ADDIS ABEBA UNVERISTY
SCHOOL OF GRAGUATE STUDIES
ADDIS ABABA INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL AND BIO ENGINEERING
ENVIRONMENTAL ENGINEERING STREAM

# STUDY OF DUCKWEED-BASED POND SYSTEM FOR THE TREATMENT OF DOMESTIC WASTEWATER

BY

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE
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**A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Degree of Master of Science in Environmental Engineering**

**Approved by the Examining Board**

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| | |
|---|---|
| APHA | American Public Health Association |
| ANOVA | Analysis of variance |
| $BOD_5$ | Five day Biochemical Oxygen Demand |
| CFU | Colony Forming Unit |
| COD | Chemical Oxygen Demand |
| CP | Crude protein |
| DM | Dry matter |
| DO | Dissolved Oxygen |
| DBWWT | Duckweed Based Wastewater Treatment |
| DW | Dry Weight |
| EC | Electrical Conductivity |
| EEPA | Ethiopian Environmental Protection Authority |
| HRT | Hydraulic Retention Time |
| Kg/ha/d | Kilogram per hectare per day |
| FC | Faecal Coliform |
| HLR | Hydraulic Loading Rate |
| L | Liter |
| $M^3$/d | Cubic meter per day |
| OLR | Organic Loading Rate |
| TC | Total Coliform |
| TKN | Total Kjedhal Nitrogen |
| TN | Total Nitrogen |
| TP | Total Phosphorus |
| TSS | Total Suspended Solid |
| USEPA | United States Environmental Protection Agency |
| WSP | Waste Stabilization Ponds |

# ABSTRACT

Existing wastewater treatment technologies such as activated sludge and tertiary nutrient removal systems are too costly to provide a satisfactory solution for the increasing wastewater problems in developing regions. Besides, these technologies do not allow for re-use of valuable nutrients contained in wastewater. In the present study, an aquatic plant duckweed (*Lemna minor*) was investigated as an alternative cost effective biological tool for the treatment of domestic wastewater to remove concentrations of organic matter, nutrients and pathogens.

Duckweed plants were inoculated into semi-batch experimental ponds to treat pretreated and untreated sewage water. The experiments were conducted under outdoor environmental conditions for a period of 21 days. Composite samples were collected under the duckweed mat every seven days. Efficiencies of duckweed for the treatment of domestic wastewater were assessed by measuring some of the physico-chemical parameters and faecal coliforms in the treated wastewater. The observations revealed mean reduction levels of 86.28% $BOD_5$, 82.26% COD, 90.18% TSS, 66.07% $NH_4^+$, 65.26% TKN, 68.84% $PO_4^{3-}$, 70.58% TP, 97.67% FC for duckweed ponds with untreated sewage and 88.14% $BOD_5$, 86.56% COD, 91.6% TSS, 85.84% $NH_4^+$, 68.20% TKN, 81.01% $PO_4^{3-}$, 74.21% TP, 98.95% faecal coliforms for duckweed ponds with pretreated sewage. Compared with the Provisional National Environmental Quality Standard, all parameters values of the duckweed effluents except faecal coliforms bacteria were within the standard limit values set by EEPA. However, faecal coliform values fulfilled WHO guideline set to ensure safe reuse of the effluents. The results showed that duckweed can be successfully used for treatment of domestic wastewater.

Keywords: Duckweed, *Lemna minor*, Wastewater, Nutrient

# 1. INTRODUCTION

## 1.1. Background

Domestic and industrial wastewater discharged without any kind of treatment is causing environmental pollution in large and small towns in Ethiopia. With increase of population, industrial and business activities, the situation will be more aggravated without adoption of an affordable and manageable safe wastewater collection, treatment and disposal system. The suitability and affordability of the conventional end of pipe system is questionable for low income countries. The increase in awareness of water pollution from different sources and the impact it has on public and environmental health draws attention to search for a feasible wastewater treatment options for developing countries like Ethiopia. Natural wastewater treatment systems are the viable options that are not only low cost but also enable us to save energy, to recover and recycle nutrients from wastewater. To this end, they are beneficial and sustainable systems that can be widely utilized in developing countries According to Nhapi (2003) wastewater treatment systems that are considered natural systems are: (1) Anaerobic pre-treatment in septic tanks, anaerobic ponds or high rate anaerobic reactors, (2) Waste stabilisation ponds, (3) Macrophyte ponds, (4) Fish aquaculture, (5) Constructed wetlands, and (6) Land treatment systems.

Therefore; to avoid diseases that are related to polluted drinking water, to recycle nutrients and to help meet the increasingly growing water demand and to supplement available water resource of the country, there is a need to use sustainable wastewater treatment options.

One of the sustainable wastewater treatment schemes comprising of anaerobic ponds as primary treatment unit followed by duckweed ponds is considered as a cost-effective way of energy, nutrients recovery and water recycling. In case of anaerobic treatment there is no need to supply mechanical aeration that is very expensive in view of its high energy and equipment need.

The anaerobic treatment is a mineralization process, which provides nutrients-rich effluent, representing a good growth medium for the production of protein-rich aquatic plants. Aquatic plants can also be used for wastewater treatment and recycling of nutrients. They are generally classified into emergent plants, plants in suspension, and floating plants.

Duckweed based wastewater treatment systems utilize a floating aquatic plants called duckweed for the treatment of domestic wastewater. Duckweed based wastewater treatment systems are efficient, low-cost, easily operated and distinguish themselves from other treatment systems by providing valuable protein rich byproduct harvested after wastewater treatment. DBWWT systems provide authentic solution to attain a healthy freshwater environment resulting in reduction of waterborne diseases and improving socio-economic aspects of the society. Duckweed systems are an ideal method of natural wastewater treatment for developing countries as it doesn't require skilled manpower, energy and high financial resources for construction and maintenance. Solids and organic materials in the wastewater are removed via sedimentation and bacterial mineralisation, while the plants are active in nutrient removal

Wastewater treatment by duckweed covered ponds has attracted the attention of researchers in various parts of the world. DBWWT systems have been applied at full-scale in Taiwan, China, Bangladesh, Belgium and the USA.

In view of this, DBWWT systems are a promising wastewater treatment technology that needs to be researched and adapted to the local environment to utilize it to its full capacity. The aim of this study is in the first instance, to provide information on the performance of duckweed-based wastewater treatment system as a sustainable solution for the treatment of domestic wastewater. The output of this study will be relevant for the development of such multifaceted and affordable wastewater technology for small and large towns throughout the country.

## 1.2. Statement of the Problem

Urban centers in Ethiopia are growing very fast in terms of population and size with increasing demand for sanitary service. Almost all urban areas in the country do not have wastewater treatment facilities. Safe collection, treatment and disposal of wastewater are becoming a major challenge for local governments. Conventional wastewater treatment systems such as activated sludge treatment systems require: high energy, high skilled man power, high capital and operational cost and thus are not affordable and sustainable.

Natural systems such as waste stabilization ponds (WSP), wetlands and duckweed pond system are some of the low cost wastewater treatment system that can be affordable to the low income tropical countries. Generally, WSP systems are considered to be ineffective in reducing of TSS and recycling of nutrients. To retain the algal biomass, it requires employing an advanced technology which is not affordable by low income countries. As a result, nutrient (N, P) incorporated in sedimented algae subsequently remineralized. The unsettled part of algae biomass leaves the system with the effluent.

Duckweed based wastewater treatment ponds are modified wastewater stabilization ponds, covered with a floating mat of aquatic plants (*Lemnaceae*). DBWWT system efficiently treats nutrient rich wastewater and recovers the nutrient through generation of a high protein and a relatively low fiber content biomass which is suitable for use as high-quality feed supplement. It creates opportunities for associated downstream pond fishery developments using duckweeds as a food source. Consequently, the wastewater treatment technology becomes sustainable through generation of income and contribution of food security.

There is a need to develop and enhance a wastewater treatment technology within the economical and technological capabilities of developing countries which aims to recover nutrient and produce an effluent with low TSS, BOD and pathogens. Therefore, this study investigates the performance of duckweed based ponds for the treatment of domestic wastewater.

## 1.3. Objective of the Study

### 1.3.1 General Objectives

The overall objective of this study is to investigate the performance of duckweed pond system for the treatment of domestic wastewater.

### 1.3.2 Specific Objectives

The specific objectives of this study were:

- Characterization of the influent domestic wastewater;
- Assessment of the efficiency of duckweed-based system for the treatment of domestic wastewater;
- Characterization of the quality of the effluent;
- Evaluate the effluent quality with respect to environmental quality requirements;

## 1.4. Significance of the Study

Duckweed based wastewater treatment system effectively recover nutrients from domestic wastewater. This enables to recycle nutrients through production of aquatic biomass called duckweed which is a fast-growing and easy to harvest plant that can be utilized for animal feed, primarily for fish and poultry. On the other hand, treated wastewater eliminates environmental pollution that would have been caused by untreated wastewater discharges. Compared with other conventional wastewater treatment technologies, DBWWT system doesn't require high technology, skilled manpower, energy and other resources so as making it a sustainable wastewater treatment system for tropical developing countries. The fact that it realizes the recovery and reuse of nutrients, people can contribute to the cost of wastewater treatment with great interest. DBWWT enables nutrient recovery from wastewater so as to utilize it for feeding of animals, thereby contributing to food security.

In addition, the output of this study will be relevant to design and use of environmentally sound duckweed based wastewater treatment ponds for small and medium towns in peri-urban areas of Ethiopia. Above all, the finding may trigger further studies and researches to develop efficient duckweed based wastewater treatment system suitable for local condition.

# 2. LITERATURE REVIEW

## 2.1 Characteristics of Duckweed

Duckweed is a small, free floating aquatic plant belonging to Lemnaceae family (Cheng et al., 2002). The Lemnaceae family consists of four genera (Lemna, Spirodela, Wolffia & Wolffiella) and 40 species have been identified (Leng, 1999). They are green and have a small size (1-3mm), with short but dense roots (1-3cm) (Altay et al., 1996). Duckweed fronds grow in colonies that, in particular growing conditions, form a dense and uniform surface mat (Hasar et al., 2000).

Duckweed found world-wide on the surface of nutrient rich fresh and brackish waters (Zimmo, 2003) but the greatest diversity appears in subtropical and tropical areas. Their habitat comprises still or slowly moving fresh or polluted waters of only a few mm to 3m depth. In particular nutrient-rich and sheltered small ponds, ditches and swamps, e.g. down-stream from sewage works, often contain duckweed (Leng, 1999).

Compared to most other plants, duckweed has (about 5%) low fiber content (Leng, 1999), since it does not require structural tissue to support leaves and stems. Due to the high protein content of duckweed (29-41%, Culley and Epps, 1973) duckweed has a high potential for protein production. The relatively high protein content of duckweed is generating interest in animal feed applications of this aquatic plant.

Figure 2.1. Pictures of *Lemna minor*

## 2.2 Factors Affecting Duckweed Growth

Duckweed reproduces both vegetatively and sexually with sporadic and unpredictable flowering. Vegetative propagation occurs through clonal budding of new daughter fronds from two pockets on each side of the mature frond (Leng, 1999). Parameters affecting duckweed growth in natural environments include temperature, light intensity, other climatic conditions, pH and availability of nutrients in the water. Under ideal conditions in terms of water temperature, pH, incident light and nutrient concentrations, biomass can double within 48 hours, competing with the most productive terrestrial plants and even exceeding biomass accumulation of field crops such as corn (Cheng and Stomp, 2009a; Leng, 1999). Inhibition occurs at high nutrient (ammonia) concentrations (Caicedo *et al.*, 2000).

### 2.2.1 Temperature and Wind

Duckweed can grow between 6 and 33°C water temperature, with a positive correlation of growth rates and increasing water temperature up to around 30°C (Leng, 1999). It is therefore less sensitive to cool temperatures than other macrophytes (Iqbal, 1999), but extreme (low and high) temperatures adversely affect its growth (Edwards, 2010). Optimal temperature lies between 20 and 30°C, which should be considered when designing duckweed ponds because in shallow waters the temperature can quickly leave this range (Leng, 1999).

Studies in temperate and cold climates have shown that under natural conditions, duckweed growth is significantly reduced or completely stopped during winter months (Leng, 1999). When the temperature drops too low, duckweed can persist until favorable conditions return by producing starch filled structures or turin which are denser than the fronds so that the plants sink to the bottom (Leng, 1999). Duckweed is sensitive to wind and is therefore not suitable to be grown in windy regions (Iqbal, 1999).

### 2.2.2 Light

The utilization rate of solar energy for wild plants is only 0.5%, and that of crops 0.5-1%. Algae and duckweeds, however have a much higher utilization efficiency in the range of 3-5% (Wang, 1991). This has attracted more and more farmers in developing countries to construct ponds to grow various species of macrophytes with high production rate. High light intensity and direct exposure to sunlight can impede duckweed growth; shading is preferred (Edwards, 2010). Duckweed tend to cover the water surface and block out the passage of light to the water below, denying algae the energy to grow and reproduce.

### 2.2.3 PH

Duckweed can survive at a pH between 5 and 9 but grows best in the range of 6.5-7.5. High pH values lead to $NH_3$ in solution which can be toxic and also lost through volatilization (Iqbal, 1999; Leng, 1999).

## 2.3 Application of Duckweed for Wastewater Treatment

### 2.3.1 Wastewater Characteristics

Sewage or wastewater is a dilute mixture of various wastes from residential, commercial, industrial and other public places. The amount and composition of wastewater produced in households is influenced by the behavior, lifestyle and standard of living of the inhabitants. The quality of wastewater is determined by many factors. Before we can decide about the line of treatment and disposal, it is essential to know its composition, quality and characteristics. The characteristics or properties of wastewater can be classified as physical, chemical and biological characteristics.

The most important physical characteristic of water is its total solids content, consisting of floating matter, matter in suspension, colloidal matter and matter in solution. Other physical characteristics are: (i) smell or odor (ii) color and (iii) temperature.

Important chemical characteristics of sewage are: (i) pH value, (ii) chloride content, (iii) nitrogen content, (iv) fat, grease and oil content, (v) sulfides, sulfates and $H_2S$ gas, (vi) dissolved oxygen, (vii) chemical oxygen demand and (viii) biochemical oxygen demand.

Biological characteristics relate to various micro organisms found in wastewater, some of which may be pathogenic. But all bacteria present in wastewater are not harmful; some of these help to treat the wastewater and reduce the cost of treatment plants.

The organic matter in sewage consists of urea from urine, proteins, carbohydrates, fats and soaps. These undergo continuous decomposition and in that process, pass through several stages, resolving into simpler elements such as nitrogen, hydrogen, carbon and oxygen with small quantities of sulfur and phosphorous. These elements ultimately combine by means of chemical and biological actions to form inorganic substance.

Wastewater can be characterized by its main contaminants (Table 2.1) which may have negative impacts on the aqueous environment in which they are discharged. The amount and composition of the wastewater are of prime importance to designers and operators of a treatment facilities. The objective of wastewater treatment is to reduce the concentrations of specific pollutants to the level at which the discharge of the effluent will not adversely affect the environment or pose a health threat.

The most important parameters in assessing the strength of a given wastewater as described by Ronald (1997) are: BOD, COD, TSS, nutrients (N, P), pathogens, pH and temperature.

Table 2.1 Typical characteristics of domestic wastewater (adapted from Metcalf and Eddy Inc., 1991)

| Parameter | Concentration | | |
|---|---|---|---|
| | Strong (mg/L) | Medium (mg/L) | Weak (mg/L) |
| $BOD_5$ | 400 | 220 | 110 |
| COD | 1,000 | 500 | 250 |
| TKN | 35 | 15 | 8 |
| $NH_3$-N | 50 | 25 | 12 |
| Total-P | 15 | 8 | 4 |
| Total Solids | 1,200 | 720 | 350 |
| Total Suspended Solids | 350 | 220 | 100 |

### 2.3.2 Duckweed Based Wastewater Treatment

Duckweed wastewater treatment systems have been studied for raw and diluted domestic sewage (El-Shafai *et al.* 2007, Korner, 2003), dairy waste lagoons (Culley *et al.* 1981), secondary effluents (Gurtekin *et al.* 2008) and waste stabilization ponds (Dalu *et al.* 2002, Zimmo *et al.* 2005). Several full-scale systems are in operation in Taiwan, China, India, Bangladesh, Belgium, and the USA (Zirschky and Reed 1988, Alaerts *et al.* 1996, Koerner *et al.* 1998).

Different authors have proposed to use duckweed ponds for the efficient and low-cost treatment of domestic and industrial wastewater at urban or rural levels (Diederik et al., 2011). The duckweed plant has been used in stabilization pond systems for wastewater treatment, which represent an important class of wastewater treatment systems in developing countries because of their cost-effectiveness (Gijzen, 2001b). Waste stabilization ponds are low cost wastewater treatment systems producing high-quality effluents that allow water reuse in irrigation (Zimmo et al. 2004).

The duckweed treatment plants installed so far almost exclusively treat domestic or agricultural wastewaters. Available literature is limited on the treatment of specific industrial wastewaters (Gijzen and Khondker 1997). Potentially, duckweed may also be applied for the treatment of industrial wastewaters, provided their nutrient content is high enough. Effluents with both a high BOD and nutrient load may require adequate primary treatment to reduce the organic load. The upper BOD limit of tolerance for duckweed growth is unknown (Gijzen and Khondker 1997). Industrial wastewaters with a high BOD load and low nutrient content are less suitable to favour duckweed growth. Mdamo (1995) reported that duckweed growth on paper mill effluents was only observed when BOD was relatively low (150 mg/L) and nutrients were added externally. High BOD removal of over 98 % was observed when 2 mg per $m^2$ of both N and P were added daily. Without the addition of nutrients, almost no duckweed growth was observed on the paper mill wastewater. Neither did wastewater with a very high BOD level (2900 mg/L) promote duckweed growth. However, Skillicorn *et al.* (1993) reported that a simple rule of thumb for dilution of primary effluent is to ensure that $BOD_5$ plug-flow treatment system is maintained below 80 mg/L.

Apart from high BOD concentrations, fatty acids, oil and grease were reported to have a negative effect on duckweed growth. This is probably due to adsorption to the plants' submerged surfaces and subsequent inhibition of nutrient uptake. Duckweed is reported to tolerate rather high concentrations of detergents (Gijzen and Khondker 1997). Skillicorn *et al.* (1993), however, suggested that high concentrations of detergents may destroy the duckweed's protective waxy coating, thereby rendering the plant more vulnerable to diseases.

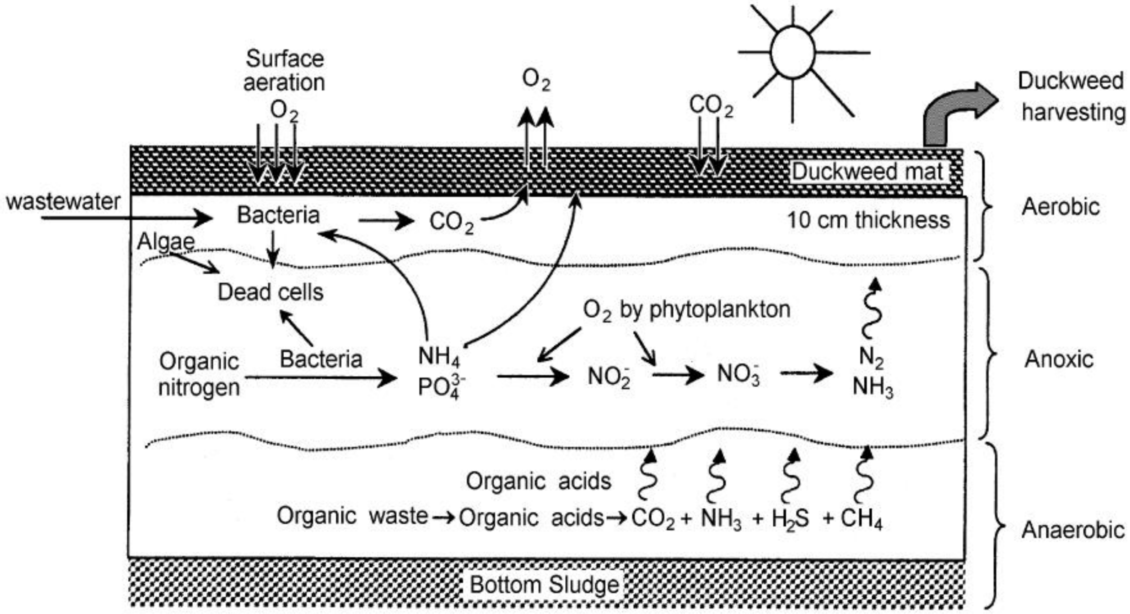


Figure 2.2. Biological Process in Duckweed Based Wastewater Treatment System

Source: Smith *et. al.* (2001)

### 2.3.1 Removal Mechanisms of Pollutants by Duckweed

The removal mechanisms of DBWWT systems for TSS, BOD, nitrogen, phosphorous, heavy metals, organic toxins and pathogen as assumed by various authors briefly described and discussed below.

#### 2.3.1.1 TSS Removal

Total Suspended Solids are removed mainly by sedimentation and biodegradation of organic particles in the pretreatment and duckweed pond system. A minor fraction is absorbed by the roots of the duckweed fronds, where organic particles undergo aerobic biodegradation by microorganisms, and part of the degraded products is assimilated by the plants. Two characteristics of duckweed treatment systems are believed to play an important role in TSS removal. A complete mat of duckweed inhibits penetration of sunlight and subsequent growth of algae. Large amounts of algae contribute significantly to TSS concentrations. Though algae take up considerable amounts of N, P and other nutrients and may, therefore, contribute to their removal, the nutrients are released again by biodegradation when algae settle, die off and become available again for algal growth.

A dense mat of duckweed can, therefore, reduce algal contribution to TSS. This is one of the reasons why a complete duckweed cover is essential for treatment efficiency of duckweed systems. Compared to facultative ponds, a second, more uncertain factor favoring sedimentation of TSS in duckweed systems is attributed to the quiescent conditions prevalent in the water column under the duckweed cover, as a consequence of the more consistent vertical temperature profile.

#### 2.3.1.2 BOD Removal

The role of duckweed in BOD removal is far from being fully understood. Generally, it can be indirect effects through provision of surface and subsurface for bacterial growth which changes the physicochemical environment in the water (Godfrey et al., 1985). The direct uptake of small hydrocarbons by duckweed as reported by Landolt and Kandeler (1987) indicates sedimentation and heterotrophic activity in the water column are probably the main mechanism in total BOD removal.

Duckweed possess a relatively small surface area for attached growth of mineralizing bacteria compared to other aquatic macrophytes with larger submerged root and leaf surfaces (Zirschky and Reed 1988). The dense cover of duckweed on the water surface would also inhibit both oxygen from entering into the water from the air by diffusion, and photosynthetic production of oxygen by phytoplankton as a result of the poor light penetration (Culley and Epps 1973). According to Zirschky and Reed (1988), BOD removal could even decrease in ponds covered with duckweed because of the limited oxygen transfer into the water.

Alaerts *et al*. (1996), however, found that with a BOD loading rate of 48-60 kg/ha·d, a water depth of 0.4-0.9 m and a HRT of about 20 days, the water column in a duckweed-covered sewage lagoon system always remained aerobic. Surprisingly, Srinanthakumar *et al.(*1983) calculated an aeration rate through the duckweed-covered surface of 3-4 $gO_2/m^2$, which is slightly higher than oxygen transfer through an uncovered surface. This leads to the conclusion that aerobic conditions occur at least in the top layer of a duckweed pond within and under the plant cover due to photosynthetic production of oxygen and surface aeration. Interesting results were also observed by Koerner *et al.* (1998) who reported that COD removal was significantly faster in the presence of duckweed than in its absence. They believe that the structure of duckweed surface and the way oxygen is supplied are important elements, since the positive influence of a living duckweed population on COD removal could not be simulated by artificial plastic duckweed surfaces and oxygen pumps.

Duckweed-covered pond system can become anoxic to anaerobic depending on the organic loading rate, water depth, HRT and the prevalent redox conditions. In this case, the main factors responsible for BOD removal in duckweed treatment systems are probably similar to the anaerobic zone of facultative ponds (Reed *et al.* 1988).

#### 2.3.1.3 Nitrogen Removal

Nitrogen in raw wastewater is mostly organic and ammonia nitrogen form (called Kjeldahl nitrogen). Mineralization of organic matter produces most of the inorganic nitrogen compounds ($N0_3^-$, $NH_4^+$) which are immediately taken up by plant or microbial biomass for the production of protein. A fraction of the total amount of organic nitrogen is re-circulated to $N_2$ via denitrification. The reuse of fixed sources of nitrogen such as nitrate, nitrite, and ammonia, therefore contributes to higher energy efficiency in biological systems.

Research on nitrogen removal in duckweed-based treatment has listed the main mechanisms of removal as: plant uptake, ammonia volatilization, ammonia assimilation into algal biomass, and biological nitrification coupled with denitrification (Zimmo et al. 2000). Körner and Vermaat (1998) claim that only a quarter of total nutrient losses were not directly or indirectly attributed to duckweed.

Existing results suggest that approx. 50 % (± 20 %) of the total nitrogen load is assimilated by duckweed, while the remaining nitrogen is removed by indirect processes other than plant uptake of which nitrogen loss to the atmosphere by denitrification and volatilisation of ammonia are suggested to play a major role (Alaerts *et al.* 1996, Gijzen and Khondker 1997, Koerner and Vermaat 1997).

Laboratory experiments were carried out by Korner and Vermaat (1998) in shallow (3.3 cm), 11-batch systems to assess the contributions of duckweed to nitrogen removal in domestic wastewater. They showed that depending on the initial concentrations, the duckweed covered systems removed 73 - 97% of the initial Kjeldahl nitrogen in three days. Also duckweed was directly responsible for 30 - 47% of the total nitrogen loss by the uptake of ammonium.

In ponds with aerobic and anaerobic environments favouring microbial nitrification and denitrification, ammonium ($NH_4^+$) is first oxidised to nitrite ($NO_2^-$), then to nitrate ($NO_3^-$) and subsequently reduced to atmospheric nitrogen ($N_2$) which is released from the system. Bacteria of the genus *Nitrosomonas* perform the first oxidation step to $NO_2^-$, whereas bacteria of the genus *Nitrobacter* perform the second oxidation step to $NO_3^-$. Denitrification microorganisms as indicated by reaction (3) below use the nitrate and nitrite as a hydrogen acceptor and release the nitrogen as $N_2$ gas.

$$NH_4^+ + 1.5\,O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + 2H+ + H2O \qquad (1)$$

$$NO_2^- + 0.5\,O2 \xrightarrow{\text{Nitrobacter}} NO_3^- \qquad (2)$$

$$NO_3^- \longrightarrow N_2 + 1.5O_2 \qquad (3)$$

### 2.3.1.4 Phosphorous Removal

Phosphorus (P) in wastewater is typically present as orthophosphate, polyphosphate and organic phosphorus compounds (Surampalli et al., 1997). It is highly mobile and present in solution, in particles and detritus, or in the cells of aquatic organisms. Like nitrogen, phosphorus is an essential macronutrient for the growth of plants and other organisms and besides other factors, responsible for eutrophication processes.

According to Metcalf & Eddy Inc. (1991) the following phosphorus concentrations are typical for domestic raw wastewater:

Total phosphorus (TP):

- Low strength wastewater: 4 mg/L

- Medium strength wastewater: 8 mg/L
- High strength wastewater: 15 mg/L

Inorganic phosphorus:

- Low strength wastewater: 3 mg/L
- Medium strength wastewater: 5 mg/L
- High strength wastewater: 10 mg/L

Phosphorus removal from aquatic macrophyte systems is due to plant uptake, microbial immobilization into detritus plant tissue, retention by underlying sediments and precipitation in the water column (Anonymus, 1998).

In a duckweed wastewater treatment system, phosphorous is normally removed by the mechanisms of: plant uptake, adsorption to clay particles and organic matter, chemical precipitation with $Ca^{2+}$, $Fe^{3+}$, $Al^{3+}$, and microbial uptake (Diederik et al.2011). Except for plant uptake, the latter three mechanisms cause storage of phosphorous in the system (Gijzen *et al.,* 2001). As no volatile intermediates such as $N_2$ or $NH_3$ as in the case of nitrogen are formed, ultimate phosphorous removal is only possible by plant harvesting and dredging of the sediment.

The plants' uptake capacity depends largely on the growth rate, harvesting frequency and available *ortho*-$PO_4^{3-}$, the favored form of phosphorous for duckweed growth (Shah *et al*., 2014). In the warmer season when the growth rate is highest, phosphorous removal rate is also highest. The uptake of phosphorous by duckweed is enhanced by frequent harvesting and adequate pretreatment of raw wastewater to release organically bound *ortho*-$PO_4^{3-}$.

Besides plant uptake, adsorption and precipitation are probably the other dominant mechanisms for phosphorous removal in a duckweed treatment system (Alaerts *et al.* 1996). Aerobic conditions contribute to the precipitation of phosphorous through oxidised forms of Fe and Al. However, phosphorous is again released under anaerobic conditions prevailing in the sediments.

#### 2.3.1.4 Duckweed for Phytoremedation

Phytoremedation is the utilization of plants accumulation capabilities to remove contamination from water, soil and air. The capacity of aquatic plants to remove pollutants from water is well documented (Demirezen *et al.*, 2004).The recent application of phytoremediation technology by duckweed in wastewater treatment and management is quite interesting and revealing. Phytoremediation systems by duckweed are one of the options that have been widely applied for combined handling of wastewater with the nutrients used for poultry and aqua-cultural projects Naphi *et al.* (2003).

Heavy metals are effectively removed from wastewater by duckweed which has the capacity to accumulate metals such as Cr, Mn, Fe, Co, Cu, Zn, Cd, Pb, Al and even Au (Leng, 1999; Iqbal, 1999). Duckweed can tolerate and accumulate with accumulation factors ranging between $10^2$ and $10^5$ $^($Landolt and Kandeler, 1987). This fact suggests a possible use of duckweed for efficient removal of metals from wastewaters. Zayed (1998) found that under experimental conditions, duckweed proved to be a good accumulator of Cd, Se, and Cu, a moderate accumulator of Cr, and a poor accumulator of Ni and Pb. The author concluded that duckweed is promising for the removal of Cd, Se and Cu from contaminated wastewater since it accumulates high concentrations of these elements. Further, the growth rates and harvest potential make duckweed a good species for phytoremediation. It should be known that duckweed produced in this way under no circumstances be used in food production (Gijzen and Khondker 1997).

As reported by Diederik et al. (2011), duckweed has a high metal uptake capacity specifically for Cr, Zn and Pb. The efficient absorption of heavy metals and other (organic) toxic compounds could be used for extraction of such toxins from industrial wastewaters. It is, however, important that the biomass is harvested at regular intervals, otherwise, the toxins will settle on the sediments with the decaying plants. The harvested plants should be burnt and/or disposed of in sealed landfills.

### 2.3.1.5 Pathogen Removal

Pathogen removal from duckweed covered lagoons treating domestic wastewater is important in order to produce an effluent quality that allows for further re-use in agriculture and/or aquaculture. The most common indicators of the level of waterborne pathogen contamination in water are the coliform group: Total Coliforms and Faecal Coliforms (USEPA, 2000).The effluent guidelines of less than 0.1 helminth eggs and less than 1000 faecal coliforms/100ml (WHO, 1989) should be met to ensure safe effluent reuse.

Duckweed restricts light penetration and may inhibit disinfection in the maturation ponds. Efficient removal of coliforms is known from conventional lagoon treatment systems without floating aquatic macrophytes. Direct sunlight and an increase in pH due to algal growth are believed to be possible factors responsible for coliform die off in such systems. These beneficial effects are not prevalent in a pond system completely covered by duckweed which cuts off light and suppresses algal growth. A study in Egypt by Dewedar and Bahgat (1995) showed no decline in faecal coliforms under a dense duckweed cover over a period of (only) 5 days. However, an analysis of studies on removal performance of *E. coli* in lagoons covered by various species of floating macrophytes (Alaerts *et al.* 1990) suggests that water temperature and hydraulic retention time are more determining factors. The effects of sunlight, pH and other parameters on bacterial and viral removal have to be investigated further in comparative studies using ponds with and without duckweed (Gijzen and Khondker 1997). Overall retention time required in a DWT system will vary depending on a range of factors including the influent nutrient levels, temperature and the discharge standards that must be met. In general, 20 days hydraulic retention time would appear to be a minimum guideline for DBWWT to achieve acceptable discharge standards and pathogen reduction in municipal sewage treatment (Skillicorn et al. 1993).

Islam *et al.* (1996), who monitored faecal coliforms in a plug-flow lagoon covered with duckweed, observed a reduction from $4.57 \times 10^4$/ml in the raw wastewater to values below $10^2$/ml after treatment with duckweed (99.78 % removal).

#### 2.3.1.6 Mosquito and Odour Control

The results of several studies on the effects of duckweed on mosquito breeding appear to be contradictory (Gijzen and Khondker 1997). Positive, negative and no effects were reported by the references in Landolt and Kandeler (1987). A positive effect of a duckweed cover on the decrease of mosquito larvae was reported by Iqbal (1999). The authors suggest that a complete duckweed cover acts either as physical barrier and hinders the mosquito larvae from reaching the surface for oxygen uptake, or that the plants release compounds which are toxic to the larvae. A possibly reducing effect of duckweed on mosquito breeding may positively contribute to the acceptance of duckweed farming systems in areas where mosquitoes are a nuisance and a vector of serious human diseases like malaria or dengue. The gaseous products resulting from anaerobic decomposition in the sediment and water column are responsible for odour development.

It is assumed that the aerobic duckweed mat acts as chemical and physical barrier against odours. Hydrogen sulphide ($H_2S$) oxidises for example to sulphuric acid ($H_2SO_4$) within the aerobic plant mat (Lemna Corp. 1994).

### 2.3.2 Design Considerations

In practice, pond depth, the organic surface loading rate, sewage temperature, and the hydraulic retention time are crucial design parameters of duckweed ponds. Type and quantity of wastewater to be treated are decisive factors in the design of duckweed treatment systems and for infrastructural requirements necessary to ensure daily nutrient inputs and use of biomass.

Metcalf and Eddy (1991) suggest that duckweed systems, exploiting mainly the wastewater treatment aspect of duckweed, can be designed as conventional stabilization ponds with the addition of a floating grid system to control the effects of wind. However, reliable design and operation guidelines aiming at the dual use of duckweed in wastewater treatment and optimum biomass production are lacking. They can be operated as batch or plug-flow (continuous flow) systems. Easy access to the pond surface for operation and maintenance should be ensured in site selection and design of a duckweed treatment pond system. Therefore, a narrow, channel-like pond design is more convenient than wider ponds.

#### 2.3.2.1 Primary Treatment of Raw Wastewater

Primary treatment of raw wastewater is essential for initial separation of some of the settleable fraction of pathogens, solids and floating material. This can be achieved in conventional deep anaerobic ponds that encourage the fermentation and breakdown of settled solids by bacterial processes into simple organic and inorganic molecules. Duckweed could enhanced primary treatment in these ponds by maintaining anaerobic conditions and reducing odour nuisance (Skillicorn et al. 1993). In addition, conventional anaerobic ponds, while effective at reducing BOD, anaerobic pretreatment promotes the release of organically bound $NH_4^+$ and $o$-$PO_4^{-3}$, the favored forms of nutrients for duckweed growth (Caicedo et al. 2000), so duckweed assimilation will enhance the nutrient removal capacity of these anaerobic systems..

High levels of ammonification occur in primary treatment systems which may be toxic to duckweed. Cheng et al. (2002) found that a range of duckweed species tested could tolerate and grow at high ammonium levels of 240 mg/L in swine wastewater - the best performer being a Queensland native, Spirodella punctata (recently renamed Landoltia punctata). Phan (2002), however, found that duckweed may need an acclimatization period to adapt to the very high N levels in raw agricultural wastewaters.

Most researchers, however, suggest that efficiency gains using DBWWT are greater in secondary and tertiary treatment of effluent where organic sludge has already been removed or converted into simple organic and inorganic molecules that can be used directly by duckweed (Alaerts et al. 1996; Caicedo et al. 2000; Smith and Moelyowati 2001). Use of conventional earthen anaerobic sedimentation ponds is an efficient, low-cost and easy manageable alternative for primary treatment, especially in low-income countries.

#### 2.3.2.2 Pond Design Consideration

As aforementioned, two basic principles for pond design and operation are used for duckweed treatment, namely plug-flow and batch systems. A plug-flow (continuous flow through) design seems to be the more suitable treatment option for larger wastewater flows originating from communities and (peri-) urban areas, as it ensures an improved and more continuous distribution of the nutrients. A plug-flow design also enhances the contact surface between wastewater and floating plants, thereby, minimizing short circuiting. To ensure plug-flow conditions, a high plug-flow length to width ratio of 10:1 or more is necessary (Hammer 1992). Alaerts *et al.* (1996) reported excellent treatment results with a length to width ratio of 38:1. Moreover, a narrow, channel-like design allows easier access to the water surface for operation and maintenance work.

In a plug-flow system, duckweed productivity, nutritional value and nutrient removal efficiency decline gradually with increasing retention time. Depletion of nutrients causes plants to visually become brownish at some stage in the plug-flow runway, to grow slower and take up less nutrients per time than plants in the initial stages of the plug-flow. Furthermore, their protein content drops and their fibre content increases. At this point, the two so far parallel running processes of efficient wastewater treatment and high duckweed production begin to diverge. Yet, if this occurs at the very end of a duckweed plug-flow system and if the required effluent standards are met, the objective of combined wastewater treatment and production of high quality feed is attained. However, reliable design guidelines are missing to dimension a duckweed plug-flow lagoon in such a way that nutrient starvation occurs at the very end of the system. The system could, therefore, either be oversized if effluent standards are already met at early fractions of total retention time, leaving most of the system's surface underused with regard to protein production, or undersized where effluent standards are not met at the end of the plug-flow.

- **Hydraulic Retention Time (HRT)**

The hydraulic retention time is the average time that water remains in the DBWWT system. HRT has major effects on the efficiency of any treatment system. A longer hydraulic residence time allows for more of the treatment processes to be completed. In duckweed ponds, the HRT is dependent on the organic, nutrient and hydraulic loading rate, depth of the system and harvesting rate (Metcalf and Eddy 1991). To ensure acceptable pathogen removal and treatment efficiency, comparatively long retention times in the range of 20 to 25 days are postulated for duckweed (plug-flow) systems (Metcalf and Eddy 1991). However, to reduce BOD to 30 or 20 mg/L depending on the influent concentration, residence time of 10 to 20 days is proposed by Smith and Moelyowati (2001). Bal Krishina *et al*. (2008) proposed a retention time of 10 days for optimum operating condition of DBWWT systems in tropical areas.

- **Water Depth**

The critical factor with respect to water depth is to ensure vertical mixing in the pond to allow the wastewater to be treated to come into contact with the duckweed fronds for nutrient uptake and BOD degradation through attached microbial populations. An outlet structure is recommended in order to vary the operating depth (Metcalf and Eddy 1991). Reported pond depths range from 0.3 to 2.7 m up to even 5 m (Lemna Corp. 1994). The majority of authors report an optimal depth ranging from 0.4 to 0.9 m, implying that a maximum depth of one meter is sufficient for acceptable temperature buffering. Higher depths are also a feasible option for systems with relatively low BOD loads, a low recirculation rate and high land costs. Shallow system depths are, however, better suited for high organic loads, a high recirculation rate and for regions with inexpensive land prices.

- **Organic Loading Rate (OLR*)***

Organic loading rate is one of the determining factors for efficiency of duckweed based treatment system. Average organic loading rates expressed in terms of BOD for plant systems without artificial aeration should not exceed 100 to 160 kg/ha·d in order to obtain effluent quality of 30 mg BOD/l or less (Metcalf and Eddy 1991, Gijzen and Khondker 1997). Odours can develop at lower loading rates, especially where the sulphate concentration in the wastewater is greater than 50 mg/L. It seems that duckweed is less suitable for the treatment of wastewaters containing high BOD loads.

#### 2.3.2.3 Wind Protection

Duckweed is very susceptible to wind drifts and water currents, which needs stabilisation of the plants on the water surface. In regions with moderate winds, drifts are prevented through floating grids dividing the pond surface into cells or compartments. Floating bamboo poles divided into small square or rectangular areas of 2 to 5 by 4 to 8 meters are most commonly used. The size of the grid is determined by mean wind conditions and, in the case of flow through systems, by maximum projected flow velocity in the system. The higher the wind and flow velocities, the smaller the cells and the higher the system's costs.

## 2.4 Harvesting and Potential Uses of Duckweed

The quantity and frequency of duckweed harvesting plays a major role in the treatment efficiency and nutritional value of the plants. Regular harvesting ensures that the accumulated nutrients or toxins are permanently removed from the system. Because younger plants show a better nutrient profile and higher growth rate than older plants, regular harvestig is important to maintain a healthy and productive crop. Laboratory results (Willet, 2005) revealed that under conditions of high nutrient loading, an increase in the cropping rate resulted in improved nutrient removal. At lower nutrient loading rates, the cropping rate should be reduced. An almost complete cover should remain on the pond surface after plant harvesting.

The standing crop density, which realises the highest duckweed productivity, will determine the harvesting frequencies and amounts. The correlation between standing crop density and absolute biomass productivity peaks at some optimal density and gradually declines as increasing density inhibits growth through crowding. Optimal standing crop densities are site-specific and have to be determined through practical experience (Willet, 2005).

Alaerts *et al.* (1996) reported a standing crop density of 1600 g (wet wt)/m$^2$ for a duckweed-covered sewage lagoon in Bangladesh. Lower standing crop densities of 400 to 800 g (wet wt)/ m$^2$ were reported by Skillicorn *et al.* (1993). Each cell should be harvested back to optimal standing crop density at rates dependent on the plants' productivity.

## 2.5 Comparison of Duckweed Systems with Other Natural Treatment Systems

In aquaculture and constructed wetlands, macrophytes (plants) are grown to suppress algal growth by shielding the water column from light, by absorbing the nutrients and by assisting the oxygen transfer into the water (Koerner et al. 2003). The floating plant duckweed (Lemnaceae), is particularly promising for aquaculture because it grows abundantly and can easily be harvested. In constructed wetlands, wastewater is made to flow either horizontally or vertically through the root zone of a permeable soil planted with vegetation. The plants, if regularly harvested, create a sink for the nutrients by their uptake and assimilation of N and P. Importantly, they also provide niches for bacteria that reduce BOD, and that enhance nitrification and denitrification. They also provide niches for predator organisms that contribute to pathogen removal. Such wetlands offer good prospects for small-scale operation in remote tropical areas.

Generally, TSS, BOD and pathogen removal undergo the same process as with WSPs and thus, duckweed-based wastewater treatment systems are enhanced lagoon systems (Bonomo *et al.*, 1997). Nutrients (N, P) are generally sequestered in the plant biomass and are removed through harvesting (Bonomo *et al.*, 1997).

Compared to water hyacinth, duckweed-based wastewater treatment systems play a smaller role in BOD removal, but are efficient in the removal of nutrients and can play a significant role in TSS reductions (Zirschikly and Reed, 1988; Brix and Schierup, 1989).

Duckweed usually forms a dense mat covering the entire water surface, which provides some special characteristics for the treatment system:

(i) Duckweed mat can prevent the growth of micro-algae by restriction of light penetration (Willet, 2005). The dense duckweed mat also prevents mosquito larvae from reaching the water surface (Culley and Epps, 1973).

(ii) Duckweed mat makes the water column largely anaerobic by restriction of gas-liquid oxygen transfer together with lacking photosynthetic oxygen by phytoplankton (Brix and Schierup, 1989). While the oxygen produced by photosynthetic on duckweed mat can be transferred into the water column to form a thin aerobic layer in duckweed root zone (Zirschky and Reed, 1988). The aerobic layer favors oxidation of rising odor gas produced from anaerobic water column below.

(iii) Evaporation from a duckweed-based pond system is restricted (Oron et al., 1984).

## 2.6 Environmental and Economic Benefits of DBWWT

Discharge of untreated wastewater in to the environment causes the deterioration of ground and surface water quality, as well as air and land pollution. The impact depends on the composition and strength of wastewater discharged into the environment.

Among others, some of the environmental and economic benefits of wastewater treatment using duckweed wastewater treatment systems are:

- Contribute to avoid or minimize pollution of ground and surface water sources caused by discharge of untreated wastewater;
- Avoidance of public health hazard from exposure to pathogen in sewage;
- Harvesting wastewater-grown duckweed helps to remove surplus nutrients, which might otherwise be released into aquatic environments by wastewater treatment plants (Oron et al., 1988);
- Duckweed systems evaporate 20% less water compared to other open water systems (Oron et al., 1986);
- In developing countries like Ethiopia where fertilizer is scarce and expensive for the small farmer, duckweed collected from local wastewater treatment ponds and wetlands can provide a cheap and effective fertilizer for rice and other crops (Ahmad et al., 1990).
- Duckweed cover restricts sunlight penetration into the water body, limiting algal development and consequently lowering of TSS levels are expected in the final effluent;

- Duckweed system may generate economic return via the commercialization of biomass for fodder, and effluent for irrigation (Willett, 2005).
- Duckweed may provide a source of mosquito anti-larval compounds that could have public health significance;

## 2.7 Limitation of DBWWT

Duckweed based waste water treatment systems have the following limitations.

- They require large areas of land that may not be available near urban areas;
- In temperate climates duckweed growth slows in the winter. This may restrict the use of such treatment systems in cooler climates;
- Duckweed-based treatment systems may be most useful in treating secondary effluents from small communities where land costs are low (Dalu and Ndamba 2003);

## 2.8 Use of Duckweed as a Food Source

If grown on domestic wastewater free of heavy metals, duckweed can be used as an animal fodder and green fertiliser (Oron, 1990; Bonomo *et al.*, 1997).

The nutrients taken up by duckweed are assimilated into plant protein. Under ideal growth conditions more than 40% protein content on dry weight basis may be achieved (Skillikorn *et al.*, 1993). Duckweed value, in terms of protein content, is similar to soybeans (Oron, 1990, 1994). Duckweed protein has a better array of essential amino acids than most vegetable proteins and more closely resembles animal protein (Hillman and Culley 1978). It is, therefore, a source of high quality protein to be exploited for domestic animal production. Duckweed grown on nutrient-rich water has a high concentration of trace minerals, K and P and pigments, particularly carotene make duckweed meal an especially valuable supplement for poultry and other animals, and it provides a rich source of vitamins A and B for humans.

Research at Louisiana State University demonstrated the value of using dried duckweed fronds as a feed source for dairy cattle and poultry (Culley et al., 1981). Research results of of Texas Tech University was indicated that duckweed species have potential as a feed ingredient for cattle, sheep, and pigs (Moss, 1999).

The value of duckweed as a source of feed for fish and poultry has been promoted by the World Bank, especially in developing countries (Skillicorn et al., 1993). Duckweed used at a level of up to 15% in broiler diets can represent an important alternative source of protein for poultry feeds in countries where soybean or fish meal is unavailable (Haustein, 1990). When dried duckweed (*Lemna* spp) was fed to crossbred meat ducks as a substitute for soybean meal there was no significant difference in the carcass traits between treatments (Bui et al., 1995).

Perhaps the most promising use of duckweed is as a feed for pond fish such as carp and tilapia. Ponds for duckweed production can be located next to fish culture ponds, eliminating the need for expensive drying to produce a dried feed. Nile tilapia and a polyculture of Chinese carps fed readily on fresh duckweed added to their ponds and the nutritional requirements of these cultured fish appear to be completely met by duckweed (Skillicorn et al., 1993).

Research on using duckweed in the diets of domestic animals has been surprisingly scarce, perhaps because of the difficulties of growing sufficient duckweed under experimental conditions.

Since the protein content of duckweed was found to be almost as high as that of soybean meal, duckweed production provided both a means of wastewater purification and a source of livestock feed as well (Johnson, 1998).

## 2.9 Criteria for Proper Wastewater Disposal

Proper disposal of sewage and other wastewater is necessary not only to protect the public’s health and prevent contamination of groundwater and surface water resources but also to preserve fish and wildlife populations and avoid the creation of conditions that could detract from the attractiveness of a community, tourist establishment, resort, and recreational areas.

As described by Salvato et. al. (2003), the following basic criteria should be satisfied in the design and operation of an excreta, sewage, or other wastewater disposal system:

1. Prevention of microbiological, chemical, and physical pollution of water supplies and contamination of fish intended for human consumption;
2. Prevention of pollution of bathing and recreational areas;
3. Prevention of nuisance, unsightliness, and unpleasant odors;
4. Prevention of human wastes and toxic chemicals from coming into contact with humans, grazing animals, wildlife, and food chain crops or being exposed on the ground surface accessible to children and pets;
5. Prevention of fly and mosquito breeding and exclusion of rodents and other animals;
6. Strict adherence to standards for groundwater and surface-water protection and compliance with federal, state, and local regulations governing wastewater disposal and water pollution control.

Table 2.2 European Community guidelines for wastewater discharged to sensitive surface water bodies based on typical raw wastewater composition.

| Variable | Raw sewage composition | EU guideline | Percentage removal (%) |
|---|---|---|---|
| $BOD_5$ (mg/L) | 250 | 25 | 90 |
| Total N (mg/L) | 48 | 10 | 80 |
| Total P (mg/L) | 12 | 1 | 90 |

Source: CEC, 1991

There are no environmental quality standards for ensuring environmental well being in the country. However, the Ethiopian Environmental Policy underlines that environmental quality standards are indispensable instruments to ensure the well being of human beings as well as other living things and therefore provisional standards were set in 2003. Table 4.6 indicates provisional quality standards for selected parameters relevant to this study.

Table 2.3 Ethiopia's EPA (2003) Provisional Discharge Standard

| Parameter | Unit | Ethiopia EPA (2003) Standard |
|---|---|---|
| $BOD_5$ | mg/L | 80 |
| COD | mg/L | 250 |
| TSS | mg/L | 100 |
| TKN-N | mg/L | 60 |
| TP | mg/L | 10 |
| $PO_4^{3-}$ | mg/L | 5 |
| $NH_4^+$-N | mg/L | 5 |
| $NO_3^-$-N | mg/L | 20 |
| FC | CFU/100ml | 400 |
| Temp | $^oC$ | 40 |
| pH | $-\log[H^+]$ | 6-9 |

# 3 MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Wastewater and Duckweed

Duckweed plants were collected from a temporary pond created for construction of a building which was receiving drains from the vicinity in front of Lideta Chruch in Addis Ababa (Fig. 3.1).

Raw domestic wastewater was collected from sewer of Kara Kore condominium houses and then batch experiments were carried out to evaluate the performance of duckweed (*Lemna minor L*) to treat the raw and settled wastewater.

### 3.1.2 Analytical instruments

Digital PH meter was used for PH measurement, Oxi-Direct BOD measuring instrument and some reagents were used to measure BOD of the wastewater samples. HACH COD reactor, multi-parameter bench photometer, and reagents were used for COD measurement. Crucible, oven, desiccators and weighing balance were used to estimate dry weight.

## 3.2 Methods

### 3.2.1 Experimental Setup

To evaluate the performance of duckweed (Lemna minor), batch experiments were carried out at the premises of Chemical and Bio Engineering School of the Institute of Technology, Addis Ababa University, in two phases as follows:

- **Phase I:** three plastic containers of 15.5 cm diameter and 25 cm depth were used to run the experiment. Two of the containers were filled with 15 liters of raw wastewater, one for duckweed (Lemna minor) treatment and the other for a control. The third one was filled with settled domestic (pretreated) wastewater. Depth of the wastewater was 20 cm while stocking density of duckweed (*Lemna minor*) was 600 g/m$^2$ fresh weight.

- **Phase II:** This is an additional experiment to investigate the performance of duckweed to treat raw domestic wastewater by increasing the surface area and reducing the depth of the experimental ponds. Plastic containers of 31 cm diameter and 14 cm depth were filled up to the depth of 10 cm with 35 liters of wastewater. Stocking density duckweed (*Lemna minor*) was 600 g/$m^2$ fresh weight.
- The experiments were run under outdoor local environmental conditions for retention time of 21 and 14 days for Phase I and Phase II respectively.

### 3.2.2 Species Identification, Acclimatization and Stocking

Duckweed species was identified as *Lemna minor L.* by the National Herbarium of Ethiopia at AAU. To avoid or reduce entrance of algae and other impurities into the experimental ponds, prior to inoculation, duckweed plants were first cleaned with tap water and then washed by distilled water. Furthermore, plants were acclimatized with a 25% diluted wastewater for a month. Then 600 g/$m^2$ fresh weight duckweeds plants were stocked in to the experimental ponds and then experiment had been run under outdoor local environmental condition.

**Figure 3.1 Photo showing duckweed plants near construction site around Lideta area, Addis Ababa**

### 3.2.3 Evapotranspiration Losses in Duckweed Ponds

The floating duckweed mat suppresses temperature and inhibits evaporation. Due to the plant direct full contact with the surface of the effluent, a larger fraction of radiation is probably returned to space (a higher albedo), as compared to open-surface water bodies (Oron et al., 1987). Water loss by evaporation from the experimental ponds was compensated for by addition of distilled water.

### 3.2.4 Sample Collection and Analysis

#### 3.2.4.1 Duckweed Sampling and Processing

Duckweed biomass was harvested every three days and duckweed density was restored to 600 g fresh weight/m$^2$. Stocking density was kept at 600 g/m$^2$ fresh weight (one layer) by harvesting the surplus every three days to prevent overcrowding and to maintain sufficient cover to minimize the development of algae in duckweed ponds (Zimmo et al., 2000).

#### 3.2.4.2 Wastewater Samples

Samples were collected from March to April 2015 for Phase I and from February to March 2016 for Phase II. Subsurface (under duckweed mat) composite water samples for physico-chemical, and bacteriological parameters were collected at 3:00 in polyethylene bottles from all sides of each pond and then thoroughly mixed. This procedure carried out every 7 days and three times within the period of the experiment. For three replicates, a total of 44 samples, 33 for phase I which includes wastewater samples from the raw, control for the raw and pretreated duckweed wastewater treatment ponds; and 9 for phase II with raw wastewater only were collected and analyzed in AAWSA Wastewater Laboratory.

#### 3.2.4.3. Analysis

All physico-chemical and microbiological analyses for COD, BOD, TSS, ammonia N, nitrite N, total Kjeldahl N, total P, phosphate, DO, Temperature, pH and faecal coliform were performed according to the Standard Methods for Examination of Water and Wastewater (APHA, 1998) as indicated below.

- Temperature and pH were measure in situ using a portable EC10 pH meter (HACH);

- $NO_3^-$-N, $NH_4^+$-N, were measured calorimetrically using DR/4000 HACH spectrophotometer;
- $PO_4^{3-}$ were determined calorimetrically with vanadomolybdate method;
- Total phosphorus was determined by phosphorus content using the per-sulphate digestion method followed by the vanadomolybdate colorimetric method (APHA, 1998);
- BOD was measured with Lovibond BOD system oxidirect;
- COD was determined using reactor digestion with potassium dichromate and colorimetric determination method;
- Microbiological quality indicator faecal Coliform was evaluated using Multiple Tube Fermentation Method (APHA, 1998);
- Organic Nitrogen was analyzed using Kjeldhal Method of nitrogen analysis;
- Duckweed protein content was estimated by analyzing organic nitrogen in the dried and powdered tissue of duckweed. The protein content was calculated based on: protein (g/g) = organic N (g/g) × 6.25 (Rusoff et al., 1980).
- Dry weight of duckweed was calculated by drying samples of the harvested duckweed at 105ºC.

After analysis of concentration of measured parameters in the influent and effluent; the removal efficiency of treatment ponds were calculated according to the following formula.

**Efficiency (%) = (A – B)/A*100**

Where,

**A** = Influent concentration (before experiment)

**B** = Effluent concentration (after experiment)

### 3.2.5 Statistical Analysis

After characterization of the effluent at the end of detention periods for each treatment levels, appropriate statistical analysis including Mean, Standard Error and Analysis of Variance (ANOVA) and multiple comparisons were performed using excel to examine the relationship among parameters.

# 4. RESULTS AND DISCUSSION

## 4.1 Influent and Effluent Wastewater Characteristics of the DBWWTs

### 4.1.1 Results of Phase I

The mean values of the various wastewater quality parameters for influent and effluent that were analyzed are summarized in Table 4.1 and 4.2 for untreated and pretreated wastewater respectively. In this thesis, the term “Untreated wastewater” refers to the raw wastewater while “Pretreated” refers to the settled wastewater.

As shown in the Table 4.1, mean concentrations of parameters measured in the influent for untreated wastewater in Phase I were: $BOD_5$ (187.8 ± 4.42), COD (310.50 ± 14.17) and TSS (205 ± 9.44) mg/L. The respective mean influent concentrations for $NO_3^-$-N, $NH_4^+$-N and TKN-N were 5.93 ± 0.18, 29.01 ± 0.45 and 41.600 ± 2.56 mg/L. Mean influent TP and $PO_4^{3-}$ concentrations were 5.47 ± 0.48, 2.92 ± 0.17 mg/L respectively.

Results indicated in Table 4.2 revealed that mean concentrations of parameters in the influent for pretreated wastewater were: $BOD_5$ (116.2 ± 9.78), COD (212.2 ± 15.05) and TSS (141 ± 8.59) mg/L. The respective mean influent concentrations for $NO_3^-$-N, $NH_4^+$-N and TKN-N were 5.83 ± 0.29, 30.06 ± 0.59 and 30.06 ± 1.17 mg/L. Mean influent TP and $PO_4^{3-}$ concentrations were 4.12 ± 0.23, 2.42 ± 0.19 mg/L respectively.

Faecal coliform concentrations were $1.68 \times 10^4 \pm 2.49 \times 10^3$ and $3.63 \times 10^5 \pm 2.49 \times 10^3$ CFU/100 ml for pretreated and untreated wastewater duckweed ponds respectively..

Table 4.1 Characteristics of the influent and effluent in duckweed based wastewater treatment ponds (mean ± standard deviation).

| Parameter | Unit | Influent | Effluent of duckweed pond | Effluent of the control |
|---|---|---|---|---|
| BOD | mg/L | 187.8 ± 4.42 | 25.77±1.44* | 44.72 ± 2.2 |
| COD | mg/L | 310.50 ± 14.17 | 55.09±7.47* | 87.19 ± 6.2 |
| TSS | mg/L | 205 ± 9.44 | 20.13±1.17* | 48.60 ± 1.5 |
| TKN-N | mg/L | 41.60 ± 2.09 | 14.45±1.20* | 28.65 ± 1.9 |
| TP | mg/L | 5.47 ± 0.48 | 1.61±0.1 | 1.21 ± 0.15 |
| $PO_4^{3-}$ | mg/L | 2.92 ± 0.17 | 0.91±0.07 | 1.66 ± 0.17 |
| $NH_4^+$-N | mg/L | 30.19 ± 0.45 | 11.57± 0.18* | 16.34 ± 0.4 |
| $NO_3^-$-N | mg/L | 5.93 ± 0.18 | 2.31±0.09* | 2.99 ± 0.13 |
| FC | CFU/100ml | 3.63 x$10^5$± 2.49 x$10^3$ | 8.60 x$10^3$ ± 8.3x$10^2$ | 3.59x$10^2$ ± 4.91 x10 |
| Temp | $^o$C | 20.04 ± 0.55 | 18.91±0.11 | 21.20 ± 0.06 |
| pH | -log[$H^+$] | 7.21 ± 0.08 | 7.50±0.07 | 7.9 ± 0.08 |
| DO | mg/L | 1.32 ± 0.37 | 4.82±0.55 | 4.55 ± 0.6 |

* shows difference in removal is statistically significant.

Table 4.2 Characteristics of the pretreated wastewater and duckweed ponds effluent (mean ± standard deviation).

| Parameter | Unit | Influent | Effluent |
|---|---|---|---|
| BOD | mg/L | 116.2 ± 19.78 | 13.78 ± 2.31 |
| COD | mg/L | 212.2 ± 15.05 | 28.5 ± 2.98 |
| TSS | mg/L | 141 ± 8.59 | 12.1 ± 1.02 |
| TKN-N | mg/L | 30.06 ± 1.17 | 9.56 ± 0.72 |
| TP | mg/L | 4.12 ± 0.23 | 1.06 ± 0.07 |
| $PO_4^{3-}$ | mg/L | 2.42 ± 0.19 | 0.59 ± 0.08 |
| $NH_4^+$-N | mg/L | 32.64 ± 1.17 | 4.6 ± 0.83 |
| $NO_3^-$-N | mg/L | 5.83 ± 0.29 | 1.74 ± 0.12 |
| FC | CFU/100ml | 1.68x$10^4$ ± 3.59x$10^2$ | 2.44x$10^2$ ± 5.59x10 |
| Temp | $^o$C | 20.21 ± 0.11 | 19.01 ± 0.44 |
| pH | -log[$H^+$] | 7.24 ± 0.13 | 7.45 ± 0.1 |
| DO | mg/L | 1.47 ± 0.35 | 5.21 ± 0.62 |

### 4.1.2 Results of Phase II

The experimental results found in Phase II using shallower duckweed ponds and higher surface area is presented in Table 4.3.The mean concentrations of parameters measured in the influent for untreated wastewater in mg/L were: BOD (211 ± 8.64), COD (344 ± 19.73), TSS (227.54 ± 8.72) with corresponding surface loading of BOD (211 Kg/ha), COD (344 Kg/ha) and TSS (227 Kg/ha). Influent concentrations for nutrients of $NO_3$-N, $NH_4^+$-N and TKN-N were 3.33 ±0.12, 22.73 ± 0.52 and 33.12 ± 3.32 with corresponding surface loading of 3.3 Kg/ha, 22.7 Kg/ha and 33.12 Kg/ha respectively.

Mean influent $PO_4^{3-}$ and TP concentrations to the duckweed experimental ponds in mg/L were 3.64 ± 0.15 and 6.3 ± 0.22 with respective surface loading of 0.125 Kg/ha, 0.033 Kg/ha, 7.75 Kg/ha and 0.142 Kg/ha.

Mean values of temperature, pH and DO were 19.20 ± 0.2°C, 6.82 ± 0.09 and 1.13 mg/L. Faecal Coliform concentrations for influent in Phase II were 3.67 $x10^4$± 2.49 $x10^2$ CFU/100 ml.

Table 4.3 Characteristics of Phase II influent and effluent wastewater from Shallow duckweed ponds (mean ± standard deviation).

| **Parameter** | **Unit** | **Influent** | **Effluent** |
|---|---|---|---|
| BOD | mg/L | 211.00 ±8.64 | 20.82 ± 3.07 |
| COD | mg/L | 344.66 ± 19.53 | 40.42 ± 3.83 |
| TSS | mg/L | 227.54 ± 11.16 | 18.60 ± 2.4 |
| TKN-N | mg/L | 33.12 ± 3.3 | 8.57 ± 1.25 |
| TP | mg/L | 6.30 ± 0.22 | 1.53 ± 0.11 |
| $PO_4^{3-}$ | mg/L | 3.64 ± 0.15 | 0.71 ± 0.08 |
| $NH_4^+$-N | mg/L | 22.73 ± 0.52 | 3.42 ± 0.5 |
| $NO_3^-$-N | mg/L | 3.33 ± 0.12 | 1.86 ± 0.11 |
| FC | CFU/100ml | $3.67 \times 10^4$ | $3.83 \times 10^2$ |
| Temp | °C | 19.20 ± 0.2 | 19.03 ± 0.31 |
| pH | -log[$H^+$] | 6.82 ± 0.09 | 7.34 ± 0.07 |
| DO | mg/L | 1.13 ± 0.22 | 6.82 ± 0.62 |

The characteristics of the influent wastewater during the two experimental phases were not significantly different ($p > 0.05$) for COD, BOD and TSS. The ratio of COD:BOD was around 1.63 during Phase I and  1.66 during Phase II. Similarly, the content of TKN and $NH_4^+$ values of the influent, the raw wastewater, were not significantly different ($p > 0.05$) between the two phases. The only parameter, which was significantly different, was TP ($p < 0.05$) with higher values recorded in the Phase II. The mean values for faecal coliform during phase I and Phase II were 3.63 x$10^5$and 3.67 x$10^4$ CFU/l00 ml respectively.

As shown in the Table 4.3, mean concentrations of parameters measured in the effluent for untreated wastewater in Phase II in mg/L were: BOD (20.28 ± 3.07), COD (40.42 ± 3.83), TSS (18.60 ± 2.4). ). The effluent concentrations for nutrients of $NO_3^-$-N, $NH_4^+$-N and TKN-N were 1.86 ± 0.11 mg/L, 4.14 ± 0.5 mg/L and 8.57 ± 1.25 mg/L.

Mean influent $PO_4^{3-}$ and TP concentrations to the duckweed experimental ponds in mg/L were 0.83 ± 0.08 and 1.53 ± 0.11. .

Faecal Coliform concentrations for effluent in Phase II were 3.83 x $10^2$ CFU/100ml. The values of temperature, DO and pH in the effluent were 19.03 ± 0.31 $^oC$, 7.34 ± 0.07 and 6.82 ± 0.62 mg/L.

**pH, Temperature and Oxygen**

As observed by Caicedo et al. (2001) pH, temperature and oxygen are more stable in duckweed ponds than in conventional ponds. Data recorded in Table 4.1 and 4.2 shows that the mean pH values of the influent wastewater were 7.21 ± 0.08 for untreated and 7.21 ± 0.13 for primary treated wastewater respectively. Mean values for the effluent were 7.45 ± 0.1, for untreated, 7.90 ± 0.08 for control and for primary treated wastewater respectively. In Phase II the mean values for Temperature, DO and pH in the effluent were 21.10 ± 0.31, 7.34 ± 0.07 and 6.82 ± 0.62.

A pH value of 7.5 was found to be the most ideal for the successful establishment of a duckweed system and optimum pond performance (Dalu & Ndamba, 2003). Hicks (1932) found that duckweed grew well at pH 6 - 7.5 with outer limits of 4 and 8. Other studies have found that duckweed growth declines as the pH becomes more alkaline (Hillman, 1976). In both untreated and pretreated wastewater, pH values of duckweed treatment ponds were found to increase in the effluent compared to that of the influent. This was caused by photosynthetic activity of the duckweed which utilized $CO_2$ and produced $O_2$. The consumption of $CO_2$ by duckweed reduced the amount of carbonic acid in the wastewater which helped to increase the pH in the treatment pond (Bal Krishna and Polprasert, 2008).

As shown in Figure 4.1, in the present experiment, the temperature in both for untreated and pretreated duckweed treatment ponds in Phase I were ranged between 18 $^{o}$C and 20 $^{o}$C while 18.5-21 $^{o}$C for the control. The observed data revealed that the temperature was within temperature tolerance limit for duckweed growth as mentioned by Culley et al. (1981) who found that the upper temperature tolerance limit for duckweed growth was around 34$^{o}$C.

The mean effluent temperature of duckweed treatment ponds were 18.91 ± 0.11 $^{o}$C and 18.87 ± 0.13 $^{o}$C for untreated and for pretreated respectively. Effluent temperature values for control were 18.91 ± 0.11 and 21 ± 0.3 $^{o}$C for untreated and pretreated respectively. In Phase I, a temperature drop of almost 1$^{o}$C was observed, while in Phase II, the temperature was slightly dropped by 0.20 $^{o}$C. The decrease of temperature values for untreated and pretreated duckweed ponds (figure 4.1) were due to duckweed plants (*Lemna minor)* which covered the surface of the water and formed shading layer which prevented light penetration, leading to reduce water temperatures.

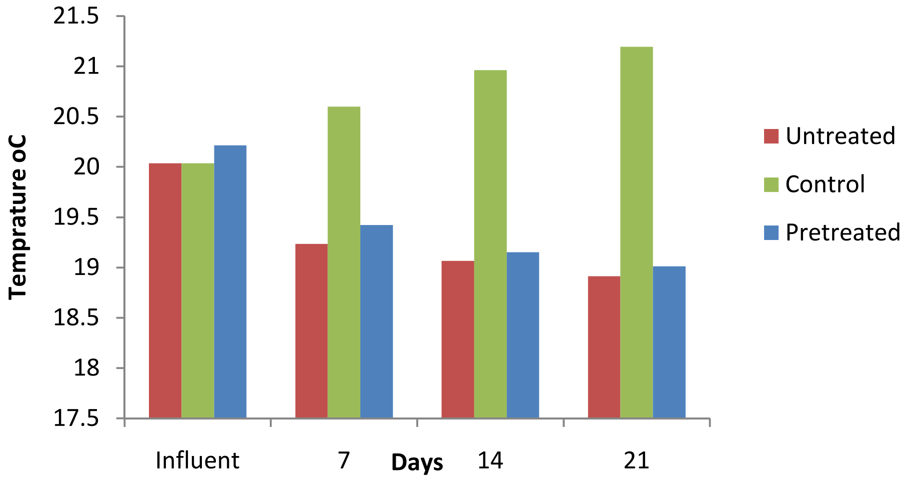


Figure 4.1 Variation of Temperature in duckweed wastewater treatment ponds.

The dissolved oxygen concentration is an important index of water quality and the average dissolved oxygen values of the treatment system registered a gradual increase from influent (1.32±0.37 mg/L to 4.82±0.55 mg/L) for untreated, from (1.47±0.37 mg/L to 5.21±0.55 mg/L) for pretreated and from (1.32±0.37 mg/L to 4.55±0.47 mg/L) for the control (Fig. 4.2).

In Phase II the mean values for DO in the influent and effluent were 6.82 ± 0.09 and 7.34 ± 0.07 mg/L respectively.

The gradual rise of oxygen levels with increase of HRT in DBWWT systems was caused by photosynthesis activity of duckweeds that indicated the effect of the treatment.

ANOVA analysis of the effluent results were showed that there is statistically significant difference ($p<0.05$) between duckweed and control effluent, and between Phase I and Phase II.

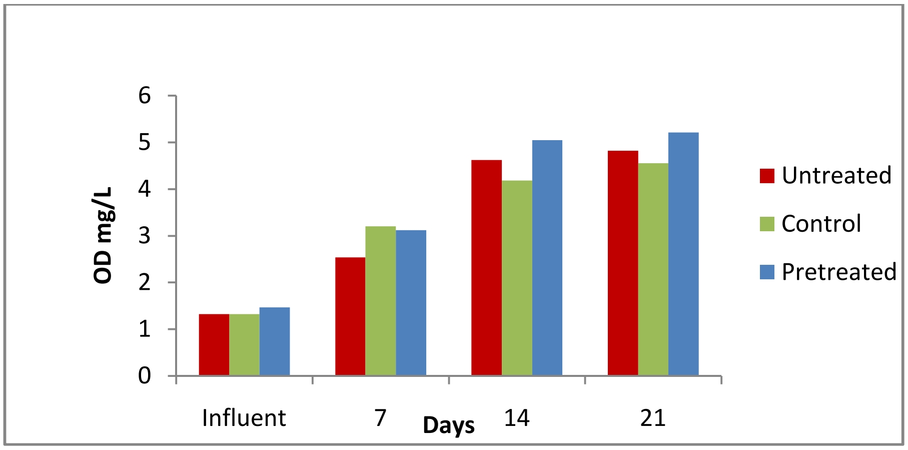


Figure 4.2 Variation of DO in duckweed wastewater treatment ponds.

## 4.2. Removal Efficiencies of Duckweed Ponds

### 4.2.1 Organic Matter and Suspended Solids Removal

BOD and COD have showed a gradual removal by prolonged treatment periods (figure 4.1 and figure 4.2). As shown in table 4.4, percent removals in Phase I for BOD and COD in a retention time of 21 days were 86% and 82% for untreated and 88% and 87% for pretreated wastewater respectively. In concurrence with the present findings, Alaerts et al. (1996) found higher removal efficiencies of BOD (95-99%) in a duckweed covered sewage lagoon with a retention period of 20.4 days. The finding of this study was higher than the observed values of Dalu and Ndamba (2002), 30-50% reduction of BOD contents when duckweeds were used for treating the wastewater in two small urban areas at Zimbabwe. Oron *et al* (1988) mentioned that the duckweed contribution for the removal of organic material is due to their ability to direct use of simple organic compounds, as well as the microbial degradation processes of organic material. Zimmo *et al* (2005) found that BOD removal efficiency was higher in duckweed than in algae based ponds.

The COD removal efficiencies were comparable with the results of Korner et al. (2003) who reported duckweed covered systems can attain COD removal rates between 50 and 95%. Lower COD values was found by Mandi (1994) at area studied on domestic wastewater (444 mg/L COD) in 14 cm deep of ponds (72.1%). Probably COD, BOD and TSS removal in duckweed ponds is mainly HRT dependent.

The data in Table 4,4 showed the removal efficiencies for Phase II were 90.13% for BOD, 88.27% for COD and 91.83% for TSS. In Phase I, these parameters were removed by 86.28%, 82.26% and 90.18%, respectively. TKN and TP removal efficiencies were 74.12% and 75.71% in Phase II and, 65.26 % and 70.60% in Phase I respectively.

ANOVA analysis indicated the efficiency of the duckweed ponds in the removal of BOD, COD and TSS in Phase II as compared to that of Phase I for untreated wastewater was significant ($p < 0.05$). The high removal of organic matter in phase II which has surface to volume ratio of 10 that was twice of the phase I was probably improved surface aeration and recirculation of wastewater to meet with duckweed fronds.

Table 4.4. Removal efficiency of DBWWTs in Phase I and Phase II

| Parameter | % Removal | | | |
|---|---|---|---|---|
| | Phase I | | | Phase II |
| | Untreated wastewater | Control | Pretreated wastewater | Untreated |
| BOD | 86.28 | 62.6 | 88.14 | 90.13 |
| COD | 82.26 | 54.12 | 86.56 | 88.27 |
| TSS | 90.18 | 61 | 91.60 | 91.83 |
| TKN-N | 65.26 | 48.2 | 68.20 | 74.12 |
| TP | 70.58 | 46.04 | 74.21 | 75.71 |
| $PO_4^{3-}$ | 68.84 | 35.27 | 81.01 | 80.49 |
| $NH_4^+$-N | 66.07 | 39.25 | 85.84 | 84.95 |
| $NO_3^-$-N | 64.54 | 36.8 | 67.74 | 44.14 |
| FC | 97.67 | 99.9 | 98.55 | 98.95 |

Figure 4.5 shows that that TSS values decreased by increasing treatment periods, reaching a concentration of 9.56 ± 0.72 mg/L and 20.13±1.17 mg/L after 21 days of retention time for pretreated and untreated wastewater respectively. The overall removal efficiencies of duckweed ponds for removal of TSS were 90.18% for pretreated and 91.61% for untreated wastewater. The result was comparable with Bal Krishina and Polprasert (2008), who

reported 90% TSS removal efficiency with similar days of retention time. Dalu and Ndamba (2002) also found a similar result, up to 90% reduction, in a three year investigation into the potential use of duckweed based wastewater stabilization ponds for wastewater treatment at two small urban areas in Zimbabwe. Van der Steen *et al*. (1999) reported better removal of suspended solids in duckweed-based system was due to suppression of algal growth by duckweed mat.

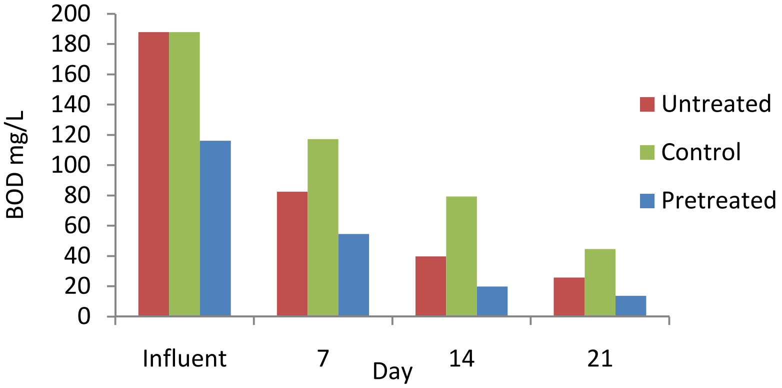


Figure 4.3 Removal of BOD in duckweed wastewater treatment ponds.

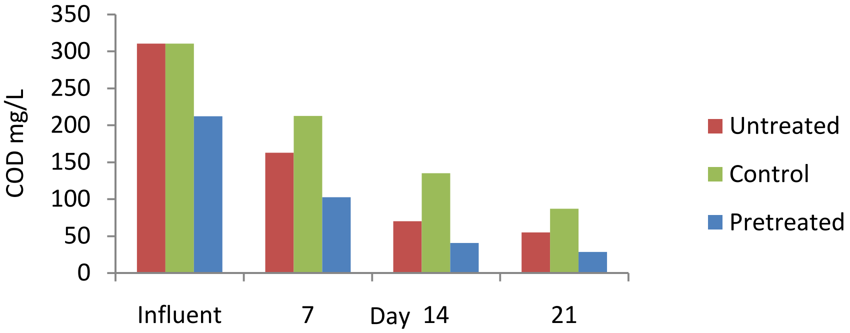


Figure 4.4 Removal of COD in duckweed wastewater treatment ponds.

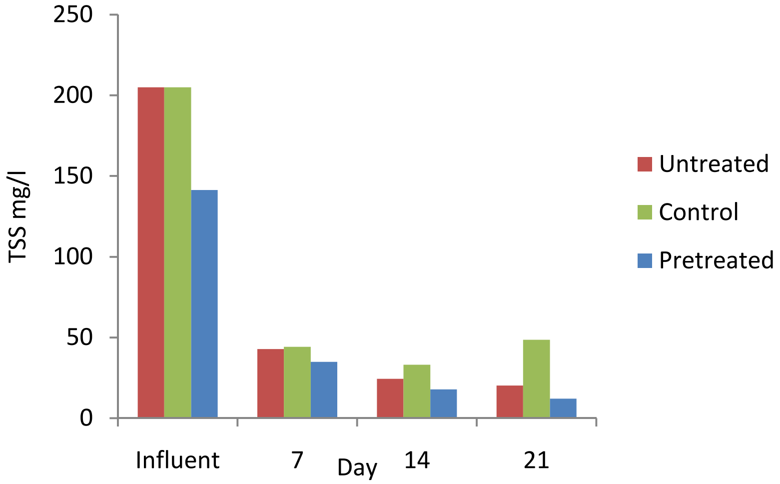


Figure 4.5 Removal of TSS in duckweed wastewater treatment ponds

## 4.2.2 Nutrient Removal

### 4.2.2.1 Nitrogen Removal

In duckweed wastewater treatment ponds, nitrogen exists in the form of ammonia, organic nitrogen, nitrite and nitrate. Several removal mechanisms exist that remove or convert one form of nitrogen to another. Removal efficiencies obtained in this study for $NO_3^-$-N, $NH_4$-N and TKN-N were 64.54%, 83.59% and 65.26% for duckweed pond with untreated wastewater and 67.74%, 84.60% and 68.20% for duckweed pond with pretreated wastewater. Table 4.3 indicates that the level of ammonium nitrogen and Kjeldahl nitrogen in the effluent of pretreated duckweed wastewater treatment pond were significantly lower ($p < 0.05$) ) than that of the system without pretreatment.

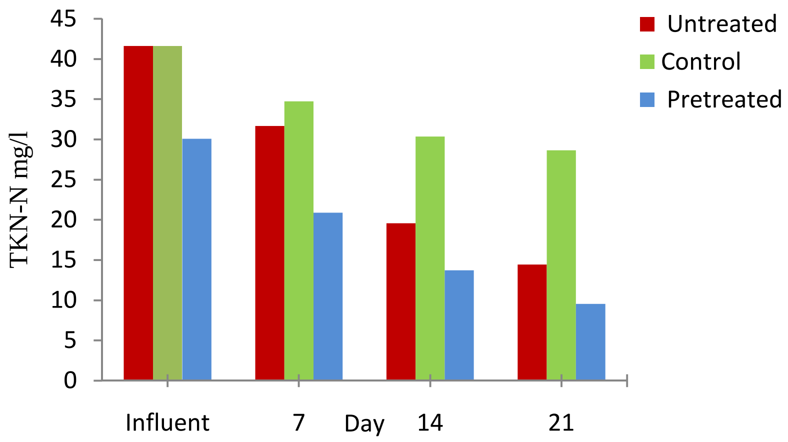
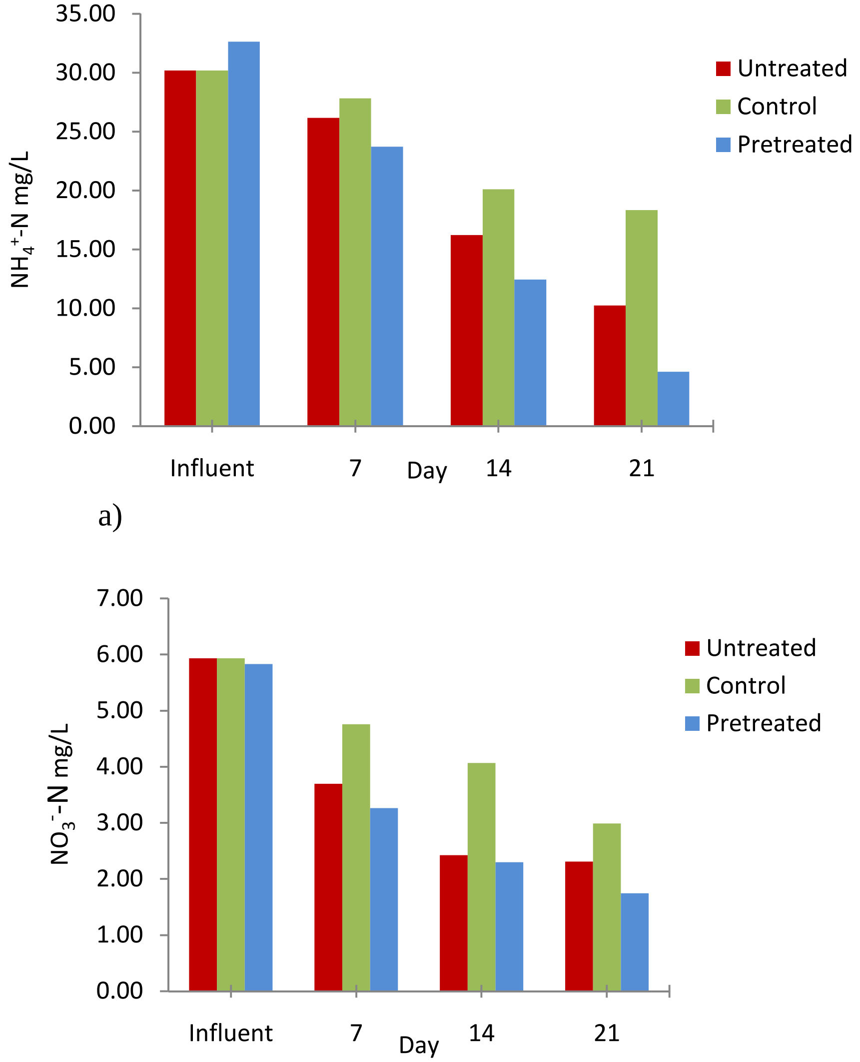


Figure 4.6 Removal of TKN in duckweed wastewater treatment ponds

Good removal efficiency of ammonia nitrogen for the duckweed treatment ponds were due to the favorable condition created by the primary treatment for hydrolysis of organic nitrogen compounds before entering the treatment pond and within the ponds (Alearts et al., 1996). Published data show that duckweed and other aquatic plants exhibit a preferential uptake of ammonia over nitrate since ammonia transformed directly into plant protein rather than having to be reduced first as in the case of nitrate (Ferguson and Bollard, 1969). According to Caiceido *et al*. (2000), duckweed had a preferential uptake of $NH_4^+$ over other nitrogen sources therefore removal of ammonium in duckweed ponds is mainly due to plant assimilation.

Efficiencies of ammonium removal found in this study showed similarity with that of Gurtekin and Sekerdag (2008). They treated secondary effluent domestic wastewater with a hydraulic retention time of 7 days with influent ammonium concentration of 11.5 and obtained ammonium concentration of 7.5 in the effluent with removal efficiency of 53%. Ndamba and Dalu (2003), reported 70% removal of Nitrate using duckweed stabilization ponds in Zimbabwe which resembles the results found in this study. The observed TKN removal efficiency is similar to removal efficiency obtained (55%) by Bal Krishina and Polprasert (2008) over a hydraulic retention time 10 days.



a)

b)

Figure 4.7 Removal of a) $NH_4^+$ & b) $NO_3^-$ in duckweed wastewater treatment ponds

Duckweed ponds removed nitrate through denitrification in the presence of organic matter, as microbes associated with attached biofilms or suspended in the water column convert nitrate in to nitrogen gas; and through duckweed plant uptake (Bitcover and Sieling,1950). Furthermore, some nitrate will also diffuse into the sediments. Greater Removal of nitrate with increase in retention time was also observed in other studies (Hammouda *et al*., 1995). The higher removal efficiencies might be due to anoxic/anaerobic conditions and organic matter available under the duckweed mat enhancing denitrification (USEPA, 2000). In his experiment Zimmo reported nitrate concentrations of 11.9 and 14.8 mg N/L in spite of the low DO concentrations in the ponds (0.5-3 mg/l).

#### 4.2.2.2 Phosphate Removal

TP and *ortho*-phosphate showed a gradual removal by prolonged treatment periods as indicated in figures 4.5 and 4.6. Percent removals were 68.84 % for $PO_4^{3-}$ (reduced from 2.92 mg/L to 0.91 mg/L) and 70.61% for TP (reduced from 5.47 mg/L to 1.61 mg/L) duckweed ponds with untreated wastewater and 75.61% for $PO_4^{3-}$ (reduced from 3.64 mg/L to 0.69 mg/L) and 81.04.5% for TP (reduced from 4.12 mg/L to 1.06 mg/L) for duckweed ponds with pretreated wastewater. Percentage removal in the uncovered duckweed ponds were 35.27% for $PO_4^{3}$ and 46.04 for TP.

ANOVA statistical analysis indicated that the difference between the duckweed covered and uncovered ponds in removal of TP and $PO_4^{3-}$ were significant (($p < 0.05$).

The statistical analysis also revealed that total-P reduction of in the pretreated and untreated duckweed ponds were found not significant ($p > 0.05$) for the study period. However, removal of $PO_4^{3-}$ was significant ($p < 0.05$).

The results achieved in this study for the removal of TP and $PO_4^{3-}$ were higher than the results obtained by Abou-El Kheir *et al*. (2007), TP and Ortho-Phosphate removal efficiencies were 48% and 64.4% respectively with *Lemna gibba.* TP removal efficiency of duckweed ponds obtained in this study for both systems falls in the range obtained (60-92.2 %) by Hammouda *et al*. (1995). Alearts *et al*. (1996) found a removal efficiency of 95 % for $PO_4{}^{3-}$ which was lower than the finding of this study.

The higher reduction of TP in pretreated duckweed pond could be attributed to duckweed uptake of the hydrolyzed organic phosphate and subsequent removal by harvesting. TP removal in duckweed ponds with untreated wastewater was lower due to the fact that part of the phosphorus was in organic form and remained in the system.

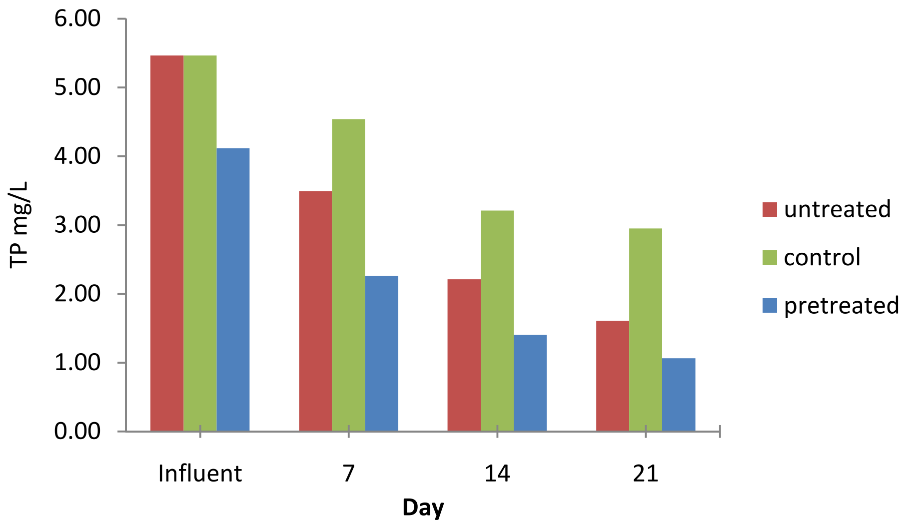


Figure 4.8 Removal of TP in duckweed wastewater treatment ponds.

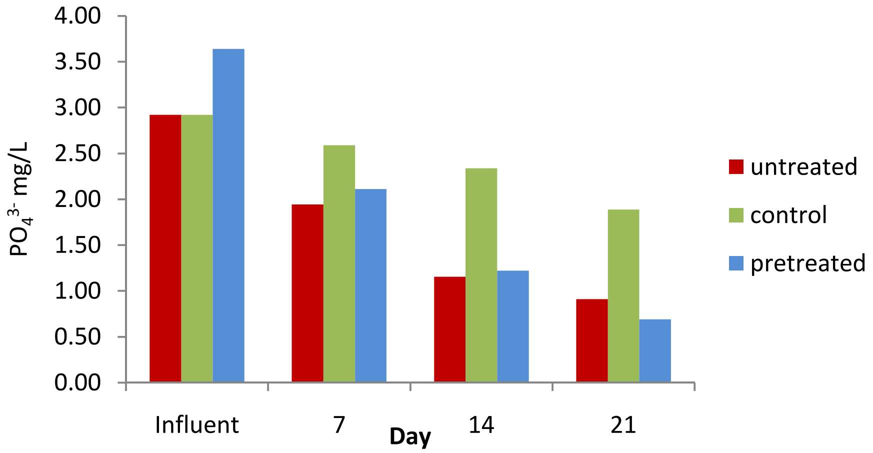


Figure 4.9 Removal of $PO_4^{3}$-in duckweed wastewater treatment ponds

## 4.3 Pathogen Removal

Indicator organism Faecal Coliform was used in this study to evaluate pathogen removal of duckweed plants. Removal of FC in pretreated, untreated and control duckweed wastewater treatment ponds in Phase I were 98.55%, 97.6% and 99.01% respectively. In Phase II, DBWWT using untreated wastewater achieved a removal efficiency of 99%. The result found in this study is in agreement with the results that were obtained by El-Shafai *et al*. (2006), 99.9% faecal coliform removal with a hydraulic retention time of 15 days.

ANOVA analysis result showed that there was significant difference (P<0.05) between the duckweed covered ponds and control. For duckweed cover ponds there were significant difference in removal of FC (P<0.05) between the two phases.

Similar to the waste stabilization ponds, removal of pathogenic bacteria is considered to be a complex phenomenon. While detention time and temperature are considered to be the most influential parameters other factors have been reported by many investigators. These include the presence of predators, high pH value and solar radiation (El-Hamouri *et al*., 1995). In WSP, the high pH value resulting from algal photosynthetic activity plays an important role in promoting faecal coliform die-off (El-Hamouri *et a*l., 1995). In this study, the removals of faecal coliforms were more efficient in the uncovered ponds than in the duckweed covered ponds with untreated and pretreated wastewater. The study showed that introduction of duckweed into the pond affects these parameters. It seemed that direct sunlight, relatively higher pH and Temperature were played a role for higher pathogen removal in control duckweed ponds. In duckweed covered ponds the main removal mechanism in addition to the effect of light was due to removal through harvesting of duckweeds.

As described by El-Shafai *et al*. (2006), the mechanisms of faecal coliform removal in DBWWTs were the result of two main processes. Firstly, the recovery of nutrients from the pond may cause a deficiency in these nutrients, which might affect the faecal coliform removal. Secondly, the adsorption of the faecal coliform to the duckweed followed by harvesting might play a role in faecal coliform removal. But probably most important to this study was that the duckweed cover protects the faecal coliform from solar radiation (Islam *et al*., 1990).

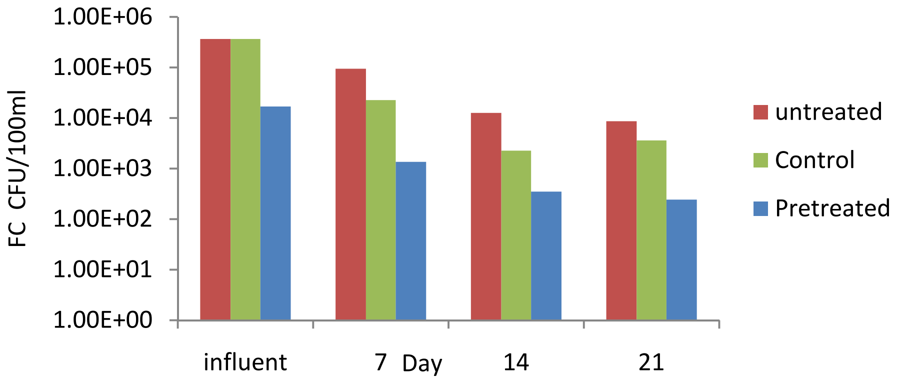


Figure 4.10 Faecal coliform removals in duckweed wastewater treatment ponds

## 4.4 Comparison of Duckweed Wastewater Treatment Pond Effluent Quality with EEPA Standard

Effluents of DBWWTs and the EEPA effluent quality discharge requirements are indicated in the Table 4.5. The data revealed that except FC for untreated duckweed, the values of all the parameters met National Environmental Quality Standard for effluent discharge to water bodies (EEPA, 2003). DBWWT with pretreated wastewater fulfill the requirements for FC. Faecal coliforms in effluent concentrations from untreated wastewater duckweed pond was not complied with EEPA effluent discharge provisional standard limit (400 CFU/100mL) even though 1.6 log reductions was observed. This might be due to shading effects of duckweed mat as UV disinfection has a direct impact on pathogen removal (El-Shafai, *et al*., 2007). However; FC in the effluent was below 1000 CFU/100ml (WHO, 1989) which ensured safe effluent reuse for irrigation.

Ammonium effluent concentration in the untreated duckweed wastewater treatment pond was 8.34 ± 0.4 mg/L which did not fulfill the required effluent standard limits (5 mg/L). Bal Krishina and Polprasert (2008) suggested that removal efficiency of FC and ammonium in duckweed ponds increases with lower organic loading and higher detention time.

Effluent of Phase II which has twice surface area compared to Phase I for untreated duckweed was fully in compliance with discharge requirement within the HRT of 14 days

EEPA's provisional environmental quality standard looks very soft with respect to BOD, COD and TSS limit values which are comparable with weak wastewater characteristics rather than treated one. On the other hand, discharge limit values of FC, 400 CFU/100ml, which is very stringent as compared to WHO (1989). EEPA provisional standard sets very high organic matter and very low pathogen which contrasts the general wastewater characteristics.

Table 4.5 Effluent Quality of DBWWTs in Comparison with Ethiopia EPA (2003) Provisional Discharge Standard

| Parameter | Unit | Effluent of DBWWT with pretreated wastewater | Effluent of DBWWT with untreated wastewater | Ethiopia EPA (2003) Standard |
|---|---|---|---|---|
| $BOD_5$ | mg/L | 13.78 ± 2.31 | 25.77± 1.44 | 80 |
| COD | mg/L | 28.6 ± 2.98 | 55.09± 7.47 | 250 |
| TSS | mg/L | 12.1 ± 1.02 | 20.13 ±1.17 | 100 |
| TKN-N | mg/L | 9.56 ± 0.72 | 14.45±1.2 | 60 |
| TP | mg/L | 1.06 ± 0.07 | 1.61±0.1 | 10 |
| $PO_4^{3-}$ | mg/L | 0.59 ± 0.08 | 0.91±0.07 | 5 |
| $NH_4^+$-N | mg/L | 4.61 ± 0.2 | 4.14±1.71 | 5 |
| $NO_3^-$-N | mg/L | 1.74 ± 0.12 | 2.31±0.09* | 20 |
| FC | CFU/100ml | $2.44 \times 10^2 \pm 55.9$ | $8.60 \times 10^3 \pm 8.3 \times 10^2$* | 400 |
| Temp | $^oC$ | 19.01 ± 0.44 | 18.91±0.11 | 40 |
| pH | $-\log[H^+]$ | 7.45 ± 0.1 | 7.50±0.04 | 6-9 |

* Parameters values found above the limits

## 4.5 Duckweed Biomass Production and Protein Content

Harvested duckweed characteristics and estimated protein content is recorded in Table 4.6. Duckweed harvesting was carried out every three days. The protein content of the dry matter achieved was 21.3% which is 213 g per Kg of dry biomass. The result was within the range of the finding of Pandey (2001), 20 - 31% protein in the dried biomass. In view of its high nutrient content, many authors were recommended to use duckweed as a food supplement for fish, poultry and cattle.

Table 4.6 Dry matter and protein content of duckweeds

| Harvested Duckweed | Values |
|---|---|
| Fresh weight | 186.45 g |
| Dry weight (DW) % | 4.21 |
| Protein content | 21.3%/DW or 213 g/Kg DW |

# 5 CONCLUSIONS AND RECCOMENDATIONS

## 5.1 Conclusions

In the present study, the experiments were conducted to find out environmental characteristics and removal efficiency of organic matter, nutrients and faecal coliforms in duckweed-based wastewater treatment systems. Removal efficiencies obtained using duckweed plants for treating domestic wastewater were $BOD_5$ (86.2-90.13%), COD (82.3-88.27%), TSS (90.18-91.83%), $NO_3^-$ (44.14 - 67.74%), $NH_4^+$ (66.07-85.840%), TKN (65.26-74.12%), $PO_4^{3-}$ (68.84 - 80.49%) TP (70.58 - 75.71%), FC (97.67 - 98.55%).

From statistical analysis done to compare removal efficiency of duckweed plants there were significant difference between ponds covered with and without duckweed. Similarly, significant difference was also observed as surface area to volume ratio was increased using untreated wastewater. The performance of duckweed was substantially improved in phase II when the pond surface area to volume ratio was doubled.

The observed values of effluent's physico-chemical quality parameters were compared with the National Environmental Quality Standard for effluent discharge to water bodies (EEPA, 2003). It was found that duckweed covered ponds with raw sewage in depth of 20 cm didn't comply the required 400 CFU/100 ml faecal coliform concentrations respectively. However; effluents quality was fully in compliance with WHO guidelines for unrestricted irrigation. On the other hand, the effluent quality from duckweed ponds with pretreated and untreated wastewater with doubled surface to volume ratio were fully complied with both the EEPA provisional standard and WHO guideline.

Effluent quality requirement was met by the effluent from the ponds without duckweed cover. In duckweed covered ponds the decrease of light penetration as a result of shading by the duckweed mat decreased the removal of faecal coliforms.

The removal of organic matters and nutrients in Phase II with a shallow duckweed treatment ponds were significantly higher than that of deeper one in Phase I. Depth of the pond was found to be critical factor to ensure vertical mixing in the pond to allow the wastewater to be treated to come into contact with the duckweed fronds for nutrient uptake and BOD degradation through attached microbial populations.

Duckweed *Lemna minor* protein content was found 213 g/Kg dry weight. The result revealed that the nutritional value of duckweed which can be used as fodder and in fish farms.

This study based on the smallest free floating aquatic duckweed plants was done under outdoor environmental conditions and it has proved that the plants were capable of treating domestic effluents. In developing countries like Ethiopia where wastewater is simply discharged to receiving water bodies; such kind of dual purpose wastewater treatment system should come to the attention of researchers, developers and planners.

## 5.2 Recommendations

This study based on the duckweed *Lemna minor* a free floating aquatic plant was done under outdoor environmental conditions and it has proved that the plants were capable of treating domestic wastewater. However, to strengthen the finding further investigation is needed as outlined below.

- This study was carried out in a batch system under outdoor environmental conditions. Therefore; it is recommended that further investigations using different species of duckweed plants on real environmental conditions.
- Duckweed plants can play essential role in wastewater treatment in Ethiopia as they are not sophisticated systems and can efficiently grow under the country’s environmental conditions, option for incorporating along with other treatment systems to enhance treatment efficiencies should be considered;
- The literature and the present study demonstrated the success to employ duckweed based wastewater ponds for domestic wastewater treatment; however, before the system can be adopted on a large scale, a further investigation at pilot level operation is recommended;

- Pilot scale operation should be undertaken over a much longer period to establish favorable conditions that will enable correct assessments of long term treatment efficiencies, optimal loading rates, optimal hydraulic retention times;
- Consideration should be given to use these plants for wastewater treatment and biomass production particularly in small towns and peri-urban areas in Ethiopia;
- The National Provisional Environmental Quality Standard for effluent discharge limit with respect to BOD (80 mg/L), COD (250 mg/L) and TSS (100) very soft as it can be characterized as weak wastewater while discharge limit for faecal coliform (400 CFU/100 ml) is very strong and can be characterize as treated wastewater in a conventional treatment plant. In view of this, it is recommended to review the National Provisional Environmental Quality Standard.

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# APPENDICES

## Appendix 1: Selected Pictures Taken During the Research Work

Duckweed mat (left) and Harvested Duckweed (right)

Experimental ponds with duckweed plant *Lemna minor.*

# Appendix 2: Laboratory Procedures

### Measurement of COD

The mg/L COD results are defined as mg of $O_2$ consumed per liter of sample. The sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O_7^{2-)}$ to green chromic ion ($Cr^{3+).}$

The COD test is specifically more suitable to measure organic matter present in industrial wastes having compounds that are toxic to biological life. However, COD results are generally higher than BOD values since the test will oxidize materials such as fats and lignin which are only slowly biodegradable. No clear correlation exists between BOD and COD in general, but at specific wastewater a correlation is possible. When once a correlation has been established, the COD measurements, which can be concluded within 3 hours, can be used to good advantage for the control and operation of those treatment plants. For typical untreated domestic wastes, the ration COD/$BOD_5$ is found to vary from 1.25 to 2.5. A higher value of the ratio indicates that the wastewater is difficult to biodegrade. For non-biodegradable wastewater, the ratio exceeds 3. The limiting value of COD of wastewater, generally specified by the authorities is 250 mg/L.

### Method

- Adaptation of the USEPA 410.4 approved method for the COD determination on surface waters and wastewater
- Oxidizable organic compounds reduce the dichromate ion (orange) to the chromic ion (green)
- The amount of remaining dichromate is determined

### Required reagent

- Reagent vial
- Deionizer water

### Materials used

- Hach DRB 200 Reactor
- Test tube cooling rack

**Measurement procedure**

- Choose a homogeneous sample
- Samples containing settle able solids need to be homogenized with a blender
- Preheat the Hach DRB 200 Reactor to 150 0c (32 0F)
- Remove the cap from two reagent vials
- Add exactly 0.2 ml of sample to one vial (sample vial)
- Add exactly 0.2ml of demonized water to the other (blank vial)
- Keep the vial at 45 degree angle while adding the water and the sample
- Replace the cap tightly and mix by inverting each vial a couple of times
- Insert the vials into the reactor and heat them for 2 hours at 150 0c
- At the end of digestion period switch of the reactor and wait for 20 minute to allow the vials to cool to about 120 0c
- Invert each vials several times while still warm, then place them in the test tube rack
- Leave the vials in the tube rack to cool to room temperature
- Do not shake or invert any more otherwise the sample may become turbid
- Select the program number corresponding to oxygen demand, chemical HR (COD) on the secondary LCD by pressing program increase or decrease symbol
- Place the blank vial into the holder and push it completely down
- Press zero and 'sip' will blink on the display
- Wait for a few seconds and the display will show '-0.0-' now the meter is zeroed and ready for measurement
- Remove the blank vial
- Place the sample vial into the holder and push it completely down
- Press read direct and 'sip' will blink during measurement
- Instrument directly displays concentration in mg/L of oxygen demand on the crystal display
- multiply the reading on the liquid crystal display by 10 to obtain the concentration in mg/L of oxygen demand

## Biochemical Oxygen Demand (BOD)

The biochemical oxygen demand (BOD) is a measure of the oxygen required to oxidize the organic matter present in a sample, through the action of micro-organisms contained in a sample of wastewater. It is the most widely used parameter of organic pollution applied to both wastewater as well as surface water. The BOD may be defined as the oxygen required for the micro-organisms to carry out biological decomposition of dissolved solids or organic matter in the wastewater under aerobic conditions at standard temperature.

### Method

**Lovibond BOD system oxidirect**

- preparing the sample
- estimate the measurement reagent and select the volume for the sample
- The sample volume is related to the expected BOD value. The oxidirect is designed to operate with the following ranges and samples volume allowing BOD measurement up to 0-4000 mg/L with out any dilution
- for 0-2000 mg/L expected value 56 ml sample volume with 3 drop ATH to avoid nitrification and 3-4 drop KOH
- for 0-4000 mg/L expected value 21.2 ml sample volume with 1 drop ATH to avoid nitrification and 3-4 drop KOH
- carry out the necessary pre treatment of the sample (setting pH value 6.5 to 7.5, filtering)
    - the optimum pH value for BOD is between 6.5 and 7.5
    - if higher or lower adjust by HCL or $H_2SO_4$ and NaOH
    - mix well and advisable to settle or filter

### Required reagent

- KOH to absorb $CO_2$
- ATH to avoid nitrification

### Materials used

- BOD bottle
- BOD sensor
- BOD bottle rack

- Incubator
- Measuring cylinder

**Measurement procedure**

- measure the sample precisely using appropriate over flow and if necessary add nitrification inhibitor (ATH)
- insert magnetic stirring rod
- place 3-4 drop of KOH solution into the seal gasket and insert gasket in the neck of the bottle
- screw the BOD sensors to the sample bottle
- place the bottle in the bottle rack
- start the measurement
- incubate the sample in accordance with the instructions $BOD_5$ for 5 days at 20 0C

## Determination of Faecal coliforms

### Method

- Multiple Tube Fermentation Method APHA, procedure 9221B and 9221E

**Materials used**

- Volumetric flask, Durham tube, spatula, loop, test tube rack, incubator, autoclave, sewage, and culture media

**Presumptive phase**

- Reagent and culture medium – lactose peptone broth

**Procedure**

- Prepare growth media 70 gm in 1 ml distilled water and Sterilize at 121 $^0$C for 15 min
- Arrange fermentation tubes in rows of five or ten tubes and add 10 ml growth media in each tube
- Inoculate each tube in a set of five with replicate sample volumes
- Mix test portions in the medium by gentle agitation
- Incubate inoculated tubes or bottles at 37 0C. After 24 + 2h swirl each tube or bottle gently and examine it for growth, gas and acidic reaction (shades of yellow color). If no gas or acidic reaction is evident, re-incubate and reexamine

at the end of 48 ± 3h. Record presence or absence of growth, gas, and acid production. Growth with acidity signifies a positive presumptive reaction

- Interpretation- production of an acidic reaction or gas in the tubes or bottles within 48 + 3h constitutes a positive presumptive reaction. Submit tubes with a positive presumptive reaction to the confirmed phase

**Confirmed phase**

- Culture medium- brilliant green lactose bile broth

**Procedur**e

- Prepare growth media 40 gm in 1 ml distilled water Sterilize at 121 $^{0}$C for 15 min
- Submit all presumptive tubes or bottles showing growth, any amount of gas, or acidic reaction with in 24 ± 2h of incubation to the confirmed phase
- Gently shake or rotate presumptive tubes or bottles showing gas or acidic growth to re-suspend the organisms.
- With a sterile loop 3 to 3.5 mm diameter, transfer one or more loop-fuls of culture to a fermentation tube containing brilliant green lactose bile broth
- Incubate the inoculated brilliant green lactose bile broth tube at 37$^{0}$C for total coliform and 44 $^{0}$C for faecal coliform. Formation of gas in any amount in the inverted vial of the brilliant green lactose bile brothfermentation tube at any time with in 48 ± 3h constitutes a positive confirmed phase or the combination of positive tubes calculate bacterial density according to the following formula

$$MPN/100ml = MPN\ value\ (from\ table)\ X\ \frac{10}{largest\ volume\ tested\ in\ dilution\ series\ used\ for\ MPN\ determinatio}$$

## NITRATE

### Method

- Cadmium reduction method ( USEPA approved for reporting wastewater analysis)

**Reagent Used**

- NitriVer 5

**Materials used**

- HACH spectrophotometer model DR / 4000

## Phosphorus

### Method

- Hach method 8190: Molybdovanadate with acid per-sulfate digestion
- Adaptation of the standard method for examination of water and waste water 20th ed. 4500-P C, vanadomolybdophosphoric acid method. A persulfate digestion converts organic and condensed inorganic forms of phosphates to orthophosphate then the reaction between orthophosphate and the reagent cause a yellow tint in the sample

### Required reagent

- Potassium per-sulfate powder pillows
- 1.54 N NaOH solution
- Molybdovanadate reagent
- Deionized water

### Materials used

- Hach reactor
- Hach spectrophotometer model DR / 4000
- Measuring cylinder
- Pipette

### Measurelment procedure

- Choose a homogeneous sample
- Preheat the Hach reactor to 150 $^0$C
- Remove the cap from two reagent vials
- Add exactly 5ml of sample to one vial (sample vial)
- Add exactly 5 ml of deionized water to the other (blank vial)
    - Replace the cap tightly and mix by inverting each vial a couple of times
- Add the content of one potassium per-sulfate reagent powder pillows for phosphorus analysis to each vials
- Replace the cap tightly and shake gently the vials until all the powder is completely dissolved
- Insert the vials into the reactor and heat them for 30 minute at 150 $^0$C
- At the end of digestion period switch of the reactor and place the vials carefully in the test tube rack and allow to cool to room temperature
- Select the program number corresponding to total phosphorus on the secondary LCD by pressing program increase or decrease symbol

- Remove the cap from the vials and add exactly 2ml of sodium hydroxide solution (1.54 N) to each vial while keeping the vial at 45 degree
- Replace the cap tightly and mix by inverting the vial a couple of time
- Remove the cap from the vial and add exactly 0.5 ml of molybdovanadate reagent to each vial while keeping the vial at 45 degree
- Replace the cap tightly and mix by inverting the vial a couple of time
- Place the blank vial into the holder and push it completely down
- Press timer and the display show the countdown prior to the measurement, alternatively, wait for 7 minute and pres zero in both case 'sip' will blink on the display
- The display will show '-0.0-' now the meter is zeroed and ready for measurement
- Remove the blank vial
- Place the sample vial into the holder and push it completely down
- Press read direct and 'sip' will blink during measurement
- Instrument directly displays concentration in mg/L of total phosphorus on the liquid crystal display
- To convert the reading to mg/L of $P_2O_5$, multiply by a factor of 0.748
- To convert the reading to mg/L of phosphorus concentration, multiply by a factor of 0.326

## Appendix 3: Laboratory result of different parameters

Table I Duckweed treatment ponds with untreated wastewater run 1

| Parameters | Unit | Time, d | | | |
|---|---|---|---|---|---|
| | | Influent | 7 | 14 | 21 |
| BOD | mg/L | 182.85 | 80.64 | 38.46 | 24.19 |
| COD | mg/L | 320.66 | 168.71 | 72.39 | 61.47 |
| TSS | mg/L | 200.88 | 42.72 | 18.36 | 20.31 |
| TKN | mg/L | 41.36 | 31.62 | 19.54 | 14.94 |
| $PO_4^{-3}$ | mg/L | 2.82 | 1.71 | 0.92 | 0.68 |
| TP | mg/L | 6.02 | 3.77 | 2.24 | 1.71 |
| $NO_3^-$ | mg/L | 6.12 | 4.02 | 2.56 | 2.39 |
| $NH_4^+$ | mg/L | 27.95 | 24.16 | 14.71 | 3.70 |
| FC | CFU/100ml | 3.65E+05 | 9.41E+04 | 1.22E+04 | 7.79E+03 |
| Temp | $^{o}$C | 20.80 | 19.40 | 19.05 | 19.05 |
| pH | -log[H+] | **7.15** | 7.25 | 7.45 | 7.45 |

Influent and effluent of DBWWT with untreated wastewater Run 2

| Parameters | Unit | Time, d | | | |
|---|---|---|---|---|---|
| | | Influent | 7 | 14 | 21 |
| BOD | mg/L | 191.25 | 85.48 | 41.22 | 27.00 |
| COD | mg/L | 294.32 | 154.45 | 67.51 | 46.56 |
| TSS | mg/L | 215.76 | 43.42 | 16.48 | 16.12 |
| TKN | mg/L | 40.35 | 29.412 | 17.46312 | 13.56 |
| $PO_4^{-3}$ | mg/L | 3.07 | 2.21 | 1.22 | 0.79 |
| TP | mg/L | 5.25 | 3.41 | 2.21 | 1.62 |
| $NO_3^-$ | mg/L | 5.76 | 3.40 | 2.33 | 2.22 |
| $NH_4^+$ | mg/L | 29.97 | 26.95 | 17.16 | 6.88 |
| FC | CFU/100ml | 3.67E+05 | 8.85E+04 | 9.34E+03 | 8.57E+03 |
| Temp | $^{o}$C | 20.80 | 19.40 | 19.05 | 19.05 |
| pH | -log[H+] | 7.15 | 7.33 | 7.45 | 7.50 |

Influent and effluent of DBWWT with untreated wastewater run 3

| Parameters | Unit | Time, d | | | |
|---|---|---|---|---|---|
| | | Influent | 7 | 14 | 21 |
| BOD | mg/L | 189.45 | 81.36 | 39.45 | 26.11 |
| COD | mg/L | 316.53 | 165.26 | 70.25 | 58.25 |
| TSS | mg/L | 198.25 | 36.63 | 14.28 | 15.24 |
| TKN | mg/L | 43.28 | 34.2 | 21.7712 | 15.36 |
| $PO_4^{-3}$ | mg/L | 2.87 | 1.93 | 1.03 | 0.83 |
| TP | mg/L | 5.15 | 3.29 | 2.18 | 1.51 |
| $NO_3^-$ | mg/L | 5.91 | 3.69 | 2.41 | 2.33 |
| $NH_4^+$ | mg/L | 29.08 | 25.48 | 14.12 | 3.70 |
| FC | CFU/100ml | 3.58E+05 | 9.88E+04 | 1.62E+04 | 9.45E+03 |
| Temp | $^oC$ | 20.80 | 19.40 | 19.05 | 19.05 |
| pH | -log[H+] | 7.32 | 7.45 | 7.65 | 7.56 |

Table II Influent and effluent of DBWWT with untreated wastewater
Run 1

| Parameters | Unit | Time, d | | | |
|---|---|---|---|---|---|
| | | Influent | 7 | 14 | 21 |
| BOD | mg/L | 130.5 | 55.89 | 20.1795 | 14.5075 |
| COD | mg/L | 210.11 | 108.89 | 41.849 | 31.13 |
| TSS | mg/L | 149.03 | 64.42 | 22.15 | 13.13 |
| TKN | mg/L | 27.25 | 19.54 | 11.63 | 8.92 |
| $PO_4^{-3}$ | mg/L | 2.43 | 1.541 | 0.89 | 0.66 |
| TP | mg/L | 3.85 | 2.11 | 1.23 | 1.05 |
| $NO_3^-$ | mg/L | 6.14 | 3.44 | 2.42 | 1.85 |
| $NH_4^+$ | mg/L | 22.42 | 20.84 | 11.85 | 7.22 |
| FC | CFU/100 ml | 1.36E+06 | 1.03E+05 | 3.29E+04 | 2.15E+02 |
| Temp | $^oC$ | 20.2 | 19.34 | 19.05 | 19.3 |
| pH | -log[H+] | 7.29 | 7.34 | 7.35 | 7.51 |

Influent and effluent of DBWWT with untreated wastewater, run 3

| Parameters | Unit | Time, d | | | |
|---|---|---|---|---|---|
| | | Influent | 7 | 14 | 21 |
| BOD | mg/L | 124.44 | 59.68 | 22.52 | 15.64 |
| COD | mg/L | 228.21 | 103.68 | 45.66 | 29.11 |
| TSS | mg/L | 132.11 | 55.51 | 19.22 | 11.09 |
| TKN | mg/L | 29.232 | 20.246 | 13.17 | 10.29 |
| $PO_4^{-3}$ | mg/L | 2.23 | 1.39 | 0.83 | 0.65 |
| TP | mg/L | 4.29 | 2.4 | 1.55 | 1.142 |
| $NO_3^-$ | mg/L | 5.76 | 3.25 | 2.36 | 1.62 |
| $NH_4^+$ | mg/L | 23.5 | 21.67 | 12.54 | 7.55 |
| FC | CFU/100 ml | 7.67E+05 | 1.38E+05 | 3.81E+04 | 3.25E+02 |
| Temp | $^{o}C$ | 20.33 | 19.52 | 19.2 | 18.5 |
| pH | -log[H+]<br>Q | 7.23 | 7.33 | 7.4 | 7.5 |

Influent and effluent of DBWWT with untreated wastewater, Run 3

| Parameters | Unit | Time, d | | | |
|---|---|---|---|---|---|
| | | Influent | 7 | 14 | 21 |
| BOD | mg/L | 93.62 | 47.93 | 17.28 | 11.2 |
| COD | mg/L | 198.33 | 95.88 | 34.75 | 25.26 |
| TSS | mg/L | 143.1 | 59.61 | 21.1311 | 12.16 |
| TKN | mg/L | 28.46 | 19.86 | 13.35 | 10.3 |
| $PO_4^{-3}$ | mg/L | 2.61 | 1.57 | 1.02 | 0.77 |
| TP | mg/L | 4.21 | 2.31 | 1.42 | 1.001 |
| $NO_3^-$ | mg/L | 5.58 | 3.1 | 2.11 | 1.76 |
| $NH_4^+$ | mg/L | 23.38 | 21.48 | 12.28 | 7.58 |
| FC | CFU/100 ml | 1.41E+06 | 1.63E+05 | 3.48E+04 | 2.53E+02 |
| Temp | $^{o}C$ | 20.11 | 19.41 | 19.2 | 19.23 |
| pH | -log[H+] | 7.2 | 7.22 | 7.29 | 7.34 |

**Appendix 4:**

Table III ANOVA for BOD, COD, TSS, TKN, pH, Temperature between Duckweed Covered Ponds with Uncovered one in Phase I.

| | | SS | Df | MS | F | P |
|---|---|---|---|---|---|---|
| BOD, mg/L | Between groups | 3322.53956 | 1 | 3322.54 | 93.30205 | 0.001 |
| | Within groups | 142.44229 | 4 | 35.61057 | | |
| | Total | 3464.98185 | 5 | | | |
| COD, mg/L | Between groups | 8664.76002 | 1 | 8664.76 | 127.4607 | 0.0004 |
| | Within groups | 271.91933 | 4 | 67.97983 | | |
| | Total | 8936.67935 | 5 | | | |
| TSS, mg/L | Between groups | 12201.61209 | 1 | 12201.61 | 630.0854 | 0.0000 |
| | Within groups | 77.46005 | 4 | 19.36501 | | |
| | Total | 12279.07215 | 5 | | | |
| TKN, mg/L | Between groups | 27858.35327 | 1 | 27858.35 | 9358.448 | 0.0000 |
| | Within groups | 11.90725 | 4 | 2.976813 | | |
| | Total | 27870.26052 | 5 | | | |
| pH, mg/L | Between groups | 0.0486 | 1 | 0.0486 | 7.009615 | 0.057127 |
| | Within groups | 0.027733 | 4 | 0.006933 | | |
| | Total | 0.076333 | 5 | | | |
| Temp, mg/L | Between groups | 7.7976 | 1 | 7.7976 | 323.105 | 0.000563 |
| | Within groups | 0.096533 | 4 | 0.024133 | | |
| | Total | 7.894133 | 5 | | | |

Table IV ANOVA for BOD, COD, TSS, TKN, pH, Temperature for Duckweed Wastewater treatment ponds between pretreated and untreated wastewater.

| | | SS | df | MS | F | P |
|---|---|---|---|---|---|---|
| BOD, mg/L | Between groups | 215.43038 | 1 | 215.4304 | 58.34228 | 0.0016 |
| | With in groups | 14.770104 | 4 | 3.692526 | | |
| | Total | 230.20048 | 5 | | | |
| COD, mg/L | Between groups | 1060.8081 | 1 | 1060.808 | 32.77197 | 0.0046 |
| | Within groups | 129.47747 | 4 | 32.36937 | | |
| | Total | 1190.2855 | 5 | | | |
| TSS, mg/L | Between groups | 1106.1838 | 1 | 1106.184 | 264.1872 | 0.0001 |
| | Within groups | 16.748485 | 4 | 4.187121 | | |
| | Total | 1122.9323 | 5 | | | |
| TKN, mg/L | Between groups | 124.801 | 1 | 124.801 | 115.9523 | 0.0004 |
| | Within groups | 4.3052535 | 4 | 1.076313 | | |
| | Total | 129.10625 | 5 | | | |
| TP, mg/L | Between groups | 0.4521015 | 1 | 0.452102 | 59.65318 | 0.0015 |
| | Within groups | 0.0303153 | 4 | 0.007579 | | |
| | Total | 0.4824168 | 5 | | | |
| $PO_4$, mg/L | Between groups | 0.0726 | 1 | 0.0726 | 5.839142 | 0.0730 |
| | Within groups | 0.0497333 | 4 | 0.012433 | | |
| | Total | 0.1223333 | 5 | | | |
| $NH_{4,}$ mg/L | Between groups | 25.422214 | 1 | 25.42221 | 17.17267 | 0.0143 |
| | Within groups | 5.9215534 | 4 | 1.480388 | | |
| | Total | 31.343767 | 5 | | | |
| $NO_3$, mg/L | Between groups | 0.48735 | 1 | 0.48735 | 46.71086 | 0.0024 |
| | Within groups | 0.0417333 | 4 | 0.010433 | | |
| | Total | 0.5290833 | 5 | | | |
| CF, mg/L | Between groups | 104332066 | 1 | 1.04E+08 | 301.5325 | 0.0001 |
| | Within groups | 1384023.9 | 4 | 346006 | | |
| | Total | 105716090 | 5 | | | |
| pH, mg/L | Between groups | 0.0042667 | 1 | 0.004267 | 0.703297 | 0.4489 |
| | Within groups | 0.0242667 | 4 | 0.006067 | | |
| | Total | 0.0285333 | 5 | | | |
| Temp, mg/L | Between groups | 0.0140167 | 1 | 0.014017 | 0.129824 | 0.7368 |
| | Within groups | 0.4318667 | 4 | 0.107967 | | |
| | Total | 0.4458833 | 5 | | | |

## Appendix 4: Emission limit values for discharges to water

Table VI General standards for industrial effluents in Ethiopia are shown below

| **Constituent group or parameter** | **Emission limit value(mg/L)** |
|---|---|
| pH | 6-9 pH units |
| BOD5 at 20$^o$C | 80 |
| COD | 250 |
| Total Kjeldhal Nitrogen (as N) | 80 |
| Nitrate (as N) | 20 |
| Total phosphate (as P) | 10 |
| Magnesium (as Mg) | 100 |
| Calcium (as Ca) | 100 |
| Chloride (as Cl) | 1000 |
| Sulphate ($SO_4$) | 1000 |
| Total Coliforms (Number per 100mL) | 400 |

**Source**: Standards for Industrial Pollution Control in Ethiopia (2003)

**Irrigation Water Standards**

While wastewater reuse for agriculture has many benefits, it should be carried out using good management practices to reduce negative human health impacts. The WHO initially published Guidelines for the Safe Use of Wastewater and Excreta in Agriculture and Aquaculture in 1989 and later revised it as “Guidelines for the safe use of wastewater, excreta and grey water, volume 2: wastewater use in agriculture” (WHO 2006). The Guidelines are set to minimize exposure to workers, crop handlers, field workers and consumers, and recommend treatment options to meet the guideline values (WHO, 2006). The Guidelines are focused on health-based targets and provide procedures to calculate the risks and related guideline values for wastewater reuse in agriculture [23].

WHO (1989) Guidelines for the safe use of wastewater in agriculture took into account all available epidemiological and microbiological data and are summarised in Table 2.4. The faecal coliform guideline (e.g. =1000 FC/100mL for food crops eaten raw) was intended to protect against risks from bacterial infections, and the newly introduced intestinal nematode egg guideline was intended to protect against helminth infections (and also serve as indicator organisms for all of the large settlable pathogens, including amoebic cysts). The exposed group that each guideline was intended to protect and the wastewater treatment expected to achieve the required microbiological guideline were clearly stated. Waste stabilisation ponds were advocated as being both effective at the removal of pathogens and the most cost effective treatment technology in many circumstances [27].

Table VII The 1989 WHO guidelines for the use of treated wastewater in agriculture

| Category | Reuse conditions | Exposed group | Intestinal nematodes[b] (arithemetic mean no. per 100mL[c]) | Feacal coliforms (geometric mean no. per 100mL[c]) | Wastewater treatment expected to achieve the required microbiologica quality |
|---|---|---|---|---|---|
| *A* | Irrigation of crops likely to be eaten uncooked, sports fields, public parks[d] | Workers, consumers, public | ≤1 | ≤1000[d] | A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment |
| *B* | Irrigation of cereal crops , industrial crops, fodder crops, pasture and trees[c] | Workers | ≤1 | No standard recommende | Retention in stabilization ponds for 8-10 days or equivalent helminth and faecal coliform removal |
| *C* | Localized irrigation of crops in category B if exposure of workers and the public does not occur | None | Not applicable | Not applicable | Pretreatment as required by the irrigation technology, but not less than primary sedimentation |

a. In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account and the guidelines modified accordingly.
b. Ascaris and Trichuris species and hookworms.
c. During the irrigation period.

d. A more stringent guideline ( 200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may cone into direct contact.

e. In the case of fruit trees, irrigation should cease two weeks before fruit is picket, and no fruit should be picked off the ground. Sprinkler irrigation should be used [27].

In Ethiopia, the standard for application of effluent to lands is given in Table 2.5.

Table VIII Controlled application of effluent to lands

| **Constituent group or parameter** | **Emission limit value(mg/L)** |
|---|---|
| pH | 5.5 - 9 pH units |
| $BOD_5$ at $20^oC$ | 500 |
| Chloride (as Cl) | 1000 |
| Sulphate ($SO_4$) | 1000 |

Source: Standards for Industrial pollution control in Ethiopia (2003)

# Declaration

I, the undersigned, declare that this thesis entitled "Study of Duckweed-Based Pond System for the Treatment of Domestic Wastewater**"** is my original work and has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been acknowledged.

Zekaryas Fanta_____
Name

____________________
Signature

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Date