and 50 cm depth, there was effect of depth on hydraulic efficiency. However, in four of the parameters compared there were no significant difference between H25/H25p and between H50/H50p, which implies there was no effect of plant efficiency.

3.2.3 Conclusion

In this study the research data demonstrates that there is effect of depth significantly on the hydrodynamics of 25 cm and 50 cm deep HSSFCW and the hydraulic efficiency of 25 cm deep beds were significantly better than 50 cm deep beds. However, there was no significant effect of plants on the hydraulic efficiency of the wetlands. The tank in series model fits well to the measured data.

3.3 Results and Discussion for treatment performance of the wetlands

Overview

This chapter presented the results of the study as it examines the pollutant removal performance at LRB, Germany for two years. The overall treatment and yearly performances of H25, H25p, H50 and H50p removal is presented in Sections 3.3.1 and 3.3.2, Effect of season with the bimonthly areal and volumetric mass performance of pollutants from Sept 2010 - Aug 2012 are reported in Section 3.3.3. In Section 3.3.4 the bimonthly areal rate coefficient at 20^0C and temperature coefficient were calculated based on the P-k-C* model and the longitudinal variation of performance on the constructed wetlands is reported in Section 3.3.5 and conclusion is provided in Section 3.3.6.

3.3.1 Overall treatment performance (inlet-outlet)

The mean values and standard deviations of pH, conductivity, dissolved oxygen, turbidity and total suspended solids for H25p, H25, H50p and H50 are shown in Table 3.4. Mean values represent period of record (POR), which was 24 months.

The pH values of the effluents of the planted beds remained the same or became slightly acidic when compared with the influent wastewater but the unplanted beds effluents were changed to a relatively more basic condition. This might be explained by the removal of ammonia by the planted beds. Besides, Sulphate reducing bacteria generates alkalinity (bicarbonate) when sulphate reduced organic acids which may raise the pH of the unplanted beds water (Sturman et al. 2008). Unplanted beds may be more anaerobic than planted beds to favour sulphate reducing bacteria.

Dissolved solids, which are quantified as total dissolved solids, contribute to electrical conductivity in the wastewater (Metcalf & Eddy 2003). From Table 3.4, electrical conductivity for H25, H50p and H50 decreased at percentage changes of 5.45%, 0.98% and 7.2% when the effluent value were compared with the influent value for the two years average. However, there was an increase of 7% for H25p.

Table 3.4: Mean values and standard deviations (number of samples in brackets) of the pH, conductivity, dissolved oxygen, turbidity and total suspend solids of H25p, H25, H50p and H50 wetlands collected at regular interval from Sept 2010 - Sept 2012.

Systems	pH		conductivity		Dissolved oxygen		Turbidity		TSS	
	C_i	C_o	µS/cm	µS/cm	C_i (mg/L)	C_o(mg/L)	C_i (NTU)	C_o (NTU)	C_i (mg/L)	C_o(mg/L)
H25p	7.4 ± 0.2 (68)	7.4 ± 0.3 (68)	1537.5 ± 283.5 (68)	1648.4 ± 333.0 (68)	0.3 ± 0.2 (68)	2.7 ± 1.4 (68)	112.8 ± 34.6(54)	124.2 ± 94.0 (54)	161.6 ± 79.8 (68)	6.8 ± 7.0 (68)
H25	7.4 ± 0.2 (68)	7.7 ± 0.3 (68)	1535.9 ± 281.6 (69)	1455.4 ± 214.0 (69)	0.3 ± 0.2 (68)	2.0 ± 1.2 (68)	112.8 ± 34.3 (54)	158.2 ± 88.6 (54)	161.9 ± 80.4 (67)	5.2 ± 2.9(67)
H50p	7.4 ± 0.2 (69)	7.3 ± 0.2 (69)	1530.3 ± 281.2 (71)	1515.5 ± 288.6 (71)	0.3 ± 0.2 (70)	1.3 ± 0.8 (70)	112.4 ± 33.8 (56)	162.6 ±115.7 (56)	162.0 ± 79.3 (69)	6.4 ±3.5 (69)
H50	7.4 ± 0.2 (68)	7.5±0.2 (68)	1531.5 ± 285.2 (69)	1429.2 ± 192.6 (69)	0.3 ± 0.2 (68)	1.3 ± 0.9 (68)	112.4 ± 34.1 (56)	178.9 ±105.8 (56)	157.4 ± 72.3 (67)	8.0 ± 9.2 (67)

The increase of conductivity of H25p was due to an increase in concentration of the effluent ion by ET. In the values of the effluent conductivity, planted beds have higher values than the unplanted beds and the H25p had the highest increase of conductivity which might indicate that the H25p may be more liable to ET than the H50p as the plant roots are fully immersed in the entire water zone. The EC and salinity reduction of constructed wetlands can be attributed to a decrease in ions such as ammonium and phosphate in the CWs as reported by Schaafsma et al. (2000).

As shown in Table 3.4, the effluent value of the dissolved oxygen concentration was 2.7, 2.0, 1.3, and 1.3 mg/L for the H25p, H25 and H50p, H50, respectively. Some general feature of the average dissolved oxygen in the beds from inlet to outlet shows that H25 and H25p have higher values than H50 and H50p while H25p had more oxygenated effluent than H25. Planted shallow beds have also shown similar trends in that shallow beds were more oxygenated than deeper beds (Garcia et al. 2004).

The average results of oxidation reduction potential in Fig 3.13 follow the same pattern as the dissolved oxygen in terms of the wetlands, the H25p had shown more positive and variable ORP followed by the H25. Both the H50 and the H50p had similar values.

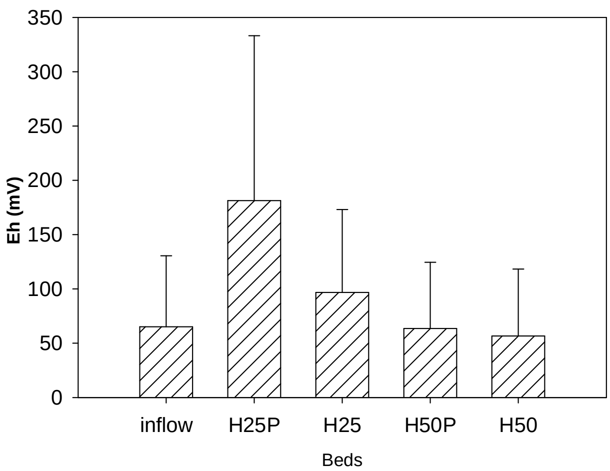


Figure 3.13: Average Eh and standard deviation of inflow, H25p, H25, H50 and H50p beds between Sept 2010-2012. E_h=E_{meas}. +211 mV at 20^0C.

As shown in Table 3.4, the outlet turbidity of the wetlands was more than the inlet turbidity in all beds except very small average positive values for H25p. The reason behind the increase of turbidity in the effluent was the formation of white cloudiness a short time after sampling. The clear effluent sample appeared to have changed into a white cloudy substance but this was not seen in the raw wastewater. Although it was not studied in this work; it may be because of hydrogen sulphide oxidation back to sulphate when the sample from the bed was exposed to the environment. This phenomenon has been observed in other HSSF wetland, treating domestic wastewater (Kadlec and Wallace 2009).

From the Table 3.4, it can be seen that, the percentage removal of TSS was almost the same for the H25p, H25, H50p and H50 beds but the removal of the H50 bed was a bit lower than the others. The reason for almost similar performance in all the beds is that the total suspended solid removal mechanism was physical in nature.

The concentration of percentage removal did not show the real performance of the wetlands because the water loss impact on the TSS mass removal based on area and volume of wetland was compared. The TSS areal mass removal rates for four beds are presented in Figure 3.14. From the results, the 50 cm deep beds had a mass removal twice as much of the 25 cm deep beds irrespective of whether the bed was planted or unplanted and the significance is shown in Figure 3.14.

From Figure 3.14, it can be concluded that the plants had no significant effect in areal mass removal of TSS; however, there was effect of depth at the same residence time. When the volumetric mass removal per day is compared statistically between H25p, H25, H50p and H50, they were not statistically different at $\alpha=0.05$ from each other, either with the presence of plant or depth.

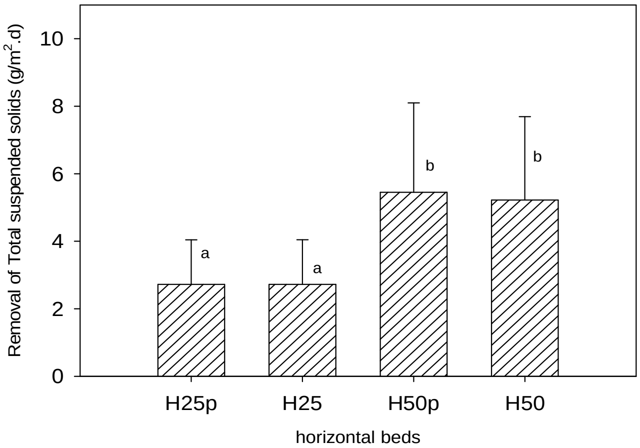


Figure 3.14: 2010-2012 average Total suspended solid (TSS) mass removal in g/(m^2d) for H25p, H25, H50p and H50, and statistical test of analysis of the significance if the wetlands TSS removal between each other. The same alphabetical code means no significant difference; different alphabetical code means there is significance difference at α=0.05.

Table 3.5 and 3.6 show the overall treatment performance based on inlet and outlet data for 25 and 50 cm deep planted and unplanted beds for total organic carbon (TOC), biochemical oxygen demand ($CBOD_5$), total nitrogen (TN), ammonia, nitrate and *E.coli*.

The $CBOD_5$ concentration removal efficiency of the 25 cm deep beds was higher than the 50 cm deep beds and again the percent concentration removal of the H25p was better than the H25. Although the difference looks small, it was an average of two years data. The $CBOD_5$ removal efficiency of the H50p was the same as H50 which might be explained by the fact that there may be no impact from the plants. Plants do not improve the BOD_5 removal (Burgoon et al. 1995). On the contrary, the areal mass removal rate of the H50p and H50 were better than H25p and H25 because the deeper beds had twice the loading as the shallow beds.

Table 3.5: Mean values and standard deviations (number of samples in brackets) of the $CBOD_5$, TOC and TN of H25p, H25, H50p and H50 wetlands collected from Sept 2010 to Sept 2012. Inlet concentration was different for some parameters because the numbers of samples were different.

Systems	Area (m^2)	Effective depth (m)	$CBOD_5$		TOC		TN	
			C_i (mg/L)	C_o (mg/L)	C_i (mg/L)	C_o (mg/L)	C_i (mg/L)	C_o (mg/L)
H25p	5.64	0.25	264.6 ± 93.7 (66)	47.5 ± 24.2 (66)	160.1 ± 49.5 (66)	34.8 ± 10.1 (66)	76.9 ± 16.5 (68)	55.4 ± 12.9 (68)
H25	5.64	0.25	269.6 ± 99.3 (67)	55.5 ± 27.5 (67)	162.4 ± 53.7 (66)	33.9 ± 10.9 (66)	77.4 ± 17.1 (68)	62.1 ± 12.8 (68)
H50p	5.64	0.50	267.3 ± 97.6 (65)	63.6 ± 22.9 (65)	162.1 ± 53.0 (68)	37.2 ± 11.1 (68)	77.3 ± 16.9 (70)	61.3 ± 12.2 (70)
H50	5.64	0.50	269.1 ± 98.2 (63)	63.5 ± 26.9 (63)	157.0 ± 47.0 (64)	35.5 ± 11.6 (64)	76.8 ± 16.9 (67)	63.1 ± 12.0 (67)

Table 3.6: Mean values and standard deviations (number of samples in brackets) of *E. coli,* ammonium and nitrate of H25p, H25, H50p and H50 wetlands collected from Sept 2010 - Sept 2012. Inflow numbers of samples were different because the measured the numbers of samples were different for operation and maintenance reasons.

systems	NH_4^+-N		NO_3^-- N		*E. coli*	
	C_i (mg/L)	C_o (mg/L)	C_i (mg/L)	C_o (mg/L)	C_i (MPN/ 100 ml)	C_o (MPN/ 100 ml)
H25p	63.5 ± 18.9 (65)	58.1 ± 20.2 (65)	0.28 ± 0.30 (66)	0.25 ± 0.27 (66)	7392238 ± 4418249 (65)	149621 ± 188029 (65)
H25		67.2 ± 22.5 (65)	0.30 ± 0.30 (65)	0.20 ± 0.21 (65)		214944 ± 197552 (65)
H50p	63.2 ± 18.9 (66)	62.8 ± 18.7 (66)	0.30 ± 0.30 (67)	0.20 ± 0.19 (67)		302568 ± 203018 (65)
H50		66.8 ±2 0.7 (66)	0.27 ± 0.30 (66)	0.25 ± 0.23 (66)		393600 ± 233270 (65)

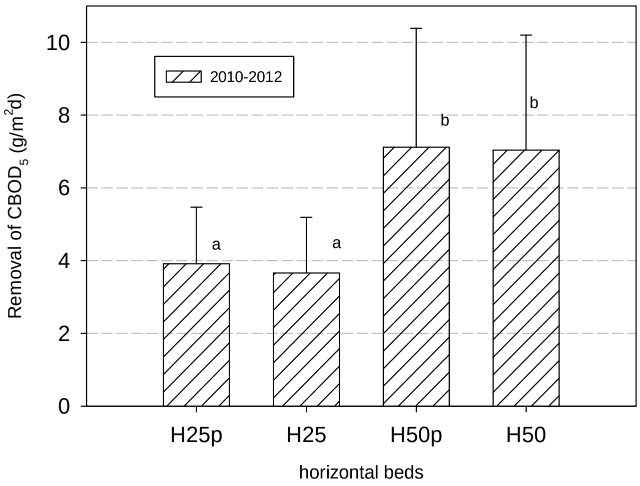


Figure 3.15: 2010-2012 average $CBOD_5$ mass removal in $g/(m^2d)$ for H25p, H25, H50p and H50, and the analysis of the significance if the wetlands $CBOD_5$ removal between each other. Same alphabetical code means no significant difference at α=0.05; different alphabetical code means there is significance difference at α=0.05

From Figure 3.15 on areal mass removal of the wetlands, it may be generalized that plants had no significant effect in removal of $CBOD_5$; however depth had an effect in removal whether it was planted or unplanted as was observed in TSS. However, when the volumetric mass removal per day is compared statistically between H25p, H25, H50p and H50, they were not statistically different at α=0.05 from each other, either with the presence of plant or depth

Although small based on the Table 3.5, the TOC percentage removal was highest for 25 cm deep beds when compared with the 50 cm deep beds. This trend was the same for $CBOD_5$.removal where shallow beds had lower effluent concentration than the deeper beds. The concentration removal performances of the unplanted beds (H25 and H50) were better than the planted (H25p and H50p) when compared for the same depth. A similar observation was made for the $CBOD_5$, analysis where the areal removal rate of TOC was highest for the 50 cm deep beds. In each group planted beds performed better than the unplanted in mass removal per unit area although not significant. This result was supported with statistical analysis of the TOC mass removal rate of all beds was compared and provided in Figure 3.16.

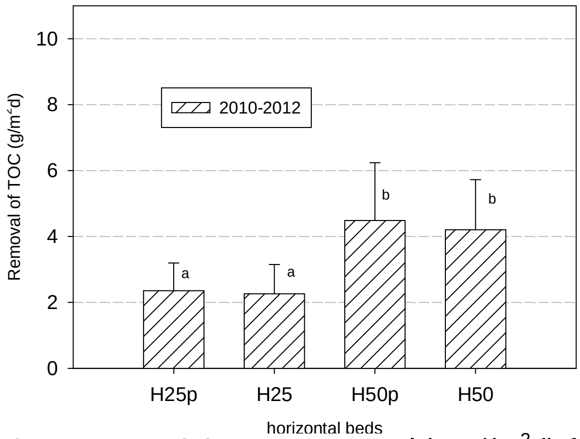


Figure 3.16: 2010-2012 average TOC mass removal in g/(m^2d) for H25p, H25, H50p and H50, and the analysis of the significance if the wetlands TOC removal differs between each other. The same alphabetical code means no significant difference at α=0.05; different alphabetical code means there is significance difference at α=0.05.

From the analysis, it is generalized that plants had no significant effect in removal of TOC; however depth has an effect in removal whether it was planted or unplanted. However, the volumetric mass removal per day is compared statistically between H25p, H25, H50p and H50, they were not statistically different at α=0.05 from each other, either with the presence of plant or depth

In Table 3.5, the TN percentage concentration removal was 28, 20, 21 and 19 for H25p, H25, H50 and H50p, respectively. From these values, it was observed that plants have shown a better concentration percentage removal advantage over the unplanted beds in 25 and 50 cm deep beds, this may be ascribed to the assimilation of nitrogen by the plants.

The comparison mass removal per unit area of TN results is shown in Figure 3.17.

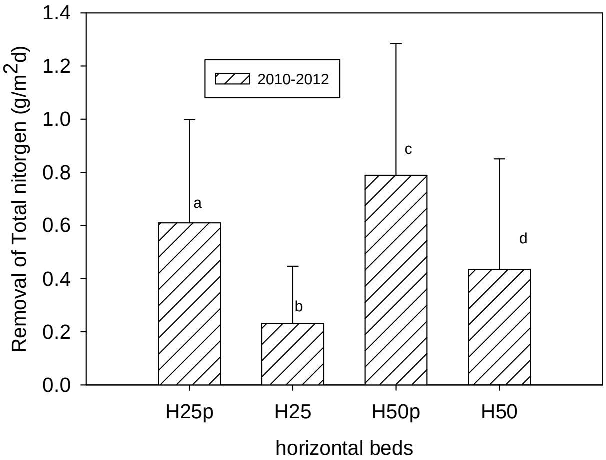


Figure 3.17: 2010-2012 average TN mass removal in g/(m^2d) for H25p, H25, H50p and H50, and the significance if the wetlands TN removal differ each other. Same alphabetical code means no significant difference at α=0.05; different alphabetical code means there is significance difference at α=0.05

Presence of plants and depth had a significant effect on the performance removal of TN in terms of the areal removal rate at α=0.05.

When the volumetric mass removal per day of TN is compared between H25p, H25, H50p and H50, they were statistically different at α=0.05 from each other, except that H25 and H50 were not different statistically showing the role of plants.

The overall ammonia nitrogen percentage removal for the two year average for the four beds was different from other parameters discussed, refer Table 3.6. H25 and H50 beds showed a net production of ammonia whereas the planted beds showed positive percentage removal and the H25p performed better than the H50p with a value of 8%. Garfi et al. (2012) reported there was no net removal of ammonium in Continental Mediterranean weather and negative mass removals are sometimes recorded. The negative removal value of ammonium for the unplanted beds and the lower overall removal of the planted beds could possibly be attributed to the decomposition of organic nitrogen in to ammonium entrapped in the wetland as particulate matter and the anaerobic condition did not allow nitrification (U.S.EPA 1993). Generally the inflow concentration was lower than the outflow concentration for unplanted beds. The areal mass removal rate was highest for H25p bed. The statistical analysis for the significance for the results is shown in Figure 3.18.

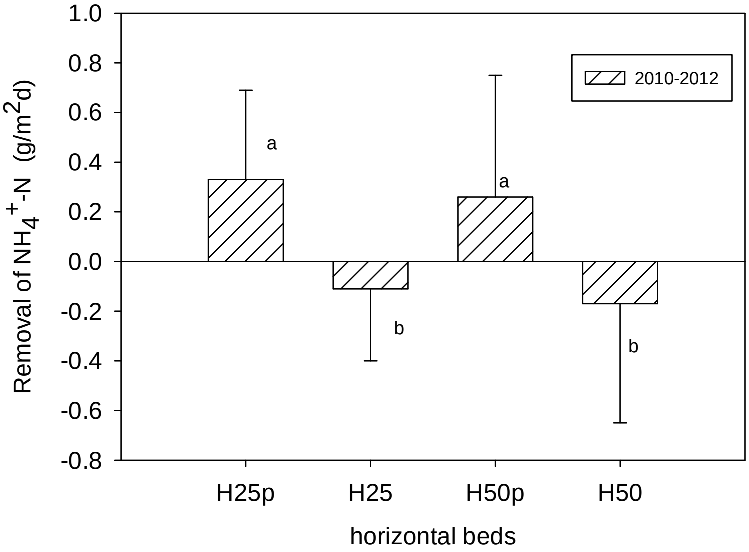


Figure 3.18: 2010-2012 average ammonium nitrogen mass removal in g/(m^2d) for H25p, H25, H50p and H50, and analysis of the significance if the wetlands ammonium nitrogen removal differ each other. Same alphabetical code means no significant difference at α=0.05; different alphabetical code means there is significance difference at α=0.05

From Figure 3.18, it is generalized that depth had no significant effect on mass removal of ammonium nitrogen but plant presence had an effect. So based on the analysis of H25p versus H50p and H25 versus H50 had statistically not different in terms of areal mass removal rate at α=0.05. The volumetric mass removal rate of the wetlands with respect to depth and plants were statistically different at α=0.05 except that H25 and H50 were not different statistically.

The nitrate nitrogen areal mass removal values were very small in magnitude and did not show a significant pattern in performance, the values are very small to discuss. The areal mass removal in decreasing order was H50p>H25>H25p>H50 from better to poor.

From *E. coli* analysis results shown in Table 3.6, the percent removal of H50 was lower than the rest of the beds. Planted beds performed better than unplanted beds.

The MPN areal *E.coli* removal rate of the 50 cm deep beds were significantly greater than that of 25 cm deep beds at α=0.05 but there were no significant difference in MPN removal per area between planted and unplanted beds (H25 and H25p or H50 and H50p). The geometric mean of the MPN removal per unit square meter per day is shown in Figure 3.19, for 25 and 50 cm deep wetlands. The deeper bed had shown higher removal rates because of the load. See Figure 3.19. Garcia et al. (2003) and Tanner et al. (1998) reported bacterial removal (Total coliform) depends on the residence time in the wetland. Although the wetlands had the same residence time, the performance of the higher loaded wetland showed higher areal removal rate.

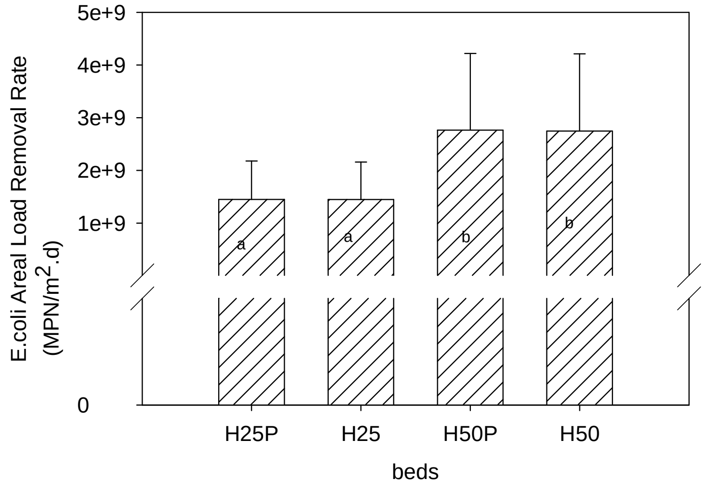


Figure 3.19: Geometric mean *E. coli* areal load removal rates (MPN/m^2.d) for the 25 and 50 cm deep planted and unplanted beds for September 2010 – 2012.

An important parameter is volumetric mass removal rate based on the volume of water treated in the wetland and there was no significant effect of depth and plants on the removal of *E.coli* at $\alpha = 0.05$.

3.3.2 Comparison of performance of wetlands in the first and second year

Overview

The purpose of this section was to compare the performance of the 25 cm and 50 cm deep planted and unplanted beds for $CBOD_5$, TOC and TN parameters using bar plots. The comparison was made between data collected during 2010 - 2011 and during the 2011-2012.

$CBOD_5$ and TOC

Figures 3.20 and 3.21 show the $CBOD_5$ and TOC areal mass removal rates for the first two years of operation for the H25p, H25, H50p and H50 beds. From the figures, the mass removal of the second year was better than the first year. This might be due to the maturation of the bed and full development of the wetland plants.

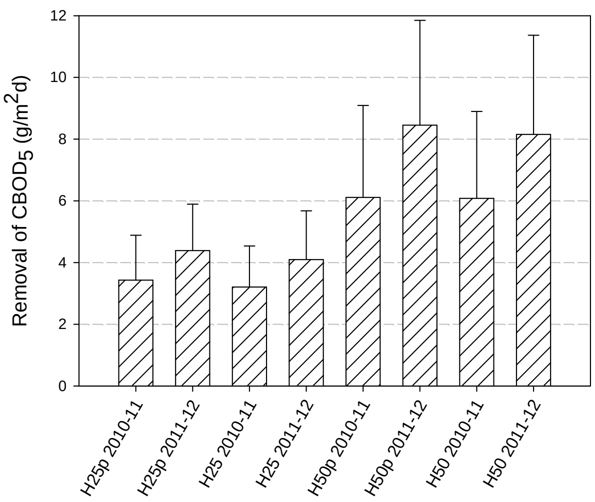


Figure 3.20: Removal of total organic carbon over the first and second year of operation of the wetlands

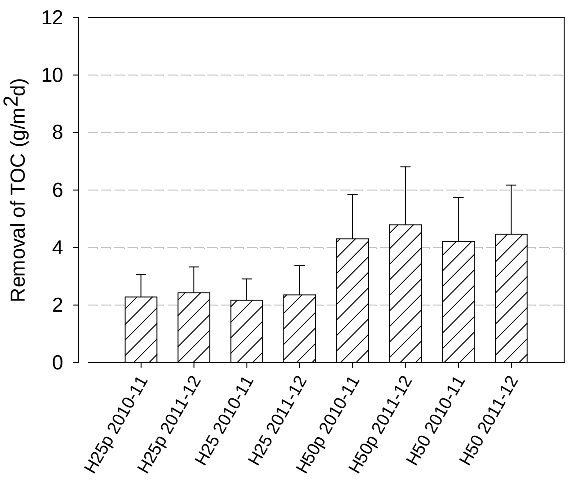


Figure 3.21: Removal of total organic carbon over the first and second year of operation of the wetlands

Total Nitrogen (TN)

Total nitrogen areal mass removal of the wetlands for 2010-2011 and 2011-2012 is shown in Figure 3.22. The figure indicates that the areal mass removal rate of the total nitrogen for 2011-2012 was lower than the 2010-2011 period as opposed to

TOC and $CBOD_5$. Nitrogen removal was highest in the first year this can be attributed to the enhanced plant uptake of nitrogen at the beginning of the growing season where they had root systems and less rhizomes as compared to the second year. The box and whisker plot of TN is shown in Figure 1, Appendix section.

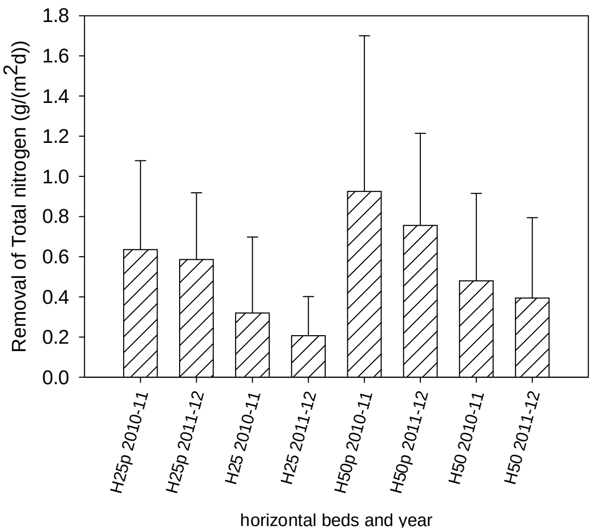


Figure 3.22: Total nitrogen mass removal rates comparing the 2010-2011 and 2011-2012 data.

Due to seasonal plant die off the first year plants could have returned nitrogen to the wastewater which might have resulted in reducing the performance of the wetland in the second year. It is important to note that the unplanted beds were showing the same trend, which means the mechanisms of nitrogen removal may not only be plant uptake but also denitrification, adsorption and volatilization.

In summary, $CBOD_5$, TOC and TN were discussed with regard to their 1st and 2nd year mass removal rate and it was found that the organic carbon removal for the 2nd year was better than the first year; however, the first year TN mass removal rate was better than the second year.

3.3.3 Effect of season on wetlands on bimonthly mass removal rate

Overview

Horizontal subsurface flow constructed wetlands have a seasonal performance change in effluent quality on a bimonthly basis. According to Kadlec and Wallace (2009) the seasonal changes are driven by plant biomass cycling, climate and water.

In this section, the seasonal trend of the H25p, H25, H50p and H50 wetlands with respect to areal and volumetric mass removal of pollutants are compared on bimonthly average basis. The analysis were conducted for months of September-October, November-December, January-February, March-April, May-June and July-August for the data collected from September 2010 to August 2012. The water quality parameters compared were TSS, $CBOD_5$, TOC, TN, NH_4-N, and *E. coli*.

3.3.3.1 Bimonthly Total Suspended Solids

In Figure 3.23 (a & b) the areal mass removal of the H25p, H25, H50p and H50 of TSS is shown. Generally, the H50 and H50p beds had areal mass removal rate that were almost twice as high as the H25 and H25p beds. The seasonal pattern followed the temperature of all the beds as seen in the Figure 3.23 (a & b).

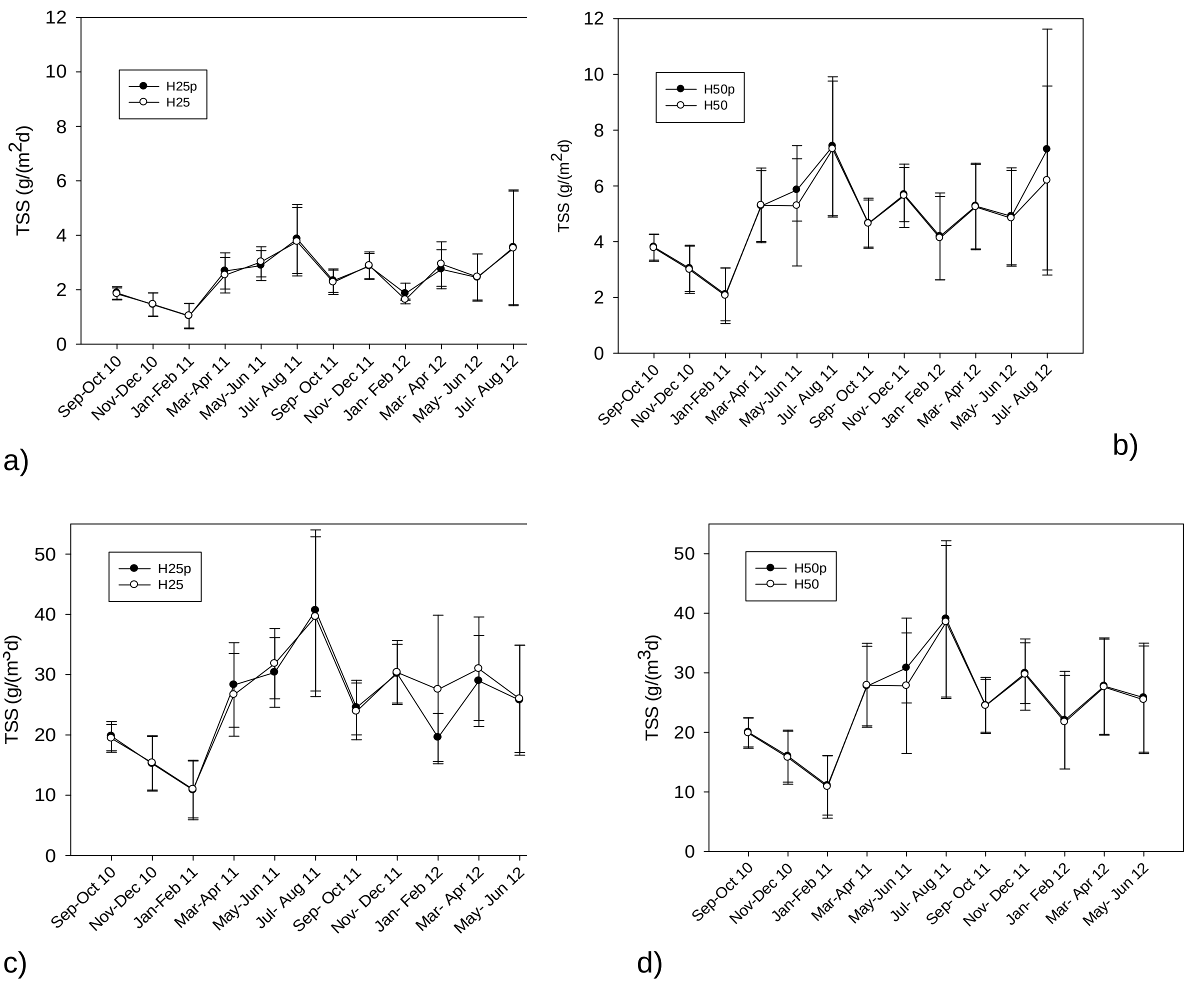


Figure 3.23: Bimonthly average and deviations of TSS mass removal (g/(m^2.d)) for a and b and mass removal (g/(m^3.d)) for c and d for beds H25p, H25, H50p and H50 from Sept 2010 to August 2012.

There was no significant effect of the plants but depth had an effect on performance. Kadlec and Wallace (2009) reported that TSS follows a gentle sinusoidal annual trend (seasonal trend) of with maximum seen in spring and summer but their explanation is based on the outlet concentration not mass removal. Since mass removal directly correlated with areal mass removal, the sinusoidal explanation works for the observed data.

When the mass removal rate per unit volume of the H25p, H25, H50p and H50 were compared as shown in Figure 3.23 (c & d) almost all the wetlands have the same mass removal pattern per unit volume.

3.3.3.2 Bimonthly Carbonaceous Biochemical Oxygen Demand

In Figure 3.24 (a & b), the average $CBOD_5$ areal mass removal of 25 and 50 cm deep planted and unplanted wetlands are presented over the two years.

The removal efficiency of all the wetlands was highest during summer (July and August) lowest in winter (January and February). These results were expected because of the temperature prevailing in the months were the main factors for the observed mass removal. Biological and chemical reactions almost doubled when temperature increased by 10^0C. However, the removal difference in a wetland over the two years was not exaggerated with the following reasons. In subsurface flow wetland particulate organic matter was trapped through the processes of sedimentation, filtration and interception. During summer, the degradation of organic matter would not be much faster based on the influent loading, this was because winter accumulated organic matter is degraded in summer and affect the performance of the summer season (Wallace and Knight 2006).

Both H25 and H25p beds had lower areal removal efficiency than the H50 and H50p. However, the effect of plants was seen at each depth, the planted systems had slightly higher mass removal than the unplanted beds although there were a few cases H50 performing higher than H50p.

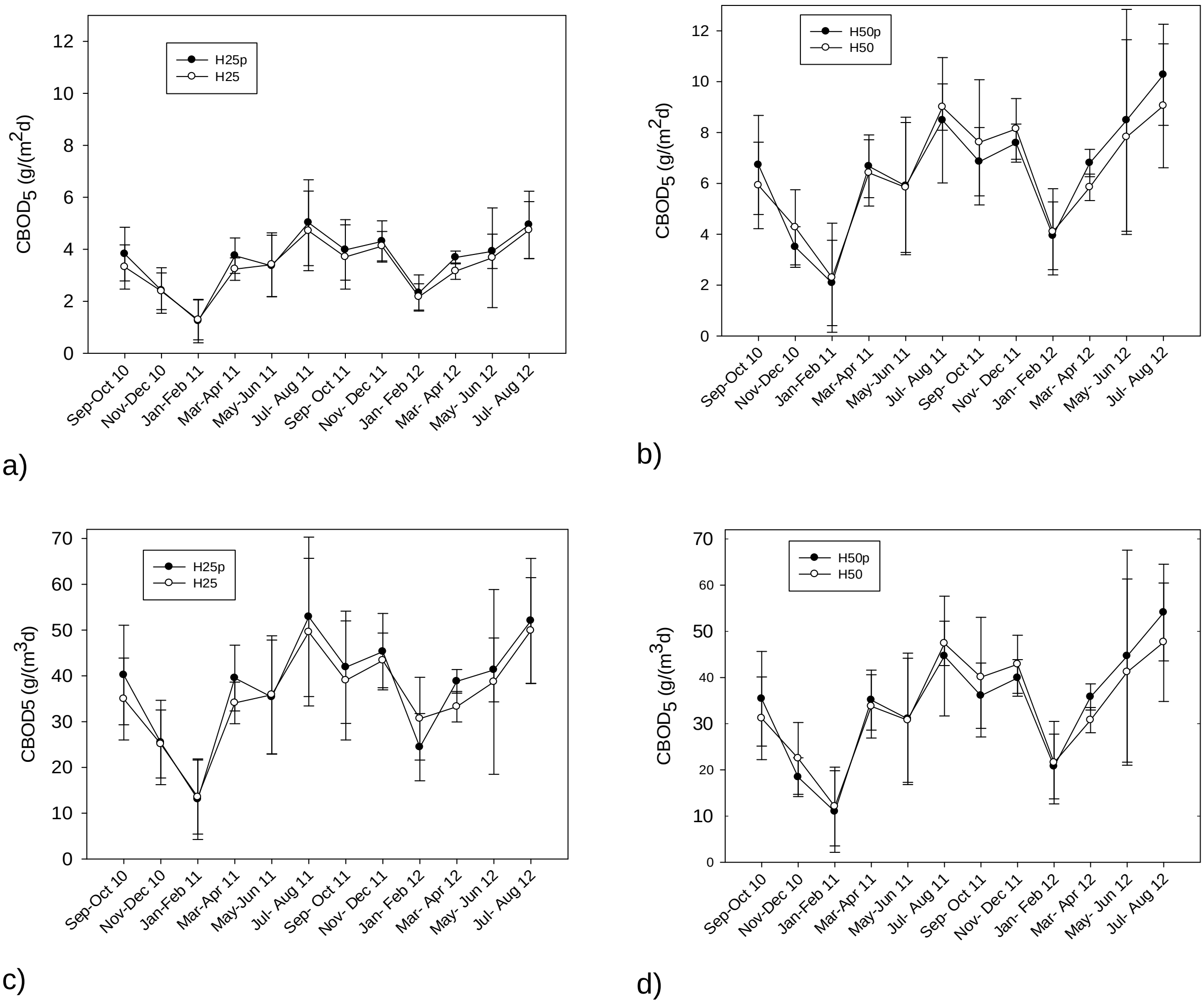
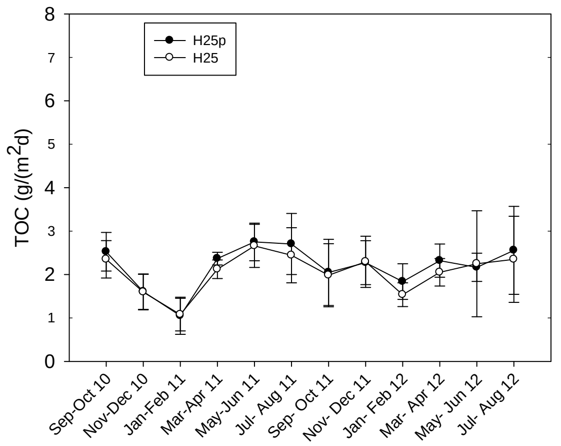


Figure 3.24: Bimonthly average and deviations of carbonaceous biochemical oxygen demand mass removal (g/(m^2.d)) for a and b and mass removal (g/(m^3.d)) for c and d for beds H25p, H25, H50p and H50 from Sept 2010 to August 2012.

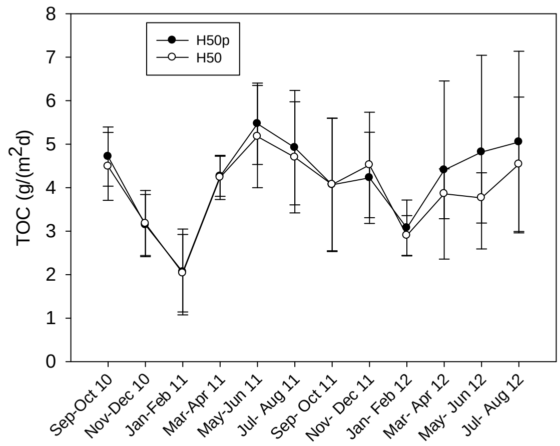
On the contrary to the areal mass removal, in Figure 3.24 (c & d), the volumetric mass removal of $CBOD_5$ showed a pattern although the H25p bed showed better removal. Generally, the performances were close to each other. Treatment wetlands have seasonally variable changes in $CBOD_5$ effluent quality which are directed by climate, plant biomass cycling and water temperatures (Kadlec and Wallace 2009). So those findings are in agreement with this general explanation. The sinusoidal seasonal trends are gentle annual cycle with minimum in winter (Kadlec and Wallace 2009).

3.3.3.3 Bimonthly Total Organic Carbon

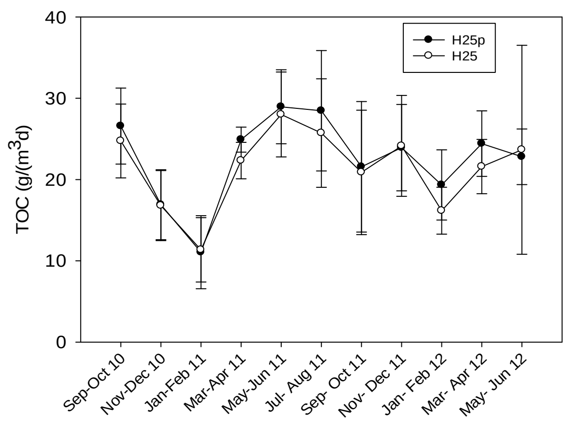
Figure 3.25 (a & b) shows the seasonal trend of the areal mass removal for TOC. From the graph it is seen that the warmer months July - August had higher average mass removal than the colder seasons and low mass removal was observed in the months Jan- Feb 2011 and 2012. H50 and H50p had higher removal rates than the H25 and H25p which means deeper beds had an advantage over shallow beds in



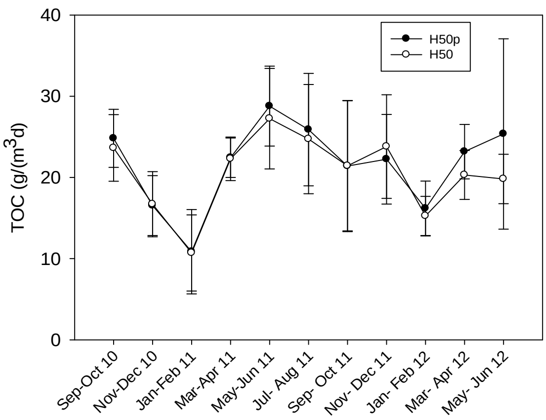
a)



b)



c)



d)

Figure 3.25: Bimonthly average and deviations of total organic carbon mass removal ($g/(m^2.d)$) for a and b and mass removal ($g/(m^3.d)$) for c and d for beds H25p, H25, H50p and H50 from Sept 2010 to August 2012.

removing TOC on an areal basis. In each case, planted beds had slightly better performance than unplanted in almost all months of analysis. In each case the colder seasons had an impact on performance. The higher areal removal rate of the 50 cm deep beds was because of the loading. Removal rate coefficients depend strongly on loading rates (Kantawanichkul et al. 2009)

Figure 3.25 (c & d) showed the volumetric mass removal rate of H25p, H25, H50p and H50 for total organic carbon in the two years bimonthly average. The pattern of the removal was the same as areal mass removal however H25p performed better than others. Generally there were lower removal efficiencies of TOC at low temperature. According to Akratos and Tsihrintzis (2007) the low efficiency of $CBOD_5$ and TOC removal at low temperature was not significant because the removal of organic matter is a result of aerobic and anaerobic processes which are working even at water temperatures of 5^0C.

3.3.3.4 Bimonthly Total Nitrogen

In Figures 3.26 (a to d), the areal and volumetric mass removal of H25p, H25, H50p and H50 are shown. All TN mass removals are positive (as opposed to ammonium nitrogen). The overall pattern of the graph has the same peak and depression points like organic carbon (TOC and $CBOD_5$) following the temperature although temperature is not a good surrogate of season. The areal mass removal of the planted beds was better than the unplanted beds. The areal mass removal of H50p was better than all the other beds in the monitoring time except at two depression points in January-February 2011 and November 2011 to February 2012 which was relatively colder.

H25p showed better performance than both the H50 and the H25 beds which means there was greater effect of plants on TN removal. H50p was also performed better than H50 which indicates the effect of plants on performance especially in warmer months. Nitrogen in HSSFCW are affected by temperature during all seasons and by additional seasonal factors like plant root oxygen release in the growing season (Garcia et al. 2010).

The performance of the H50 beds was better than the H25 beds and the performance of the H50p beds were better than the H25p bed especially in warmer months which indicate there was impact of depth on performance on TN.

As in Figure 3.26 (c & d), the volumetric mass removal of H25p in all the seasons was higher than the other beds. This might be ascribed to the high volumetric efficiency of the shallow beds along with the presence of the plants. The volumetric mass removal of the shallow planted bed may be favoured by the high oxidation reduction potential which might improve nitrogen removal.

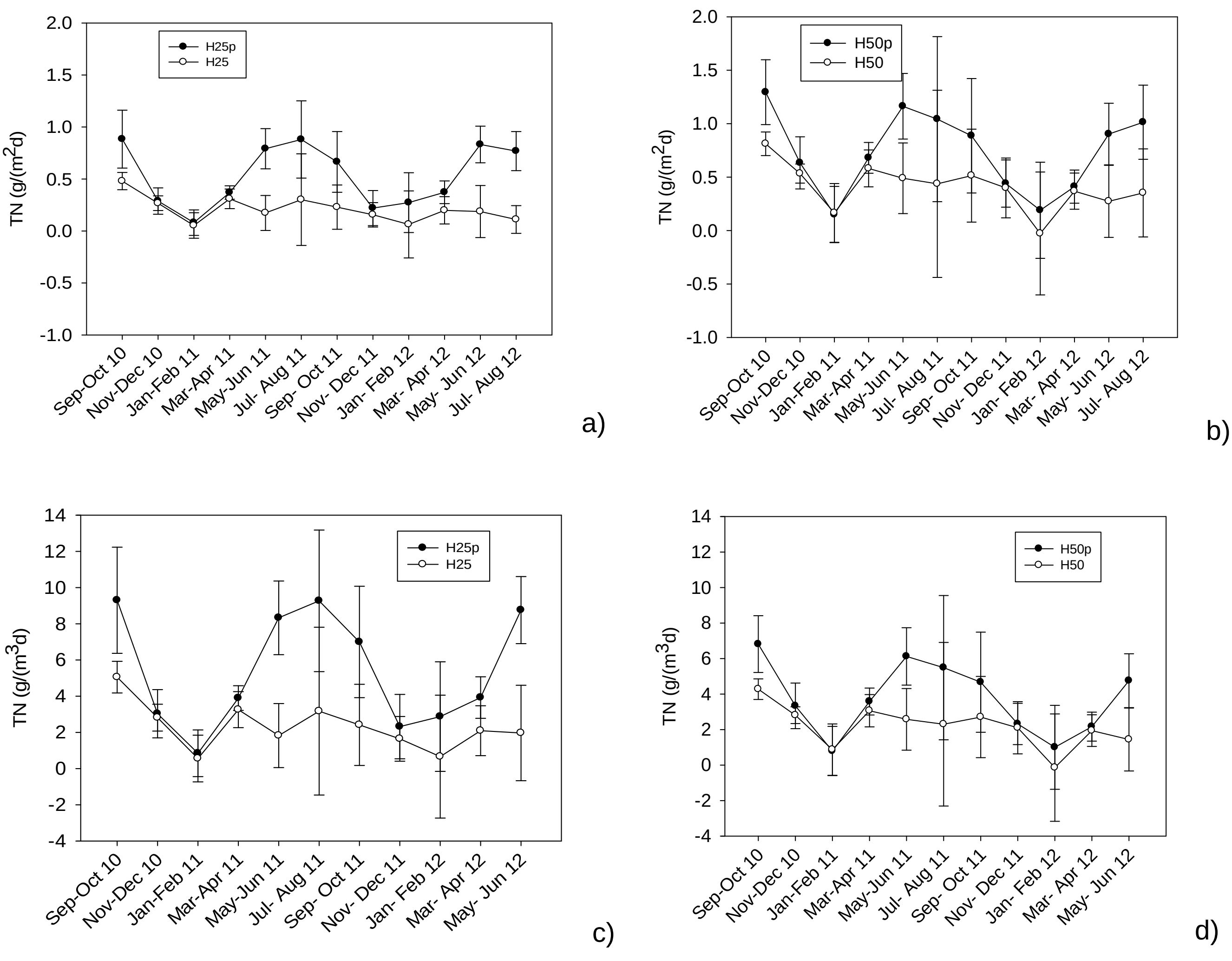


Figure 3.26 : Bimonthly average and deviations of total nitrogen mass removal (g/(m^2.d)) for a and b and mass removal (g/(m^3.d)) for c and d for beds H25p, H25, H50p and H50 from Sept 2010 to August 2012.

3.3.3.5. Bimonthly Ammonium nitrogen

The pattern of areal and volumetric mass removal of ammonia of the H25p, H25, H50p and H50 beds over the two years is presented in Figure 3.27 (a - d). The areal and volumetric mass removal rates of H25p were lower than the H50p between September - December 2010 but this was reversed to the opposite from May-September 2011 and from February to August 2012. This implies that mass removal of ammonium was dependent on depth and availability of plants. In both areal and volumetric mass removal, H25p had negative values in January-February 2011 and November-December 2011 whereas H50p displayed ammonium production in January-February 2011 and from November 2011 to April 2012.

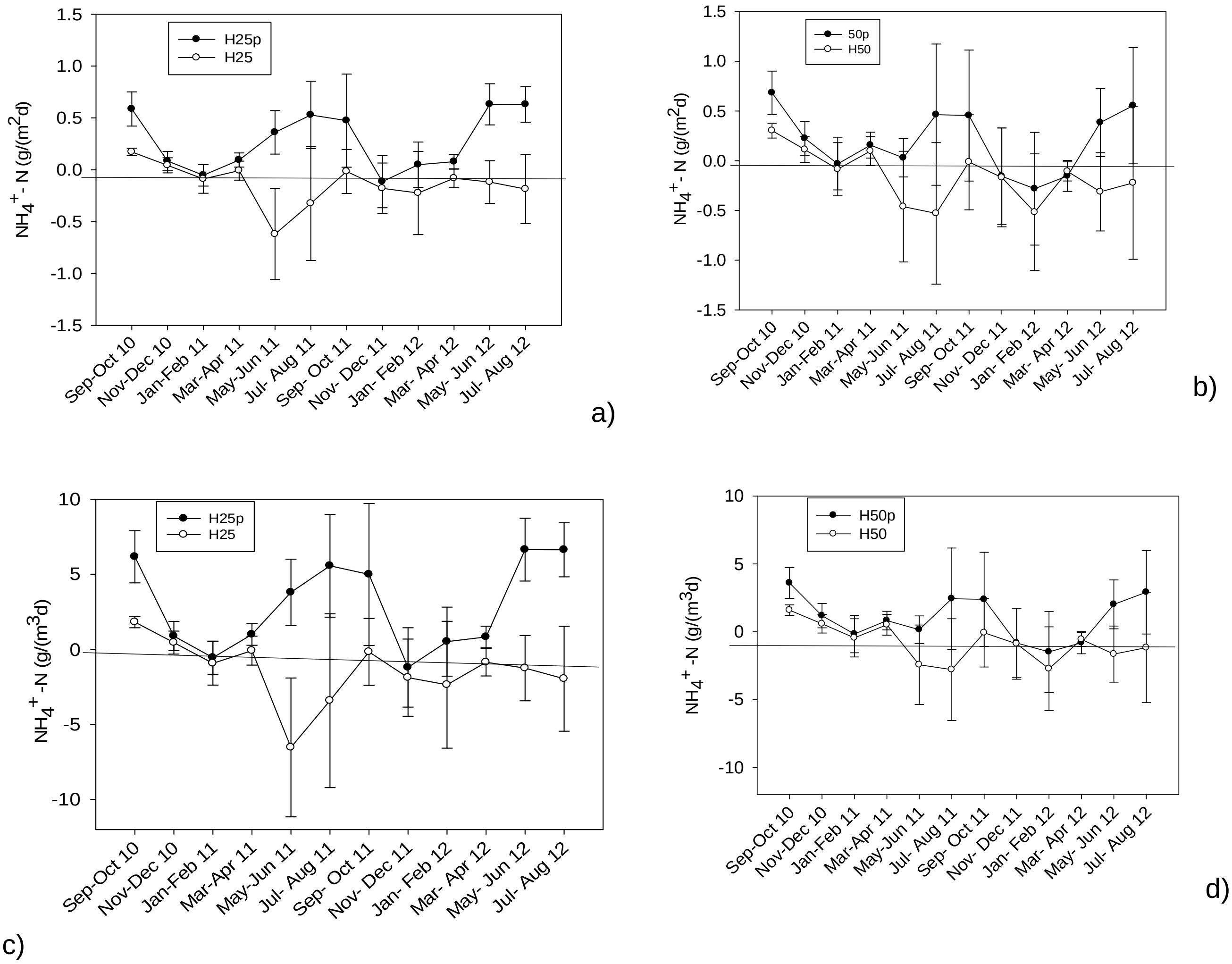


Figure 3.27: Bimonthly average and deviations of ammonium nitrogen mass removal (g/(m^2.d)) for a and b and mass removal (g/(m^3.d)) for c and d for beds H25p, H25, H50p and H50 from Sept 2010 to August 2012.

This more production of ammonium in warm months may be related to the decomposition of stored nitrogen containing organic matter. Akratos and Tsihrintzis

(2007) gave the explanation that the production of ammonium or a higher concentration of ammonia in the effluent than influent at low temperatures for the planted beds was because of the production of ammonium and insufficient nitrification of ammonia. Garfi et al. (2012) reported no net removal and sometimes mass loading of the effluent more than the influent or negative values. For total nitrogen and ammonia from Figure 3.26-3.27, the dependence on temperature is clearly seen because plant uptake and the bacteria responsible for nitrogen removal are temperature dependent. The microorganisms responsible for nitrogen removal functions optimally above 15^0C (Kuschk et al. 2003) and the role of plants on nitrogen removal supported by different authors (Newman et al. 2000, Yang et al. 2001, Vymazal 2002, Kuschk et al. 2003, Jing and Lin 2004) depends on temperature.

From Figure 3.27, the ammonia mass removal for H25 and H50 was negative therefore it is production from January-February 2011 until the end of measurement, August 2012. The production of ammonium increased in warmer months (from May to August 2011) in unplanted beds may be organic matter which is converted to ammonia but oxygen availability in the subsurface environment is not high enough to support nitrification. The net positive increase of ammonia in the wetland could be the anaerobic decomposition of organic nitrogen trapped in the bed and the insufficient oxygen to further oxidize into nitrate as the system is anaerobic (U.S.EPA 1993).

3.3.3.6 Bimonthly *E. coli*

Figure 3.28 (a - d) shows the areal and volumetric MPN removal rate of *E. coli* analysis result conducted from Sept 2010- August 2012. The areal MPN removal was better for the deeper beds and it was shown that in all cases the trend of removal increase from the colder to the warmer summer months. The volumetric mass removal of the H25 and H25p were slightly better than the H50 and H50p beds. In both areal and volumetric results, the effects of plants were not clearly observed although the planted beds showed better result at some points in the figure. In the Figure 3.28 a and b, the variance of the 50 cm deep beds were more than the 25 cm deep beds which this may be related to their high removal rates.

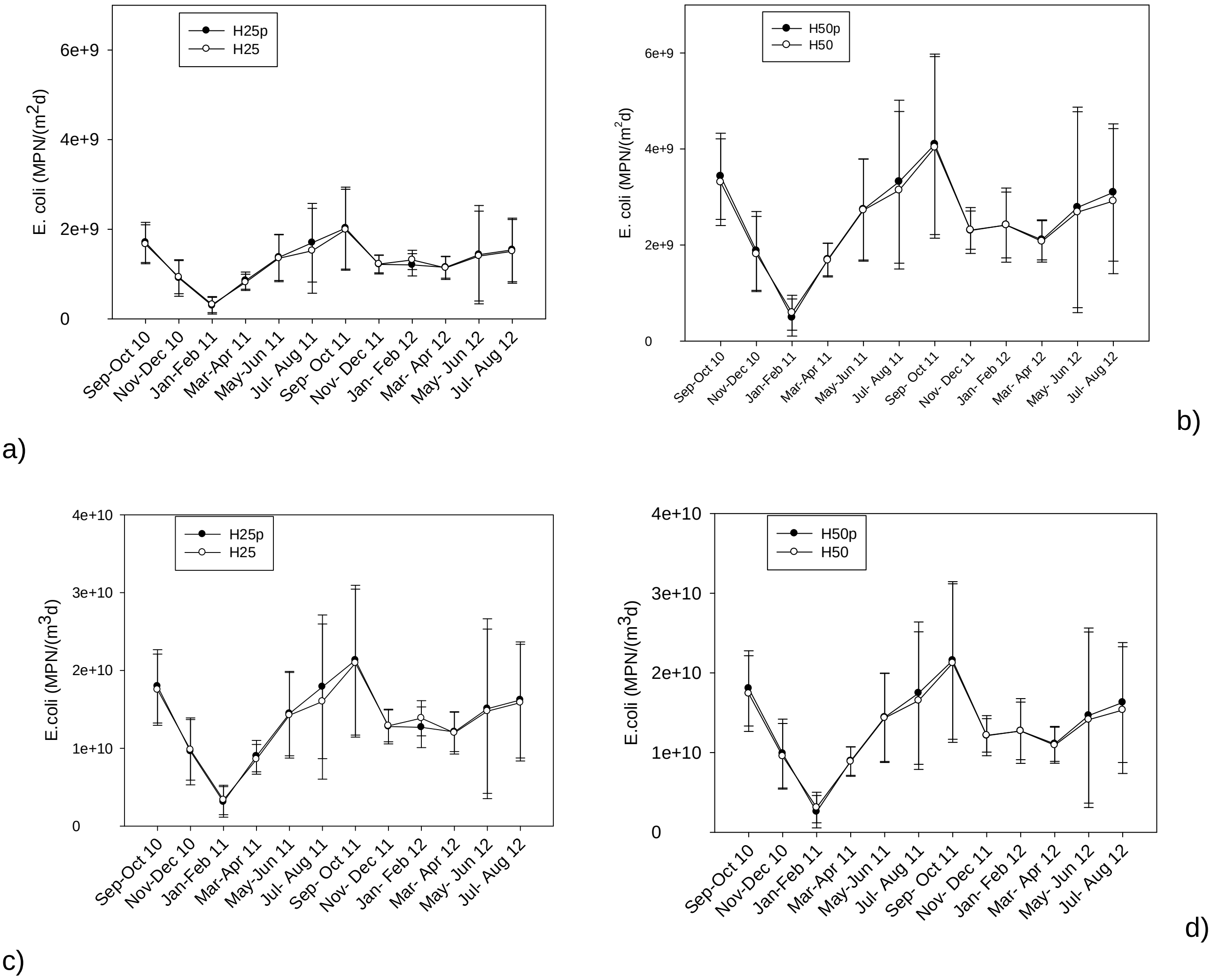


Figure 3.28: Bimonthly average and deviations of *E.coli* MPN removal (MPN/(m^2.d)) for a and b and MPN removal (MPN/(m^3.d)) for c and d for beds H25p, H25, H50p and H50 from Sept 2010 to August 2012.

In summary, there was a seasonal effect the performance of the 25 cm and 50 cm deep, planted and unplanted beds almost for all the parameters compared. This means that season had a great influence on the performance of the wetlands. In the literature, authors do not share the same opinions, some reported the influence of temperature and the others reported no influence of temperature on the performance of horizontal subsurface flow wetlands (Vymazal and Kröpfelová 2008). For instance Hook et al. (2003) pointed out that the wetland followed seasonal patterns in a temperature controlled experiment whether it was planted or unplanted. The experimental results of this study support Hook`s results.

3.3.4 Removal rate coefficient

Tracer data provides volumetric efficiency, detention time, shape parameter (N) and variances in the wetlands. Tracer testing outputs in addition to offering information about the hydraulics of a system, are used to model and calculate rate coefficients for use in effluent concentration prediction, and in the sizing of a wetland bed (Kadlec and Wallace 2009). The tracer values combined with k values give the wetland effluent concentration. Eq. 2.43 is a P-k-C* model used to calculate the rate coefficient using P and τ obtained from the tracer testing. N is the maximum bounding of P, as P ≤ N (Kadlec and Wallace 2009).

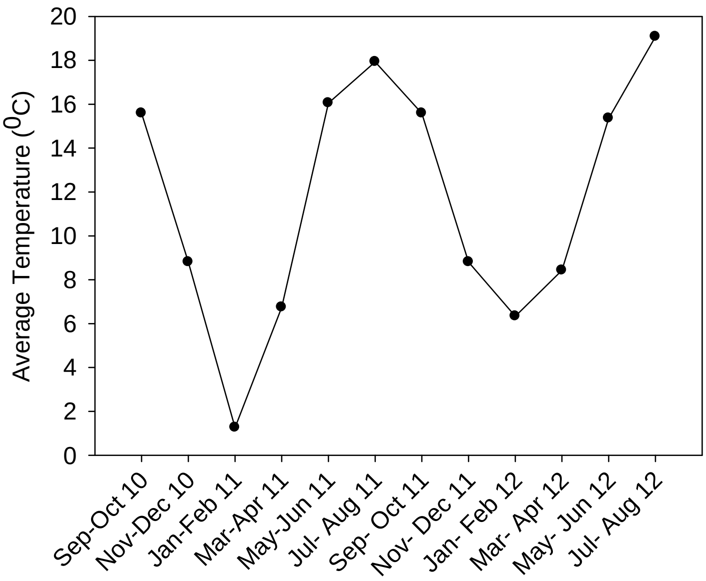


Figure 3.29: Bimonthly average water temperatures sample for the H25p, H25, H50p and H50 collected when sampling.

The areal rate coefficient of H25p, H25, H50p and H50 were carried out using the P-k-C* model (Eq 2.43) for the bimonthly averaged inlet and outlet concentration between Sept 2010 - August 2012. The bimonthly average water temperature of the measurement is shown in Figure 3.29. Areal rate coefficients at 20^{0}C and temperature factors were calculated for $CBOD_5$, TOC, TN and *E. coli* for each wetland using Eq 2.43 and Eq 2.36 and the predicted effluent concentration presented. The excel sheet is shown in Table 1, Appendix section. The areal rate coefficients of the four water quality parameters are presented from Figure 3.30-3.33. It is important to observe that in all cases the rate coefficients were not constant for a specific wetland. Rate coefficients vary with depth, pollutant concentration and hydraulic loading (Kadlec 2000).

The $CBOD_5$ values used for the calculation were averaged bimonthly from data collected during Sept 2010 – August 2012. The background concentration, C* represents the biogeochemical background (speciation considered in P) may be considered as free parameter C*>0, or it may be selected to be the lowest concentration ever measured in a comparable situation, such as at far down gradient in impacted pristine systems (Kadlec and Wallace 2009). The P-value which includes KVD and DTD is less than N calculated from the tracer analysis and C* are assumed (Ci> C*) and taken from Kadlec and Wallace (2009) as shown in Table 2.5. The source of C* includes plants and internal microbial autotrophic production (Garcia et al. 2010).In this work, the background concentration (C*) was used from Table 2.5.

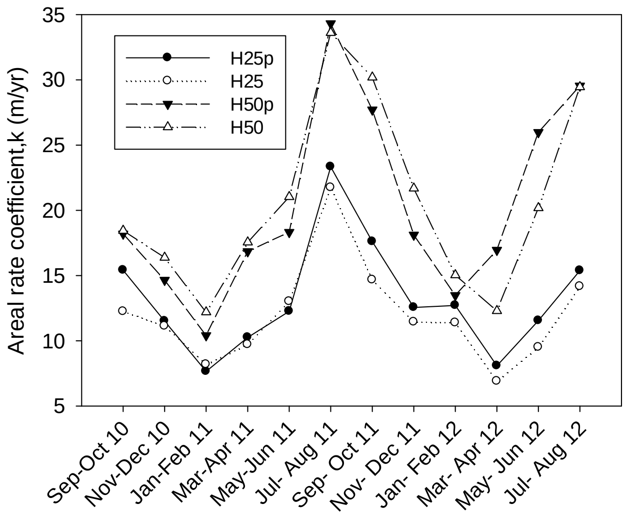


Figure 3.30: $CBOD_5$ areal rate coefficient calculated on a bimonthly basis from Sept 2010-August 2012.

From Figure 3.30, shows the effect of depth on the rate is clearly seen and little difference between H25 and H25p or H50 and H50p beds. The more wastewater addition as inflow implies more $CBOD_5$ so rate calculated for deep wetland had relatively higher values. Areal removal rate coefficients are generally higher at high loading rates (Kantawanichkul et al. 2009). However, the volumetric rate coefficient calculated (not shown here) has higher values for the 25 cm deep bed wetlands and had high rate as in the Equation, $k_v = \frac{k}{\varepsilon h}$ (Kadlec and Wallace 2009).

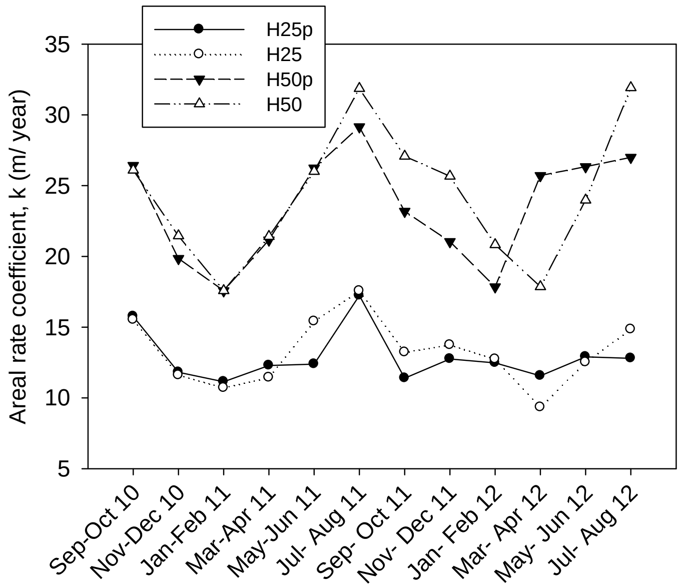


Figure 3.31: TOC areal rate coefficient calculated on a bimonthly basis from Sept 2010-August 2012.

In the same manner as $CBOD_5$, TOC also showed similar rate coefficients in the measured bimonthly data, as shown in Fig 3.31. The rate coefficients of deeper beds clearly pronounced when compared with 25 cm deep beds that the effect of plant.

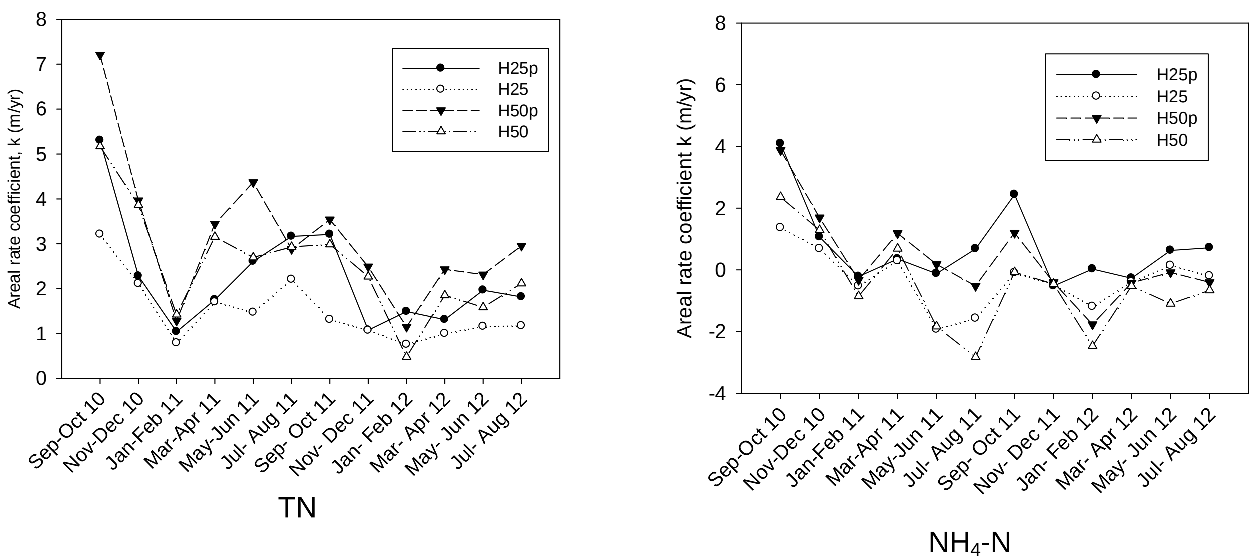


Figure 3.32: Total nitrogen and ammonium nitrogen areal rate coefficient calculated bimonthly basis from Sept 2010 - August 2012. TN= total nitrogen; NH_4-N= ammonium nitrogen

Total nitrogen and ammonium nitrogen areal rate coefficients were different from the k_A rate coefficient pattern of TOC and $CBOD_5$ as shown in Figure 3.32. For TN the k_A rates for H50p were better followed by H25p and H50 follows by interchanging positions in the figure but H25 had the lowest performance in all ranges. In the ammonium mass removal H25p outperformed the other beds and followed by H50p. The performances of the unplanted beds were not as good as the planted.

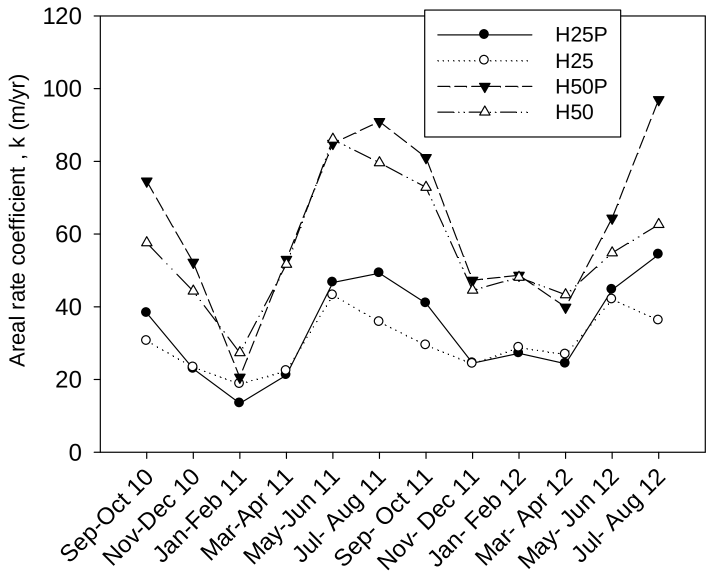


Figure 3.33: *E. coli* areal rate constant and average temperature calculated bimonthly basis from Sept 2010 - August 2012.

For *E. coli* the areal removal rate coefficient is shown in Figure 3.33 indicates that depth has an effect on the rate but there was also a planted related effect in most cases.

There was a strong dependence of the k-rate on season. The driving forces of seasons are plant biomass cycling, climate and water temperatures (Kadlec and Wallace 2009). The areal k-rates of the deeper beds were almost twice as much as the shallow beds; which can be explained by the fact that H50 was loaded with twice as much wastewater as the H25 beds which contributed to the high areal removal rate. Kadlec and Wallace (2009) showed graphically from different works that the value of k_A for $CBOD_5$ is nearly proportional to hydraulic loading and inversely proportional to the hydraulic retention time. The rate constant are not constant but depends influent concentrations, hydraulic loading rates and water depths (Headley et al. 2005).

The areal and volumetric rate coefficient at 20^0c and temperature coefficients of the four H25p, H25, H50p and H50 were calculated for the $CBOD_5$, TOC, TN and *E. coli* using bimonthly average inlet and outlet data analysis as shown in Tables 3.7. The areal rate coefficient of the $CBOD_5$ and TOC shows the effect of depth and unplanted beds show a higher removal rates for planted beds in each depth. The volumetric rate coefficient of the organic carbon (TOC and $CBOD_5$) was very close which means the effect of the vegetation and depth is not seen but the unplanted systems have a slightly higher value than the planted. The 50 cm deep bed rate coefficient values are close to the literature values because most of the literature k_A values were calculated for 50 cm deep or more. The 25 cm deep bed was less than those identified in the literature. The other important point for the high rate coefficients of the 50 cm deep bed had twice as much as the 25 cm deep. The rate coefficients are nearly correlated to the hydraulic loading rate (Kadlec 2000, WEF 2001). The hydraulic loading rate of 25 cm deep bed was half of the 50 cm deep beds. Generally, the literature value of rate coefficients for BOD_5 at 20^0C vary widely from 0.06 - 6.1 m/d and Kv of 0.11-6.11 d^{-1} according to the review (Rousseau et al. 2004). First order rate coefficients are not constant and depend on many factors like influent concentration, water depth, plant species and hydraulic loading rate (Garcia et al. 2010).

The effect of both depth and plants were seen for TN and *E.coli* areal rate coefficients. When the volumetric rate constant of TN and *E. coli* were compared, the situation changed to the opposite.

As reported by Kadlec and Wallace (2009), calculation of θ values using P-k-C* model value ranged from 0.891-1.14. The 50th percentile of the Arrhenius temperature factor for $CBOD_5$ is 0.981 and the 50th percentile for areal rate coefficient was 37 m/year. The temperature factor of this study (Table 3.7) was also in the range of the values reported by Kadlec and Wallace (2009). The authors also mentioned 1.14 is an extreme condition to happen practically and θ <1 means the performance of the bed is worse at high temperature.

Table 3.7: Areal and volumetric rate coefficients at 20^0C and temperature coefficient of HSSFCW calculated for $CBOD_5$, TOC (p=6, C*= 5) TN (P= 4, C*= 1), *E.coli* (P=6, C*= 0) using *P-k-C** model. The 50 and 25 cm deep beds were working at the same hydraulics detention time.

	beds	**H25p**	**H25**	**H50p**	**H50**
	k_A (m/d)	0.048	0.042	0.083	0.082
$CBOD_5$	k_v d^{-1}	0.508	0.442	0.434	0.432
	θ	1.045	1.041	1.051	1.05
	k_A (m/d)	0.039	0.044	0.079	0.085
TOC	k_v d^{-1}	0.406	0.463	0.416	0.445
	θ	1.012	1.027	1.025	1.031
	k_A (m/d)	0.008	0.005	0.01	0.008
TN	k_v d^{-1}	0.087	0.048	0.055	0.04
	θ	1.052	1.021	1.031	1.018
	k_A (m/d)	0.182	0.125	0.281	0.216
E. coli	k_v d^{-1}	1.912	1.314	1.48	1.137
	θ	1.074	1.037	1.066	1.045

The areal rate coefficients calculated at 20^0C for TN range from 2-4 m/year from shallow to deep beds, as indicated in Table 3.8. The median annual reduction of total nitrogen in HSSF wetlands is 8.4 m/year which is more than the analysis in the work; however, the result was in agreement for the 20^{th} percentile which is 3.3 according to Kadlec and Wallace (2009). The 50^{th} percentile for temperature factor for total nitrogen removal rate constants in HSSF wetlands is 1.005 but as in indicated in Table 3.7 it ranged from 1.02-1.05 which is a little bit higher than the literature. However, it matched with the 80^{th} percentile (Kadlec and Wallace 2009). In Garcia et al. (2010) review, they have reported a value of total nitrogen areal rate coefficient between 0.007-0.1 m/d.

Measured and predicted concentration with P-k-C*

In Figure 3.34-3.35, the $CBOD_5$ and TN values of the four wetlands concentration at inflow and outflow and the predicted effluent concentration of the wetlands using the P-k-C* model. From the graph, the P-k-C* model predicts a relatively good fit for $CBOD_5$ and TN. The ammonium nitrogen prediction also indicated in Figure 3.36 for comparison purposes. Similar figure for total organic carbon is shown in Figure 2, Appendix section.

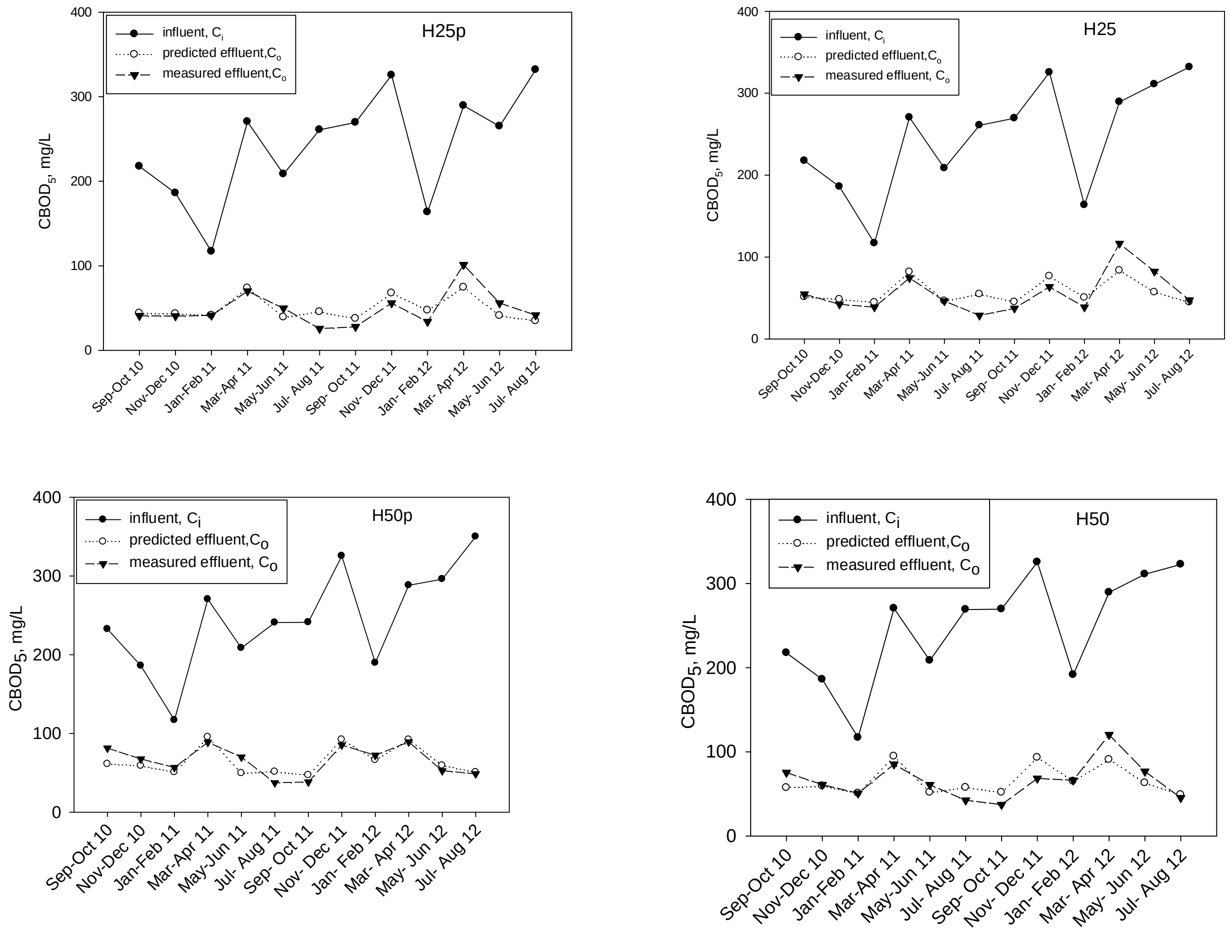


Figure 3.34: Predicted and measured $CBOD_5$ using the P-k-C* model.

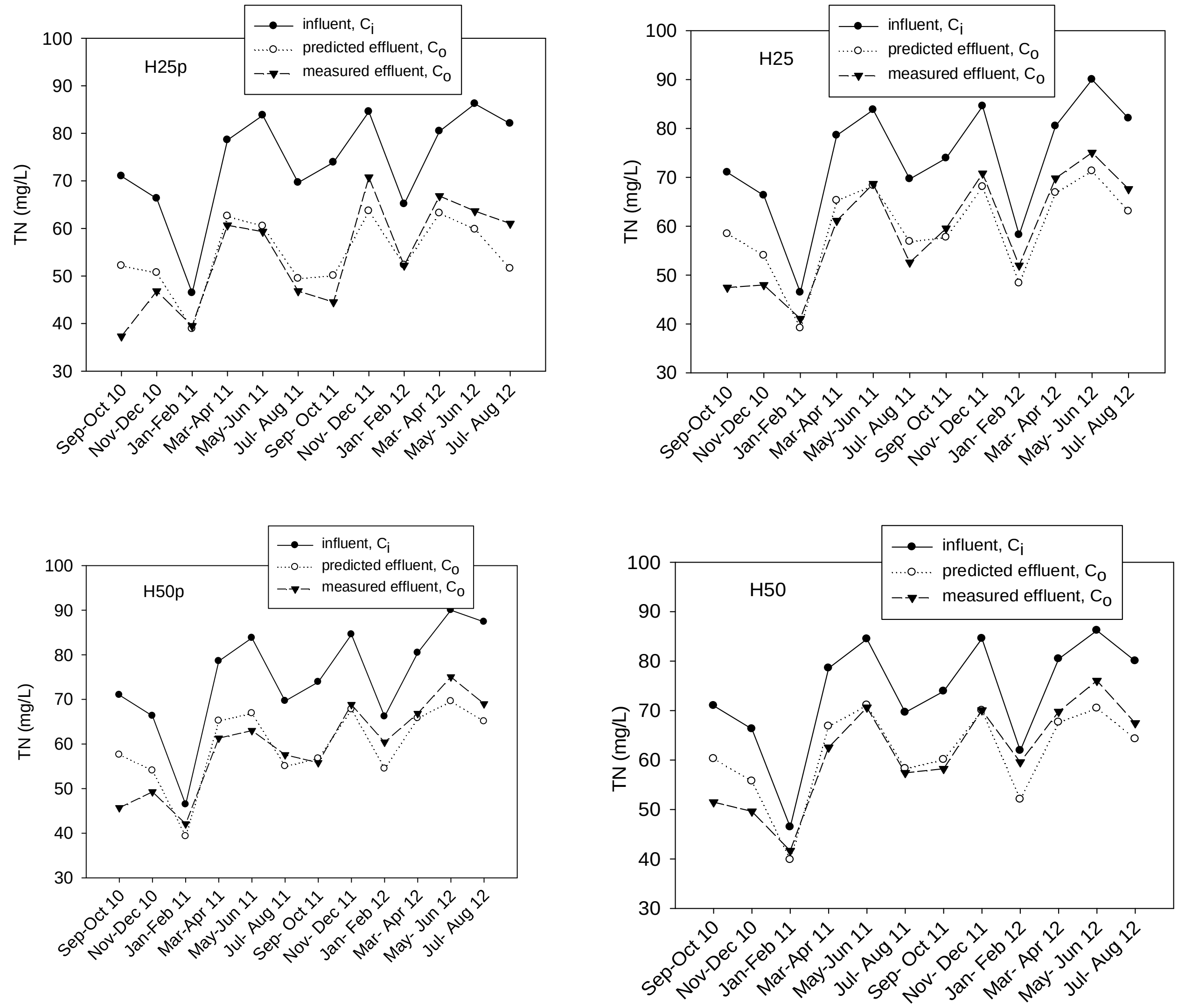


Figure 3.35: Predicted and measured TN using the P-k-C* model.

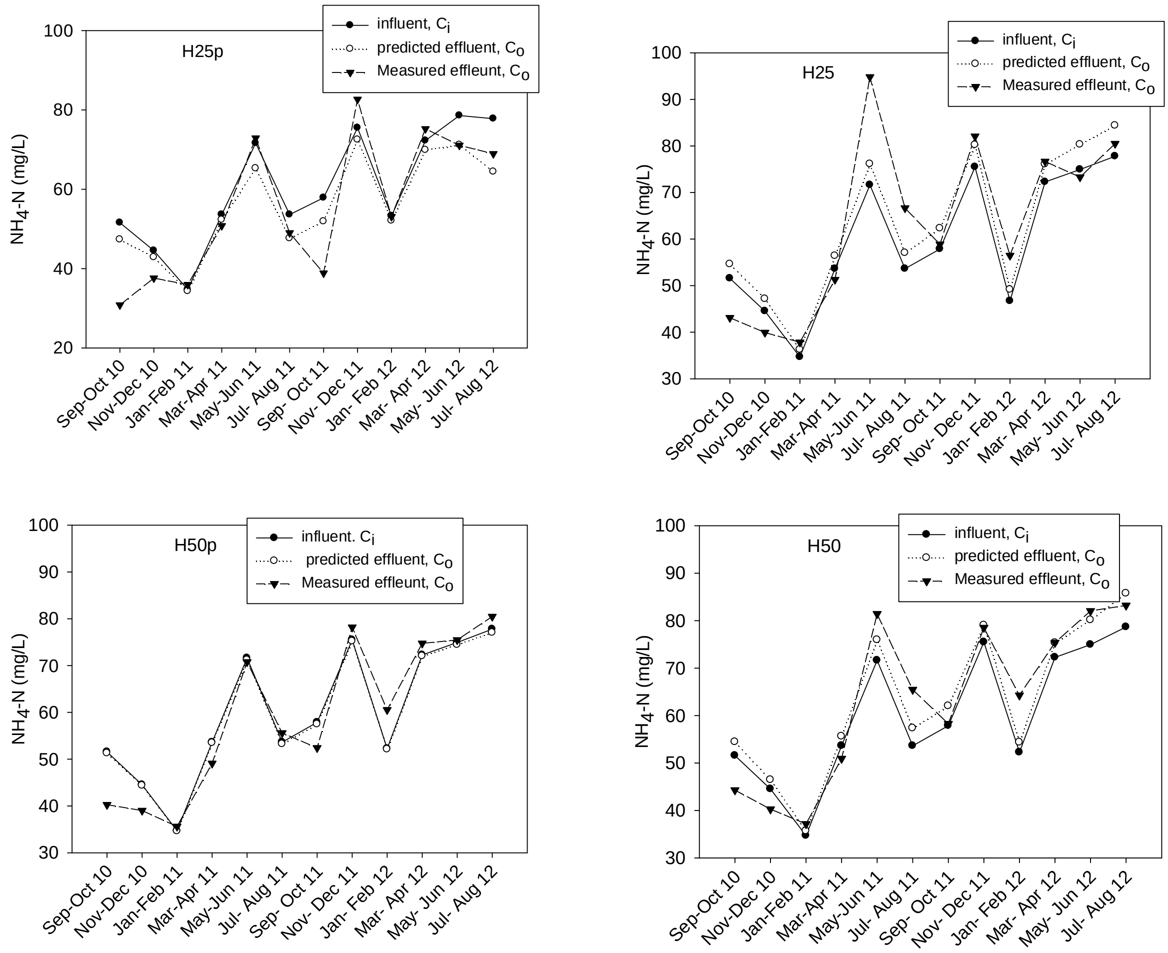


Figure 3.36: Predicted and measured NH_4^+-N using the P-k-C* model.

Figure 3.34 to 3.37 were included here to show how far the P-k-C* model predicts the performance of the wetlands in four of the wetlands.

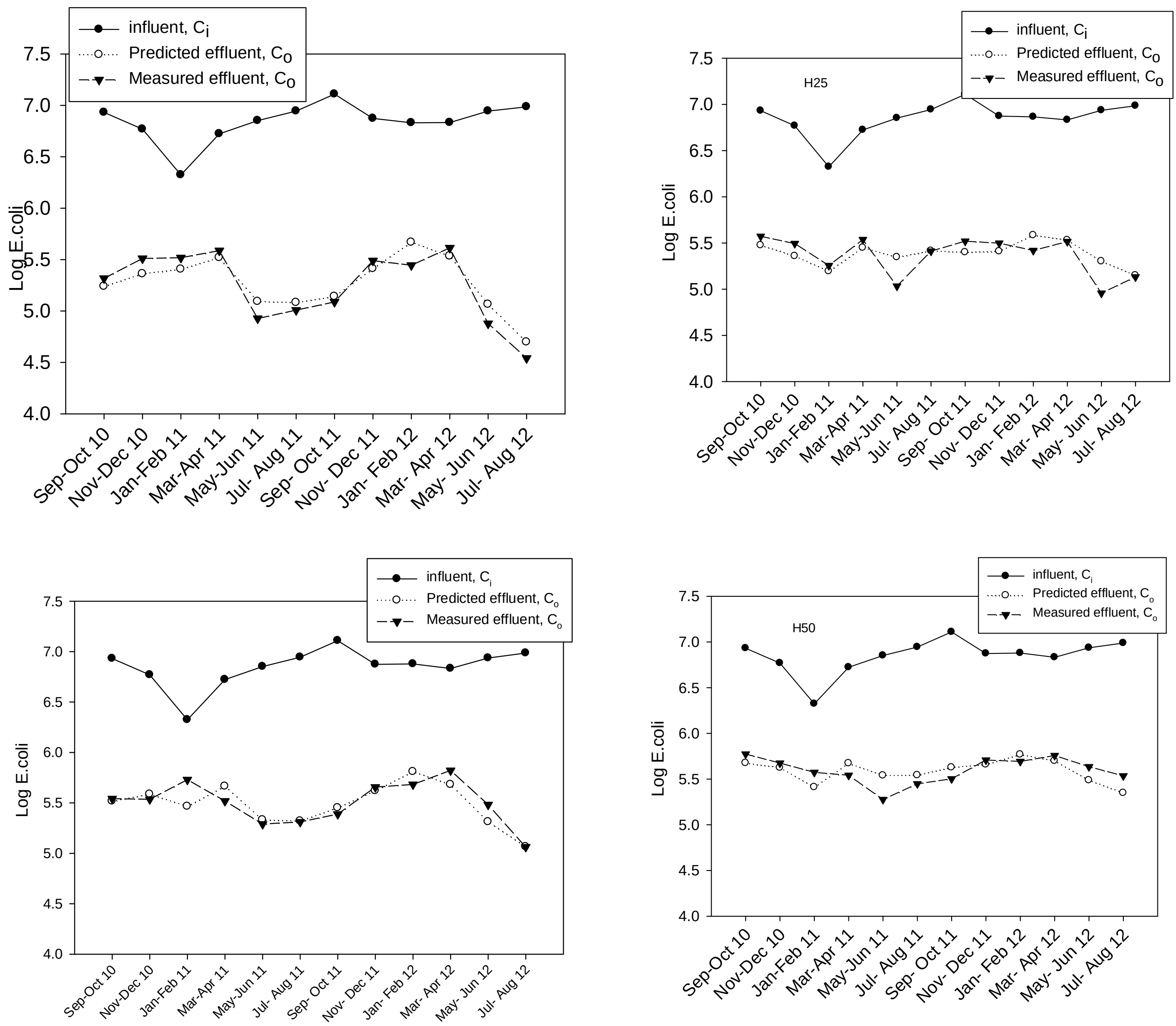


Figure 3.37: Predicted and measured *E.coli* using the P-k-C* model.

In conclusion, areal rate coefficients were calculated using P-k-C* model using the bimonthly average inlet and outlet concentration from Sept 2010 to August 2012. The rate coefficients are related to the water temperature. There was no observed impact of the plants on organic carbon removal. On the contrary, effects of plants observed for the total nitrogen and *E. coli* removal. The k_A was proportional to hydraulic loading rates or inversely proportional to detention time.

3.3.5 Longitudinal concentration profiles

Overview

Internal samples were collected from a fractional distance of 0, 12.5%, 25%, 50%, 75%, 100% (which coincides with nHRT (days) of 0, 0.7, 1.4, 2.7, 4, 5.4) for TOC, TN, NH_4-N, DO and Eh parameters and 0, 12.5%, 50%, 100% for TSS and $CBOD_5$ from Sept 2010- Sept 2011. In this section, the longitudinal profile of the average concentrations and the standard deviation of TSS, $CBOD_5$, TOC, TN, NH_4-N, DO and Eh against nHRT is plotted from Figure 3.38-3.44. The slope of each parameter was compared between the four wetlands with ANCOVA and the results are presented in Table 3.9.

3.3.5.1 Total suspended solids

Figure 3.38 shows the total suspended solids removal longitudinally from the inlet to the outlet. In the Figure, the sample analysis for TSS was conducted for fractional distance of 0.0 %, 12.5 %, 50 % and 100 %, or for nominal detention time of each wetland. The amount of suspended solids increased at nHRT of about 0.7 more than the raw wastewater then declined to almost a constant value from nHRT of about 2.8 until it came out as effluent. This trend was the same for H25, H25p, H50p and H50. However, there was a difference in TSS values at nHRT of 0.7 for the planted and unplanted beds, H25 and H50 showed TSS values more than 100 mg/L for the H25p and H50p. This might be because the unplanted beds released entrapped particulates more easily than the planted beds during sampling. This same trend was observed $CBOD_5$, TOC and TN removal in all wetlands as well. The low concentration of suspended solids at the 0.7 fractional distances for the planted beds might be because the suspended solids removed before this point of the unplanted beds and/ or plant roots might make a contribution by strongly holding the suspended matter.

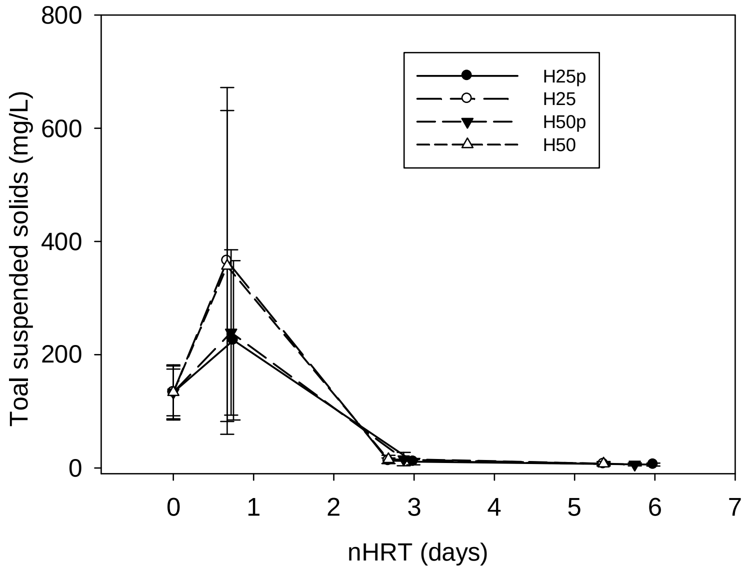


Figure 3.38: Longitudinal total suspended solids versus nominal hydraulic detention time

This may indicate the root systems affect on the distribution of suspended solids. The higher suspended solids in the beginning of the bed may be an indication of clogging occurring. This observation is supported by several researchers who have found that clogging was the most severe within the first 1/4 to 1/3 of the system and the hydraulic conductivity (which is associated suspended solids accumulation) was found to be less restricted and fairly uniform over the remaining length of the system (Crites and Tchobanoglous 1998, U.S.EPA 2000, Headley et al. 2005). These results demonstrated that the TSS which is responsible for clogging was occurred almost at the same location irrespective of the depth or presence of plants. Additional internal sampling points would be required to exactly locate the peak of solids removal along the beds.

3.3.5.2 Organic matter

Figure 3.39 and 3.40 show the TOC and $CBOD_5$ concentration against the nominal detention time of the wetlands as days. As it was with TSS, the $CBOD_5$ concentration increased at nHRT of 0.7 from the inlet; however, the highest concentration of TOC was 1.4 of the distance along the bed from the inlet. For $CBOD_5$ and TOC, the large proportion of the organic carbon greater than 40% as COD is usually in particulate form and so removal is by sedimentation and filtration in the beginning of the bed (Caselles-Osorio et al. 2007). This might be due to the fact that the TOC analysis was different from $CBOD_5$ but in agreement with total nitrogen results. In both cases the effect of plants was seen that unplanted beds have shown high concentration at the point of analysis.

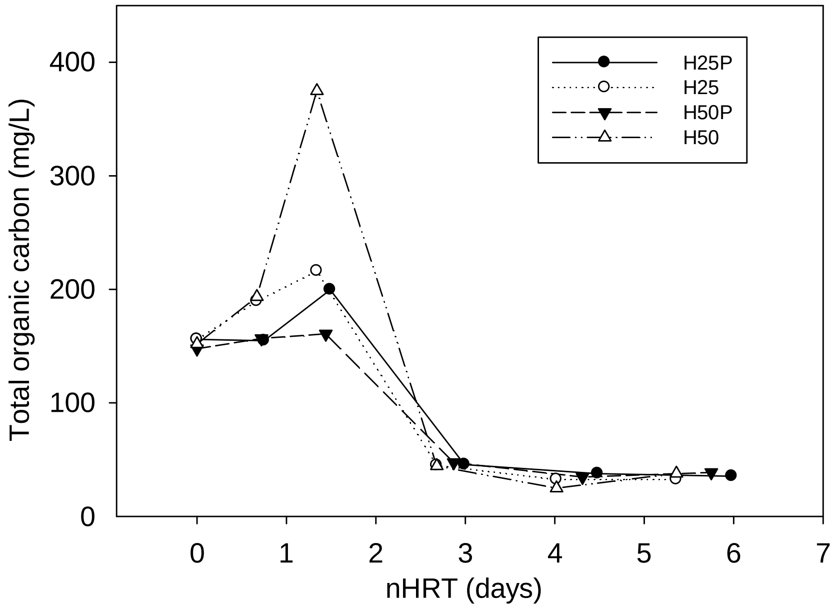


Figure 3.39: Longitudinal TOC concentration versus nominal hydraulic detention time. The error bars left out purposely to properly see the variation between the wetlands concentration.

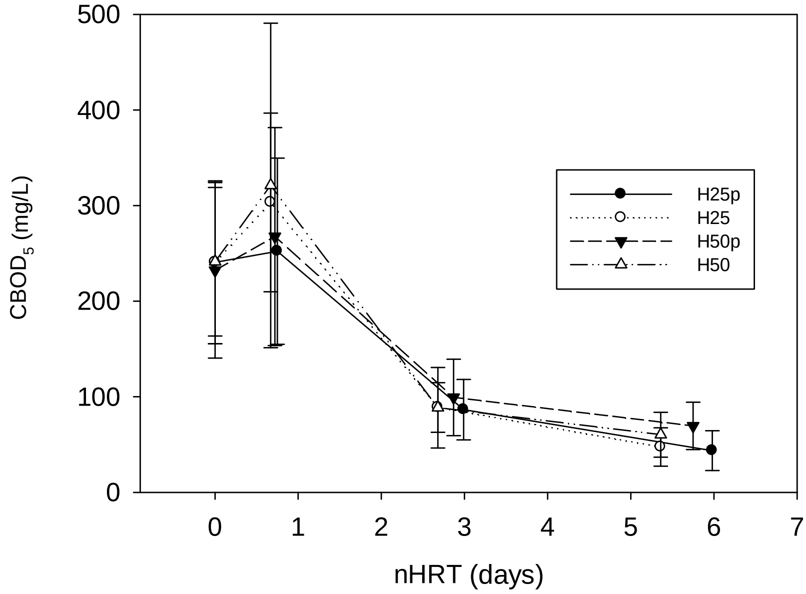


Figure 3.40: Longitudinal $CBOD_5$ concentration versus nominal hydraulic detention time

The H25p concentration was lower than that of the rest of the beds for $CBOD_5$ in the three points of measurement may because of the nature of the biochemical processes in the beds. Garcia et al. (2005) reported that the TOC removal efficiency of shallow beds was biochemical processes.

3.3.5.3 Total Nitrogen

The longitudinal TN concentration profiles for Sept 2010 - 2011 is shown in Figure 3.41. The TN concentration increased from the inlet point to the nHRT of 1.4 down the bed, with the planted beds performing better when compared to the unplanted beds, H50p had the lowest total nitrogen concentrations in the middle but at the final outlet H25p had the lowest concentration. The peak at 1.4 and then at 0.7 observed here are like the trend of TOC because both are analysed with the same pre-treatment and instrumentation simultaneously.

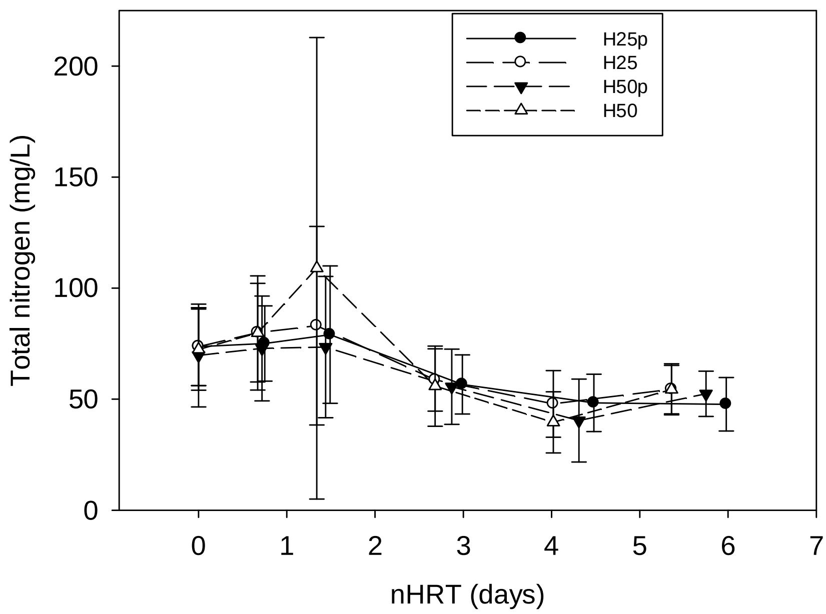


Figure 3.41: Longitudinal TN versus nominal hydraulic detention time

At the end of the beds (the final effluent), the planted beds have performed better than the unplanted beds. The order of performance is H25p>H50p> H50 ≈ H25.

3.3.5.4 Ammonium nitrogen

Longitudinal ammonium nitrogen removal in the beds did not show the same pattern as that of TSS, $CBOD_5$, TOC and TN as shown in Figure 3.42 H50 and H50p showed low concentrations at the first two sampling points and this pattern changed in the H25 and H25p which had shown low concentrations and finally the planted beds were performing better than unplanted beds especially the H25p. The effluent concentration which was assumed to be the sum or integrated form of the beds wastewater showed that H25p>H50p>H50>H25 in the order of performance.

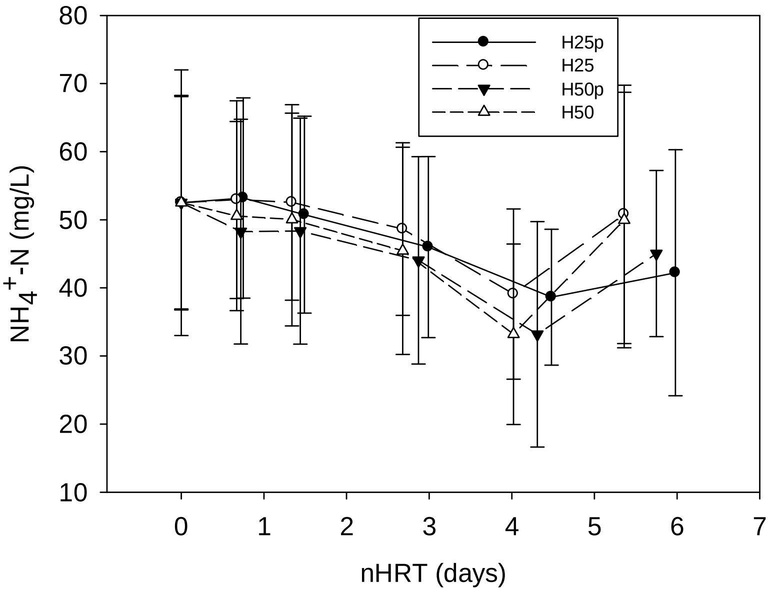


Figure 3.42: Longitudinal ammonium nitrogen versus nominal hydraulic detention time

The deviation between the 75% fraction point and the outlet in Figure 3.41 and 3.42 from the overall profile, with a small increase TN and NH_4^+-N concentration might be due to the outlet of the system contributing to some mixing. The effluent water had passed through a collection pipe located at the bottom of the beds and then flowed through an outlet chamber and a length of pipe before reaching the outlet sample collection point whereas the internal sample was collected from a sample well.

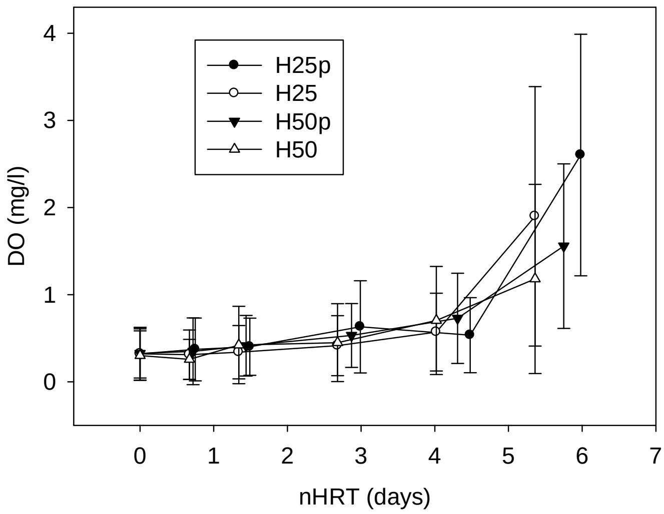


Figure 3.43: Longitudinal DO versus nominal hydraulic detention time

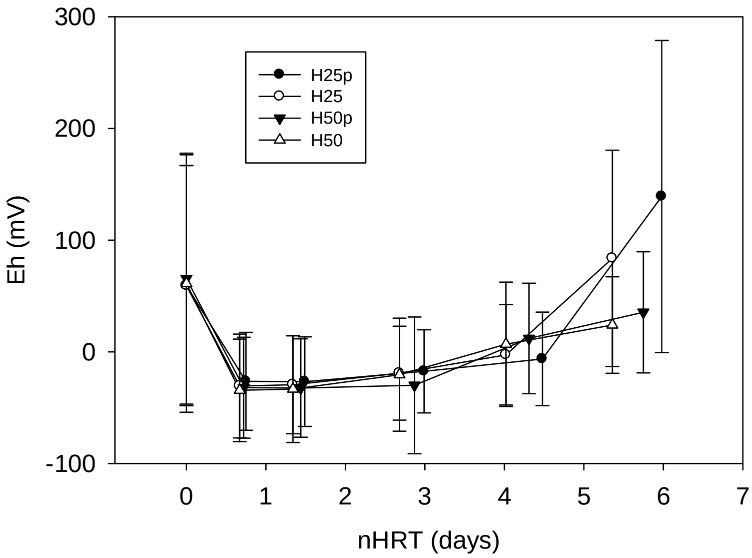


Figure 3.44: Longitudinal ORP versus nominal hydraulic detention time

In Figure 3.43 and 3.44 the longitudinal profiles of the dissolved oxygen and ORP are shown, respectively. Generally it can be observed that the ORP values were first decreased at the point where high accumulation of solid and organic matter is observed in the wetland. Then as the organic matter and nitrogen removal declined slowly, the Eh and DO values increase with the H25p showing higher values than the other beds at the outlet and the H25p roots are in contact with the all water being treated as opposed to H50p. ORP usually increase from inlet to outlet due to progressive degradation of pollutants (García et al. 2003, Headley et al. 2005).

3.3.5.5 Comparison of longitudinal pollutant concentration removal rate between wetlands

Linear regression equations describing the relationship between pollutant concentration and nominal hydraulic detention time (nHRT) were derived for H25p, H25, H50p and H50 wetlands (Table 3.8). In all of the parameters except ORP, the concentration was log transformed to fit in a linear regression.

Table 3.8: Linear regression between water quality parameters concentration and nHRT for H25p, H25, H50p, H50 and analysis results of ANCOVA testing to determine if regression equation slope differ significantly between the wetlands.

Parameter	dependent variable	wetland	slope	y-intercept	r^2	ANCOVA significant difference (α=0.05)
TOC	log(C)	H25p	-0.128	2.202	0.863	NO
		H25	-0.147	2.25	0.791	
		H50p	-0.137	2.25	0.791	
		H50	-0.172	2.31	0.68	
TN	log(C)	H25p	-0.0348	1.848	0.892	NO
		H25	-0.0348	1.859	0.787	
		H50p	-0.0378	1.863	0.696	
		H50	-0.042	1.865	0.564	
NH_4^+-N	log(C)	H25p	-0.0179	1.707	0.644	NO
		H25	-0.008	1.698	0.158	
		H50p	-0.0179	1.712	0.511	
		H50	-0.0168	1.705	0.201	
DO	log(C)	H25p	0.044	-0.486	0.743	NO
		H25	0.13	-0.609	0.800	
		H50p	0.112	-0.539	0.938	
		H50	0.12	-0.539	0.938	

The ANCOVA analysis indicated that the slopes of the linear regression equations were not significantly different ($p>0.05$) between H25, H25p, H50p and H50 beds for measured parameters: TOC, TN, NH_4^+-N and DO. The calculated F value for the H25, H25p, H50 and H50p were smaller than the critical value. The slopes were not different (Zar 2010). The elevation of the regression equations were not checked because all parameters have the same inflow source which means they have the same initial concentration or y-intercept. This implies that the apparent rate of pollutant concentration removal was not statistically significant for four of the wetlands for the parameters measured. These results suggest that depth of bed and plants are not important for the rate of concentration removal of the pollutants studied here. The apparent lack of any effect of depth on treatment performance observed in the present was in agreement with other work of Headley et al. (2005) although their study was done in one wetland sampled at different depths.

3.3.6 Summary

In this section the performance of H25, H25p, H50 and H50p wetlands were compared at the same detention time based on two year analysis from Sept 2010 to August 2012.

The concentration removal of the H25p and H25 beds were better than H50 beds and the H25p beds showed smaller effluent concentration than the H25 beds. When the overall average mass removal per unit area per day was compared, deeper beds showed an advantage over the shallow beds irrespective of presence of plants, with exception of total nitrogen and ammonium nitrogen. Plants do not have significant difference in areal mass removal of $CBOD_5$, TOC, and TSS in H25/H25p and H50/H50p. Areal mass removal of deeper beds was almost twice as much as shallow beds because the loading of deeper beds were twice the shallow beds. The volumetric mass removal of the deeper and shallow beds was almost the same for TSS, $CBOD_5$ and TOC water quality parameters. However, for TN there was significance difference in mass removal between the wetlands. However, the ammonia mass removal (NH_4-N) was statistically different with plant but statistically similar with depth.

When the first and second year mass removal rate were compared, the areal removal of the second year was more than the first year for TOC and $CBOD_5$ but the first year performance was better than the second year for TN.

Bimonthly values of the areal mass removal of the H25p, H25, H50p and H50 on TSS, $CBOD_5$, TOC, TN, and NH_4^+-N indicated that the removal follows the trend of the temperature. Areal mass removal of TSS, $CBOD_5$ and TOC of the deeper beds was twice the shallow beds in the range of measurement so removal depends on depth. The volumetric removal was almost the same irrespective of depth or availability of plants. On the contrary, the mass removal of nitrogen depends on plants rather than depth in regard to both volumetric and areal mass removal. H25p was better performing than the other beds. Unplanted beds had negative values.

k_A was also calculated on a bimonthly basis to show the trend for $CBOD_5$, TOC, TN and *E. coli* and the areal rate constant was also dependent on temperature and deeper beds had higher values for the organic carbon and *E. coli* and the pattern is changed for total nitrogen.

From the inlet-outlet and intermediate sampling points profiles of H25p, H25, H50p, and H50 indicated higher concentrations in the first part of the bed because of the filtration of the pollutants at that point and the pollutants $CBOD_5$, TOC, TSS and TN concentration declined in all wetlands irrespective of the presence of plants or depth. Kadlec (2003) observed that total organic carbon concentration decreases rapidly near the inlet and little additional removal occurs later on. Analysis of ANCOVA on the profile of the wetland, indicated that the rate of pollutant concentration reduction between the four wetlands was not significantly different ($p>0.05$) for TOC, TN, ammonium nitrogen and DO. Thus, it can be concluded that that the rate of decomposition of contaminants as wastewater moved through the HSSFCW was approximately similar between planted, unplanted, deep and shallow for TOC, TN, and ammonium nitrogen water quality parameters.

3.4 Results and discussion on the nitrification potential of root and gravel

Overview

The rate of ammonia removal or the formation of nitrate potential (nitrification potential) of root and gravel biofilm samples collected from H25p, H25, H50p, H50, HAp and HA were analyzed based on nitrate/nitrite formation or ammonium removal. In this Section, first the nitrification results of the gravel of the inlet and outlets are reported and discussed, followed by the comparison of the results of the nitrification potential of root of the planted beds for samples from May 2011. Finally, nitrification potential of gravel sampled in February 2011 and May 2011 from 12 points in 6 beds is compared.

3.4.1 Nitrification potential of inlet and outlet of passive and aerated beds

Nitrification is the microbial transformation of ammonium ion into nitrate. Nitrification potential measurement gives a direct evidence for existence of nitrifying bacteria and a rough estimate of their density (Faulwetter et al. 2009). The activities of nitrifying bacteria and their numbers are correlated positively (Bodelier et al. 1996). Hydraulic levels or depth considerably affect different aspects of microbial activity including potential nitrification (Truu et al. 2009). In order to obtain an indicator of the nitrification potential of the attached gravel and root biofilms with respect to depth, plant and aeration, analysis were conducted from roots and gravel collected from inlet and outlet side of H25p, H25, H50p, H50, HAp, and HA. Each gravel sample was incubated with nitrification buffer and the resulting nitrate concentration at 0, 2, 4, 8 and 24 hours of measurement was averaged and plotted, the slope of the regression curve is the nitrification potential, refer Figure 3.45. Steeper slope indicates high nitrification potential and the presence of high amount of nitrifying bacteria in the gravel biofilm.

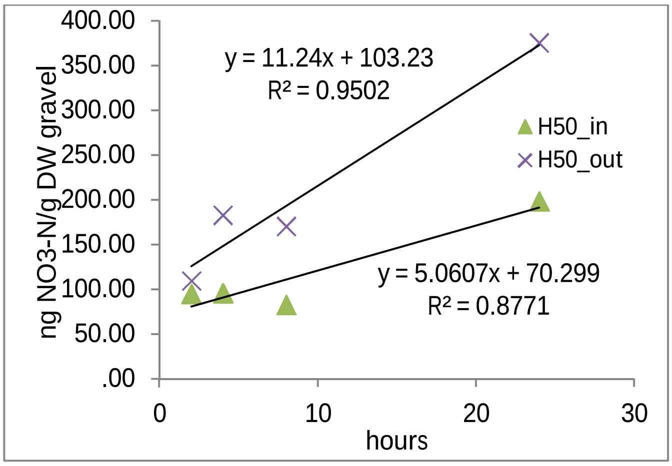


Figure 3.45: Example of nitrification potential plotted for H50 beds gravel biofilm collected from the inlet and outlet.

Figure 3.46 and 3.47 show the nitrification potential of the inlet and outlet side of the same wetlands, respectively. From the figures, the nitrification potential of the outlet of the H25p, H25, H50p and H50 was higher than their inlet within a wetland. This showed higher nitrifying bacteria biofilm were available closer to the outlet part of the beds than at the inlet where anaerobic behaviour prevails. According to Garcia et al (2003) and Nurk et al. (2005) nitrification activity was found to increase along the distance from the inlet to the outlet of the HSSFCW when purifying municipal wastewater due to progressive biodegradation of pollutants. On the contrary, the inlet of the aerated beds had higher nitrification potential than the outlet for both planted and unplanted beds. This was due to the higher rate of aeration at the inlet and the continuous availability of ammonium ion from the inflow wastewater and the decline of ammonia substrate for nitrifying bacteria in the direction of the outlet. Application of oxygen to wetland increases the biological ammonia oxidation or nitrification (Palmer et al. 2009). Density of nitrifying bacteria is related to the availability of oxygen and ammonia in the system (Bastviken et al. 2003).

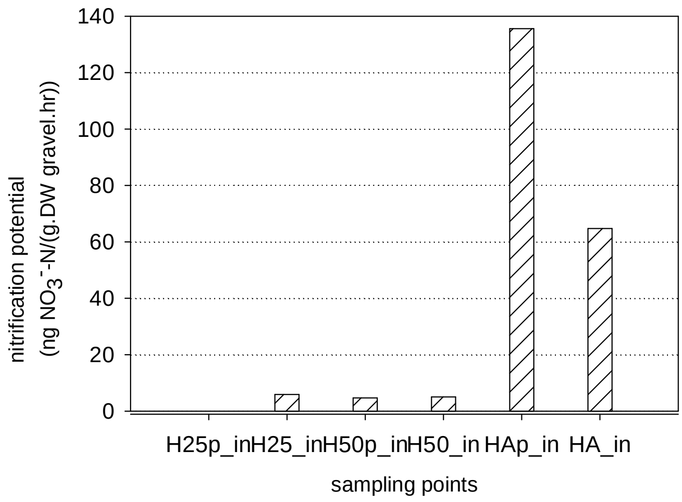


Figure 3.46: Nitrification potential of gravel as (ng NO_3-N/ (g dry weight gravel hour)) against the beds gravel sample. All samples collected were from inlet side of the wetlands sampled May 2011.

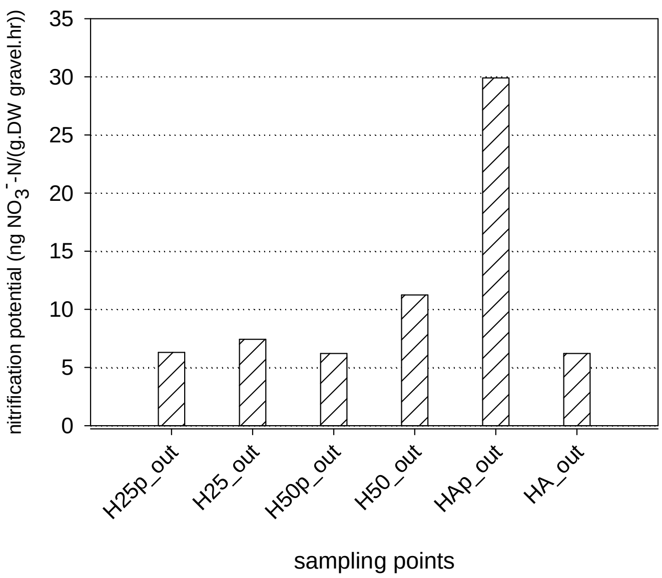


Figure 3.47: The nitrification potential of gravel collected from the 6 beds and 12 sampling points from the outlet.

Table 3.9: Nitrification potential of the gravel of H25p, H25, H50p, H50, HAp and HA. It is the average of three replicates.

gravel sample	ng N /(g DW gravel.hr)
H25p_in	-0.5
H25p_out	6.3
H25_in	5.9
H25_out	7.4
H50p_in	4.7
H50p_out	6.2
H50_in	5.1
H50_out	11.2
*HAp_in	135.6
HAp_out	29.9
*HA_in	64.7
HA_out	6.2

**HA= horizontal aerated bed, HAp=horizontal aerated planted*

As shown in Table 3.9, the nitrification potential of gravel biofilm collected from 12 places which enable to compare the nitrifying condition within wetland and between the wetlands. The nitrification potential of the inlet of the non aerated wetlands ranges from 0 to 5.9 and at the outlet from 6.2 to11.2 ngN/(gDW.hr). The nitrification potential of the outlet is higher than the inlet. It is also interesting that the unplanted beds had the highest values than the planted on gravel biofilm. For the sake of comparison the nitrification potential of agricultural soils (1.2-1900µg/(g.dw.d)) (Gorra et al. 2007) and aquatic sediments (1-1200 µg/(g.dw.d)) (Kurola et al. 2005, Hoffman et al. 2007). In this work the aerated beds are closer to the soil and sediments results.

Generally, the nitrification potential of the gravel of H25, H25p, H50 and H50p beds showed almost the same trend. Based on the gravel analysis, the nitrification potential of the H25, H50 were higher than the H25p and H50p when the inlet and outlet side were compared at the same depth. The higher nitrification potential of the H25 and H50 might be that all the available attached nitrifying bacteria located on the gravel but in the H25p and H50p they had additional attachment sites on the plant roots. Therefore, overall the H25p and H50p had higher nitrifying bacteria than the H25 and H50, at the same depth. The number of ammonia oxidizing bacteria are much higher in planted systems (Kyambadde 2004, Kantawanichkul et al. 2009).

In the aerated beds the inlet nitrification potential ranges from 65 to 136 and the outlet nitrification potential ranges from 6 to 30 ngN/(gDW.hr). The nitrification potential of the inlet was greater than the outlet for the planted and unplanted beds within each wetland. The nitrification potential of the planted was higher than the unplanted beds in contrast to the non aerated beds which may mean that the plant roots provided favourable conditions for nitrifying bacteria proliferation. The higher oxygen and ammonium supply created favourable conditions for nitrifying bacteria functions.

Since nitrifying bacteria are obligate aerobes, nitrifying activity in the rhizosphere of plants is possible if there is excess oxygen and no shortage of ammonia (Bodelier et al. 1996). The gravel collected from the inlet side of the aerated planted bed contained partially decomposed organic matter attached to it as opposed to the 25 and 50 cm deep beds which the gravel looked washed clean. As it was reported in Bastviken et al.(2003) different surfaces have different capacity in supporting nitrifying bacteria. The attached organic matter on the gravel favours this situation. The nitrate and nitrite production rate during the first 24 hours of incubation was high slope for the aerated beds.

3.4.2 Nitrification potential of root

For comparison of the nitrification potential of gravel and root samples collected from H25p, H50p and HAp planted beds, the results are shown in Figure 3.48. Root samples were collected from the same site with gravel. The aerated root samples collected appeared to be different physically from the H25p and H50p. Although it is not our primary objective, it is important to explain about the roots of the aerated beds. The aerated bed roots were white and thick (spongy) throughout and had no finer parts like the H25p and H50p and floated in water. The floating behaviour might be from the porous nature of the root and this behaviour was more exaggerated for roots sampled at the outlet side of the bed and for the phragmites that were observed to have yellow leaves. For the aerated bed all root parts were incubated because there were not enough fine root hairs for incubation at the same area of sampling. One hypothesis is that artificial aeration of the bed from the bottom might have pushed against gravitational growth of the root and also the aeration discouraged the formation of slimes around the root as appeared to occur naturally, in the beds without aeration. Secondly, there may have been a shortage of nutrients likes ammonia and iron which might precipitate out. For the incubation of H25p and H50p, finer parts of the root which was believed to be active in oxygen transfer and as the microbial biofilm habitat was selected and rhizomes were excluded.

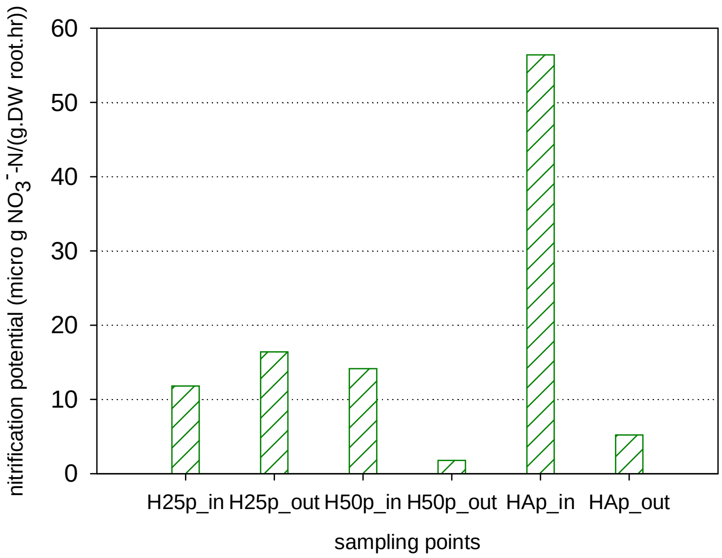


Figure 3.48: Nitrification potential as (µg NO_3-N/ (g dry weight root hour)) against the beds.

In Figure 3.48, the nitrification potential of the root H25p_in and H25p_out was 11.8 and 16.4, H50p_in and H50_out was 14.1 and 1.9, µg NO_3-N/ (g dry weight root hour), respectively. The nitrification potential of the aerated beds, HAp_in and Hap_out was 56.4-5.2 µg NO_3-N/ (g dry weight root hour). The nitrification potential of the root was measured in micrograms and the gravel was measured in nanograms. In the same way as the gravel sample, the nitrification potential of the outlet greater than the inlet in H25p but this was reversed for the H50p. The inlet nitrification potential of the aerated beds was greater than the outlet roots in the same patterns as the gravel analysis before.

The result of the nitrification potential of the roots was higher than the gravel which might be explained by the high surface area of the root and the high weight and low surface area of the gravel. Besides, roots produce exudates which might be favourable for biofilm formation to support attachment of bacteria like nitrifying bacteria and roots also have air supplies. Bastviken et al.(2003) explained that different surfaces have different capacity in supporting nitrifying bacteria. Gagnon et al (2007) reported that microbes are present on wetland substrates and more on root surface or correlated with the presence of plant roots and with depth. Bodelier et al. (1996) reported that roots have high nitrifying bacteria than on the bare sediment. Generally from the root and gravel nitrification potential sum, H25p had higher nitrification potential than H50p at the outlet side and at the inlet side the H50p nitrification potential was more than the inlet of the H25p. This might indicate that the shallow beds had a high nitrification potential in the direction of the effluent and which means high dissolved oxygen concentrations availability than the H50p bed. This is supported with high oxidation reduction potential profile of the H25p beds towards the outlet than the H50p, H50 and H25 beds. Roots of emergent macrophyte *Glyceria maxima* had a high activities and numbers of nitrifying bacteria (Bodelier et al. 1996).

Therefore, there may be more nitrifying bacteria found at the outlet part of H25p. The root nitrification potential of the inlet of the H50p and aerated beds was greater than the outlet. The explanation previously given for the gravel potential of the aerated plant bed might hold true for the observations made for the root; however, the high nitrification potential of the inflow side than the outflow side of H50p is not clear.

3.4.3 Effect of temperature on the nitrification potential of gravel

Figure 3.49 shows the nitrification potential of the gravel sample collected in February and May 2011. The results were presented in terms of the reduction of ammonia as opposed to the previous presentations because the low nitrate production concentration measurement was difficult than ammonium loss in February 2011.

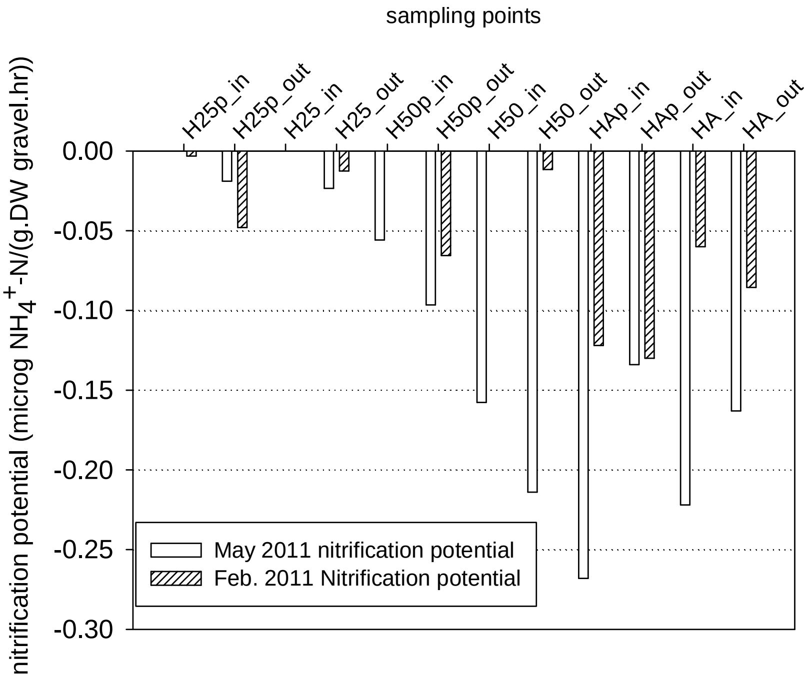


Figure 3.49: Nitrification potential (µg NH_4^+-N/(g.DW-gravel.hr)) of gravel against gravel sample collected in February and May 2011.The negative values are meant negative slope or indicates ammonium concentration removal and the more negative values indicates higher rate of removal of ammonium or have high nitrification potential.

As shown in Figure 3.49, the nitrification potential of the aerated beds excels the passive beds. Except H25p and H25 inlet side, the nitrification potential of all the samples measured in May 2011 was higher than in February 2011 because of the temperature. Nitrification activities and numbers are higher in spring and summer for the root zone of *Glyceria maxima* (Bodelier et al. 1996). The pore water temperature of February during sampling was 3-4^{0}C but it was 20^{0}C in May 2011. According to the review of Truu et al. (2009), nitrification is regulated by temperature because ammonium oxidizers grow faster than nitrite oxidizers above 15^{0}C. Nitrifying bacteria activities reduced at low temperature (Kuschk et al. 2003) and early studies by Brodrick et al (1988) reported that nitrification is inhibited around 6-10^{0}C. Therefore, at low temperature there would be low activities or low number of nitrifying bacteria and during the experiment small number of bacteria oxidize small amount of ammonia resulted in low nitrification potential on the opposite side at high temperature, high mass of nitrifying bacteria in the biofilm so higher nitrification potential observed when they are supplied with excess substrate and oxygen.

3.4.4 Summary

The nitrification potential (ammonium removal rate) experiment is an easy experiment used to predict the nitrifying bacteria distribution and with this indicate oxygen distribution in the wetland. From the nitrification potential experiment of the 25 and 50 cm, 100 cm deep beds, the following conclusions was drawn. There was a clearly visible high nitrification potential difference between the aerated and the non - aerated beds. Planted aerated beds had higher nitrification potential (with the sum of root and gravel biofilm) than unplanted aerated beds and both planted and unplanted aerated systems had higher inflow side nitrification potential than outflow side within the wetland because may be the less amount of ammonia at the outlet might not encourage growth of nitrifying bacteria relatively. When the passive H25p, H50p, H25 and H50 were compared, planted beds had a higher nitrification potential than the unplanted and most of the nitrifying bacteria were found on the root surface of the plants rather than the gravel surface. When the H25p and H50p were compared, the nitrification potential of the outlet side of H25p was higher than the H50p and the inlet side nitrification potential of the H50p was higher than H25p. The result from the Nitrification potential experiment showed that seasonal variation affects the distribution of nitrifiers in the wetlands and therefore in May 2011 the nitrification potential was generally higher than February 2011.

The higher nitrification potential identified for the planted beds and for higher temperature implies higher nitrifying bacteria which favours nitrate formation in the wetlands a requirement for denitrification. This is in support for the higher nitrogen removal measured in the previous and later chapter at the planted beds and at higher temperature conditions.

B. Arba Minch pilot system, Ethiopia

4 Effect of depth and plants on pollutant removal from anaerobic pond effluent

Overview

As a continuation of the research in Germany, the effect of depth and plants in removing pollutants in semi arid climatic conditions was conducted in Arba Minch, Ethiopia. This section describes the performance of 25 and 50 cm deep constructed wetlands filled with gravel media and planted with *Phragmites australis* from July 2012 to March 2013*.* It was found from the previous study in Germany that the areal mass removal rates of the 50 cm deep beds were higher than the 25 cm deep beds when the 50 cm deep beds were loaded at a rate twice as high as the 25 cm deep beds. The results from Germany indicated that the increased loading was the reason for high areal mass removal rate for organic carbon. Therefore, the monitoring study in Arba Minch was conducted to provide further investigation on the same detention time for 25 and 50 cm deep beds and also to investigate whether the same loading rate on the wetlands has an effect on performance, thus the 50 cm deep beds had twice as much detention time as the 25 cm deep beds. The main aim was to study the performance of wetlands (two separate experiments) at the same detention time and at the equal hydraulic loading of 50 cm and 25 cm deep planted and unplanted HSSF wetlands.

4.1 Methods

4.1.1 Site description

Experiments were carried out from July 2012 to March 2013 at the Arba Minch university demonstration site, south of Ethiopia located at N $06^0 2´$ and E $37^0 33´$ at an elevation of 1202 m mean above sea level. The site is located 500 km South of Addis Ababa.

According to the national metrological agency of southern zone (NMASZ, 2006) the town receives mean annual rainfall of 863.7 mm. The climate is characterized by bi-modal distribution, with two rainy and two dry seasons occurring intermittently with erratic rainfall. The first rainy season falls mainly in April and May, and the second, mainly in October. Minimum and maximum average air temperature varies from

17.4^{0}C to 30.5^{0}C and is recorded with an annual average temperature of 24^{0}C based on 37 years of meteorological data from 1974 to 2011.

4.1.2 Construction of the wetlands

The wetlands were constructed with concrete at the bottom and hollow concrete block for the walls and an approximate 1% slope at the bottom. All sides of the bed and the bottom were plastered with cement. Leakage was tested by keeping water within the system for two weeks and monitoring the water level change, when the level was lowered or the walls were wetted from outside, the inside plaster was redone until satisfactory sealing achieved. The outlet and the level of the effluent were controlled with pipes typically used for drip irrigation. Each concrete tank had a surface area of 1.2 m x 0.4 m and a depth of 40 and 60 cm for shallow and deep beds. The wetted depths of the shallow and deep beds were 25 and 50 cm, respectively. Figure 4.1 - 4.3 shows the picture and scheme of the wetlands.

Figure 4.1: Photo of 25 cm and 50 cm deep planted and unplanted wetlands at Arba Minch, Ethiopia. Endemic *Phragmites australis* was used in the system.

The four pilot scale units (Figure 4.1) were built to receive effluent from a primary treatment anaerobic pond at Arba Minch University, Arba Minch, Ethiopia. The wastewater received by the wetland was mainly domestic in nature.

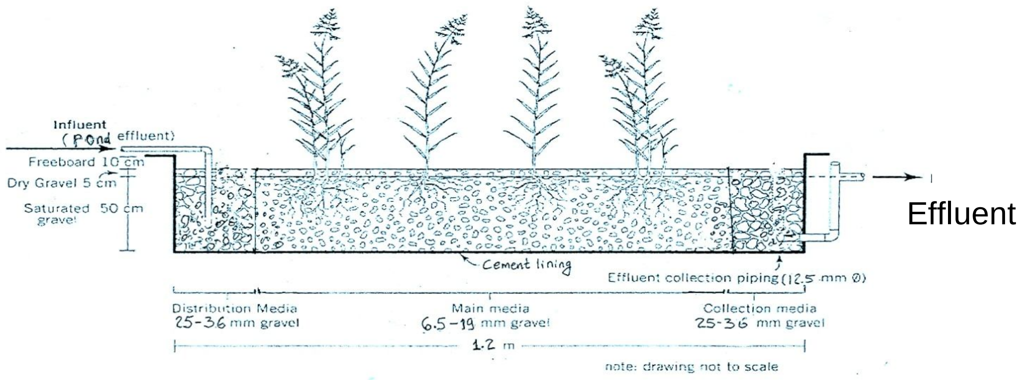


Figure 4.2: Scheme of 50 cm deep constructed wetland with a total surface area of 0.48 m^2

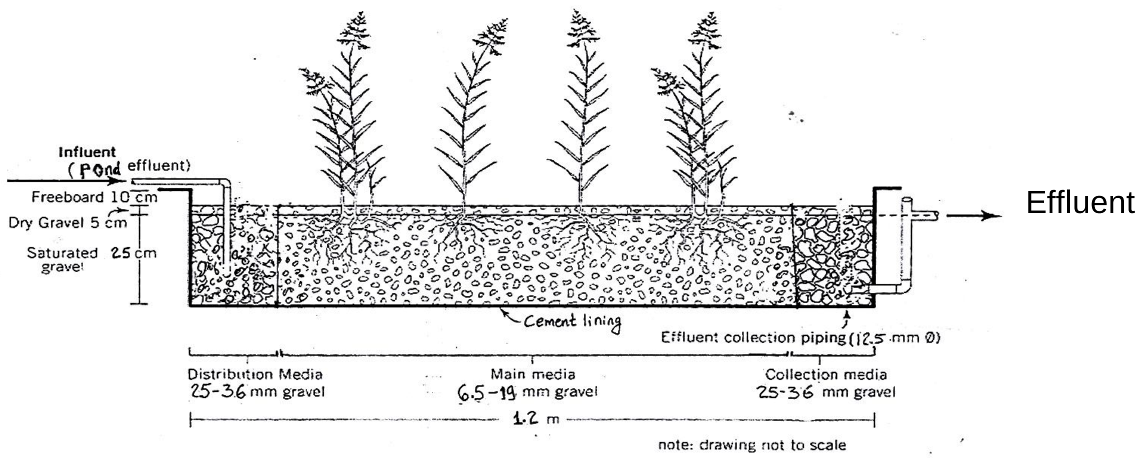


Figure 4.3: Scheme of 25 cm deep constructed wetland with a total surface area of 0.48 m^2.

The beds were loaded from June 2012 to March 2013. Wastewater was initially dosed slowly via gravity from a Jerry can at a rate of 8 and 16 L d^{-1} for the 25 and 50 cm deep wetlands, respectively from June to October 2012. From January to March 2013, the wastewater feeding was done at a rate of 35 litres per day at two equal applications at 8 am and 5 pm. Influent and effluent samples were taken for analysis regularly and the inflow and outflow volumes were measured manually every day.

4.1.3 Media

Plants were grown in 25 cm and 50 cm deep 1.2 m x 0.4 m wide concrete basin in an open field. The main bed was filled with 6.5-19 mm diameter alluvial gravel and the 10 cm closest to the inlet and outlet in each side were filled with 25-36 mm alluvial gravel up to a depth of 30 and 55 mm, respectively and wastewater was filled up to a depth of 25 and 50 cm, respectively. Preparation of the main media was done by sieving with diameters of 6.5 and 19 mm, the media was also washed before it was placed into the wetland beds. The porosity of the main gravel was 38% and had a uniformity coefficient of 1.49.

4.1.4 Planting

Phragmites australis was collected from Kako and Omo River sides 750 km to the south of Addis Ababa. The identity of the plants was confirmed by the Addis Ababa University National Herbarium, Addis Ababa, Ethiopia. The stems of the plant were planted in water saturated wetland gravel bed for about 2 weeks before wastewater was added to the system.

4.1.5 Flow and weather measurements

Wastewater collected from primary treated anaerobic pond effluent was supplied at 5 pm each day from a jerry can with a 6 mm drain pipe from the inflow side of the bed. The jerry can containers placed at the effluent side of each wetland bed were used to measure daily outflow volumes. Effluent collecting jerry cans were cleaned regularly to avoid algae and slime formation. The ET was calculated using Eq 2.18. Metrological data was collected from the Ethiopian meteorology station located at about 1 km away.

4.1.6 Sampling

From July - October 2012, wastewater samples were collected every 15 days after the establishment of the wetland from the inflow and outflow of the pilot wetland and transported to the laboratory for analysis. Samples for physicochemical analysis was analyzed in a water quality laboratory located about 0.6 km from the research site. Most of the parameters were analysed on the day of sampling otherwise they were stored at 4^0C in a refrigerator and analysed as soon as possible. From February-March 2013, sampling was undertaken every week.

Plant biomass: After 1 year of growth, all stems were cut at the gravel surface and their bases washed to remove any adhering sediments. All shoots were counted before cutting them. H25p and H50p wetland roots were excavated and the gravel sorted and wet-sieved to recover below-ground plant material. In the H50p bed the below ground biomass was classified into three classes: 0-20, 20-40 and below 40 cm depths; all H25p below ground mass was collected in one. All above and below ground biomass samples were then dried in the sun and finally oven dried at 70^0 C for 48 hours and weighed with modification from Headley et al.(2012).

4.1.7 Analytical procedures

$CBOD_5$ (un-seeded) analyzed using mercury free WTW oxitop OC100 with controller. N-allyl thiourea was added as a nitrification inhibiter at a rate of 1 drop per 50 ml sample.

COD was analysed with an air cooling condenser with the open reflux method. Concentrated sulphuric acid and potassium dichromate were used with silver catalyst and mercury sulphate chloride inhibiter in the digestion. The excess dichromate ion was titrated with ferrous ammonium salt using ferroin as an indicator (APHA et al. 1999).

TSS were measured gravimetrically by taking measured volume of sample and filtering through 1.5 μm pore diameter glass microfiberfilter. 934-AH™ and then oven dried at 103^0C according to APHA et al. (1999). The empty filter was washed with distilled water and dried at 103^0C before being used for the sample analysis.

Subsamples were filtered with a 25 mm syringe and 0.45 μm nylon membrane filter for nitrate, nitrite, ammonium and phosphate analysis. Nitrate (LCK 339, 0.23-13.5 mg/L NO_3^--N); nitrite (LCK 341,0.015-0.6 mg/L); ammonium (LCK 303, 2-47 mg/L NH_4^+-N, and LCK 304, 0.015-2 mg/L NH_4^+-N) and dissolved reactive phosphate (LCK 349, 0.05-1.5 mg/L PO_4^{3-}-P) analysis were conducted using HACH DR 2800 spectrophotometer following the HACH LANGE procedures. Samples with high concentration were diluted to the measuring range of the reagent and instrument.

Water temperature, DO, conductivity, pH were measured using a portable HQ40d meter (HACH) onsite and in the laboratory. Oxidation reduction potential (ORP) was measured using HACH Sension1 meter with a combination ORP electrode after calibration. The ORP electrodes were standardized against a ferrous–ferric standard. All parameter readings with electrode including ORP were recorded after stable reading was established.

TKN (ammonia and organic nitrogen) were analysed by digestion with strong sulphuric acid and neutralizing with strong base and finally made basic with sodium hydroxide. A twenty-five ml sample of the raw wastewater and 50 ml sample of the effluent were digested in strong acid in the presence of copper catalyst until the volume was reduced, bubble formed and black colour changed to clear. After digestion the solution was neutralized with 45% sodium hydroxide to make the medium basic and promote ammonia evaporation. The mix was connected to a distillation setup with cooling system and the distillate was collected in boric acid solution with an immersed delivery. The solution collected was titrated with standard 0.02 N sulphuric acid until a pink colour was observed. The concentration calculation was based on the volume and concentration of the acid used for the titration. Total N was calculated as the sum of TKN and NOx-N, and organic Nitrogen as TKN less ammonium and theoretical NBOD as 4.3 times TKN (Kadlec and Knight 1996). Ammonium nitrogen was also measured by distillation method (APHA et al. 1999).

Areal mass removal rates for $CBOD_5$, COD, TSS, TKN and ammonia were calculated using Eq. 3.2. Volumetric mass removal was calculated by dividing the areal removal by the depth and porosity of the wetlands (mass unit volume of water not per unit volume of bed).

4.1.8 Bacteriological and *Ascaris lumbricoides* egg analysis

The concentration of *Ascaris lumbricoides* eggs in the inflow and outflow of the wastewater was conducted using the modified Bailenger method applied to wastewater Ayres and Mara (1996). Two litres of sample was used from the inflow wastewater and 10 litres of water was taken from the treated effluent for the analysis. The samples were first kept for sedimentation for 4 hours and then siphoned from the top part of the sample up to a 500 ml sample volume. The sediment was then transferred into smaller containers and further concentrated with centrifuge while washing with 0.1% Tween 80 when transferring. The final volume was transferred into a McMaster slide for microscope count. The number of eggs L^{-1} in wastewater was subsequently determined by the McMaster slide in a microscope.

Total coliforms bacteria was analysed with nutrient pad with Lauryl sulphate medium (Teepol from Sartorius). One ml of 1000 times serially diluted sample was filtered into a 0.45 μm pore filter and placed in a nutrient pad on petridish incubated at 36^0C for 18-24 hr. Total coliform bacteria forming 1-2 mm diameter yellow colonies surrounded by yellow zone were then counted.

Enterococci bacteria was analysed with a nutrient pad with a culture medium (Azide from Sartorius™). One ml sample of 1000 fold serially diluted sample was filtered with 0.45 μm pore filter and placed in a sterile distilled water wetted Azide nutrient on Petri-dish incubated at 36^0C for 40-48 hrs. Enterococci form red, pink or reddish brown colonies with a diameter of 0.5-2 mm were counted. Enterocci are considered indicator organisms of faecal contamination. Enterococcus spp. took the place of faecal coliform as the new federal standard for water quality at public salt water beaches and *E. coli* at fresh water beaches (U.S.EPA 2004). Jin et al. (2004) suggests that enterococci provide a higher correlation than faecal coliform with many of the human pathogens often found in city sewage. In the bacteriology analysis the Sartorius™ company procedures were used.

4.1.9 Evaluation of rate coefficients and θ from the P-k-C* model

The P-k-C* model was applied to calculate rate coefficient (k) at 20^0C and θ for water quality parameters of $CBOD_5$, TN, NH_4^+-N and TKN for average in and outflow concentrations July-October 2012 and for Feb- March 2013 in Arba Minch wetland. The C* (Table 2.4) were taken from literature Kadlec and Wallace (2009) and P was assumed to be 3 since tracer results were not available. θ and k at 20^0C were put into the combined Eq 2.43 and Eq 2.36 using *SOLVER tool from MS Excel*™ 2007 to minimize the sum of squares errors (SSQE) between the measured and predicted concentration values of the effluent. Detailed explanation is given in section 3.1.5.

4.1.10 Statistical analysis

Statistical procedure was carried out using SPSS® statistics version 16 software package (IBM Corporation, USA). Linear mixed model (LMM) with repeated measures methods was used to check the significance of performance with areal and volumetric mass removal rate of H25, H25p, H50 and H50p beds for TSS, $CBOD_5$, COD, TKN and NH_4^+-N. LMM with repeated measures was chosen rather than ANOVA because the data do not satisfy independence which is the fundamental assumption of ANOVA. The significance were checked at $\alpha=0.05$.

4.2 Results and discussion weather and water balance

Overview

The study in Arba Minch, Ethiopia in total took approximately one year starting from the planting of the *Phragmites australis* to the final monitoring day. Two different amounts of wastewater were loaded onto the 25 and 50 cm deep wetlands during the study period: July - October 2012 with same detention time and from January - March 2013 with same inflow rate feeding. From July to October 2012 eight and sixteen litres per day of wastewater were loaded for 25 and 50 cm deep wetted depths, respectively, and 35 litres per day was loaded from February - March 2013 for all wetlands. In this Section, the water quality monitoring results of the two loading approaches are presented separately with the first being between July – October 2012 and the second between February - March 2013. In the result and discussion, the water balance of the wetlands is reported for both situations is presented then the pollutant removal rates of the major water quality parameters, areal and volumetric mass removal of the wetlands were discussed with relevant tables and graphs for each monitoring times. Finally, the biomass production of the wetlands was measured and discussed and the possibility of reuse of the treated wastewater and the conclusions are provided.

4.2.1 Weather summary

Arba Minch is located in dry climatic conditions of the southern rift valley. The average temperature in Arba Minch is $24^{0}C$ and the maximum was up to $36^{0}C$ during Feb – March 2013 besides the air was dry from January to March, refer Figure 4.4. The Figure shows the average monthly relative humidity from Jan 2011 to March 2013. The relative humidity of the town was around 40% between Jan- March and it was between 60 and 70 % in the rest of the year.

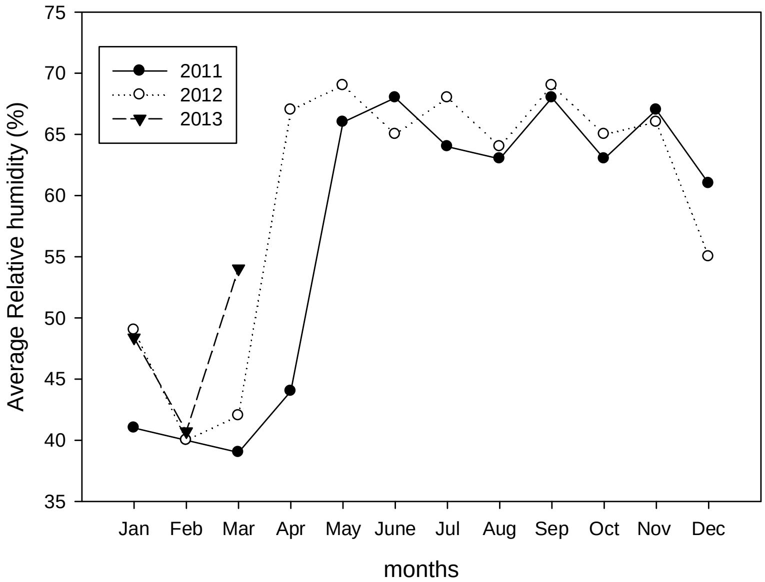


Figure 4.4: Relative humidity of Arba Minch, Ethiopia from January 2011 - March 2013.

Figure 4.4 and 4.5 are presented here in order to explain the influence of the weather on wetland performance at the site. Although the average monthly relative humidity of March 2013 was 54, most of the humidity data of the first two weeks of the month ranged from 30 - 40% and it increased later in the second half of the month because of the start of the rainy season. The rainfall pattern of the experimental site is bimodal peak in around April and October, refer to Figure 4.5.

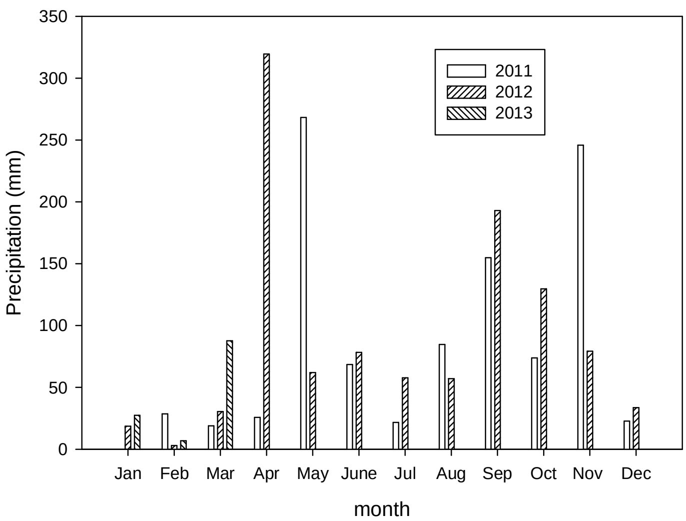


Figure 4.5: Rain patterns of Arba Minch from January 2011 - March 2013. Data was collected from the local metrology station located 1 km from the experimental site.

4.2.2 Water balance and evapotranspiration

In Table 4.1 the inflow, outflow and ET values of the monitoring from July - October are presented. In the Table, Qo is based on 7 day averages. inflow, outflow and the ET value of the wetland during February- March 2013 are also presented.

Table 4.1: Arba Minch wetland average inflow, outflow and ET calculated from the measured flows during monitoring in mm/day. ET was calculated using Q (unplanted)- Qo (planted) equation 2.18.

<table>
<tr><th rowspan="2">Treatment systems</th><th rowspan="2">Area of wetland, m²</th><th colspan="3">July- October 2012 (mm/day)</th><th colspan="3">Feb- March 2013 (mm/day)</th></tr>
<tr><th>q_i</th><th>q_o</th><th>ET</th><th>q_i</th><th>q_o</th><th>ET</th></tr>
<tr><td>H25</td><td>0.48</td><td rowspan="2">16.7</td><td>16.6</td><td>0.1</td><td rowspan="4">72 - 76</td><td>66.7</td><td>0.2</td></tr>
<tr><td>H25p</td><td>0.48</td><td>5.2</td><td>11.4</td><td>14.0</td><td>52.6</td></tr>
<tr><td>H50</td><td>0.48</td><td rowspan="2">33.3</td><td>32.6</td><td>0.7</td><td>71.3</td><td>0.5</td></tr>
<tr><td>H50p</td><td>0.48</td><td>14.7</td><td>17.9</td><td>0.3</td><td>71</td></tr>
</table>

From July to October 2012 the ET of the planted beds was 11.4 (68% of influent flow) and 17.9 mm/d (54% of influent flow) for H25p and H50p, this respectively implies that the H50p loses more water than H25p beds. However, there was a situation where the H25p bed was not producing effluent more often than the H50p. When the influent wastewater is compared with ET, the H25p was losing higher percentage of water than the H50p.

During February and March of 2013, monitoring was undertaken at the same inflow rate for deeper and shallow beds, the ET rates of H50p was about 93% of the influent flow and that of H25p was 72 % of the influent flow which is very high. Garfi et al. (2012) reported an ET of up to 95% of the influent flow at Mediterranean weather for wetlands. Ranieri (2003) reported an ET losses of up to 40 mm/day for a 2000 m^2 wetland planted with *P. australis* in the hottest summer days in South East Italy (semi arid conditions). The high ET rates in the Arba Minch study were largely a function of the small plot of the wetlands which likely led to enhanced ET rates due to oases and clothesline effects. The clothesline effect contributes to high ET because the plant (in this case *Phragmites*) height in the H25p and H50p wetlands was greater than that of the surroundings and the oasis effect was also contributing as the wetland vegetation had higher soil water (saturated media) availability which implies different moisture conditions (Allen et al. 1998). Refer Figure 4.4. The clothesline and oasis effects contributes to the peak K_c (coefficient) values exceed the values of 1.20-1.40 where, $ET=K_c*ET_0$. This is actually working for areas less than 2000 m^2, in comparison to this area the wetland had only an area of 0.48 m^2. However, the ET measurements from small plots and vegetation stands should not be extrapolated to larger stands or regions as an overestimation of regional ET may occur. Nevertheless, the ET measurement showed us high water loss in the region of study, it may be advisable based on ET to use unplanted wetland system to save water for reuse. U.S.EPA (2000) reported that unplanted systems have been found to perform as well as planted systems in regard to $CBOD_5$ removal.

4.3 Result and discussion on performance at the same detention time

Overview

The monitoring of the Arba Minch wetland working at the same detention time was conducted between July - October 2012. All the common water quality parameters were monitored from inflow and outflow of H25, H25p, H50 and H50p wetlands. The result of pH, conductivity, dissolved oxygen, oxidation and reduction potential, BOD_5, TSS, Kjeldahl nitrogen, ammonia, nitrate, nitrite, phosphate and *Ascaris lumbricoides* are presented from Section 4.3.1 to Section 4.3.9.

4.3.1 pH, conductivity, DO and ORP

The pH of the outflow of the H25, H25p, H50, and H50p is shown in Table 4.2. The Table shows that the values were 7.94, 7.50, 8.06 and 7.77, respectively. Generally all the differences were small, it was seen that the H25p and H50p have lower pH than the inflow pH values, this may be because of a reduction in the ammonium. However, the H25 and H50 beds showed higher pH than the inflow 7.78. In the case of the increase in pH of the unplanted beds, the alkalinity production due to sulphate reduction bacteria explained in section 3.3.1 can be a reason.

In the case of the conductivity values, the planted beds showed higher concentrations than the unplanted beds. But the difference was not big because of the relatively low temperature conditions and the presence of agricultural crops nearby the wetland between July-October 2012 might have minimized effect of the oasis and clothesline effects. Besides, the relative humidity between July - October in the area was around 60-70 % in contrast to the relative humidity of Feb- March 2013 which was about 40% as shown in Figure 4.4.

In Table 4.2, the dissolved oxygen values of the effluent of H25 and H50 beds were higher than the H25p and H50p beds at the same depth; however, the oxidation reduction potential of the planted beds was higher than in the unplanted beds although the difference was small. In addition, the oxidation reduction potential of the H25p beds was higher than the H50p. The oxygen and the ORP values of the wetlands were higher than the horizontal subsurface flow wetlands due to the oxygen supplied by the drawdown of water level and fill created by ET and feeding, respectively. Zhang et al. (2012) reported ET causes water level fluctuation and favour oxygenation of the HSSFCW in tropical environment. Additional dissolved oxygen and ORP may be ascribed to the source of the inflow wastewater which was from effluent of anaerobic pond in open air conditions, prevailing water temperatures and relatively low load favouring oxidation.

4.3.2 Carbonaceous Biochemical Oxygen Demand

From Table 4.2 shown, the $CBOD_5$ of the wetlands effluents were below 30 mg/l with an average inflow concentration of 92 mg/L. From the results, the percentage concentration removal of the unplanted beds was higher than the planted beds when compared with in the same depth. The higher effluent concentrations of the planted beds were influenced by ET and internal input. The $CBOD_5$ of the H25 beds were lower than H50 because shallow depth favoured by air diffusion for oxidation of organic matter.

When the areal mass removal rates of the wetlands are compared as shown in Figure 4.6, H50 (2.4) and H50p (2.6) removed almost twice as much of the $CBOD_5$ than the H25 (1.2) and H25p (0.96). The result was a statistically different effect of depth on areal mass removal at α=0.05. The areal mass removal rate of the 50 cm deep beds were twice that of the 25 cm deep beds which is correlated to the high load of the 50 cm deep beds. The areal mass removal rate of wetlands increase with the increase of the mass loading rate (Tanner et al. 1995).

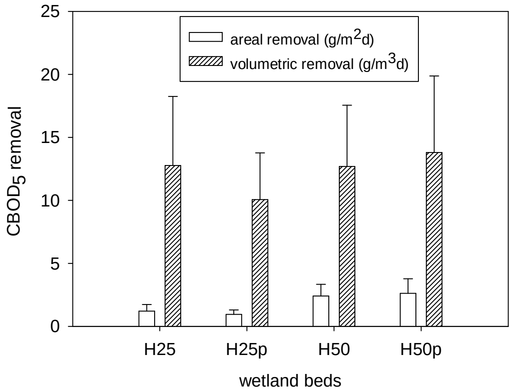


Figure 4.6: $CBOD_5$ areal and volumetric mass removal from July - October 2012 conducted at the same detention time.

Depth was not affecting the areal mass removal rate. But there was small difference on the presence of plants although not statistically significant. The volumetric mass removal rate was 12.8, 10.0, 12.7, and 13.8 g/m^{3}d for H25, H25p, H50 and H50p, respectively were not significantly different between all beds.

4.3.3 Total suspended solids

The areal and volumetric mass removal of TSS is presented in Fig 4.7. The TSS mass removal for H25, H25p, H50, H50p 1.0, 1.0, 2.0 and 2.2 (g.TSS/m^{2}d), and 10.3, 10.8, 10.3 and 11.4 g TSS/(m^{3}d), respectively.

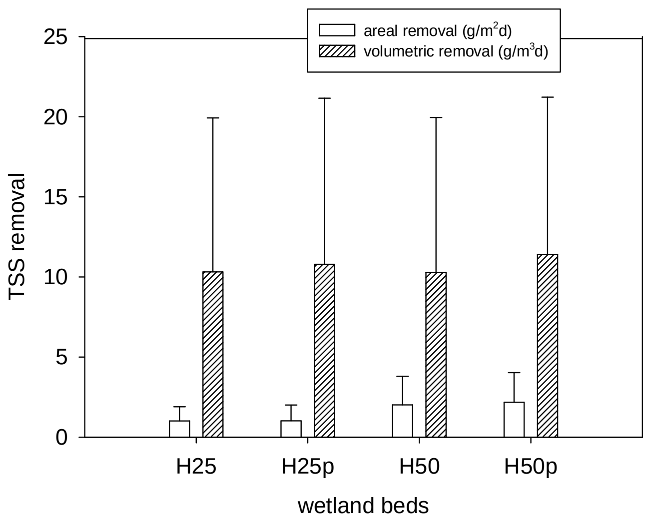


Figure 4.7: TSS areal and volumetric mass removal from July - October 2012 conducted at the same detention time.

However, there was no effect of plant and depth significantly at α=0.05 at areal and volumetric mass removal rate.

Table 4.2: Concentration of the inflow and outflow values of the 25 and 50 cm deep beds with inflow rate of 8 and 16 litre/ day from July - October 2012. ORP was not changed to standard conditions. Average temperature of the water temperature was 22.5^{0}C. (pH, conductivity, DO, Redox potential, n=8; TSS, $CBOD_5$, n=9; PO_4^{3-}-P, n=5; NH_4-N, n=7; TKN, n=6)

parameter	influent	H25	H25p	H50	H50p
pH	7.79 ± 0.31	7.94 ± 0.28	7.50 ± 0.25	8.06 ± 0.23	7.77 ± 0.18
Cond.(µs/cm)	1305	1025	1386	1043	1358
DO, mg/L	3.1 ± 1.9	5.4 ± 1.3	4.9 ± 1.6	5.5 ± 1.0	5.3 ± 1.2
ORP	107.5 ± 45.3	125.2± 35.4	141.2 ± 36.5	118.5 ± 39.4	130.4 ± 35.1
$CBOD_5$, mg/L	92 ± 141	19 ± 25	28 ± 47	20 ± 30	27 ± 32
TSS, mg/L	62 ± 51	5 ±2	10 ± 5	5 ± 3	9 ± 5
PO_4^{3-}-P, mg/L	10.97 ± 2.65	5.65 ± 1.33	3.57 ± 2.38	4.49 ± 2.07	3.33 ± 0.73
TKN, mg/L	56.8 ± 25.2	11.4 ± 6.3	5.4 ± 4.6	17.0 ± 5.0	12.4 ± 8.1
NH_4^+-N, mg/L	50.2 ± 20.4	7.9 ± 2.6	0.02 ± 0.01	9.7 ± 3.7	4.7 ± 1.0
NO_3^-- N, mg/L	0.34 ± 0.09	3.01 ± 2.49	0.30 ± 0.08	0.88 ± 0.65	0.29 ± 0.04
NO_2^- -N, mg/L	0.03 ± 0.03	0.61 ± 0.6	0.02 ± 0.01	0.21 ± 0.30	0.04 ± 0.03
A. lumbricoides egg	35 ± 30	← Not detected →			

4.3.4 Total Kjeldahl nitrogen

TKN based on Table 4.2 values of the inflow was 56.8 mg/L and the percent concentration removal was 80%, 91%, 70%, 78% for H25, H25p, H50 and H50p, respectively. The removal of nitrogen is high because of the prevailing temperature. Garfi et al. (2012) reported the ammonia removal as much as 99 % in at Barcelona in summer Mediterranean environment. In contrast to the other parameters, shallow beds had a higher concentration removal than deep wetlands and the H25p had the highest concentration removal. Depth and plant effect can be observed in TKN. Nitrogen removal in subsurface flow constructed wetlands is affected by temperature, vegetation type, the properties of the medium and hydraulic detention time (Kuschk et al. 2003, Akratos and Tsihrintzis 2007). Akratos and Tsihrintzis (2007) showed the significance of nitrogen removal rates at temperature more than 15^0c when compared with those observed at lower temperature.

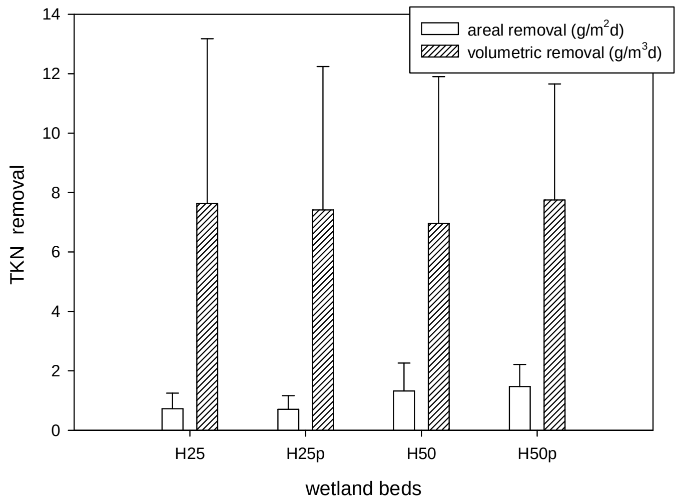


Figure 4.8: TKN areal and volumetric mass removal from July - October 2012 conducted at the same detention time.

Referring Figure 4.8, the areal mass removal of the wetlands was compared H50 (1.3) and H50p (1.4) beds had values twice as much of the H25 (0.7) and H25p (0.7) beds in performance and the planted beds were better than the unplanted beds in the 50 cm beds. The TKN areal mass removal rate of the deeper beds was different significantly at α=0.05. The 25 cm deep beds were loaded twice as much as that of 50 cm deep beds. In support to this Tanner et al. (1995) reported that removal rates increased with an increased mass loading rates. Here depth does not matter but loading rate. From the volumetric mass removal rate H25 (7.5), H25p (9.2), H50 (7.0) and H50p (7.8), the plants showed higher removal; however, all were not significantly different at α=0.05.

4.3.5 Ammonium and nitrate

Ammonium concentration percentage removal for H25, H25p, H50, H50p beds were 84.2%, 99.9%, 80.7%, and 90.6%, respectively. The removal of ammonium of the planted beds was higher than the unplanted beds. The major pathway ammonia is removed as nitrogen gas is by biological nitrification/ denitrifcation microbiological processes and plants assimilate about 10 percent of the nitrogen removed by wetlands (U.S.EPA 1993). In Table 4.2, the ammonium removal was high and the net nitrogen denitrified is the difference of inflow and outflow concentration of TKN and deducting 10% of the TKN removed assimilated in planted beds and net nitrate produced and the calculated denitrified nitrogen was 42.2, 46.3, 39.7, 40 mg/L for H25, H25p, H50 and H50p beds, respectively. Denitrification is one of the major organic matter removal mechanisms and nitrification was favoured by presence of oxygen and high ORP conditions. The oxygen required for nitrification of ammonia is from diffusion from air water interface and/or root oxygen release and therefore the planted beds were better than the unplanted beds. H25p was better removed ammonia because of the root and wastewater in contact. Denitrification estimated to be highest in shallow beds (27 cm deep beds) (García et al. 2004). Additional oxygen was supplied for nitrification in the planted bed by the drain of the water level by ET. Zhang et al. (2012) reported ET causes water level fluctuation and favour ammonia or TKN removal in tropical environment.

The ammonium removal of the H25 was better than H50 although they were operated at the same residence time because the air diffusion at air water interfaces on the shallow beds.

As shown in Figure 4.9, the areal mass removal rate of the H50 (1.3) and H50p (1.5) beds were higher than H25 (0.7) and H25p (0.8) but they were not significantly different at $\alpha=0.05$. Similarly, the volumetric mass removal ranged from 7.1 to 9.2 for the four wetlands was not different significantly. Most studies have shown that planted wetland systems achieve higher treatment efficiency than unplanted filters for nitrogen compounds. The vegetation had mostly a positive effect, i.e., supports higher treatment efficiency for nutrients such as TKN, NH_4 and TP. This could be due to plant uptake and increase of oxygen supply to plant roots as compared to unplanted filters.

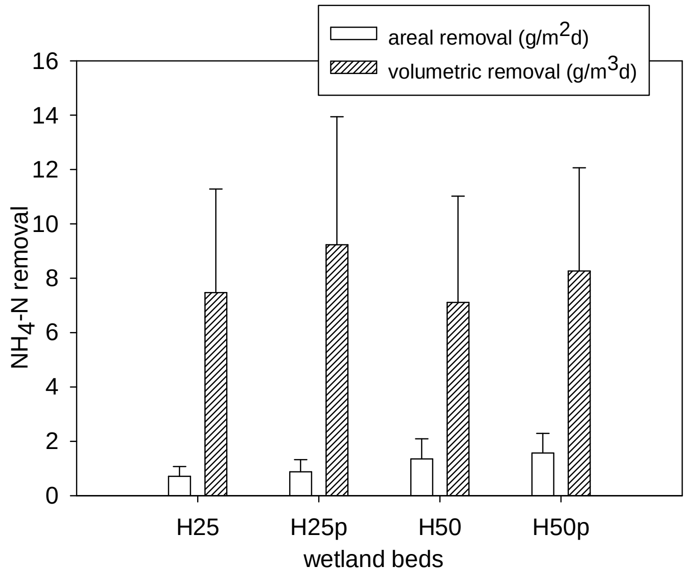


Figure 4.9: NH_4-N areal and volumetric mass removal from July - October 2012 conducted at the same detention time.

When nitrate and nitrite concentration values are considered, from Table 4.2, it was found that unplanted beds had higher nitrate concentrations than planted beds in their effluent, in their respective depth.

3.3.6 Phosphate

Phosphate is one of the nutrients required for plant growth and does not have a phase transformation mechanism like nitrogen. The mechanism of phosphorous removal in wetlands could be plant/microbial uptake, precipitation and complexation, fragmentation and leaching, mineralization and burial (Vymazal 2007, Garcia et al. 2010). As the values indicated in Table 4.2 planted beds had higher concentration removal when compared to the unplanted beds.

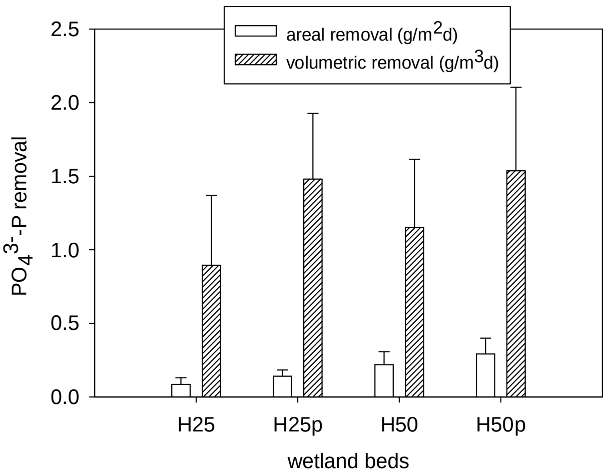


Figure 4.10: PO_4^{3-} - P areal and volumetric mass removal from July - October 2012 conducted at the same detention time.

When areal mass removal is compared among the wetlands as shown in Figure 4.10, H25 (0.09), H25p (0.14), H50 (0.22), H50p (0.29) there was statistically significant difference of depth and plant on the phosphate ion removal except H25 and H25p bed at α=0.05. However, there was significant effect of plants in volumetric mass removal rate but there was no depth effect at significance level of α=0.05.

4.3.7 *Ascaris lumbricoides* egg

Besides the study of physico-chemical removal of pollutants with respect to depth and plant effect and relevance to developing country, the helminth removal analyses of the wastewater were conducted using *Ascaris lumbricoides* as surrogate, results in Table 4.2. There are several kinds of helminthiases in wastewater, *Ascaris lumbricoides* ova is the most common and is endemic in Ethiopia and most developing countries. *Ascaris lumbricoides* is the most prevalent parasitic infection (Kadlec and Wallace 2009). From the experimental results of the *Ascaris lumbricoides* eggs analysis, it was found that there was a complete removal of the egg from the effluent of four of the wetlands. Ascaris level of the effluent wastewater had an average of zero (not seen) egg count. Helminth egg removal in HSSFCW is efficient (Mara and Horan 2003). This meets the WHO guideline for irrigation water which is less than one helminth egg per litre (WHO 2006b). This might suggest that the wetland in association with the anaerobic pond has the potential to remove helminth egg irrespective of the depth and presence of wetland plants. In other research work, the removal rate of planted gravel beds in tropical and subtropical regions (Mexico and two UK sites) dosed with settled sewage had 100% *A. lumbricoides* removal in both sites (Rivera et al. 1995). The same authors reported the removal of Giardia as 100%. HSSF CW is effective in reducing the number of eggs of the parasites in the range of 79-100% presumably because of settling, filtration, interception and predation (Kadlec and Wallace 2009). In lagoon, most of the egg removal is by sedimentation and high concentrations of protozoan cysts and parasite eggs are detected at bottom of in the biosolids (Bouhoum et al. 2000).

4.3.8 Removal rate coefficients at the same detention time

In Table 4.3, first order areal and volumetric rate coefficients and temperature factor were calculated based upon the period of record for average input and output from July – October 2012 for Arba Minch wetland. The results were in the range of the literature values (Kadlec and Wallace 2009). The k rate coefficient $CBOD_5$ of the 50 cm deep beds were twice as much of 25 cm beds and their k_V was almost the same. k_A and k_v for unplanted beds were better than planted. The value of the rate coefficient of the planted beds were less than unplanted, not because of the wetland were performing badly but because of the high net loss of water from the wetland and concentration of the effluent was considered in the calculation. This has to be considered especially in arid and hot climates as the effect might be there for nitrogen compounds but their performance increased with plants (Kadlec and Wallace 2009).

Table 4.3: Areal and volumetric rate coefficients at 20^0C and temperature factor of HSSFCW calculated for $CBOD_5$, TN, TKN & NH_4^+-N using *P-k-C** model. The 50 and 25 cm beds were at the same hydraulics detention time.

Parameter		H25	H25p	H50	H50p
$CBOD_5$	k_A (m/d)	0.043	0.029	0.084	0.065
	k_A (m/yr)	15.7	10.4	30.8	23.7
	k_v (d^{-1})	0.453	0.3	0.444	0.342
	θ	0.987	0.99	0.972	0.946
TN	k_A (m/d)	0.024	0.051	0.039	0.06
	k_A (m/yr)	8.6	18.8	14.1	21.8
	k_v (d^{-1})	0.248	0.542	0.901	0.315
	θ	1.043	1.041	1.046	1.028
TKN	k_A (m/d)	0.033	0.078	0.049	0.077
	k_A (m/yr)	12.1	28.3	18	28.3
	k_v (d^{-1})	0.348	0.817	0.26	0.408
	θ	1.082	0.956	1.034	0.963
NH_4^+-N	k_A (m/d)	0.042	0.07	0.073	0.126
	k_A (m/yr)	15.4	25.5	26.8	46.1
	k_v (d^{-1})	0.445	0.736	0.386	0.665
	θ	1.004	1.05	0.997	0.976

The k_A of NH_4^+-N and TN of the 50 cm deep beds was higher than the 25 cm deep beds. The k_V was almost the same. k_A and k_v of planted beds were better than unplanted although ET. This is because nitrogen affected by microbial, plant uptake and ET. For TKN, k_A and k_v of the planted better than unplanted beds but the k_A of the H25p and H50p were the same.

4.3.9 Summary

When the performance of H50 and H50p at (q=33.3 mm/d) and H25 and H25p (q=16.6 mm/d) wetlands were compared with respect to effect of depth and plant impact at the same detention time in semi arid conditions the following output were concluded:

- Areal mass removal of the $CBOD_5$, TSS, TKN, NH_4^+-N and PO_4^{3-}-P of the 50 cm deep beds were higher than the 25 cm deep beds and their volumetric mass removal rates were not significantly different. However, there was an effect of the presence of plants on the volumetric mass removal of TN,TKN, PO_4^{3-}-P and NH_4^+-N, although not significant.

- The removal of *A. lumbricoides* was 100% whether the HSSFCW was shallow, deep, planted or unplanted.
- The k_A coefficient $CBOD_5$ of the 50 cm deep beds were twice as much of 25 cm beds and their k_V was almost the same. k_A and k_v of unplanted beds were better than planted.
- The k_A of NH_4^+-N, TN of the 50 cm deep beds was higher than the 25 cm beds. The k_V was almost the same. k_A and k_v of planted beds were better than unplanted.
- For TKN, k_A and k_v of the planted better than unplanted beds.

4.4 Results and discussion on performance at the same hydraulic loading rate

Overview

In this section the performance of the H25, H25p, H50, H50p beds were evaluated from February – March 2013, at the same hydraulic loading rate and nominal detention time of 1.3 and 2.6 days for 25 cm and 50 cm deep wetlands, respectively. The same amount of load was added to compare the performance with respect to depth and plants. The result of pH, conductivity, dissolved oxygen, ORP, COD, BOD_5, TSS, Kjeldahl nitrogen, ammonia, nitrate, nitrite, phosphate, total coliform and enterococci are discussed from Section 4.4.1 to Section 4.4.11. The temperature was very high and dry season in the area.

4.4.1 pH, conductivity, DO, ORP

Referring to Table 4.4, the pH of H25, H25p, H50 and H50p effluents were modified when compared with the influent wastewater values. The effluent pH of the H25 and H50 beds were increased more than the influent and on the contrary the pH of the H25p and H50p decreased in comparison to the influent pH. The pH of H50p was lower than the H25p which may be because the H50p bed had a high nitrate concentration. Nitrification consumes alkalinity and produces H^+ ion which results in a reduction in alkalinity and pH (Kadlec and Wallace 2009). In this work, the decomposition of the macrophyte at high temperature conditions might be responsible to the pH modification of the planted beds as well. In the case of the increase in pH of the unplanted beds, the alkalinity production due to sulphate reduction bacteria explained in section 3.3.1 can be a reason.

Table 4.4 presents the conductivity results, the values of the H25 and H50 beds were lower than the inflow wastewater. However, the H25p and H50p conductivity values were higher with average values of 10184 μs/ cm and 17034 μs/cm for H25p and H50p, respectively. The increase of conductivity can be attributed to high water loss due to ET because of the prevailing temperature during the experiment, when the clothesline and oasis effects may have occurred between February and March 2013. The relatively low electrical conductivity of the H25p when compared with

H50p can be attributed to the detention time of H50P being twice that of H25p high ET. Total ionic salts may have been altered by physical processes of dilution and the concentration may have been affected by precipitation and evaporation which are known to be major influences (Kadlec and Wallace 2009). Therefore, electrical conductivity may be an indicator of the influence of ET on the wetland.

From Table 4.4, the average oxygen concentration in the inflow wastewater was 2.2 mg/L which was higher than fresh wastewater because the wastewater was collected from primary treated anaerobic pond but the average water temperature was 25^0C. The DO of the effluent of the treated wastewater was about twice that of the influent and H25p beds had more dissolved oxygen than the H50p bed. This might be due to the air/water interface and effect of plant root.

The ORP shows the state of the medium in the anaerobic, aerobic or anoxic conditions. From Table 4.4, the overall inflow ORP was -53.6 mV and the average effluent ORP of all the four beds were very close to each other but the planted beds had higher values in the same depth. The positive values of the effluent ORP happens eventually as the wastewater was getting closer to the exit. For instance, in one internal ORP profile analysis of the H50p bed (37, -199.3, 3.5, -11.7 mV for raw wastewater, inlet side, middle and exit side, respectively) first decreased as far as -200 mV and increased gradually to the effluent side. The relatively higher ORP of the H50p in Table 4.4 might be due to the higher hydraulic detention time and ET than was occurring in the H25p. Higher ET caused water levels to decrease, resulting in higher ORP values (Burgoon et al. 1995). The water levels drop because of the feeding was every 12 hours interval.

4.4.2 Total suspended solids

When the TSS of the influent and effluent were compared in Table 4.4, the concentration of the effluent for the H25p and H50p was higher than H25 and H50 in relation to their respective depths. However, the TSS concentration of the H50p effluent was more than the H25p bed because of the concentration influenced by the ET caused a greater detention time in H50p. Therefore, the removal percentage of TSS of the unplanted beds was higher than the planted. A white precipitate also formed when the effluent of planted wetlands water was kept at room temperature which contributed to high TSS. Therefore, plants had a negative effect on TSS removal or introduce organic matter and 50 cm beds produced more suspended solids than shallow beds at the same inflow rate.

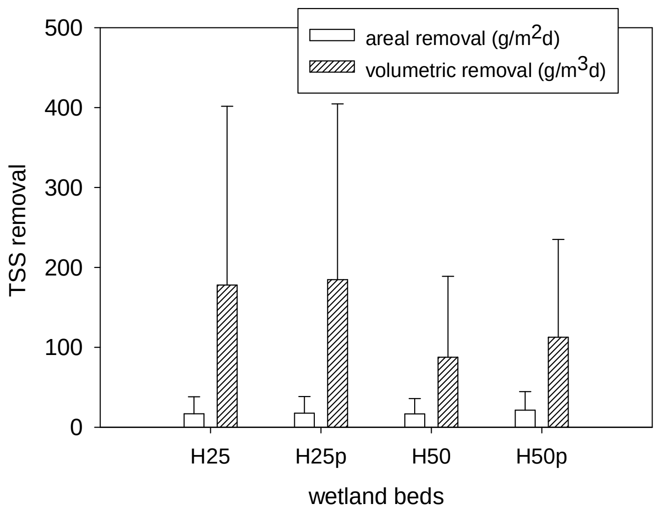


Figure 4.11: TSS areal and volumetric mass removal from February- March 2013. Inflow was 35 litres per day for both 25 and 50 cm wetted wetland depths.

From Figure 4.11, the areal mass removals of TSS for all beds were compared, they were not statistically different at α=0.05. And the same was true for the volumetric mass removal although the 25 cm deep beds had TSS relatively higher values.

Figure 4. 12: The effluents of H25, H25p, H50, H50p and inflow from left to right, in the order.

As shown in Figure 4.12, the H25p and H50p beds effluent has more orange colour than the inflow wastewater and the unplanted effluents, and the colour was most pronounced for H50p. The colour was not removed when filtered with a 0.45 μm filter which might indicate that the soluble matter was dissolved plant extract or it is the true colour of the water not apparent colour which is not an input from suspended matter (APHA et al. 1999). Decomposed wetland plants (organic compounds) result in the “tea” coloured effluents (Tanaka et al. 2011). However, when the same sample was kept at room temperature, a white precipitate was formed but this phenomenon was not observed when the sample was kept in a refrigerator. The colour of the effluent might have come from organic matter that was falling into the media and degraded.

4.4.3 Carbonaceous Biochemical Oxygen Demand

As shown in Table 4.4 the $CBOD_5$ concentration removal of the H25, H25p, H50, and H50p were 69%, 58%, 72% and 20%, respectively, which indicates that the concentration removal of the planted beds was lower than the unplanted beds. This was because the planted beds might have added new carbon load from the debris of the plant and the concentration of the pollutant increased by ET. The deep colour and turbidity supports this explanation. Although plants are helping in the pollutant removal they also have the potential to reintroduce new pollutants to the environment called internal loadings (Kadlec and Wallace 2009, Garcia et al. 2010). Compared to other forms of wastewater treatment systems, in planted wetlands BOD is produced from plant decay (U.S.EPA 1993). The percent concentration removal of the H50 was higher than the H25 beds because the residence time of the H50 was twice as much as H25 bed.

When areal mass removal rate is compared in Figure 4.13, the planted beds showed slightly more removal but generally they were not different significantly from each other. In addition, although the volumetric mass removal rate of the shallow bed was higher than the deeper beds, they were statistically not different at $\alpha=0.05$.

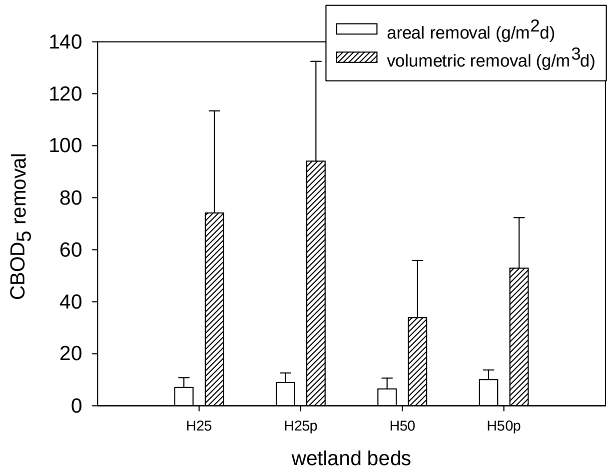


Figure 4.13: $CBOD_5$ areal and volumetric mass removal from February - March 2013. Inflow was 35 litres per day for both 25 and 50 cm wetted wetland depths.

Table 4.4: Overall treatment performance for inlet and outlet data of pH, DO, conductivity, ORP, $CBOD_5$, COD, TSS, PO_4^{3-}-P, TKN, NH_4^+-N, NO_3^-- N & NO_2^- -N for 25 cm & 50 cm deep planted and unplanted beds averaged from February - March 2013. n=5, inflow=35 L/day. The average wetland water temperature was 25.2^{0}C. ORP was not converted to standard conditions.

parameter	influent	H25	H25p	H50	H50p
pH	7.8 ± 0.3	8.1 ± 0.2	7.7 ± 0.2	8.2 ± 0.1	7.5 ± 0.1
Cond. (µs/cm)	2411.4	2018.8	10184.0	1998.8	17034.0
DO, mg/L	2.2 ± 2.18	3.6 ± 2.46	4.3 ± 1.08	3.9 ± 1.96	4.1 ± 0.55
ORP	-53.6 ± 155.5	12.1 ± 71.6	39.1 ± 36.1	39.1 ± 28.6	52.7 ± 24.2
TSS, mg/L	280 ± 297.8	45.2 ± 21.7	186.8 ± 54.4	48.6 ± 35.7	255.6 ± 129.7
$CBOD_{5\,,}$ mg/L	228.7 ± 225.2	69.9 ± 75.9	96.9 ± 51.4	64.2 ± 46.5	183.8 ± 64.9
COD, mg/L	304.0	134.4	555.2	105.6	984.0
PO_4^{3-}-P, mg/L	23.3 ± 7.7	18.0 ± 3.3	9.5 ± 5.9	16.5 ± 1.6	6.4 ± 1.8
TKN, mg/L	159.6 ± 62.7	77.5 ± 15.2	31.1 ± 7.5	84.1 ± 9.2	46.5 ± 17.3
NH_4-N, mg/L	130.8 ± 49.0	67.8 ± 14.6	6.6 ± 6.4	71.4 ± 12.9	2.36 ± 2.6
NO_3^--N, mg/L	0.79	0.79	154.57	0.92	197.8
NO_2^- -N, mg/L	0.06 ± 0.05	0.10 ± 0.06	0.51 ± 0.33	0.10 ± 0.02	0.99 ± 0.74

4.4.4 Chemical Oxygen Demand

As shown in Table 4.4, the COD concentration of H25p and H50p were compared among the wetlands and it was found that the COD of the effluent was higher than the influent wastewater. When H25p and H50p beds were compared, the COD values of the H50p bed were higher than the H25p. This could be due to the high detention time of the H50p and high ET and equal influent loading as was discussed for the previous parameters, $CBOD_5$ and TSS. The percent concentration removal of the H50 was higher than the H25 beds because the residence time of the H50 was twice as much as H25 bed. In the planted beds, drain because of ET and filling while loading supports oxygenation of the bed but did not help the COD and BOD removal rather it increased its concentration. Although it was not to this extent draining by ET did not help COD removal according to Zhang et al. (2012) supports our results.

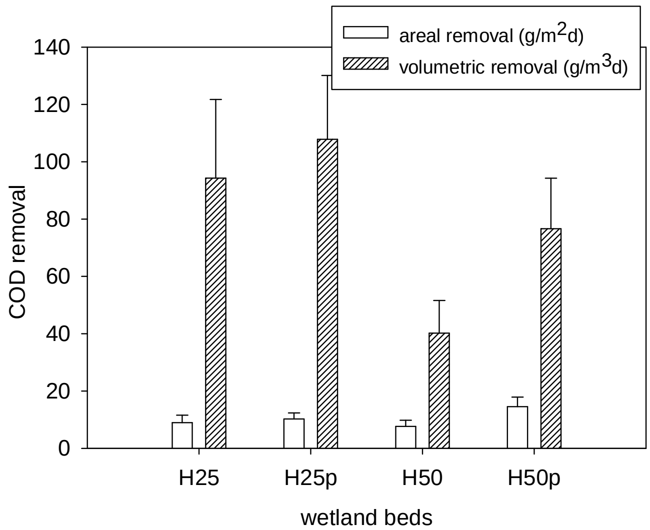


Figure 4.14: COD of areal and volumetric mass removal from February - March 2013. In flow was 35 litres per day for both 25 and 50 cm wetted wetland depths.

From Figure 4.14, both the areal and the volumetric mass removal of COD were not statistically different at $\alpha=0.05$ for the compared depth and plant effect. However, the 25 cm deep beds volumetric mass removal rate was greater than 50 cm deep beds.

The ratio of COD to BOD_5 in domestic wastewater is normally between 1.5 to 2 (APHA et al. 1999); however, this ratio was 5.4 for H50p and 5.7 for H25p beds. The reported settled sewage mean ratio of COD to BOD_5 is 1.88 to 2.04 (Mara and Horan 2003). On the other hand, the calculated COD:$CBOD_5$ ratio was 1.32, 1.6, and 1.65 for the influent wastewater, H25 and H50 effluent, respectively. This is in agreement with the literature (APHA et al. 1999). These results indicated that the high ratio (COD:$CBOD_5$) of the planted beds had internal input from the macrophytes or there was a significant impact from the ET. As a supportive observation to this statement, the wetland one year old had new shoots which were growing and old ones which were dying. Figure 4.12 shows of the planted beds potentially due to vegetation decay.

4.4.5 Total Kjeldahl Nitrogen

A TKN concentration from Table 4.4 shows that H25p had greater removal when compared to the rest of the wetlands. The concentration percentage removal was 80.5>70.9>51.4>47.3 for H25p>H50p>H25>H50 in the order shown. The 25 cm deep planted bed was effective in reducing the concentration and showing better performance than the unplanted counterparts. The percent concentration removal of the H25 was better than the H50 because the better air/ water exchange or diffusion of oxygen favours nitrogen removal via nitrification.

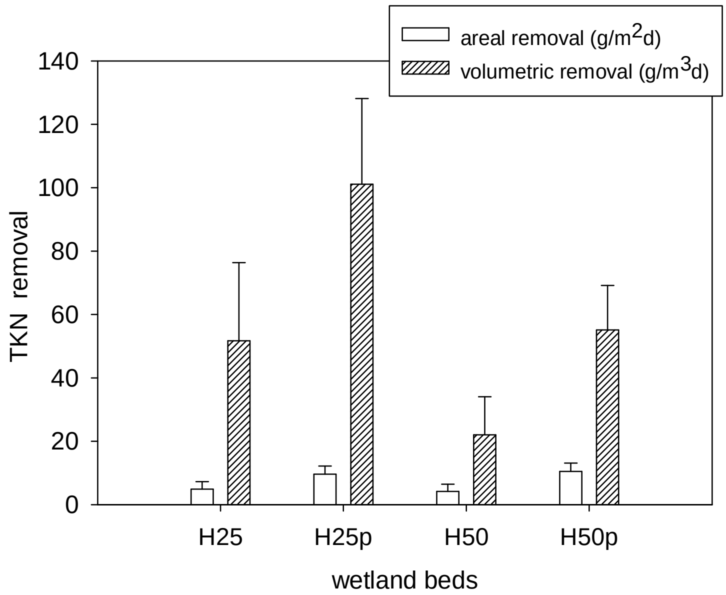


Figure 4.15: TKN of areal and volumetric mass removal from February - March 2013. Inflow was 35 litres per day for both 25 and 50 cm wetted wetland depths.

From Figure 4.15, the areal and volumetric mass removal rates are presented. The effect of plants on the mass areal and volumetric removal of TKN was significantly different at $\alpha=0.05$. However, there were no effect of depth at $\alpha=0.05$ between H25 and H50 and between H25p and H50p on the areal mass removal rate. The volumetric mass removal rate was significantly different with the depth of the wetlands.

4.4.6 Ammonia

From Table 4.4, the ammonia concentration percentage removal was 48.2, 95, 45, and 98.2, for H25, H25p, H50 and H50p, respectively. The concentration percentage removal of ammonia in the planted beds was higher than the unplanted beds. The ammonium removal was high because of Nitrification. Nitrification was very much enhanced in the planted beds due to the high ET lowered the water level in the bed and favoured oxygenation of the medium. The effect of ET in nutrient removal in tropical wetland was reported in support of this result by (Zhang et al. 2012).

The net ammonium denitrified is the difference of inflow and outflow concentration of TKN deducting 10% removed TKN assimilated in planted beds and net nitrate and nitrite produced gave 82.1, -38, 75.3, -96 mg/L for H25, H25p, H50 and H50p beds, respectively. Based on these results, the planted beds produced extra nitrate in the effluent when mass balance checked which showed introduction of nitrogen from another source, plant decomposition is more likely as it was seen from deep orange colouration of the planted effluent. The net positive increase of nitrate beyond the amount of inflow TKN in the inflow could be also the anaerobic decomposition followed by nitrification of organic nitrogen trapped in the bed (U.S.EPA 1993). However, the inability of the denitrification of nitrate produced is because the high dissolved oxygen in the wetland is preferred electron acceptor than nitrate. Denitrifying bacteria are facultative aerobes that use nitrate when there is no oxygen as electron acceptors (Garcia et al. 2010). However the unplanted beds showed higher dentitrification than the planted.

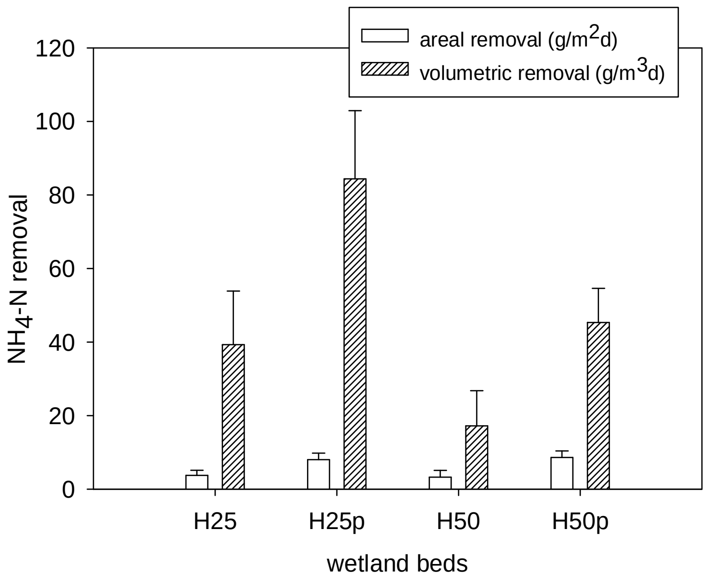


Figure 4.16: Ammonium nitrogen of areal and volumetric mass removal from February-March 2013. In flow were 35 litre per day for both 25 and 50 cm wetted wetland depths

The percent concentration removal of the H25 was better than the H50 because the air/ water exchange or diffusion of oxygen in H25 (shallow depth) favours ammonium removal via nitrification.

Referring Figure 4.16 and statistical result, the areal mass removal of the planted beds were higher than the unplanted wetlands significantly at $\alpha=0.05$, however, they were significantly not different with depth (H25/H50) and (H25p/H50p). The volumetric mass removal rates of all beds were significantly different with depth and plants.

4.4.7 Nitrate and nitrite

In Table 4.4, the concentration of nitrate were 154.5 mg/L for H25p and 197.6 mg/L for H50p and 0.79, 0.92 mg/L for H25 and H50 beds, respectively. The high nitrate concentration for H25p and H50p is correlated to tidal flow by ET of the wetlands. The tidal flow created in the system was because it was loaded on two occasions to fill and drain down by ET during the day favouring aeration. The effect of ET in nutrient removal in tropical wetland was reported in support of the high nitrification in the planted beds due to bed aeration by drawdown of the water level (Zhang et al. 2012). According to Garcia et al. (2010), the non uniform distribution of ORP conditions can be caused by different factors in the presence of plant root systems and the fluctuation of the water level due to ET and tidal flow conditions. This was responsible for nitrification. However, the areal mass removal rate was almost a zero increase in nitrate-N.

The nitrate amount from the H25p and H50p effluent is very high for irrigating purposes. This result indicates that the high concentration of the effluent will be harmful for plants irrigated with this water. Usually, wastewater is required as a supplement to irrigate land during water stressed periods so the planted beds irrespective of depth made the water less useful under some circumstances. The increased salinity levels may reduce the fertility of the land within a short time. Nitrogen is a plant nutrient and available in the form of nitrate, ammonium and nitrite for plant uptake. If excessive quantities of nitrogen are present or applied, the production of several commonly grown crops may be upset because of over-stimulation of growth, delayed maturity or poor quality (Ayers and Westcot 1985). Sensitive crops may be affected by 5 mg/L nitrogen concentration but most are relatively unaffected until nitrogen exceeds 30 mg/L (Ayers and Westcot 1985). As a solution, may be the hydraulic load of the wetland has to be increased in order to reduce the effect of increase in concentration of nitrate in the effluent by ET or unplanted beds might be used.

Although it was not like nitrate, the nitrite value increased to a maximum of 2 mg/L for the H50p. Nitrite is an intermediate and less stable form of nitrogen and has a harmful effect on the environment. The majority of the plant biomass might be returned back to the system as organic nitrogen increases nitrogen. This undergoes ammonification and nitrification and denitrification involves the intermediate nitrite involvement (Kadlec and Wallace 2009).

4.4.8 Phosphate

Phosphate is one of the most important plant nutrients. From Table 4.4, the amount of phosphate in the influent wastewater was 23.3 mg/l and the H25 and H50 beds effluents had 18 and 16.5 mg/L, respectively whereas the H25p and H50p beds had 9.5 and 6.4 mg/l in their effluent. This indicates that the phosphate concentration was reduced in the wetlands. It may also be due to the plants absorbing the phosphate for their growth or it might have precipitated or adsorbed to the substrate as there was no other sufficient source and phase transformation of phosphate in the system. Besides the effluent concentration of the H25 was higher than the H50 beds because the residence time of the H50 was twice as much as H25 bed. H50p has higher phosphate removal than H25p because the H50p had twice the residence time of H25p affected by high ET which favours oxidized condition and enhanced precipitation of phosphate such as iron. The presence of plants favours the removal phosphate at high ET conditions (Zhang et al. 2012).

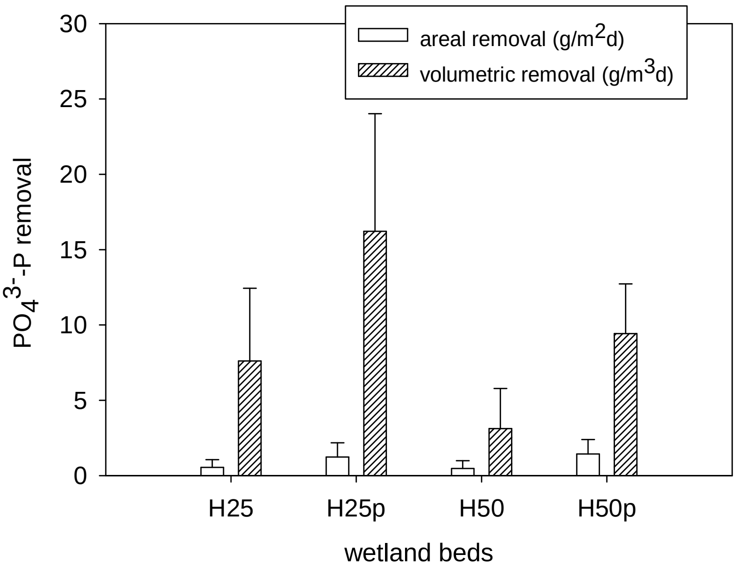


Figure 4.17: PO_4^{3-}-P of areal and volumetric mass removal from February - March 2013. In flow were 35 litres per day for both 25 and 50 cm wetted wetland depths

In Figure 4.17 the areal and volumetric mass removal of phosphate are presented and indicate that the planted beds had significantly greater than the unplanted beds. There was no significant difference of effect of depth in areal and volumetric mass removal of phosphate except between volumetric mass removal of H25p and H50p beds.

4.4.9 *Enterococci* and Total coliform

Enterococci and total coliform were analyzed using membrane filtration methods with Azide and Teepol nutrients at 36^{0}C. The log mean concentration of *Enterococci* of the influent, H25, H25p, H50 and H50p were 5.46, 5.06, 5.05, 5.53 and 3.26, respectively. The log concentration removal of the H25, H25p were below 1 and H50 effluents were increased more than the influent. Log *Enterococci* removal of H50p was 2.2 because of the increased HRT by ET. From the results, it can be generalized that the *Enterococci* removal efficiency was poor for the wetlands and below log 1 except for H50P. The *Enterococci* log reduction in HSSFCW with mixed vegetation is in the range of 0.3 to 2.4 at the same load and detention time (Kadlec and Wallace 2009). But log reduction of 2.4 was reported for the bed depth of 0.3 m and 1.5 log reductions were reported for a depth of 0.6 meters.

The log transformed concentration of total coliform values of 5.79, 5.01, 5.15, 5.85, and 4.77 were for inflow, H25, H25p, H50 and H50p, respectively. The total coliform log removal of the H25 and H25p beds were below 1 and the H50p bed was 1.02. H50 effluent showed an increase in concentration of the total coliform in the same manner as for *Entrococci*. However, from the results, it was hardly possible to conclude which wetland system was more efficient in removing the indicator bacteria. One reason may be the low detention time.

According to Vymazal et al. (2008), the log removal of total coliform based on 75 entries is 0.93 and *E. Coli* log removal based on the 48 data entries is 0.76. These show that bacterial removal in HSSCW is low. Which this possibly explain the low removal of the *Enterococci and* Total coliforms*.* The low residence time was responsible for the reuslts. The removal of indicator bacteria, Faecal coliform is dependent on the hydraulic residence time (Tanner et al. 1998, García et al. 2003).

4.4.10 Removal rate coefficients at the same loading rate

In Table 4.5, the rate coefficient were calculated based upon the average of the influent and effluent concentrations during hot temperature conditions and the net loss of wetland water was not considered which is indicated by the negative values of the COD and TN rate coefficient values for the planted beds and high load of wetlands. TN values reported here calculated from TKN, ammonium, nitrate and nitrite concentrations.

Table 4.5: Areal and volumetric rate coefficients at 20^0C and temperature coefficient of HSSFCW calculated for BOD_5, COD, TN, TKN and NH_4^+-N using P-k-C* model. The 50 and 25 cm beds were working at the same hydraulics loading rate. The experiment was conducted at highest temperature season in Arba Minch.

Parameters		**H25**	**H25p**	**H50**	**H50p**
$CBOD_5$	k_A (m/d)	0.136	0.085	0.168	0.036
	k_A (m/yr)	49.8	30.9	61.4	13
	k_v (d^{-1})	1.437	0.891	0.886	0.188
	θ	0.967	0.981	0.948	0.883
COD	k_A (m/d)	0.079	-0.381	0.13	-0.467
	k_A (m/yr)	28.9	-139	47.5	-170.4
	k_v (d^{-1})	0.832	-4.009	0.685	-2.457
	θ	0.986	0.689	0.956	0.732
TN	k_A (m/d)	0.052	-0.009	0.045	-0.028
	k_A (m/yr)	19	-3.4	16.3	-10.2
	k_v (d^{-1})	0.549	-0.098	0.235	-0.146
	θ	1.023	1.023	1.026	1.007
TKN	k_A (m/d)	0.054	0.147	0.043	0.115
	k_A (m/yr)	19.7	53.8	15.7	42
	k_v (d^{-1})	0.569	1.55	0.226	0.606
	θ	1.019	1.018	1.035	0.998
NH_4^+-N	k_A (m/d)	0.057	0.401	0.063	0.431
	k_A (m/yr)	20.8	146.2	23.2	157.4
	k_v (d^{-1})	0.601	4.217	0.334	2.269
	θ	0.99	0.988	0.958	1.061

From the Table 4.5, the $k_{A,20}$ rate coefficients for COD, $CBOD_5$ and TKN of H25p was higher than the H50p and of H50 was greater than H25, which is the direct effect of ET. The rate coefficients of the planted beds for TN and TKN were greater than the unplanted at the same depth. The rate coefficient of the planted for the COD and TN was negative because the effluent was more than the influent due to ET and introduction of pollutants. High nitrate production is responsible for the high effluent TN. Planted beds had a higher removal rate for TKN and NH_4^+-N but the opposite was true of the unplanted beds which had high kinetic coefficients for COD and

$CBOD_5$. The additional pollutant made the rate coefficient comparison for planted systems unreliable. Some comparison of $k_{A,20}$ values from the literature are, Rousseau et al. (2004) k_A of 0.06 to 1.00 m/d and k_V of 0.17-6.11 day^{-1} for BOD_5, Villasenor et al. (2011) 0.22 to 0.83 d^{-1} for COD which indicates rate coefficient is not constant. k_A, k_V and θ are not constant because they lump a large number of internal and external characteristics which are the complex interaction in constructed wetland and external like influence of weather conditions (Rousseau et al. 2004).

4.4.11 Summary

Four of the beds were supplied with the same flow rate, thus creating equal hydraulics loadings, 1.3 days and 2.6 days detention time for 25 cm deep and 50 cm deep beds, respectively. The monitoring results are summarized as follows:

- The concentrations of the COD, $CBOD_5$ and TSS in the planted beds effluent were higher than the unplanted beds.
- Plants had a strong effect on the removal of TKN, ammonia and phosphate In regard to outlet concentration.
- Planted beds had shown increased nitrate in the effluent but the mass removal rate was almost zero.
- The areal mass removal of COD, TSS, $CBOD_5$, TKN, ammonium nitrogen and phosphate were performing almost equally in all the wetlands. There was the effect of depth, with the deeper bed performing much more poorly by about a factor of two when volumetric mass removal was calculated for all parameters. This difference being a result of the hydraulic load variation.

Garcia et al.(2005) had done similar experiment with 27 and 50 cm deep wetlands and loaded at the same HRT in Spain at air temperature range 6.1 - 25^0C, and found that COD, BOD_5, NH_4^+-N and phosphate of the shallow bed showed better concentration percent removal. In this experiment, the result indicated that at the same loading for H25p and H50p percent removal of the shallow beds were better only for $CBOD_5$, TKN and TSS. The main reason could be that the average working temperature of the water in Arba Minch was 25.2^0 C and with range of air temperature 18^0C to 35^0C. So the analysis was conducted based on mass removal basis to take care of the ET effect. The areal mass removal of $CBOD_5$, COD, TKN, TSS and phosphate of the H50p was a little bit more than the shallow beds and nitrite and nitrate concentration of H50p were more than H25p. The difference observed between this result and Garcia et al (2005) could be the working high temperature which enhanced tidal flow.

4.5 Plant biomass

At the end of the experiment the total phragmites biomass was determined after removing the entire biomass and drying, the results are presented in Table 4.6. The density of the vegetation was higher at the inlet side of the treatment systems. Above ground stem densities was 852 stems/ m^2 for the H25p and 841 stems /m^2 for H50p. These values were twice as much as reported for the same type of wetland systems in Germany (Nivala 2012), which were 400 and 430 stems/m^2 for 25 cm and 50 cm deep beds, respectively.

Table 4.6: Arba Minch wetland above and below ground biomass of *Phragmites australis*, planted in March 2012 and collected on 14 March 2013.

Description		H50p, g/m^2	H25p, g/m^2
Number of plant stems/m^2		842	852
Above ground biomass		15583.3	13458.3
Below ground biomass	0-20 cm	5141.7	---
	20-40 cm	875	---
	40-50 cm	125	---
	0-25 cm	---	5150

H25p and H50p were planted in March 2012 from the stems of *Phragmites australis*. The growth of the plants increased throughout the year and second generation were starting to grow around January 2013. The results of biomass measurement of *Phragmites australis* in non tropical climates ranged from 1800-9900 g/m^2 dry weight Vymazal and Kröpfelová (2008) indicates that it was less than the values in Table 4.6. This may be because of the continuous development of the plants in this experiment. The ratio of above ground to below ground biomass was 2.61 for H25p and 2.54 for H50p systems, which was in the range of emergent plants ratios reported by Tanner (1996) which was 0.35 to 3.35.

From Table 4.6, the below ground biomass was 5150 g/m^2 for H25p and 6141.7 g/m^2 for H50p wetland systems based on the actual whole biomass analysis. From H50p roots data, the distribution of the plant roots was found in the depth ranging from 0-20 cm, 83% of the below ground mass. The remaining 14 % was found in 20-40 cm interval and only 2% for roots mass collected below 40 cm. This was in agreement with researchers who report that about 85% of the below ground biomass resided in the top 20 cm possibly due to a reduced need to seek nutrients (Headley et al. 2003, Headley et al. 2005). U.S.EPA (2000) investigations of root depth and flow pathways have found that the roots do not fully penetrate to the bottom of the media and there was substantially more flow under the root zone than through it.

4.6 Reuse of constructed wetland effluent in Arba Minch study

When wastewater reuse is considered, quantity and quality of the wastewater after treatment with the wetland should be taken into consideration. According to the experimental results in Arba Minch the quantity of the wastewater was very much reduced by ET to use it for irrigation especially in dry seasons (January- March 2013). This time of the year is a critical time in which the prevalence of high temperature, no precipitation or lowest river flow and conflict for the irrigation water increased among the farmers and pastoralists. The plant biomass may however present a good source of animal feed.

The possibility of reuse of the wastewater effluent in irrigation from the wetland effluent was reduced because the water quality changes in conductivity and nitrate during the water stressed times of the year. Conductivity (salinity) of the water increased by 10 fold and other water quality parameters like nitrate also increased. The measured conductivity was more than 3 dS/m and NO_3^--N was more than 30 mg/L which is more than the FAO irrigation water guideline shown in Table 4.7 make the effluent unsuitable for irrigation. Salinity is an impediment for most crops although there are a few crops can grow with highly saline water. Salinity can also permanently damage the usefulness of the soil for farming. Excessive salinity stunts the crop by reducing the availability of soil-water, slowing crop growth and restricting root development (Allen et al. 1998). Draining down of salinity is impossible in the current context as there is not enough technical capability or additional water supplies to do that in this part of Ethiopia.

Table 4.7: Guidelines for interpretation of water quality for irrigation (Ayers and Westcot 1985).Other parameters were excluded as they were not analyzed in this experiment

Potential irrigation problem	**units**	**Degree of restriction on use**		
		None	Slight to moderate	Severe
Conductivity	dS/m	<0.7	0.7 – 3.0	> 3.0
NO_3^--N	mg/ L	<5	5-30	>30

As a general conclusion, although it is dangerous to conclude with 0.5 m^2 wetland but indicating, for semi-arid areas like Arba Minch planted wetlands are not an appropriate wastewater treatment Technology for reuse application. So since the performance of the unplanted subsurface wetlands is good, they can be used for treatment in that area besides waste stabilization ponds which have water loss by ET. Coleman et al. (2001) also reported that gravel alone provides significant wastewater treatment although vegetation further improved treatment efficiencies. Subsurface flow wetlands without plants shallow or deep have a major advantage in such cases to prevent vectors breeding, reduce human exposure to contamination and protect water from evaporation. The health of workers and consumers of the products irrigated by the treated wastewater can be protected when subsurface wetlands are used. Direct contact from contaminated surfaces, accidental ingestion of wastewater, consumption of raw vegetables irrigated with wastewater, and long term exposure in the vicinity of spray irrigation are the exposure routes for the pathogens (Bitton 2005). Unsafe water and poor sanitation, including inadequate sanitation facilities responsible for 80 percent of diseases in developing countries (UN 2013). There are also reports for instance Nokes et al. (1992) found that helminth reduction programmes in schools can have a dramatic impact on health and learning among school children.

4.7 Clogging

As shown in the internal analysis (LRB) in Figure 3.38 and 3.39, an increase in concentration of the total suspended solids in the first half of the wetland was observed. This point will be the clogging point, loss of conductivity. When the 25 and 50 cm deep beds loaded at 72 mm/day (Arba Minch), the performance of the two depth wetlands was not different; however the 25 cm deep beds will be clogged faster because of the limited void volume. This will result to the reduction of hydraulic conductivity because of the entrapment of suspended matter. Hydraulic conductivity decrease in HSSFCW after some time of operation because of clogging (Garfi et al. 2012).

Plant roots also contribute for reducing conductivity of water in wetland. Since all the root of the macrophytes were located in the entire volume of the 25 cm deep bed, given the same time and same volume of wastewater, the 25 cm deep planted beds obviously clogged faster than 50 cm deep planted beds. Although Fisher (1990) reported that hydraulic conductivity problem arises by biomat formation in the gravel, the presence of plant roots and rhizomes in the HSSFCW beds have a negative effect on the hydraulic conductivity of the medium and favours bottom short circuiting in deeper beds (50 cm in this case) (Breen and Chick 1995, Tanner and Sukias 1995). Therefore, the 25 cm deep planted beds can be exposed to surface flooding as there is no chance for bottom short cutting as 50 cm deep beds (no media without plant root in 25 cm beds).

4.8 Summary

The side by side comparison of the 25 and 50 cm deep planted and unplanted gravel beds were studied from July 2012 - March 2013. From July – October 2012, four of the beds were working with the same detention time. From Feb- March 2013, four of the beds were working with the same flow rate, thus creating equal hydraulic loading rate, but a doubled retention time in the 50 cm deep beds.

Most of the results in this chapter are presented in areal and volumetric mass removal rates rather than concentration. In the parameters analysed like $CBOD_5$, COD the use of concentration as indicator of performance is misleading because of the prevailing evapotranspiration. Because of ET losses, concentration is a poor indicator of performance (Burgoon et al. 1995). In that case areal load removal rate is quite useful although it is weak in increasing with increase of hydraulic loading without delivering lower concentration at the outlet (Headley et al. 2013).

The main results summarized at the same detention time are:

- pH from the planted effluent was lower than the influent and the unplanted effluent was greater than the inflow. Conductivity of the planted beds effluent was greater than the influent.
- DO of the unplanted bed were higher than the planted but ORP of the planted beds greater than unplanted beds
- The phosphate removal of the planted beds was higher than the unplanted because of the effect of the plant.
- Areal mass removal of the $CBOD_5$, TSS, TKN, ammonium nitrogen and phosphate of 50 cm deep bed was better than the 25 cm deep bed , by about a factor of 2. However the planted beds were better than unplanted beds for TKN, ammonium nitrogen and phosphate although it was not significant.
- Ascaris *lumbricoides* ova were 100% removed irrespective of the wetland planted, unplanted, deep or shallow wetlands.

The main results summarized at the same hydraulic loading are:

- pH and conductivity were the same like the case when the wetlands were working at the same detention time, but conductivity of the planted beds were higher than unplanted beds by about a factor of 9 of the inflow conductivity.
- The dissolved oxygen and ORP of the planted bed were higher than the unplanted.
- The areal mass removal rate of COD, TSS, $CBOD_5$, TKN, ammonium and phosphate were performing almost equally in all the wetlands. There was a very strong effect, with deeper bed performing much more poorly by about a factor of two when volumetric mass removal was calculated for all parameters. This difference can be attributed to the increased hydraulic load.
- Plants had a strong effect on the removal of TKN, ammonia and phosphate when compared with the outlet concentrations.

- Plants had a strong effect in increasing the nitrate and nitrite concentration but the mass removal was almost zero
- High ET in the planted wetlands may result in increased pollutant concentrations which might not be good if reuse is a priority and the region is semi arid. However, the ET results obtained in this experiment cannot be extrapolated for the region because of exaggerated values due to the small size of the wetland but it is a good indicator.
- The biomass production of the wetlands for the deep and shallow beds were almost the same, 15 kg/m^2 and 83% the underground biomass (root) of the H50p was found within top 20 cm depth.

5 Synthesis, Conclusions and Recommendations

5.1 Synthesis

Overview

Experiments were conducted on the effect of depth and plants in 25 and 50 cm deep horizontal subsurface flow constructed wetlands in Langereichenbach, Germany (LRB) and Arba Minch, Ethiopia. In Germany, four systems with a surface area of 5.64 m^2 and depth of 25 and 50 cm deep with the same detention time were monitored from September 2010 - September 2012. During this time, tracer studies, performance of removal of main water quality parameters and nitrification potential of the wetland components were conducted. In Arba Minch, four constructed wetlands with a surface area of 0.48 m^2 and depth of 25 and 50 cm deep were built. The four constructed wetlands were monitored first with the same detention time and later with the same hydraulic loading rates from July 2012 to March 2013.

The main aim of the research was to determine the effect of depth and plants on the performance removal of pollutants under different climatic condition, same retention time and same hydraulic loading rates. The main findings of the research are summarized.

Effect of depth on hydraulics in HSSFCWs

From the tracer experiments conducted at LRB, parameters delay time (t_d), normalized variance (σ_θ^2), normalized detention time (λ_t), normalized peak time (λ_p), and number of tanks in series (NTIS) were compared statistically for the H25, H25p, H50 and H50p beds. The research data demonstrates that there was effect of depth on the hydrodynamics of 25 cm and 50 cm deep HSSFCW and the hydraulic efficiency of 25 cm deep beds were significantly better than 50 cm deep beds.

Effect of plants on hydraulics in HSSFCWs

Based on the tracer studies, the planted beds had shown higher detention time and relatively higher NTIS than the unplanted beds. So plant roots had an effect in modifying the hydraulic characteristics of the wetlands. However, at the same depth, there was no significant effect of plants on the hydraulic efficiency of the wetlands based on the parameters t_d, σ_θ^2, λ_t and λ_p.

Effect of climate on hydraulics in HSSFCWs

Since tracer studies were not conducted in Arba Minch it is not possible to compare with enough evidence, however the following qualitative explanation can be given.

In semi arid climate the hydraulics of the small-scale HSSFCW were sometimes changed into a tidal effect because of the high ET which occurred most of the year. This increased the detention time of the wastewater and increased the concentration of some pollutants (like $CBOD_5$, COD) in the effluent when compared results from the temperate climate. This situation also increased the oxidation of nitrogen into nitrate and nitrite. The effect was much exaggerated for the planted beds.

Effect of depth on treatment performance in HSSFCWs

The effect of depth on the treatment performance of H25p, H25, H50 and H50p compared in Table 5.1 and 5.2. Table 5.1 summarizes the effect of depth on the pollutant areal and volumetric mass removal of the wetlands working at the same detention time and Table 5.2 summarizes the effect of depth on the pollutant areal and volumetric mass removal of the wetlands working at the same hydraulic loading rates.

Table 5.1: Summary of the **effect of depth** on areal and volumetric mass removal rates at the same hydraulic detention time. Symbols: Significantly different (**+**), significantly not different (**-**).

LRB	Areal mass removal rate		Volumetric mass removal rate	
	H25 - H50	H25p - H50p	H25 - H50	H25p - H50p
TOC, $CBOD_5$, *E.coli*	+	+	-	-
TN	+	+	-	+
NH_4-N	-	-	-	+
Arba Minch				
$CBOD_5$, PO_4^{-3}-P	+	+	-	-
TSS, NH_4^+-N	-	-	-	-
TKN	+	-	-	-

Areal mass removal rates at the same hydraulic detention time

In LRB

- TSS, $CBOD_5$, *E.coli*, PO_4^{-3}-P and TN were statistically different with depth in the performance of the wetlands. The areal mass removal of $CBOD_5$, TOC and *E.coli* were different with 25 and 50 cm deep beds because of the hydraulic loading rate were different.

- Ammonia mass removal rate was not significant depth wise.

In Arba Minch

- $CBOD_5$, PO_4^{-3}-P were significantly different depth wise and TKN was significantly different with unplanted beds but was not different with planted beds
- TSS and NH_4^+-N were not significantly different depth wise

Volumetric mass removal rates at the same hydraulic detention time

In LRB

- TSS, $CBOD_5$, *E.coli* and PO_4^{-3}-P were not different from each other in performance because the root system and the air contact did not increase removal rates.
- TN, and NH_4^+-N were statistically different between H25p and H50p but they were the same when comparison was made between H25 and H50 beds. The higher efficiency of H25p for TN and NH_4^+-N might be because of the interaction between the wastewater and the oxygen transferred from the atmosphere and/or through the plant roots was highest near the upper surface of the bed (close to the atmosphere and where the majority of plant roots occur). In the 50 cm deep beds a greater proportion of flow can bypass beneath the bulk of the roots and beyond the influence of atmospheric diffusion. In another words, the majority of water treated by H25p and H50p were different in such a way that the water treated by H25p was in contact with the roots which favours removal but the water treated with H50p only 50% of the water is in full contact with the root therefore performance would be less.

In Arba Minch

- $CBOD_5$, PO_4^{-3}-P, TSS, NH_4^+-N and TKN were not significant different.

Table 5.2: Summary of the **effect of depth** on areal and volumetric mass removal rates at the same hydraulic loading rates based on Arba Minch experiment. Symbols: Significantly different (**+**), significantly not different (**-**).

	Areal mass removal		Volumetric mass removal	
	H25 - H50	H25p - H50p	H25 - H50	H25p - H50p
TSS, COD, $CBOD_5$, NH_4^+-N	-	-	-	-
TKN	-	-	+	+
PO_4^{-3}-P	-	-	-	+

Depth wise areal mass removal rate at the same hydraulic loading rate

- TSS, COD, $CBOD_5$, TKN, NH_4^+-N and PO_4^{-3}-P were not different with depth in the performance of 25 and 50 cm deep beds.

Depth wise volumetric mass removal rate at the same hydraulic loading rate

- TSS, $CBOD_5$, COD and NH_4^+-N were not different significantly when compared between H25/H50 and H25p/H50p.
- TKN volumetric removal rates were significantly different with respect to depth irrespective to available plant or not but phosphate was significantly different for the planted beds only.

Effect of plants on treatment performance in HSSFCWs

The effect of plant on the treatment performance of H25p, H25, H50 and H50p compared in Table 5.3 and 5.4. Table 5.3 summarizes the effect of plants on the pollutant areal and volumetric mass removal of the wetlands working at the same detention time and Table 5.4 summarizes the effect of plants on the pollutant areal and volumetric mass removal of the wetlands working at the same hydraulic loading rates.

Table 5.3: Summary of the **effect of plant** on areal and volumetric mass removal rates at the same hydraulic detention time. Symbols: Significantly different (**+**), significantly not different (**-**).

LRB	Areal mass removal		Volumetric mass removal	
	H25-H25p	H50-H50p	H25-H25p	H50-H50p
TOC, $CBOD_5$, *E.coli*	-	-	-	-
TN, NH_4^+-N	+	+	+	+
Arba Minch				
TSS, $CBOD_5$, TKN, NH_4^+-N	-	-	-	-
PO_4^{-3}-P	-	+	+	+

Areal mass removal rate at the same HRT

In LRB

- TOC, $CBOD_5$, and *E.coli* were not different with plants in their performance
- TN and NH_4-N were different significantly when compared between wetlands because of the contribution of the planted beds to the medium which favoured relatively higher ORP.

In Arba Minch

- TSS, $CBOD_5$, TKN and NH_4^+-N were not different with plants in their performance but PO_4^{-3}-P removal rate was significantly different with the 50 cm deep beds but not in 25 cm deep beds

Volumetric mass removal rate at the same HRT

In LRB,

- o TOC, $CBOD_5$, and *E.coli* were not different with plants in their performance but NH_4-N was significantly different when compared in the 25 cm and 50 cm beds.

In Arba Minch,

- o TSS, $CBOD_5$, TKN and NH_4^+-N were not different with plants in their performance but PO_4^{-3}-P was significantly different.

Table 5.4: Summary of the **effect of plant** on areal and volumetric mass removal rates at the same hydraulic loading rate (q) based on Arba Minch experiment. Symbols: significantly different (**+**), significantly not different (**-**).

	Areal mass removal		Volumetric mass removal	
	H25-H25p	H50-H50p	H25-H25p	H50-H50p
TSS, COD, $CBOD_5$	-	-	-	-
TKN, NH_4^+-N, PO_4^{-3}-P	+	+	+	+

Volumetric and areal mass removal rate at the same hydraulic loading rates

- TSS, COD, and $CBOD_5$ were not different with plants and unplanted beds in their performance
- Performance of planted beds was significantly greater than unplanted beds for TKN, NH_4^+-N, and PO_4^{-3}-P.

Effect of climate on treatment performance in HSSFCWs

Climate had a strong influence on the performance of the HSSFCW systems in this study. The concentration percent removal of $CBOD_5$ and TSS was less in semi-arid climate of Arba Minch, Ethiopia than in the temperate climate of Langenreichenbach, Germany because of the high rates of evapotranspiration observed in the Arba Minch systems. Removal of the nitrogeneous compounds was also observed to be higher in the Arba Minch study, in part due to the significantly warmer water temperatures throughout the course of the study.

In order to compare the performance of the HSSFCWs at Arba Minch and LRB, temperature corrected, first order areal rate coefficients were calculated for $CBOD_{5,}$ TN and NH_4^+-N for H25, H25p, H50 and H50p working at the same residence time (Table 5.5). The k_A were calculated using P-k-C* data fitting exercise and used here as means for common comparison of the systems at the two research sites. From the results, the rate coefficients of $CBOD_5$ were similar for H25 in Arba Minch with H25 in LRB and H50 in Arba Minch with H50 in LRB. So based on the unplanted beds, it is possible to conclude that there was no climatic effect on the performance of $CBOD_5$ removal. Kadlec and Wallace (2009) explain, based on the evidence collected from a review of available data, that $CBOD_5$ removal rate coefficient are not necessarily improved or affected by temperature. Further, Akratos and Tsihrintzis (2007) also report that organic anaerobic and aerobic bacteria are working as low as 5^0C to remove carbonaceous compounds. However, the k_A values of the planted beds at Arba Minch and LRB were not the same. The comparatively low rate coefficient of planted bed at Arba Minch is ascribed to the net loss of water which affects the wetland effluent concentration and biases the comparison on a concentration basis.

Table 5.5: First order $\mathbf{k_A}$ (m/yr) for $CBOD_5$, TN and NH_4^+-N at 20^0C for the four wetlands based up on period of record (POR) input-output analysis. The values in the table have been temperature corrected to 20^0C using Arrhenius equation. The beds were loaded at 16-17 mm/d for H25 and H25p and 32-34 mm/d for H50 and H50p. Negative values indicate net concentration increase.

	Location	**H25**	**H25p**	**H50**	**H50p**
$CBOD_5$	LRB	15.3	17.6	30	30.1
	Arba Minch	15.7	10.4	30.8	23.7
TN	LRB	1.6	3	2.8	3.8
	Arba Minch	8.6	18.8	14.1	21.8
NH_4^+-N	LRB	-0.5	1.2	-1.1	0.1
	Arba Minch	15.4	25.5	26.8	46.1

When TN k_A (20^0C) was compared between LRB and Arba Minch, Arba Minch results show higher values than LRB because of the effect of climate. The same trend was observed with ammonia in the unplanted beds where k_A for ammonia was negative (due to production of ammonia) at LRB as opposed to the high k_A for the planted and unplanted beds in Arba Minch. Different studies have shown that climate affects wetland performance, especially in regard to nitrogenous compounds (Cerezo et al. 2001, Kadlec and Wallace 2009, Taylor et al. 2011) because plant removal and nitrogen degrading bacteria are not are not particularly active during cold temperatures. The high removal rates for ammonia at Arba Minch could be due to aeration of the thin water films on the gravel, which is created by water level drop due to ET. The effect of ET in nutrient removal in tropical wetlands has been reported, and supports the high nitrification rates observed in the planted beds at Arba Minch due to bed aeration by drawdown of the water level (Zhang et al. 2012).

As a conclusion, the effect of climate on the rate coefficients of nitrogenous compounds was high and favouring the semi arid climate irrespective of depth. Areal removal rate coefficients for $CBOD_5$ were similar for both Arba Minch and LRB systems, for both shallow and deep beds.

Effect of hydraulic loading rate on treatment in HSSFCWs

Table 5.6 is presented to explain the effect of depth on k_A at 20^0C at the same hydraulic residence time (HRT) and at the same hydraulic loading rate (HLR). When comparing the k_A of H25 with H50 bed and H25p with H50p at the same HRT, 50 cm deep beds showed k_A values that were twice as high as 25 cm deep beds for $CBOD_5$ and NH_4^+-N. This is because of the hydraulic loading rate. However, k_A values were almost the same for TKN.

Table 5.6: Comparison of the $\mathbf{k_A}$ (m/d) for $CBOD_5$, TKN and NH_4^+-N at 20^0C for the H25, H25p, H50 and H50p beds in Arba Minch working at the same hydraulics residence time (HRT) of 6 days and the same hydraulic loading rate (HLR) of about 72 mm/day.

		H25	**H25p**	**H50**	**H50p**
$CBOD_5$	Same HRT (≈ 6 days)	0.04	0.03	0.08	0.07
	Same HLR (≈72 mm/d)	0.14	0.09	0.17	0.04
TKN	Same HRT (≈ 6 days)	0.03	0.09	0.05	0.08
	Same HLR (≈72 mm/d)	0.05	0.15	0.04	0.12
NH_4^+-N	Same HRT (≈ 6 days)	0.04	0.07	0.07	0.13
	Same HLR (≈72 mm/d)	0.06	0.4	0.06	0.43

When the same comparison is made at the same HLR (72 mm/day), both 25 cm and 50 cm deep beds had almost the same k_A for $CBOD_5$, NH_4^+-N and TKN. This shows that areal rate coefficient is affected by hydraulic loading rate.

The 25 cm deep beds have shown the same or better areal mass removal rate than the 50 cm deep beds for organic carbon and nitrogenous compounds at the same loading rate. In other words, the 25 cm deep beds performed equally with 50 cm deep beds at half HRT of the 50 cm deep beds. This might be due to the higher volumetric efficiency, plant root effect and influence of the water- air interface.

5.2 Conclusions

The main conclusions resulting from this study are summarized as follows:

- Tracer test using bromide and fluorscein dye in Germany showed that 25 cm deep beds had a higher hydraulic efficiency than 50 cm deep beds irrespective of presence of plants.
- Areal mass removal rates of the wetlands depend on the hydraulic loading rate. Deep beds performed better at the same hydraulic residence time, but the effluent concentration of the 25 cm deep beds were always lower in magnitude than the 50 cm deep beds.
- Areal rate coefficients (k_A) calculated using the P-k-C* model showed seasonal trends.
- Performance of the wetlands at LRB over two years followed seasonal trends and the effect of plants in the removal of nitrogen was also observed.
- In an attempt to compare the effect of climate by the areal rate coefficient, same depth and plant type, same operation conditions were compared for $CBOD_5$ and nitrogen at temperate (LRB) and semi arid (Arba Minch) conditions. For $CBOD_5$, unplanted beds (H25 (LRB) versus H25 (Arba Minch) and H50 (LRB) versus H50 (Arba Minch)) showed the same k_A in both places. However, the K_A of the nitrogen was greater in Arba Minch for the H25, H25p, H50, H50p beds (e.g., between H25 in Arba Minch and H25 in LRB).
- When the 25 and 50 cm deep planted and unplanted beds were working at the same residence time, there was no observable difference on the rate of change of concentration of pollutants against nominal hydraulic residence time from the inlet to outlet for H25, H25p, H50 and H50p beds for TN, TOC, NH_4^+-N & DO.
- The nitrification potential of passive beds (H25p, H25, H50, H50p) and aerated beds (HAp, HA) were analysed in order to determine the distribution of nitrifying bacteria in the media. It was found that aerated beds had shown higher nitrification potential than the passive beds and in all cases planted beds had higher nitrification potential because of the root and gravel surface. Nitrification potential was found highest at higher temperature condition.

- When the 25 and 50 cm deep planted and unplanted beds were loaded at the same hydraulic loading rates, both 25 cm and 50 cm deep planted and unplanted beds have shown no significant difference in their areal mass removal rates for organic matter and nitrogen.
- At the Arba Minch site, *Ascaris lumbricoides* egg was removed in all wetlands completely, irrespective of the depth or presence of plant.
- The biomass production of the wetlands for the deep and shallow beds at Arba Minch were almost the same, 15 kg/m^2 and 83% of the underground biomass (root) of the H50p was found within top 20 cm depth.
- Evapotranspiration was highest in dry seasons in planted beds in Ethiopia resulted in concentrated effluent (e.g high nitrate and salinity) and loss of quantity of water. Evapotranspiration encourage aeration of HSSFCW beds, however.

5.3 Recommendations

In the present study, encouraging results were obtained on the effect of depth and plants on the wetlands from Arba Minch and LRB, especially the results from Arba Minch can be used for semi-arid climate where data is scarce. Based on this studies, recommendations for the academic community and user of the technology are provided here.

In order to obtain refined results for Arba Minch, further long term research on the performance of the wetlands with respect to all parameters using larger sized wetland is required. For LRB research site, study of the effect of depth and plants at the same HLR is important to see the performance at temperate conditions. Besides, further analysis of nitrification potential using the surface area of the root and gravel is recommended for better results.

For the user of the technology in semi-arid climate: since the performance of the 25 cm deep beds were significantly not different with the 50 cm deep beds at the same HLR, so 25 cm deep beds can be chosen for application and their construction cost is obviously cheaper. However, evapotranspiration was highest and exaggerated during dry seasons in planted beds in Arba Minch, Ethiopia which resulted in concentrated effluent (decrease in quality) and reduced quantity of water for reuse so unplanted beds is recommended. Furthermore, it is worthwhile to use HSSFCW as one part of the treatment system to reduce helminth infection before applying wastewater for reuse in irrigation.

Appendix

Appendix 1: Box and whisker plot of Total nitrogen

The box and whisker plot for the nitrogen concentration for 25 and 50 cm deep planted and unplanted beds. The plot was intended to show the distribution of the pollutants as a representative example for water quality parameters measured. The distribution of the concentration in 2010-2011 was more dispersed than the 2011-12 data. It may be possible to conclude that performance was more reproducible in the second year as the wetland was getting more mature.

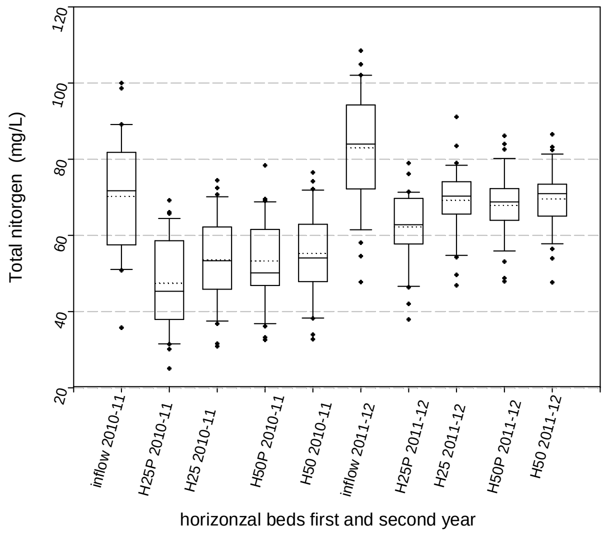


Figure 1: Box and whisker plot for total nitrogen concentration for 25 and 50 cm deep planted and unplanted beds from Sept 2010 to Sept 2012.

Appendix 2: Effluent prediction using P-k-C* model excel sheet and inflow, measured and predicted

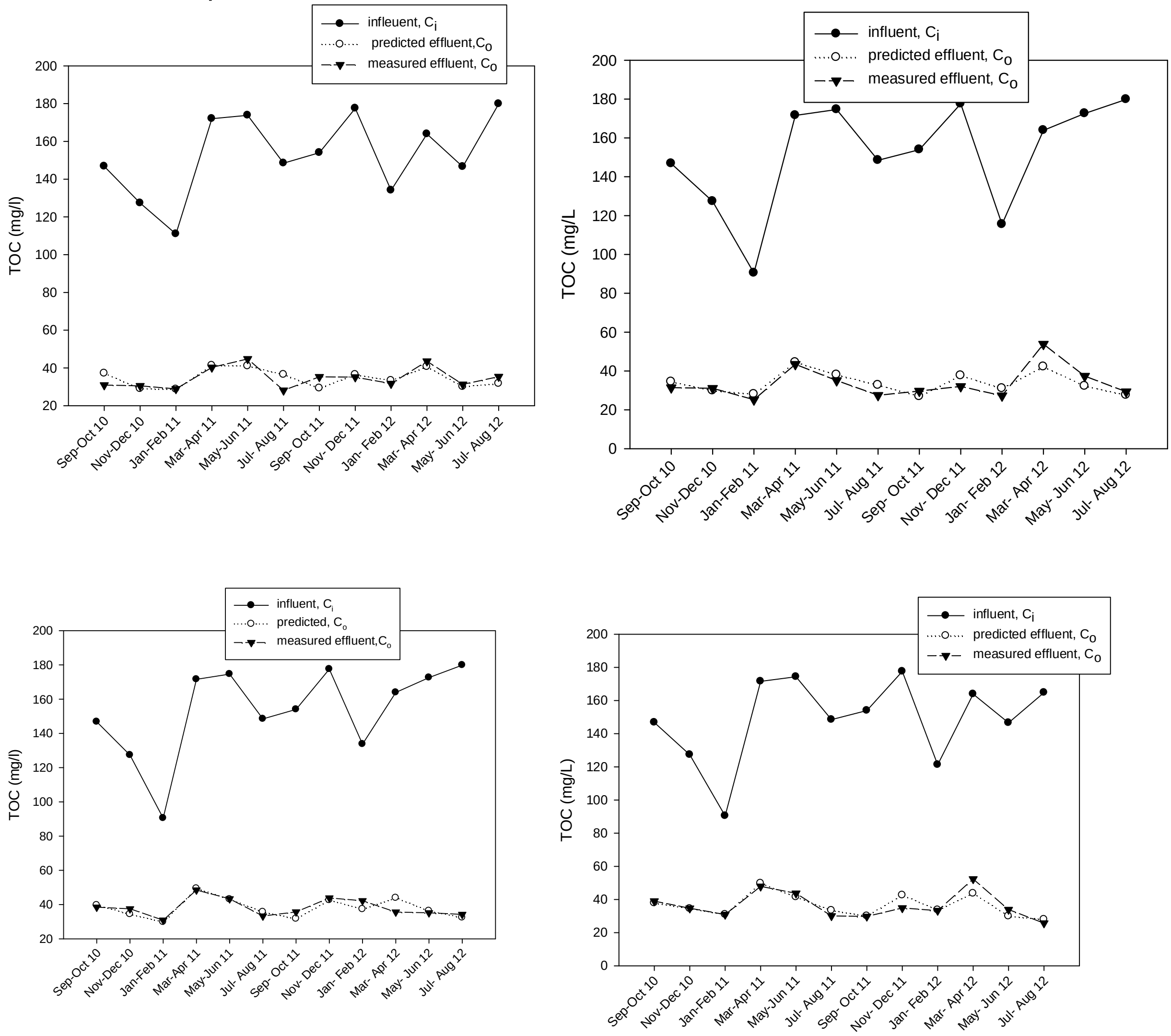


Figure 2: Total organic carbon results of P-k-C* predicted and measured effluent concentrations at LRB. The prediction was good when it compared with the measured effluent.

Table 1: Excel sheet of the calculation of evaluation of P-k-C* model

Calculating K20 and θ with PKC* model by excel

C*	5	mg/L	K20	0.0483	with solver
p	6	unitless	θ	1.0450	with solver

Date	T ^{0}C	Ci (mg/L)	Co (mg/l)	q (m/d)	Ka (calculated)	Denom-inator	Co predicted	SSE
Sep-Oct 10	15.8	217.60	40.86	0.020	0.0400	5.4682	43.8795	9.12
Nov-Dec 10	9.2	186.00	40.53	0.017	0.0299	4.7370	43.2099	7.21
Jan-Feb 11	1.0	116.93	41.23	0.017	0.0209	3.0731	41.4213	0.04
Mar-Apr 11	6.7	270.40	69.90	0.018	0.0269	3.8723	73.5384	13.24
May-Jun 11	16.0	208.50	49.78	0.020	0.0405	5.9396	39.2617	110.71
Jul- Aug 11	17.9	260.83	25.53	0.020	0.0440	6.3228	45.4618	397.14
Sep- Oct 11	15.8	269.50	27.88	0.016	0.0400	8.1272	37.5450	93.35
Nov- Dec 11	9.2	325.40	55.80	0.016	0.0299	5.1155	67.6333	140.03
Jan- Feb 12	6.0	163.50	33.85	0.018	0.0261	3.7388	47.3931	183.41
Mar- Apr 12	8.9	289.40	101.20	0.019	0.0296	4.0962	74.4309	716.59
May- Jun 12	15.5	265.00	55.82	0.017	0.0395	7.2306	40.9585	220.86
Jul- Aug 12	19.4	331.9	41.7	0.016	0.0470	10.9968	34.7258	48.02
SSE								1939.71

Note: Substitute k from equation 2 into equation 1 then calculate Co; this value and measured C outlet concentration were compared with SSE optimized using solver substituting k20 and Ɵ The aim is to get smaller SSE

$$\text{sum of square errors (SSE)} = \text{minimum} = \sum_{i=1}^{n} (C_{\text{measured}} - C_{\text{Calculated}})$$

$$\frac{Co - C^*}{Ci - C^*} = \left(1 + \frac{k}{qp}\right)^{-p}$$

$$C_0 = C^* + \frac{C_i - C^*}{\left(1 + \frac{k}{pq}\right)^{p}} \cdots (1)$$

$$k = k_{20}\,\theta^{(t-20)} \quad \cdots (2)$$

where, k = areal rate constant

q = hydraulic load

p = no of tanks inseries

Ci = inflow concentration

Co = outflow conc

c^* = background conc

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