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NITROGEN REMOVAL FROM DOMESTIC WASTEWATER USING CONSTRUCTED WETLAND HAVING DIFFERENT WATER LEVELS

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ABSTRACT

The ability of LSCWs in treating domestic wastewater to prevent the environmental pollution and to keep the surface water quality was investigated. It is generally efficient in removing TSS, COD, BOD and bacteria. Excess nitrogen in water creates a potential problem to the environment and living things. It is a source of Eutrophication in water bodies ultimately it causes toxicity to fish and aquatic plants. In drinking water, nitrogen is also a cause of blue baby syndrome (methemoglobinemia) for infants. The aim of this research is to find an optimum condition for removal of nitrogen from domestic wastewater through microbial nitrification-denitrification pathways by using different water levels in Vertical flow Constructed Wetlands. TN, BOD, NH_4-N NO_3-N, pH, conductivity and temperature were measured weekly for both influent and effluents for the LSCWs. The data were analyzed using SPSS to see the significance between factors. COD removal in 7cm, 14cm and 21cm bucket were 81%, 71% and 70.8% respectively, and TN removal was 38.9% 32.3% and 25.5% respectively. From the results obtained, the water level with 7cm has the best performance in removing nitrogen to 14cm and 21 cm water levels.

Keywords: Microbial Nitrification-Denitrification, Wetlands, Eutrophication.

1. INTRODUCTION

A constructed wetland (CW) is defined as a wetland specifically constructed for the purpose of pollution control and waste management, at a location other than existing natural wetlands. It contains complex biological processes that mimic the natural self cleansing system. Wetlands provide services of great value to society. Such as: control floods, protect coastal zones, control water qualities and they host a great diversity of species. The cultural and economic importance of wetlands to indigenous communities is beyond words. That is why a special international treaty, the Ramsar Convention on wetlands, has been established by governments and NGOs. More action will be needed in order to secure wetlands and their values for our generation as well as for those to come.

Wetlands are found all over the world, from the polar regions to the tropics. In several regions, wetlands are deteriorating rapidly. Seeing that they are the most threatened ecosystems of our planet, action to preserve wetlands is very essential; the sooner the better. People around the world are relying on wetlands for their economic, cultural as well as spiritual well-being. That is why currently special attention is given to safeguard the existence of wetlands worldwide. The current scenario of population in the world dramatically increases. The increase in a number of populations is the primary cause of environmental pollution. Human waste disposal to the environment pollutes water, air and soil. Specially, water is easily contaminated by human development such as urbanization, agriculture, recreational areas, municipal wastes etc. The most important pollutants of water or nutrients, organic matters and heavy metals.

Wastewater treatment for removal of Nitrogen is important because nitrogen compounds are the major pollutant that create a potential hazard to living things and the ecosystem. For example, high nitrogen concentrations in water can directly affect human health, as when nitrate in drinking water causes methemoglobinemia in infants, commonly known as 'blue baby syndrome' (Crites and Tchobanoglous 1998). However, the primary impact of nitrogen is due to its role as a limiting nutrient in many aquatic environments. Elevated nitrogen inputs in water bodies can cause eutrophication. Eutrophication due to nitrogen inputs have been implicated in loss of species diversity (Preston et al. 2003) and increased occurrence of harmful algal blooms such as red tide, which threaten both human and ecosystem health (Anderson et al. 2002, Huang et al. 2003). Excessive nutrients in aquatic systems cause eutrophication, which can lead to decreased dissolved oxygen levels and fish kills (Cook, 2001).

A “constructed wetland” is a wetland specifically constructed for the purpose of pollution control and waste management, at a location other than existing natural wetlands (EPA, 1993). CWs remove nitrogen from waste water through two pathways: Storage (assimilation or adsorption) in the system, and removal through denitrification and ammonia volatilization (Donald, 1990). Optimization of denitrification reliability and efficiency will help to ensure that constructed wetlands are an economically feasible treatment technology.

2. MATERIAL AND METHODS

2.1 STUDY AREA: LABORATORY SCALE CONSTRUCTED WETLANDS, LSCWS

The laboratory scale constructed wetlands (LSCWs) were started in October 2008 within UNECSO-IHE laboratory (Green house room). From the three grain sized sands available, the sand with low hydraulic conductivity and low porosity was selected as a substrate bed to the wetlands. A column measurement was used to determine the hydraulic conductivity and porosity of the sands. For this activity a 10cm diameter and 90cm height PVC tube were installed. The length of the tube filled with sand was 70cm. The results of the column test where indicated in the table 1 below. From the result depicted on the table 1 the sand with the slower hydraulic conductivity and the medium porosity and retention time was selected to fill the buckets. There for the grain size of the sand in the LSCWs were from 0.8 to 1.2mm diameter.

The experimental setup for the LSCWs is shown in figure 1. The main elements of the setup are 12 buckets filled with coarse sand at different water levels. The water levels 7cms, 14cms and 21cms respectively. The average dimension of one bucket is 27cms in diameter and 33cms height. The buckets are a little bit smaller at the bottom than at the top. The sand level inside the bucket is 27cm. Every day 1.9L of artificial wastewater was fed to each bucket intermittently. The volume of wastewater added was enough for complete saturation of the sand. It was obtained by subtracting the volume of the water used for saturation from the total volume of the sand. (Saturation volume 7.6L and Retention time is 4 days). The buckets were planted with the shoots of the *Phragmites australis*, which were transferred from Dr. Bruggen garden and transplanted at an average with a density of 9 seedlings per bucket, in November 2008.

Table 1: Sand size available in UNESC-IHE Environmental Engineering Laboratory

Sand Grain size	Retention time	Porosity(Vv/V)	Hydraulic conductivity
1.0 to 2.0 mm	2.5 minutes	low	25 seconds
0.8 to 1.2 mm	10 minutes	medium	50 seconds
0.5 to 1.0 mm	15 minutes	high	1.5 minutes

To make the room comfortable to the plants growth, the temperature, humidity and the intensity of the light energy were under controlled conditions. The light intensity in the temperature room was the same everywhere. It was measured in $\mu E/m^2/s$. The average light intensity was $60\mu E/m^2/s$ which is very suitable to plant growth. The humidity was measured using hydrometer and kept the air moisture between 45 to 60%. The temperature of the room was also controlled by a special thermometer. The range of temperature was maximum 28 and minimum 22^0C.

A vertical position PVC pipe was installed in each bucket. COD was added through the pipe when there were deficiencies in the root zone of the wetland. At the inner part of the wetlands, DO was measured through the pipe. The tube was also used to re-aerate the wetland root zone so that facilitates nitrification. Sometimes samples were taken from the inner wetland by the PVC tube. For microbial nitrification and denitrification to take place inside the treatment wetlands, a commercial sludge from Hoek van Holland wastewater treatment plant was inoculated to each of the investigated wetlands. To consolidate the microbial activities, the sludge was inoculated twice. A total of 350ml of sludge was added to each bucket.

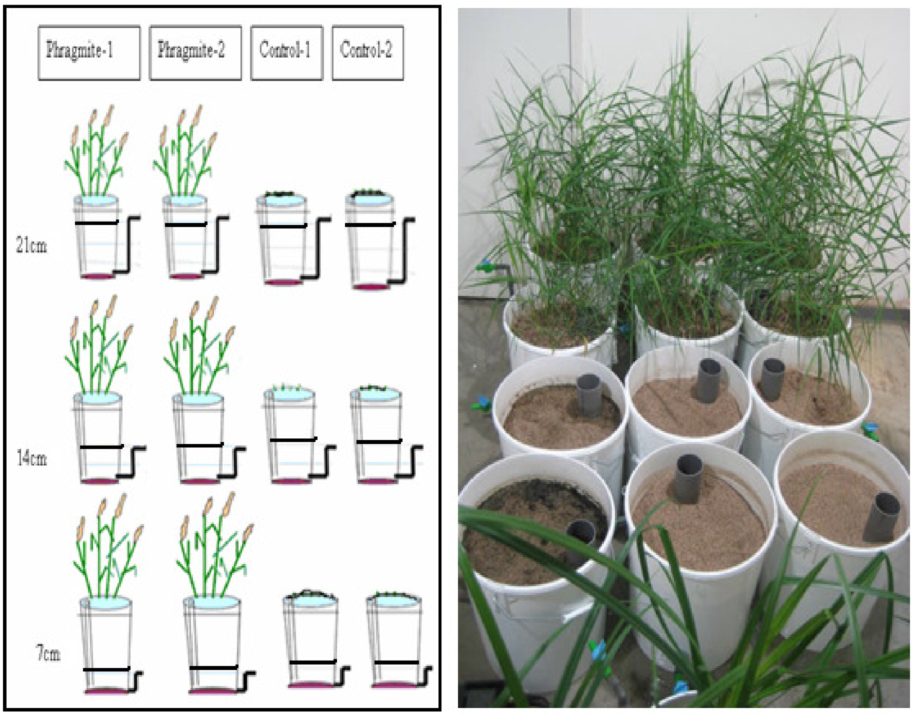


Figure 1: Laboratory Scale Constructed Wetlands scheme according to water Levels

(a) Synthetic Domestic Wastewater

Artificial wastewater was preferred to natural wastewater for experimental work because the concentration didn't vary so much. The synthetic influent used in the CWs under study, was slightly modified because some of the chemicals were not available.

(b) Measured Influent Compositions

All ingredients were weighed using an analytical balance. The chemical compounds were weighed on the 0.01g balance but trace metals were weighed in 0.0001g analytical balance precisely. Ingredients were mixed in beaker contained warm water to make it homogenous. A 20L stock solution of synthetic wastewater was stored in a cool room(4^0c) The stock wastewater was diluted 10 times before feeding the wetland vegetations.

(c) Sampling Of Water From Laboratory Scale Constructed Wetlands

The influent and effluent water samples of the pilot- scale constructed wetlands were taken periodically once a week to evaluate the treatment performances of the wetlands from November through March 2008. Water samples were taken to the Environmental Engineering laboratory in UNESCO-IHE.

(d) Analytical Methods and Equipments

A brief description of the methods and equipments used to measure parameters of concern for this research work is presented in the following subsections. Chemical analyses were performed following the methods described in Environmental chemistry (Kruis, 2005). Temperature, conductivity, pH, NH_4^+-N, TN, NO_3^--N, COD, BOD and TKN were monitored. For each of the parameters, samples were analyzed in duplicates in order to minimize errors. The samples were only filtered for ammonium and nitrate analyses. The samples were kept in cold room at 4°c.

- *EC, pH, Temperature and DO*

Measurement of pH was carried out by using METROHM-691 pH meter (Swiss made) which was calibrated prior to the measurement. A sample of about 120 ml volume was collected in a plastic cup from the influent and effluent and was placed on a magnetic stirrer to ensure uniformity. Then, the meter probe (electrode) was immersed in the sample after rinsing it thoroughly by spouting demineralized water from a plastic wash bottle. The stable final reading was then taken.

The electrical conductivity of influent and effluent water was measured with conductivity meter. The meter probe was immersed in the sample, stirred to ensure uniform mixing and a stable reading obtained was then recorded.

Dissolved oxygen (DO) was measured using WTW oxi 340 oximeter microprocessor with electrode E096. It was used to measure DO in the influent, effluent and in the vertical position tube located in each of the CWs. A special sampling tube was also prepared to measure DO inside the sand. During measuring DO, the probe of the oxygen meter was placed in the small plastic cap and the tape of the sampling point would slowly open to avoid contact of the DO probe with air. The reading of DO didn't stabilize quickly. A stable reading obtained was then recorded.

Micro logger METEX M4650 was used to measure the light intensity of the room. The light intensity in milli volt was converted into $\mu E/m^2/s$ by the relation:

2.64 milli volt = 1000 $\mu E/m^2/s$ *in air and:*
2.50milli volt = 1000 $\mu E/m^2/s$ *in water*

Using the above relation the light intensity inside the room was maintained from 55 to 70 $\mu E/m^2/s$. Using the range of light intensity, the wetlands were illuminated for 16 hours per day.

The length of the light intensity helped the *Phragmites* for more biomass production in the plants. A timer was installed to switch on and off the light.

- *Ammonia Nitrogen, NH_4-N*

Ammonia measurements were carried out by using Dichloroisocyanurate method. Salicylate and dichloroisocyanurate reagents were added to undiluted samples and measured the absorbance through the spectrophotometer at wave length of 655nm as indicated in laboratory manual, Environmental Chemistry, Selected Analytical Methods, UNESCO-IHE, and LN168/07/1.

- *Nitrate Nitrogen, (NO_3-N)*

Nitrate was measured using DIONEX, ICS-1000 Ion Chromatography system coupled with ISA-100 Automated Sample Injector. One mL filtered sample was used for the analysis of nitrate in the Ion Chromatography system. Milli-Q and other control sample of known concentration were always analyzed prior to the real sample measurement. In the machine, one sample needs about 8 minutes to analyze the nitrate concentration with a single injection.

- *Nitrite Nitrogen, (NO_2-N)*

Nitrite-Nitrogen was measured by adding color reagent to the undiluted samples and measured the absorbance through spectrophotometer against the wavelength of 543nm, as described in Standard Methods for the Examination of Water and Wastewater and Environmental Chemistry, Selected Analytical Methods, UNESCO-IHE, (Kruis, 2007).

- *BOD Analysis*

BOD_5 was measured using DO meter and Winkler bottles. DO measurements had taken twice per sample during BOD_5 measurement. The first DO was taken at sample collection from the wetlands and the second was after five days of incubation at 20^0C. Initial and final DO readings were taken and BOD concentrations were calculated using the formula given below.

$$BOD_5 = \frac{(B - S) * V_b}{C}$$

Where: B = Dissolved Oxygen blank (mg/L)
S = Dissolved Oxygen sample (mg/L)
V_b = Volume of Sample bottle (ml)
C = Volume of sample (ml)

- *COD Analysis*

COD Measurements were done using the closed reflux method described in Standard Methods for the Examination of Water and Wastewater and Environmental Chemistry, Selected Analytical Methods, UNESCO-IHE, (Kruis, 2007).

- *Total Kjeldahl Nitrogen (TKN)*

Kjeldahl Nitrogen was determined using the macro Kjeldahl method as described in the Standard Methods for Examination of Water and Wastewater chapter 4500-N org B and Environmental Chemistry, Selected Analytical Method, UNESCO-IHE (Kuris, 2007).There were two processes for TKN analysis. These were destruction and distillation. The destruction took about four hours followed by distillation for about two hours.

TKN was calculated using the following formula:

$$TKN = \frac{\mu g\ NH_4^+ - N\ in\ 50mL\ end.volume}{mLsample} * \frac{mL\ end.volume.distillate}{mL\ distillate\ transferred\ to\ 50mL\ flask}$$

Estimate Nitrification Activities In The Soils

The procedures were:

- Two 20 g soil samples were weighed. One of the 20 g sample was used to determine nitrification activity. The other 20 g sample was used to determine the dry weight of the sand and the sands were placed on a piece of aluminum foil of known weight.
- 20 g of soil samples, 90 ml of phosphate buffer and 0.2 ml of ammonium sulfate solution were added to a 125 ml flask (total volume should be about 100 ml).
- The flasks were placed on a rotary shaker for 5 min.
- The flasks were removed and allowed to stand for approximately 5 minutes.
- The filtrate samples were kept in a refrigerator to slow down nitrification.
- In the next session samples were analyzed for nitrate concentration after 48-hour of incubation using IC machine.
- The potential nitrifying bacteria were estimated by the increment of the nitrate concentration between the first and the second sessions of the analysis.

Estimate Denitrification Activities In The Soils

Chemicals and materials used for denitrification activities were 250 mL bottle, filter and funnel, phosphate buffer solution, sodium nitrate solution, glucose and nitrogen gas. 90ml of phosphate buffer solution, 1 mL (0.45 g in 50 ml) sodium nitrate solution and 1mL (0.6 g in 50 ml) of glucose were mixed with 20g of sand. The prepared sample was bubbled with nitrogen gas to create anoxic environment in the bottle. Nitrate was analyzed before bubbling of nitrogen gas and three days after bubbling of nitrogen. The presence of denitrify bacteria was determined by the concentration of nitrate reduced after 3 days.

Removal Efficacy of the wetlands

Average removal efficacy and specific pollutant load were calculated with the following Formulas:

Removal efficacy: $100 \times (C_{in} - C_{out}) / C_{in}$ [%]
Where: C_{in} = concentration of given component in inflow [g/m^3]
C_{out} = concentration of given component in outflow [g/m^3]

Specific pollutant load: $(C_{in} \times V_{av}) / N_{theo}$ [g/m^3 per capita]

Where: V_{av} = average volume of incoming wastewater
N_{theo} = theoretic number of residents (Vav / PE; where PE = 150 l per capita)

2.2 DATA ANALYSIS

To determine whether the treatment performances of the wetland with *Phragmites* and without *Phragmites* at different water levels are statistically different, one-way ANOVA and t-test at a significance level of 0.05 was applied to the removal efficiencies calculated from the data for a monitoring period from January to March 2008 for each of the water quality parameters. These analyses were conducted by using a sub-program of Microsoft Office Software EXCEL XP. The statistical results were presented in the following form: t-test result as (t-value, df and p-values) and ANOVA result as (One-way ANOVA;$F_{0.95}$ (d.f.:dN); p) where $F_{0.95}$ = 95n % confidence limit; d.f.: degree of freedom; dN = sample size; $p>0.05$ non significance in the related section of result and discussions.

3. RESULTS AND DISCUSSION

3.1 Laboratory scale Constructed Wetlands-LSCWs

In the climatic room containing the wetlands, the buckets were identified using *Phragmites* and controls (without plants) in different water levels. Table 3 shows the identification of the wetlands under investigations.

Table 3: The names and classifications of the LSCWs using *Phragmites* and Blank

Planted wetlands	Un-planted wetlands
Phragmites 7cm	Control 7cm
Phragmites 14cm	Control 14cm
Phragmites 21cm	Control 21cm

3.2 The Physical Characteristics Of The Effluent Water: Temperature, Ph, DO And EC

The temperature for the LSCWs was 21.5^{0}C. The temperature was constant because the room was under controlled conditions of temperature and humidity. The pH of the wetland varied from 7.3 to 7.8 with mean value 7.5 ± 0.1 to 7.5 ± 0.3. The pH didn't decrease much the reason could be the weakness of the nitrification rate in the system. DO in the wetland was very low as it was depicted in table 4. They were almost the same in all of the three water levels. This could affect the healthy process of the wetland system because nitrification depends on the oxygen level. Organic matter containing nitrogen could not be converted to nitrite if oxygen was deficient. Denitrification reaction could also be affected indirectly by oxygen concentration, because if there is no oxygen nitrification is affected and if there is no nitrification denitrification will be handicapped

Table 4: The mean temperature, DO, pH and EC in *LSCWs* with *Phragmites* & blank

Water Levels	*Phragmites* Average temperature 21.5^{0}C			Control wetland (without *Phragmites)* average temperature is 21.5^{0}C		
	DO in mg/L	Ph	EC in µS/cm	DO in mg/L	pH	EC in µS/cm
	Mean ± Std	Mean ± Std	Mean ± Std	Mean ± Std	Mean ± Std	Mean ± Stdev
7cm	0.3 ± 0.1	7.5 ± 0.1	568 ± 8.5	0.3 ± 0.1	7.7 ± 0.2	570 ± 29.8
14cm	0.3 ± 0.1	7.5 ± 0.1	534 ± 23	0.3 ± 0.2	7.7 ± 0.1	530 ± 28.4
21cm	0.3 ± 0.1	7.2 ± 0.3	554 ± 6.5	0.4 ± 0.1	7.7 ± 0.1	546 ± 16.9
Influent	8.7 ± 0.3	4.6 ± 0.15	398 ± 11.4	8.7 ± 0.3	4.6 ± 0.15	398 ± 11.4

3.3 Nitrogen

Table 5: Range, mean and removal efficiency (RE%) ,(A- with *Phragmite* & B-blank)

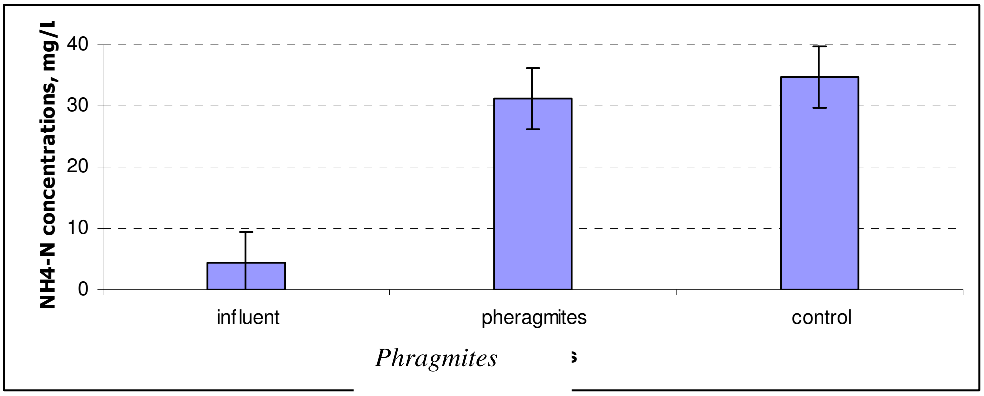
A. Wetland water levels	Parameter	Minimum	Maximum	Mean + stdev	RE %
7cm	NH_4-N	24.4	33.9	29 ± 3.5	-
	NO_3-N	0.03	0.95	0.32 ± 0.35	-
	COD	27	100	51 ± 26	81
	BOD	20.02	43.2	28.1 ± 7.4	88.4
	TN	14,8	37.5	28.8 ± 8.8	38.8
14cm	NH_4-N	27.4	35.6	31.3 ± 2.9	-
	NO_3-N	0.02	0.21	0.06 ± 0.06	-
	COD	38	150	78 ± 42	71
	BOD	29.05	62.25	41.2 ± 11.5	83
	TN	18.6	36.4	31.9 ± 6.3	32.3
21cm	NH_4-N	28.9	36.8	34 ± 3.1	-
	NO_3-N	0.01	0.11	0.04 ± 0.03	-
	COD	45	105	65 ± 23	76
	BOD	28.3	69.6	59.9 ± 18.3	75.3
	TN	28.1	37.2	35.1 ± 3.4	25.5
B. Wetland water levels	Parameters	Minimum	Maximum	Mean ± stdev	RE %
7cm	NH_4-N	32.1	37.5	34.7 ± 2.3	-
	NO_3-N	0.01	1.2	0.4 ± 0.4	-
	COD	39	156	65 ± 44.8	72.9
	BOD	26.2	76.8	44 ± 17.7	79.7
	TN	34.7	39.9	36.8 ± 2	21.6
14cm	NH_4-N	32.7	36.6	34.3 ± 1.4	-
	NO_3-N	0.02	0.18	0.09 ± 0.07	-
	COD	74	58.8	67 ± 52	72
	BOD	21.5	65	42 ± 14.8	81.2
	TN	34.8	37.8	36.1 ± 1.3	23
21cm	NH_4-N	34.4	38.4	35.3 ± 1.9	-
	NO_3-N	0.01	0.25	0.09 ± 0.1	-
	COD	98	56.9	91 ± 50	63.8
	BOD	27.1	90.6	64 ± 25.7	71.6
	TN	35.7	38.2	37 ± 1	21.2

3.4 Ammonium Nitrogen

Ammonium in the influent was very low ranging from 3.5 to 5.2 mg/L with a mean value of 4.4 ± 0.5 mg/L. But the effluent ammonium concentration in different wetlands varies from 25.9 to 36.2 with mean ± stdev (31.7 ± 3.9). By hydrolysis and mineralization, organic nitrogen is converted to ammonium nitrogen (Paredes et al., 2007). This indicates the organic matter in the wastewater applied to the wetlands was reduced to ammonium. One-way ANOVA with post hoc test showed that the *Phragmites* 14 cm deep wetland has no significant difference from *Phragmites* 7cm and *Phragmites* 21cm wetlands. The *Phragmites* 7 cm deep wetland had a significant difference to the *Phragmites* 21cm deep ($F_{0.95}$ (2; 14) = 0.038; $P<0.05$). The highest water level has high ammonium concentration than the lowest; the reason might be the slow nitrification rate in the 21 cm water level. Dissolved oxygen level in the highest water level was low as it was always saturated with water. Plants and microorganisms consume nitrogen from organic matter if it is in the form of ammonium ion. Even though the nitrification activity in the LSCWs was very weak, the *Phragmites* 7cm depth wetland has shown little nitrification.

In LSCWs, the NH_4-N effluent concentrations at *Phragmites* 7cm water level (table 15) varied from 24.4 to 33.9 mg/L with the average of 29.9 ± 3.5 mg/L. *Phragmites* 14cm wetland ranged from 27.4 to 35.6 mg/L with an average of 31.9 ± 2.5 mg/L and the range of *Phragmites* 21cm was from 28.9 to 36.8 with an average of 33.9 ± 3.1mg/L. There should be an absence of nitrifying bacteria in the system or the oxygen level was low.

After the independent sample t- test analysis, the wetland with *Phragmites* have a significant difference from a control wetland in NH_4-N concentrations ($t = -3.441$, $df = 82$ and $P < 0.05$). Even if the wetland plants did not mature enough, they have little contribution in wetland chemistry not directly by plant uptake for biomass formation but by re-aerating and growing bacterial population in the wetlands.



Phragmites

Figure 2: NH_4-N variation in the influent and effluent at LSCWs

The ammonium concentration in *Phragmites* and control wetlands had visible significance difference. The presence of plants in the wetland plays an important role in transforming ammonium to other forms of nitrogen. The *Phragmites* either supply oxygen to the wetland through the root zone or uptake of nitrogen for biomass production. At this time the nitrogen in the form of the organic matter will be converted to ammonium and subsequently to nitrite and nitrate.

Throughout the monitoring periods, the ammonium concentration in the influent was smaller than the effluents. In the artificial wastewater the ammonium concentration was very low. The nitrogen in the wastewater was existing in the form of organic matter. The organic matter was decomposed to ammonium in the system.

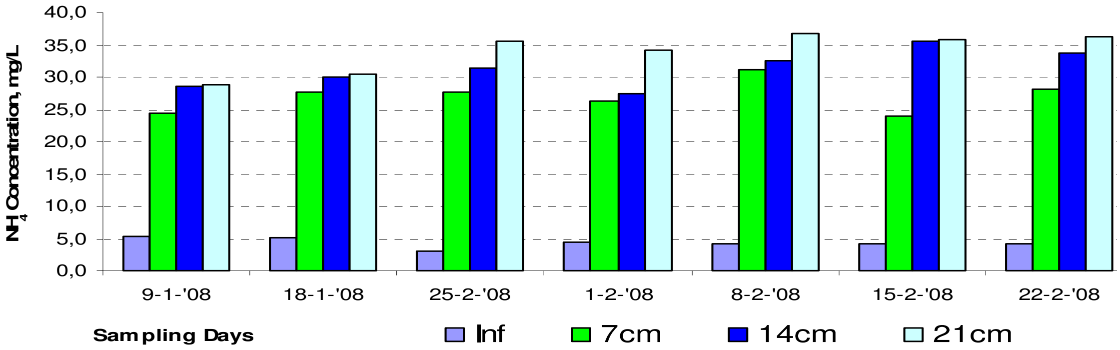


Figure 3: NH_3, mg/L in different wetlands containing Phragmites

Nitrogen removal performance of subsurface flow constructed wetlands treating ammonium-reach wastewaters were often relatively poor and has proven difficult to predict accurately (IWA, 2000). This problem also exists in the LSCWs. The oxidation of ammonium to nitrite requires 2 moles of bicarbonate for every mole of ammonium (Paredes et al., 2007). This was essential for the nitrification, because a low content of alkalinity can cause a reduction in the pH and a complete stop of the reaction. Additionally, oxygen available inside the wetland was primarily used by the heterotrophic microbes to remove organic matter, and the ammonia oxidizers would become oxygen-limited.

3.5 Nitrate nitrogen

In order to have efficient nitrogen removal, most of the biodegradable carbon has first to be removed from the wastewater, enabling the nitrifying bacteria to convert ammonium to nitrate easily (Haberl et al., 1995). At higher organic loads to the wetlands, only suspended solid and carbon removal can be obtained, whereas at lower loads nitrification and denitrification can take place (Vymazal et al., 1998).

During the monitoring period, the NO_3-N effluent concentrations of the *Phragmites* 7cm deep wetland ranged from 0.03 to 0.95 mg/l (0.32 $\pm$ 0.35 mg/L), *Phragmites* 14 cm wetland varied from 0.02 to 0.21 mg/L (0.06 $\pm$ 0.07 mg/L) and the range of *Phragmites* 21 cm wetland (fig 14) was 0.01 to 0.09 mg/L (0.04 $\pm$ 0.03 mg/L). The wetland with the *Phragmites* 7cm water level has relatively better value. There was a scarcity of dissolved oxygen level in the wetland system. Even though NH_4-N concentration in the wetlands was sufficient but the absence of oxygen high NH4-N concentration in the wetland inhibits the nitrification activity (Joseph , 2005).

The effect of water level on NO_3-N concentration in the investigated wetlands was tested using t-test. The result indicated that there was no significant difference between *Phragmites* 14cm and *Phragmites* 21 cm (t-test, t=1.333, df =26 and p = 0.194). On the other hand *Phragmites* 7cm was significantly different from Phragmites 14 and Phragmites 21 cm in regard to nitrate concentrations (t-test, t =2.665, df = 21.558, $p < 0.05$ and t = 3.658, df = 21.338, $p < 0.05$) respectively. Even though the dissolved oxygen level in the wetlands were very small, comparatively water level at 7cm has a good performance in reducing NH_4-N to NO_3-N. One-way ANOVA with post hoc test for multiple comparisons indicated that there was no significant difference in nitrate concentration between the three water levels of the control wetlands (without *Phragmites*) $P > 0.05$.

The t-test was also used to investigate for differences in nitrate concentration between the different water levels (7cm, 14cm and 21cm) in the control wetlands. The results showed that there was no significant difference in the concentration of nitrate between the three control water levels. The t-test result (t=1.184, df =26 and P= 0.247) indicates no significant difference between control 7cm and control 14cm. Similarly, the result (t=1.966, df = 26 and P = 0.60) also indicates no significance difference between control 7cm and control 21cm. The final t-test result, (t= 0.975, df = 25.99 and P = 0.338) also show there is no significant differences between control 14cm and 21cm. A successful constructed wetland for treatment of N must provide suitable conditions for both nitrification and denitrification. The BOD_5 of the wastewater must be less than 20 mg/L before significant nitrification can occur (Reed, et al., 1995). Temperature and water retention time in the wetland are also an important factor for the rate of nitrification.

In figure 4, at the beginning of the monitoring period the NO_3-N concentration was very small and did not show a positive trend. After the wetlands were getting matured, the production of nitrate was improved from time to time accordingly. The process of denitrification in the system could reduce the amount of NO_3-N inside the wetlands. Research in the area proved that nitrate produced can subsequently be reduced to nitrogen gas by biological denitrification if there is a readily available carbon source (Haberl et al., 1995; Vymazal et al., 1998).

Extremely high concentration of ammonium nitrogen and nitrous acid are reported to be inhibitory (substrate inhibition) to the nitrification process. Although effective nitrification has been reported in systems with residual oxygen as low as 0.5 mg/l, DO concentration below 1.5 mg/L are reported to limit the nitrification process (Hammer, 1994).

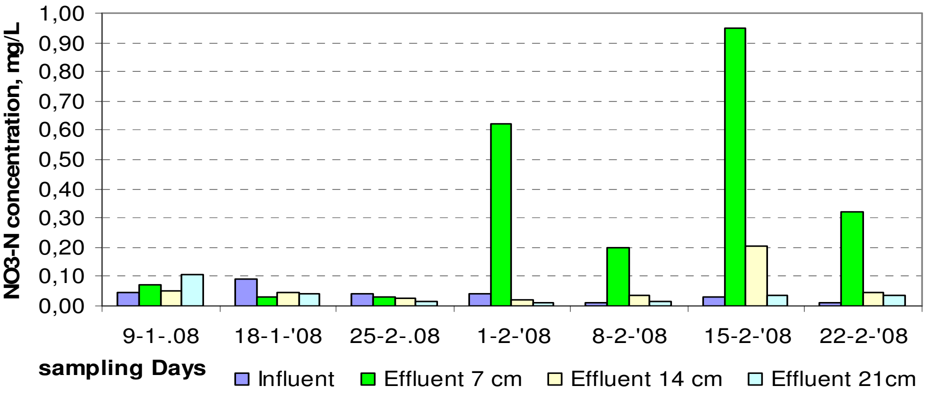


Figure 4: Shows the trends of nitrate concentration in different water levels

3.6 Total Nitrogen

Nitrogen in wastewater exists in many forms. Each of the nitrogen forms is interconvertible and they are components of the nitrogen cycle. From table 10, the total nitrogen in the effluent of *Phragmites* 7cm ranged from 14.8 to 37.5 mg/L with an average of 28.8 ± 8.8 mg/L, in *Phragmites* 14cm effluent the range was from 18.6 to 36.4 mg/L (31.9 ± 6.3 mg/L) and at 21 cm *Phragmites* wetland the range was 28.1 to 37.2 mg/L with average 35.1 ± 3.4 mg/L.

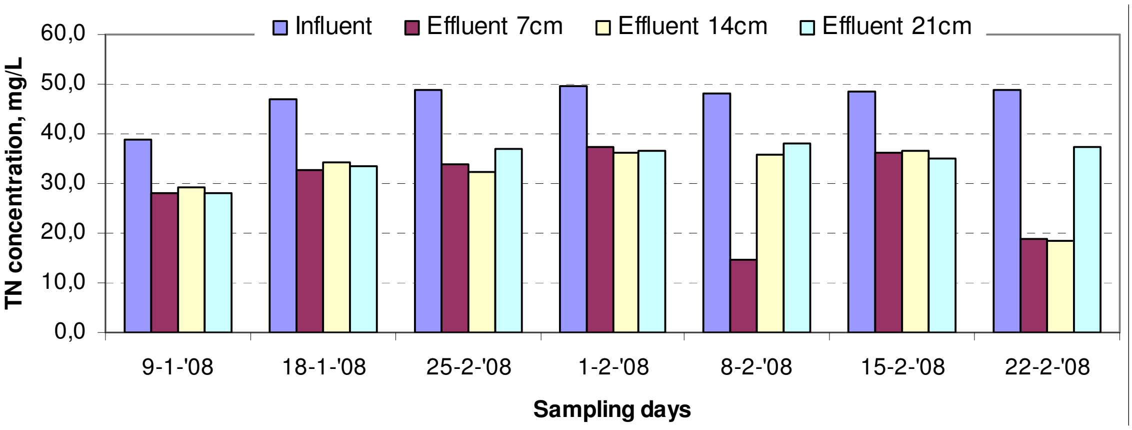


Figure 5: TN (mg/L) in the influent and effluents of the different water levels

From the independent sample t-test result, there was no significant difference in total nitrogen concentrations between the three *Phragmites* vegetative wetlands. But there was a difference in the removal efficiencies between the water levels. The removal percentage of *Phragmites* 7cm, 14 cm and 21cm was 40%, 35% and 29%, respectively. The independent sample t-test result showed that there was a significant difference in TN concentration between plant system and control wetlands ($t = -2.420$, $df = 60.691$ and $P < 0.05$).

3.7 Organic Matter - Chemical Oxygen Demand (COD)

During the first few months of the monitoring period, the COD removal was mostly very weak since the plant root zone and the biological degradation by bacteria was not well established in those periods. But the removal of COD improved over time in the wetland systems.

In the LSCWs COD concentration varies in the three water levels. In *Phragmites* 7cm wetland the COD range was from 27 to 100 mg/L (51 ± 24 mg/L), *Phragmites* 14 cm varies from 38 to 150 mg/L (78 ± 42mg/L) and in *Phragmites* 21cm it was from 45 to 105 mg/L(65 ± 23mg/L). In both water levels COD was reduced above 70% compared to the effluent concentrations.

Independent sample t-test was used to see the significance of the *Phragmites* water levels (7cm, 14cm and 21cm) in relation to COD concentrations. Results showed that there was no significance difference in the mean of COD concentrations among the *Phragmites* wetlands. The t-test result between *Phragmites* 7cm and 14cm was (t = -0.819, df = 22 and P =0.426). Since P > 0.05 there has been no significant difference between the two wetlands. Similarly, P was greater than 0.05 in the other water levels. Even though the results indicated that there is no significance difference between the three wetlands, but COD was removed in the both wetlands effectively.

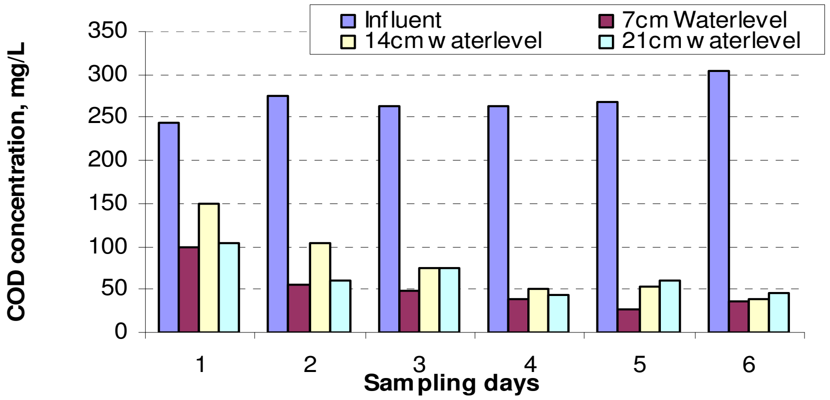


Figure 6: COD (mg/L) in the influent and effluents of different water levels

3.8 Biological Oxygen Demand (BOD$_5$)

The measured average concentration of BOD$_5$ in the effluents of *Phragmites* 7cm, *Phragmites* 14 cm and *Phragmites* 21 cm were 28, 1 ± 7.4 mg/L, 59.9 ± 18.3 mg/L and 41.2 ± 11.5 mg/L, respectively. The treatment efficiency of wetlands for BOD$_5$ is very high (Kadlec and Brix, 1995). The LSCWs has also high removal efficiency of BOD$_5$. The average BOD$_5$ removal in *Phragmites* 7cm, 14cm and 21cm wetlands were 75.3%, 83 % and 88.4%, respectively. Relatively the *Phragmites* 7cm wetland has the highest removal efficiency.

One-way ANOVA with post-hoc tests was used to scrutinize for differences in BOD$_5$ concentration between the different wetland water levels (*Phragmites* 7cm, *Phragmites* 14cm, and *Phragmites* 21cm).The results showed that there was a significant difference (F0.95(38,14)=1.037), P = 0.896 between the groups.

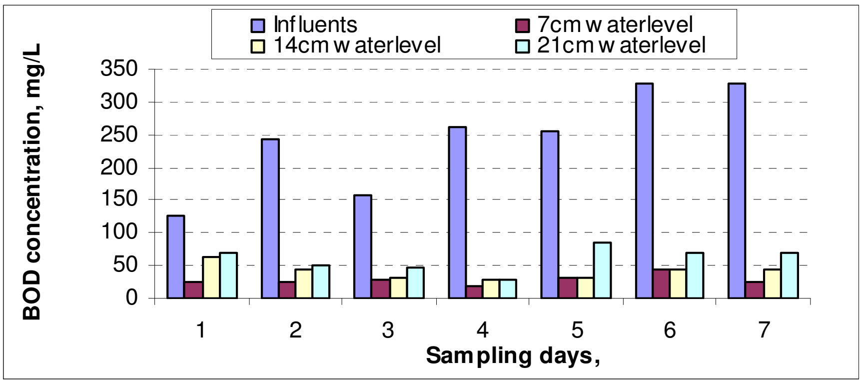


Figure 7: BOD$_5$ of influent and effluents at different water levels, LSCWs

3.9 Comparison of LSCWs with others

(a) Ammonium-Nitrogen

The ammonium–nitrogen concentration in the influent in ZIN wetlands for 2007 was higher than 2008 treatment. Samples in 2007 were taken during summer season. But the NH4-N influent concentration of ZIN 2008 was comparable to the Czech Republic and Austria. In most countries (table 6), there was a reduction of ammonium-N in the effluents, but in the different water levels of the LSCWs ammonium-N concentration was increased in the effluents. The reason in this case was the influent for the LSCWs was prepared artificially in the laboratory. The nitrogen in the artificial wastewater was not in the ammonium form. It is in organic nitrogen form. Therefore, the treatment wetlands in the laboratory convert the organic nitrogen into ammonium-N by hydrolysis and bacterial actions.

Table 6: NH_4-N Influent and effluent concentration (mg/L) and concentration based removal efficiency (%) of VF CWs.

Countries	Influent	Effluent	RE%
[1]Austria	72	4,5	93,7
[1]France	25	18	28
[1]Czech republic	55	8,5	84,2
[2]METU, Turkey	26,4	11,4	55,9
[3]ZIN New 2007	87,2	0,6	99,3
[3]ZIN old 2007	87.2	0.9	97
ZIN Old 2008	62,8	0,21	99
ZIN New 2008	62,8	2,7	95,8
LSCW 7cm	4,4	29	
LSCW 14cm	4,4	31,3	
LSCW 21cm	4,4	34	

Sources: Vymazal et al. (1998), Korkusuz (2004), Shresta (2007)

The removal efficiency of ZIN in 2007 and 2008 was higher than any other country. But in the laboratory scale contracted wetlands rather there was a production of ammonium. In comparison ZIN 2008 with ZIN 2007 there is no significant difference in removing ammonium. But if a closer look is made, the new ZIN effluent 2008 a little bit lower removal percentage than the others. Raising the water level of the new effluent to 1meter high may create anaerobic condition inside the wetland to convert nitrate into nitrogen gas by denitrification pathway. The higher removal efficiency of NH_4-N in 2007 and in 2008 in both new and old wetlands is due to the low loading rate of BOD_5 in the wetlands. The oxygen found in the wetland is used for oxidation of NH_4-N to NO_3-N rather than to oxidize BOD_5.

(b) Nitrate-Nitrogen

The NO_3-N concentration in all wetlands was very low. Low NO_3-N concentration is the characteristic property of most influent wastewater. Nitrate is formed after the biological oxidation of NH_4-N by *Nitrosomonas* bacteria. In all influent wastewaters there are low NO_3-N concentrations.

Table 7: NO_3-N Influent and effluent concentration (mg/L) and concentration based removal efficiency (%) of VF CWs.

Countries	Influent	Effluent
[1]Czech republic	0,7	24,4
[1]France	0	5
[2]METU, Turkey	1,47	8,38
[3]ZIN New 2007	0,4	71,3
[3]ZIN old 2007	0,4	63,5
ZIN Old 2008	0,17	55
ZIN New 2008	0,17	46,5
LSCW 7cm	0,04	0,32
LSCW 14cm	0,04	0,06
LSCW 21cm	0,04	0,04

Sources: Vymazal et al.(1998), Korkusuz (2004), Shresta (2007)

ZIN 2007 and 2008 at both new and old effluents there is a higher NO3-N concentration comparing to other countries. ZIN wetlands have the better nitrification potential than the other countries. In comparing new and old effluents of 2008 the result of NO_3-N concentration, the new effluent with the higher water level has a little bit lower nitrate-N concentration. The reason could be the creation of the anaerobic environment in the wetland that reduced NO_3-N to N_2 gas.

(c) Total Nitrogen

The total nitrogen concentration in the influent of ZIN is almost comparable to Czech Republic influent. But the TN in the influent of the LSCW is very low. The removal of TN in most wetlands, are very low and variable according to the characteristics of the incoming wastewater concentration. ZIN wetlands have a relatively lower removal efficiency than the Czech Republic and the LSCWs. In comparison between the old and the new wetlands of ZIN, the new effluent with a higher water level has a higher TN removal efficiency. Even though the removal efficiencies of the LSCWs were very close to each other, the wetland with the lower water level (7cm) has the highest removal efficiency. In the LSCWs every condition between the wetlands are the same, but the only difference between them is the depth of the water level. The water level with 7cm, the lower water level, can create anaerobic conditions better than the other water levels. This implies that at 7cm water level the condition for denitrification was better than at the other water levels.

Table 8: TN Influent and effluent concentration (mg/L) and concentration based removal efficiency (%) of VF CWs.

Wetlands	Influent	Effluent	RE%
[1]Czech Republic	81,9	47,6	43,6
ZIN old 2008	76,9	56,6	24,3
ZIN new 2008	76,9	50,2	32,3
LSCW 7cm	47,1	28,8	38,9
LSCW 14cm	47,1	31,9	32,3
LSCW 21cm	47,1	35,1	25,5

Source: Vymazal et al. (1998)

(d) Organic Matter, COD

The mean influent COD concentration of the LSCWs is lower than ZIN 2008 (new and old) and ZIN 2007 treatment. This could be the source of potential problems for denitrification in the laboratory scale wetlands. The ZIN 2007 and 2008 treatments have higher COD influent concentration than the Belgium, Austria and Turkey. Does this lower concentration of COD in the influent really affect the treatment efficiency of the wetlands?

Table 9: COD Influent and effluent concentration (mg/L) and concentration based removal efficiency (%) of VF CWs.

Countries	Influent	Effluent	RE%
[1]Austria	325	33	90
[1]Belgium	168	60	64
[2]METU, Turkey	251	143	49
[3]ZIN New 2007	417	49	88
[3]ZIN old 2007	417	62,9	88
ZIN old 2008	379	39.8	89.3
ZIN new 2008	379	21.4	94.6
LSCW 7cm	270	51	81
LSCW 14cm	270	78	71
LSCW 21cm	270	75	70,8

Sources: Vymazal et al. (1998), Korkusuz (2004), Shresta ,(2007)

From table 22, ZIN 2007 and ZIN 2008 treatment has the highest removal efficiencies than others. The LSCWs have a higher removal efficiency than the METU, Turkey and Belgium. Even though the difference is very small, the LSCW with the smaller water level (7cm deep) has the highest removal efficiency of COD than 14cm and 21cm water levels. Similarly, ZIN new (1 meter height) effluent has a better COD removal efficiency. This indicates creating an anaerobic zone is useful for removal of COD. In all LSCWs as well as in ZIN the wetland with anaerobic environment has the better removal efficiency for COD, NO_3-N & TN.

(e) Comparison between Phragmites and Carex Pendula

Similar experiments were also done by Wasala in 2008 using *Carex pendula* as a wetland plant. The results of the experiments were very near to each other. Even though the wetlands have different plants, the other condition remains the same in both wetlands. This indicates that nitrogen removal by plant uptake is very little in both plants but plants have a significant influence on the nutrient removal. The wetlands under investigation were not fully matured. This could have an impact on the performance of the wetlands to visualize the difference between the phragmites *and Carex pendula.*

Table 10: Comparison of wetlands with Phragmites to Carex pendula

	Phragmites			*Carex pendula*		
	7cm	14cm	21cm	7cm	14cm	21cm
Ave $_{\text{NH4-N (mg/l)}}$	29.9 ± 3.5	31.9± 2.5	33.9± 3.1	32.9.± 3.3	31.9± 2.4	32.8± 3.5
Ave $_{\text{NO3-N mg/l}}$	0.32± 0.35	0.06± 0.07	0.04± 0.03	0.63± 0.91	0.04± 0.05	0.04± 0.04
Ave $_{\text{COD (mg/l)}}$	51 ± 24	78 ± 42	65 ± 23	71 ± 42	60 ± 32	70 ± 35
Ave $_{\text{BOD(mg/l)}}$	28.1 ± 7.4	59.9± 18.3	41.2± 11.5	32.9± 14	36.8± 23.2	41.4± 31.4
Ave $_{\text{TN (mg/l)}}$	28.8 ± 8.8	31.9± 6.3	35.1 ± 3.4	35.4± 3.7	33.8± 2.5	36.2± 3.1
TN removal %	38.8	32.3	25.5	27.1	30.5	25.6
N-removal due to denitrification	28.5	22.8	16.2	13.0	14.2	8.2
COD removal %	81	71	76	76.6	80.1	77.0
BOD removal %	88.4	83.0	75.3	86.2	84.5	79.7
Potential nitrification (mg/kg/day)	1.5	0.8	1.1	1.8	1.0	0.4
Potential denitrification (mg/kg/day)	26.5	26.01	24.39	23.4	24.8	25.0

Relatively, the nitrogen removal by *phragmite* is higher than the *catex pendula*. High ammonium concentration in *carex pendula* than phragmites indicates that better nitrification was taken place in phragmites. Reed contributes to wastewater cleaning processes in many different ways: increasing the permeability and porosity of the substrate; creating oxygenated microsites within reducing conditions by releasing oxygen from the roots (Csilla, et al, 2005).

4. CONCLUSIONS AND RECOMMENDATIONS

The study focused on the potential use of Vertical Flow Constructed Wetlands in removing nitrogen from domestic wastewater at different water levels. It demonstrates that the aerobic and anaerobic conditions are an important focus in removing nitrogen from domestic wastewater in the treatment wetlands. Similarly, in LSCWs there was a significance difference between the three water levels in removing nitrogen from the constructed wetlands was absorbed. COD and TN removal was higher in *Phragmites* 7cm water level than *Phragmites* 14cm and 21cm water levels. COD removal in 7cm, 14cm and 21cm bucket were 81%, 71% and 70.8% respectively, and TN removal was 38.9% 32.3% and 25.5% respectively. From the results obtained, the water level with 7cm has the best performance in removing nitrogen to

14cm and 21 cm water levels. This is due to the denitrifying are active in the anaerobic 7cm water level to undergo denitrification.

The oxygen concentration in the LSCWs was very scarce. The organic materials that contain nitrogen in the wastewater were converted to ammonia by hydrolysis and breaking of big molecules. Since oxygen in the wetlands was very low, ammonium-N didn't undergone oxidation. As a result the nitrification process became very low. The accumulation of ammonium-N in the LSCWs inhibits the nitrification.

The main objective of this research is to find an optimum condition for nitrogen removal from domestic wastewater by wetlands using different water levels. In most wetland nitrogen removal is very low and variable. According to the EU directive for wastewater discharge to the environment, the discharge limit for TN is 15 mg/L for a population equivalent between 10000-100000 P.E. (EU, 1998). The results of TN from the LSCWs are above the EU discharge limit. Therefore, to meet the EU discharge limit, the following points are recommended.

- Effect of plants can be seen if the wetland is matured. At the early stage of the plant growth the effect on nitrification/denitrification may not be observed. So, more time is needed for plant growth.
- The artificial wastewater feed into the wetlands should have enough carbon sources. In the AWW prepared for the LSCWs, there was a shortage of ammonia. Because all the nitrogen source in the wastewater was in the form of organic matter.
- The water level for 14cm is difficult to differentiate from 21cm and 7cm. It is better to increase the gap between the water levels.
- The LSCWs experiment was done in the confined room. The climate room didn't represent exactly the real natural condition. The model wetlands could be constructed outside the room.

REFERENCES

Abira M. A., 2008. A pilot constructed treatment wetland for pulp and paper mill wastewater. Unpublished PhD dissertation.

Anderson, D. M., P. M. Gilbert, et al. (2002). "Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences." Estuaries 25(4B): 704-726.

Bastviken, S. (2006). Nitrogen removal in treatment wetlands- Factors influencing spatial and temporal variations. Linkoping Studies in Science and Technology. Linkoping, Sweden, Linkoping University,Institute of Technology**:** 34.

Brix, H. (1998). Constructed wetlands for wastewater treatment in Europe._. Leiden, Backhuys publishers.

Bruggen, J. J. A. v. (2007). Wetland for Water Quality. Delft, UNESCO-IHE Institute for Water Education.

Christina, L. (2005). Nutrient Removal using a constructed wetland in South Quebec. Department of Bioresource Engineering Montreal, McGill Univeristy**:** 101.

Cooper, P. F. (!996). A review of the design and performance of vertical flow and hybrid reed beed treatment systems,, Water sci Technol.

Csilla, T., M. G. Tamás, et al. (2005). "The use of the reed (Phragmites Australis) in wastewater treatment on constructed wetlands." Acta Biologica Szegediensis Volume 49(1-2): 81-83.

Dupoldt, C., R. Edwards, et al. (1993). A Handbook of constructed wetlands, http://www.epa.gov/owow/wetlands/pdf/hand.pdf**:** 52.

Environment Canada. 2001. Threats to sources of Drinking Water and Aquatic Ecosystem Health in Canada. National Water Research Institute, Burlington, Ontario. NWRI Scientific Assessment Report Series No. 1.72p.

Hammer, D. A. (1989). Constructed wetland for Wastewater Treatment. Chelsea, Michigan, Lewis publishers, INC.

IWA., 2000. In: Cooper P (Ed), Specialist group on the use of macrophytes in water pollution control, IWA Newsletter No. 22, pp 1 – 68.

Joseph, K. (2005). Optimizing Processes for Nitrogen Removal in Nakivubo Wetlands, Uganda. Stockholm, Royal Institute of Technology**:** 57.

Kadlec, R. H. and R. L. Knight (1996). Treatment Wetlands. Boca Raton, CRC Press, Inc.

Kelderman, P., H. J. Lubberding, et al. (2006). Eutrophication, Case Stady. Delft, UNESCO-IHE Instutute for water education**:** 35.

Kruis, F. (2007). Environmental Chemistry: Selected Methods for Water Quality Analysis Delft, UNESCO-IHE.

Meuleman, A. F. M. (1999). Performance of treatment wetlands. Utrecht, Utrecht University.

Plessis, C., K. Kinney, et al. (1998). "Denitrification and nitric oxide reduction in an aerobic toluene-treating biofilter." Biotechnol.Bioeng 58: 408-415.

Ramsar (2006). The Ramsar Convention Manual: A Guide to the convention on Wetlands. Switzerland, Ramsar Convention Secretariat.

Reddy, K. R. and W. H. Patrick (1984). Nitrogen transformations and loss in flooded soils and sediments, CRC Crit Rev Environ Sci Technol

Reed, S. C., R. W. Crites, et al. (1995). Natural Systems for Waste Management and Treatment. New York, McGraw-Hill.

FAO(1992), Wastewater treatment and use in agriculture- irrigation and drainage, page 47.

Sedlak, R. (1991). Phosphorus and Nitrogen Removal from Municipal Wastewater New york, Lewis Publitior.

Shresta (2007). Possibilities for Recycling Domestic Wastewater with Vertical Flow Constructed Wetlands. Environmental Science. Delft, UNESCO-IHE Institute for Water Education. Master of Science: 72.

UN (2005). Investing in Development: A Practical Plan to Achieve the Millennium Development Goals._MDG Summery Task Force Reports. New York, UN Millenium Project.

USEPA (2000). Constructed Wetlands Treatment of Municipal Wastewater Manual(EPA625R99010), . National Risk Management Research Laboratory, office of research and development. USEPA. Cincinnati,Ohio.

Vymazal J., 1995. Algae and element cycling in wetlands, Boca Raton, FL, USA; CRC Press-Lewis Publishers,

Vymazal, J. (2007). "Removal of nutrients in various types of constructed wetlands." Science of The Total Environment 380(1-3, 15 July 2007): 48-65.

Wasala, W. M. (2008). Use of constructed wetlands for Wastwater Treatment emphasizing on Optimization of Nitrogen removal. Environmental Science. Delft, UNESCO-IHE institute for Water Education**:** 53.