PHYTOREMEDIATION OF INDUSTRIAL EFFLUENT FROM PAPER RECYCLING INDUSTRY USING *CERATOPHYLLUMDEMERSUM, LUDWIGIA ABYSSINICA* AND *HYDROLEA GLABRA* IN CONSTRUCTED WETLAND

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER DEGREE IN WATER RESOURCES AND ENVIRONMENTAL ENGINEERING

DEPARTMENT OF WATER RESOURCES AND ENVIRONMENTAL ENGINEERING,
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DECLARATION

I declare that the work in this thesis, entitled **"PHYTOREMEDIATION OF INDUSTRIAL EFFLUENT FROM PAPER RECYCLING INDUSTRY USING *CERATOPHYLLUM DEMERSUM, LUDWIGIA ABYSSINICA* AND *HYDROLEA GLABRA* IN CONSTRUCTED WETLAND**"has been carried out by me in the Department of Water Resources and Environmental Engineering, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Udeme Emmanuel JOHNSON ………………………. ……………………

Signature Date

CERTIFICATION

This dissertation entitled "PHYTOREMEDIATION OF INDUSTRIAL EFFLUENT FROM PAPER RECYCLING INDUSTRY USING *CERATOPHYLLUM DEMERSUM, LUDWIGIA ABYSSINICA* AND *HYDROLEA GLABRA*IN CONSTRUCTED WETLAND" by UDEME EMMANUEL JOHNSON meets the regulations governing the award of the degree of M.Sc. in Water Resources and Environmental Engineering of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Engr. Dr. S. B. Igboro……………….... ………………

Chairman, Supervisory Committee Signature Date

Engr. Prof. Charles U. Okuofu……………….... ………………

Member, Supervisory Committee Signature Date

Engr. Dr. M. A. Ajibike....………………. ……………...

Head of Department Signature Date

Prof. S. A. Abdullahi...……………… ……………… Dean, School of Postgraduate Studies Signature Date

DEDICATION

This dissertation is dedicated to God Almighty who has sustained me through my programme in A.B.U, Zaria, to my darling Mother, Mrs Gloria E. Johnson (JP) for her relentless support and to my Late Father, Elder Emmanuel E. Johnson, you will never be forgotten. May his gentle soul rest in peace, Amen.

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ABSTRACT

Paper and pulp mill produce large amount of wastewater containing different chemicals and various heavy metals causing water and soil pollution, which are also toxic to human. Various processes have been developed to reduce heavy metal pollution problem involving conventional technologies which have been quite expensive in comparison to wetlands. Three aquatic plants were planted in three identical laboratory scale vertical subsurface flow wetland (cells) of dimensions 0.40m x 0.20m x 0.20m in depth, length and width respectively. The base of the cells were filled with gravels of <20mm size to the depth of 0.10m, followed by sand of <0.5mm to the depth of 0.20m and then covered with gravels of <20mm size, with the fourth wetland void of plant which served as control. The Paladin factory wastewater was introduced into the cells from a storage tank and operated with retention time of 7, 14, 21 and 28 days using two operational methods (batch and continuous flow). The effluent samples were collected at the end of each retention time and analysed for Biological Oxygen Demand, Chemical Oxygen Demand, pH, Lead, Chromium, Manganese and Zinc. The results showed that *Ludwigia abyssinica* had the highest removal efficiency for both the batch and continuous flow with BOD, COD, Lead, Chromium, Manganese and Zinc having 85.1%, 81.5%, 72.8%, 90.7%, 93.3%, 92.1% and 82.0%, 77.8%, 69.4%, 64.6%, 80.4%, 57.5% respectively. *Hydrolea glabra* was also effective in its removal efficiency for the batch and continuous flow of BOD, COD, Lead, Chromium, Manganese and Zinc by 80.6%, 65.1%, 63.1%, 78.8%, 90%, 65.9%, and 76.1%, 58.7%, 54.9%, 52.1%, 53.3%, 65.9% as compared to *Ceratophyllum demersum* having 74.6%, 61.4%, 48.5%, 56.4%, 81.0%, 58.7%, and 65.7%, 57.7%, 38.8%, 35.6%, 31.2%, 52.5% respectively, which performed poorly. The control had 70.2%, 57.1%, 27.5%, 33.1%, 38.2%, 56.0% for the batch and 62.69%, 52.9%, 18.5%, 26.7%, 25.2%, 48.8% for the continuous flow, which indicated that substrate played a vital role in the removal efficiency. The values of pH for both the batch flow and continuous flow was between 6.03 to 6.11 showing that the constructed wetland has a high buffering capacity. The overall results show that the constructed vertical subsurface flow wetland using the three aquatic plants treated Paladin wastewater to WHO standards.

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ABBREVIATIONS

Abbreviations	Descriptions
BF	Batch Flow
BOD	Biological Oxygen Demand
C.D	Ceratophyllumdermersum
CF	Continuous Flow
COD	Chemical Oxygen Demand
H.G	Hydroleaglabra
HRT	Hydraulic Retention Time
HSSF	Horizontal Subsurface Flow
L.A	Ludwigiaabyssinica
PVC	Polyvinyl Chloride
SSF	Subsurface Flow
VSSF	Vertical Subsurface Flow

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Water in many areas of the world is polluted with toxic metals due to large-scale industrialization, population explosion and the production of a variety of chemical compounds have caused severe environment al pollution. Unlike the organic wastes, metals such as Arsenic, Selenium, Zinc, Chromium, Manganese, Lead, Mercury and Cadmium are non-biodegradable and need to be removed from the environment due to their effect on human health (Jing *et al*., 2015; Alluri *et al*., 2007).

Paladin is a paper recycling industry in Kaduna state with the mission of converting "Waste into Wealth" through waste paper recycling. It recycles waste into reusable paper product (tissue paper) which generate a considerable amount of wastewater that is eventually discharged into Romi stream in Southern Kaduna state. The effluent resulting from these activities are produced in an effort to improve human standard of living but ironically, their unplanned intrusion into the environment can reverse the same standard of living by impacting negatively on the environment (Nayyef *et al*., 2012; Asamudo *et al*., 2005; Xiaomei, 2004).

Phytoremediation is a technology that uses various plants to degrade, extract and immobilize contaminants from the environment specially soil and water (Xin *et al*., 2016; Prasad and Maiti, 2016; Ugya *etal*., 2015; Ajibade *et al*., 2013; Gupta *et al*., 2012; Akinbile and Yusoff, 2012; Kumar *et al*., 2012). It can be used for a wide range of organic (Cluis *et al*., 2004) and inorganic contaminants (Vamerali *et al*., 2010). It is also referred to as green technology and can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air (Gratao *et al*., 2005; Salt *et al*., 1998).

1.2 STATEMENT OF RESEARCH PROBLEM

To remove toxic metals, industries need to employ several physico-chemical processes, which often require high capital and recurring expenditure (Kapoor *et al*., 1999). The prevailing purification technologies used to remove the contaminants are costly and sometimes non-ecofriendly. Therefore, to overcome these problems of heavy metal and organic toxicants removal, environmental and public health engineers have been searching for an inexpensive and efficient technology (Verma *et al*., 2005).

1.3 JUSTIFICATION OF RESEARCH

Therefore, this research is oriented towards low cost and eco-friendly technology involving the use of plant species for controlling heavy metal pollution generated by paper recycling industry which will be beneficial to surrounding communities.

1.4 AIM AND OBJECTIVES OF RESEARCH

The aim of this research is to treat effluent resulting from the activities of Paper Recycling Industry, Kaduna using vertical sub-surface flow wetland as a method of heavy metals removal.

To achieve this aim, the objectives are

- To determine the quality of effluent discharged from Paper Recycling Industry.
- To design a vertical sub-surface flow wetland.
- To construct a vertical sub-surface flow wetland to treat Paper recycling effluent.

1.5 SCOPE OF STUDY

The study will involve the use of effluent resulting from the activities of paper recycling industry into Romi stream in Southern Kaduna and three locally sourced aquatic plants namely; *Hydrolea glabra* (False fiddleleaf), *Ludwigia abyssinica* (Primrose willow) and *Ceratophyllum* d*emersum*(Hornwort).

CHAPTER TWO

LITERATURE REVIEW

2.1 WATER POLLUTION

Pollution is the introduction of contaminants into the natural environment that cause adverse change. There are several hazardous effects that accompany pollutions as well as economic harm that can be caused by it.

Water pollution (either by natural means or as a result of human activities) has become a general issue that requires swift action. According to Mellem (2008), Many industries discharge their untreated wastewater and other industrial wastes containing various proportions of heavy metals depending on what the industry produces, into natural water systems and on lands with both aquatic and terrestrial habitats becoming progressively polluted due to the discharge of these generated industrial pollutants. As a result of ineffectiveness in the purification systems, the wastewater leads to the accumulation of toxic products in the receiving wastewater bodies with harmful consequences on the ecosystem (Aghalino and Eyinla, 2009).

Water pollution is one of the major threat to public health especially in developing and under developed countries as drinking water quality in these countries are poorly managed and monitored (Mwegoha, 2008; Azizullah *et al*., 2011). In general, water pollution has served impacts on the quality of fresh water and aquatic system with decreasing availability of safe and healthy drinking water due to pollution, in terms of quality and quantity which has been a major health concern in Africa and Nigeria in particular (Ugya *et al.,* 2016). Based on this, the EPA (US Environmental Protection Agency) has therefore, proposed the need for its removal from industrial effluents before finally releasing the industrial wastewater into the environment.

2.2 PAPER RECYCLING

Recycling of paper is a form of converting waste papers into reusable material which involves five basic steps carried out by variety of methods. Therefore, the final effluent is a combination of wastewater from each of the unit processes and the methods employed. These basic steps involved in paper recycling are;

2.2.1 Sourcing, Collection and Transportation: Recycling center collects and wraps in tight bales, the recovered paper from homes, offices, institutions and transports them to the paper mill.

2.2.2 Storage: The recovered papers are put into warehouses and sorted out. When the paper mill is ready to use the paper, forklifts move the paper from the warehouse to large conveyors.

2.2.3 Re-pulping and Screening: The paper moves by conveyor to a pulper which chops the recovered paper into small pieces. Heating by the impellers break the paper more quickly into fibers slurries. The pulp is forced through screens containing holes of different sizes and shapes. The screens remove small contaminants like pins, bits of plastics, glasses, coarse sand etc.

2.2.4 Deinking and Refining: Deinking is a process of pulp laundering to remove printed inks by washing process. During refining, the pulp is beaten to make recycled fibers swell, making them ideal for papermaking.

2.2.5 Papermaking: The pulp is mixed with water and chemicals to make it 99.5% water. This watery pulp enters the headbox and sprayed on a huge flat wire screen in the paper making machine. Drying is carried out by a heated cylinder, the sheet is passed round the cylinder and held in heated contact with the heated surfaces by means of dryer felt and the finished paper is wound into rolls and removed from the machine (Greentumble, 2018).

2.3 HEAVY METALS

Heavy metals are defined as metallic elements that have a relatively high density compared to water (Fergusson, 1990). With the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure (Duffus, 2002). Unlike other pollutants, they are difficult to degrade, but can accumulate throughout the food chain, producing potential human health risks and ecological disturbances (Akpor and Muchie, 2010).Although heavy metals are naturally occurring elements that are found throughout the earth's crust, most environmental contamination and human exposure result from anthropogenic activities such as mining and smelting operations, industrial production and use, domestic and agricultural use of metals and metal-containing compounds (He, *et al*., 2005; Goyer, 2001; Herawati *et al*., 2000; Shallari *et al*., 1998). Natural phenomena such as weathering and volcanic eruptions have also been reported to significantly contribute to heavy metal pollution (Fergusson, 1990; Bradl, 2002; He *et al*., 2005; Shallari, 1998; Nriagu, 1989). Industrial sources of heavy metals include metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, plastics, textiles, microelectronics, wood preservation and paper processing plants (Arruti, *et al*., 2010; Strater, 2010; Pacyna, 1996). However, their multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment.

Increased level of heavy metals in the environment causes some toxic effects in the biota such as the damaging of cells as well as the inhibition of enzyme activity (Jadia and Fulekar, 2009). The deadly effect of metallic toxins on health intensely depends on their absorption, distribution, excretion and metabolic rate in living organisms. These toxins can get into the human body through skin, lungs and the gastrointestinal tracts and once they get to the bloodstream, they may be carried to the target organs (such as the liver or kidney) or some may be deposited in bones or muscles. Mathias and Cummings (1973) ascertained that heavy metals have the ability to generate different health difficulties to humans by taking in the contaminated water or the edible aquatic resources. Some heavy metals are known to cause neurological disorder, kidney damage, gastro-enteritis, and high blood pressure. In addition, due to the non-biodegradable nature and the long biological half-lives required for their elimination from biological tissues, the presence of heavy metals in the biota has therefore, become a great concern (Olatunji *et al*., 2009). Generally, considering all the effects of these toxic substances it can be inferred that their presence in the environment presents more harm than good on the receiving ecosystem.

2.3.1 LEAD

Lead which is one of the oldest metals known to man is a naturally occurring bluish-grey metal present in small amounts in the earth's crust. Although lead occurs naturally in the environment, anthropogenic activities such as fossil fuel burning, mining, and manufacturing contribute to the release of high concentrations. Exposure tolead occurs mainly via inhalation of lead-contaminated dust particles or aerosols, and ingestion of lead-contaminated food, water and paints (ATSDR, 1999). Adults absorb 35 to 50% of lead through drinking water and the absorption rate for children may be greater than 50%. Lead absorption is influenced by factors such as age and physiological status. In the human body, the greatest percentage of lead is taken into the kidney, followed by the liver and the other soft tissues such as heart and brain, however, the lead in the skeleton represents the major body fraction (Flora *et al.*, 2006). The nervous system is the main vulnerable target of lead poisoning. Headache, poor attention span, irritability, loss of memory and dullness are the early symptoms of the effects of lead exposure on the central nervous system (CDC, 2001; ATSDR, 1999). Acute exposure to lead induces brain damage, kidney damage, and gastrointestinal diseases, while chronic exposure may cause adverse effects on the blood, central nervous system, blood pressure, kidneys, and vitamin D metabolism (ATSDR, 1999; U.S. EPA, 2002; Litvak *et al*., 1998; Hertz-Picciotto, 2000).

2.3.2 CHROMIUM

Chromium (Cr) is a naturally occurring element present in the earth's crust, with oxidation states (or valence states) ranging from chromium (II) to chromium (VI) (Jacobs *et al*., 2005).Chromium enters into various environmental matrices (air, water, and soil) from a wide variety of natural and anthropogenic sources with the largest release coming from industrial establishments. Chromium concentrations range between 1 and 3000 mg/kg in soil, 5 to 800 μg/L in sea water, and 26 μg/L to 5.2 mg/L inrivers and lakes. Occupational and environmental exposure to Cr(VI)-containing compounds is known to cause multi-organ toxicity such as renal damage,allergy and asthma, and cancer of the respiratory tract in humans (WHO, 1988; Goyer, 2001). Accidental or intentional ingestion ofextremely high doses of chromium (VI) compounds by humans has resulted in severe respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, and neurological effects leading to death or in patients who survived because of medical treatment (ATSDR, 2008).

2.3.3 MANGANESE

Manganese is an important metal for human health, essential for development, metabolisim, and the antioxidant system. Nevertheless, excessive exposure or intake may lead to a condition known as manganism, a neurodegenerative disorder that causes dopaminergic neuronal death and symptoms similar to Parkinson's disease (Avila *et al*., 2013). According to results from Bouchard *et al*., (2010), 'Higher levels of exposure to manganese in drinking water are associated with increased intellectual impairment and reduced intelligence quotients in school-age children'. However, human body can recover from certain adverse effects of over exposure to manganese when stopped, then the body can clear the excess (Devenyi *et al*., 1994).

2.3.4 ZINC

The most common element that is found in the earth's crust is zinc and is found in all three spheres of earth that is atmosphere, hydrosphere and lithosphere and we can say that zinc has shown its presence in the biosphere and is present in all foods. The main use of zinc is that it is used as anti-rusting agent that helps to prevent rust and corrosion which otherwise cause damage to steel and iron. Zinc is an essential nutrient needed by humans and animals. For normal protein, nucleic acid and membrane metabolism, zinc plays an important role. Zinc also plays an important role in growth and division of cells. Zinc deficiency may have an impact on carcinogenesis, though the direction of the influence seems to vary with the agent (Fong *et al*., 1978; Wallenius *et al*., 1979).Therefore, certain levels of zinc intake are recommended.

2.4 WASTEWATER TREATMENT

Wastewater has been posing serious environmental problems in cities, particularly in underdeveloped countries and its management and treatment technology needs to be suitable and sustainable (Ajibade *et al*., 2013; 2014). Wastewater treatment involves the process of removing contaminants from sewage. It involves physical, chemical, and biological processes to remove these contaminants and produce environmentally safer treated wastewater. The main aim of wastewater treatment is to remove as much of the contaminants as possible before the effluent is discharged back to the environment. Wastewater collected from domestic and other sources must ultimately be returned to receiving water or land. Hence, there is need to always determine which contaminants to be removed and to what extent should they be removed. Wastewater treatment involves three stages such as primary, secondary and tertiary treatment.

2.4.1 PRE-TREATMENT

Pre-treatment removes all materials that can be easily collected from raw sewage before they damage or clog the pumps and sewage lines of primary treatment clarifiers. Objects commonly removed during pre-treatment include trash, tree limbs, leaves, branches, and other large objects.

The influent in sewage water passes through a bar screen to remove all large objects like cans, rags, sticks and plastic packets, carried in the sewage stream. Pre-treatment may include a sand or grit channel or chamber, where the velocity of the incoming sewage is adjusted to allow the settlement of sand, grit, stones, and broken glass (Srivastava and Abbasi, 2013).

2.4.2 PRIMARY TREATMENT

When wastewater arrives at the treatment facility, it goes through the primary treatment process. The suspended solids from the wastewater are separated to some extent through a separation process achieved by screening and sedimentation. Primary treatment consists of temporarily holding the sewage in a quiescent basin where heavy solids can settle to the bottom while oil, grease and lighter solids float to the surface, the settled and floating materials are removed and the remaining liquid may be discharged or subjected to secondary treatment (Srivastava and Abbasi, 2013). The treatment unit used to settle raw wastewater is referred to as primary sedimentation tank. Sedimentation is the oldest and most widely used process in the effective treatment of wastewater (Shun, 2007).

2.4.3 SECONDARY TREATMENT

Secondary treatment is directed toward the removal of organic matter and residual suspended solids. Conventional secondary treatment is defined as a combination of processes customarily used for removal of these constituents which includes biological treatment by activated sludge, fixed-film reactors, or lagoon systems and sedimentation (Metcalf and Eddy, 1991). Secondary treatment is typically performed by indigenous, waste-borne micro-organisms in a managed habitat and may require a separation process to remove the micro-organisms from the treated water prior to discharge or tertiary treatment. At this stage, most of the biodegradable organic matter is removed but the non-biodegradable or refractory organic material or may be some end products of biological decomposition. After secondary treatment, effluent usually has little BOD_5 and a low suspended solids value (Sriu and Pydi, 2011; Tilley, 2011).

2.4.4 ADVANCED WASTEWATER TREATMENT

Advanced wastewater treatment which is also known as tertiary treatment is adopted for achieving better water quality with the aim of removing toxic and trace elements, reducing health effects and environmental degradation. In this present century, it is highly essential to develop suitable, inexpensive and rapid wastewater treatment techniques which would enable water reuse and reduce stress on natural sources. Advanced wastewater treatment systems like microfiltration, ultrafiltration, reverse osmosis, activated carbon adsorption, and sand filters are being employed. Advanced oxidation processes (AOP) which uses combinations of ozone, ultraviolet (UV) light, and hydrogen peroxide that generate highly reactive hydroxyl radical (OH), is another technology for water treatment which ensures the treatment of wastewater that are toxic or resistant to biological treatment (Sarria *et al*., 2004; García-Montaño *et al*., 2006). For the treatment of wastewater, none of the technologies alone can be used or is efficient for exclusion of all pollutants to produce treated effluent that would meet the standards. A novel and hybrid waste water treatment technology using conventional treatment methods along with advanced wastewater treatment methods appear to be the best. The innovation of advanced technologies for waste water management is highly essential and phytoremediation with special reference to industrial wastewater treatment is novel treatment employed for treatment of domestic wastewater, conservation of soil, sediment and water that would explore in future (Lakshmi *et al*., 2017).

2.5 PHYTOREMEDIATION

The term 'Phytoremediation' consists of two words: Phyto derived from the Greek means - plant and remedium derived from Latin means able to cure or restore (Vamerali *et al*., 2010). Phytoremediation can be defined as the process which uses green plants for the release, transfer, stabilization or degradation of pollutants from soil (Elekes *et al*., 2014; USEPA. 2001; Paz- ferreiro *et al*., 2014).

The advantage of phytoremediation is its capability to clean soils that are contaminated with heavy metals, as well as to produce bioenergy. The harvested biomass crops can be converted into bioenergy by using different energy recovery techniques (anaerobic digestion, incineration, gasification and production of biodiesel) (Ginneken *et al*., 2007). Several plant roots can absorb and immobilize metal pollutants, whereas other plant species have the ability of metabolizing or accumulating organic contaminants. Phytoremediation processes are most effective wherever contaminants are present at low to medium levels, as high contaminant levels can reduce plant and microbial growth and activity (USEPA, 2010). Diverse relationships and relations between plants, microbes, soils and contaminants make phytoremediation processes possible.

2.6 MECHANISMS OF PHYTOREMEDIATION

Utilizing the ability of certain trees, shrubs, and grass species to remove, degrade, or immobilize harmful chemicals can reduce risk from contaminated soil, sludges, sediments, and ground water through contaminant removal, degradation, or containment (Zavoda *et al*., 2001). In general, phytoextraction and phytovolatization are considered as the main options for the removal of heavy metals and other elemental compounds, whereas phytodegradation and phytostabilisation are applied mostly to organic contaminants (Meagher, 2000; Guerinot and Salt, 2001). Phytoremediation offers a cost-effective, nonintrusive, and safe alternative to conventional cleanup techniques (Glick, 2003). Phytoremediation can be accomplished by phytoextraction, phytodegradation, phytostabilization, phytovolatization and rhizofiltration.

2.6.1 PHYTOEXTRACTION

This method of phytoremediation involves the uptake of contaminants through the roots, with the contaminant being translocated to the aerial portions of the plant (Gleba *et al*., 1999). After a period of growth, the plant is harvested thereby removing the contaminant from the soil (Cluis, 2004). Plant roots generally contain higher metal concentrations than the shoots despite the translocation mechanisms, but an upper limit to the metal concentration within the root can occur. As a plant-based technology, the success of phytoextraction is inherently dependent upon several plant characteristics. The two most important characteristics include the ability to accumulate large quantities of biomass rapidly and the ability to accumulate large quantities of environmentally important metals in the shoot tissue (Cunningham and Ow, 1996; Deepa *et al*., 2006). Phytoextraction should be viewed as a long-term remediation effort, requiring many cropping cycles to reduce metal concentrations (Kumar *et al*., 1995) to WHO standard. The time required for remediation is dependent on the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years. This technology is suitable for the remediation of large areas of land that are contaminated at shallow depths with low to moderate levels of metal- contaminants (Kumar *et al*., 1995; Blaylock *et al*., 1997).

2.6.2 PHYTODEGRADATION

The plant takes up the contaminant through its roots from where the contaminant is translocated to the aerial portions of the plant. Phytodegradation is also known as phytotransformation, and is a contaminant destruction process. Plant-produced enzymes metabolize contaminants which may be released into the rhizosphere, where they can remain active (Singh *et al*., 2003). When the phytodegradation mechanism is at work, contaminants are broken down after they have been taken up by the plant. As with phytoextraction and phytovolatilization, plant uptake generally occurs only when the contaminants' solubility and hydrophobicity fall into a certain acceptable range. Phytodegradation has been observed to remediate some organic contaminants, such as chlorinated solvents, herbicides, and munitions, and it can address contaminants in soil, sediment, or groundwater (EPA, 2000).

2.6.3 PHYTOSTABILISATION

Phytostabilization (also called phytorestoration) is a plant-based remediation technique that is aimed at reducing the risk of metal pollutants by stabilizing them through formation of a vegetative cap at the plant rhizosphere, where sequestration (binding and sorption) processes immobilize metals so as to make them unavailable for livestock, wildlife and human exposure (Munshower, 1994; Cunningham *et al*., 1995; Wong, 2003). Contrasting other phytoremediative techniques, the aim of phytostabilization is not to remove metal contaminants from a site, but moderately to stabilize them and decrease the risk to human health and the environment. Being less expensive, less environmentally evasive and simple to execute, phytostabilization is considered to be more beneficial than other soil-remediation practices (Berti and Cunningham, 2000). Advantages related with this technology are that the occurrence of plants reduces soil erosion and decreases the amount of water available in the system (USEPA, 2000). Phytostabilization has been used to treat contaminated land areas affected by mining activities and superfund sites. Normally, the role of plants in this technique is to lessen the amount of water percolating through the soil matrix that will finally lead to the formation of toxicants (hazardous leachates) and avoid soil erosion and transport of toxic metals to distinctive areas. The plants selected for this technology should follow certain criteria: (1) effectiveness of translocation of heavy metals from root-to-shoot system should be low; (2) they must have a fast growth rate and resistance to heavy metals; and (3) there should be cost-effective management. Basically, this technique is not only applicable at sites with high organic load and porosity but is also efficient for a wide range of surface contamination sites (Berti and Cunningham, 2000). One of the drawbacks of this technique is that it is not applicable to those areas which are heavily contaminated because such conditions become an obstacle in plant growth and development (Berti and Cunningham, 2000).

2.6.4 PHYTOVOLATIZATION

Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere

(USEPA, 2000). Phytovolatization is primarily a contaminant removal process, transferring the contaminant from the original medium (ground water or soil water) to the atmosphere. However, metabolic processes within the plant might alter the form of the contaminant, and in some cases transform it to less toxic forms. Unlike other remediation techniques, once contaminants have been removed via volatization, there is a loss of control over their migration to other areas (Whitacre, 2010).

2.6.5 RHIZOFILTRATION

Rhizofiltration is a phytoremediative technique designed for the removal of metal contaminants from aquatic environments. The process involves the growth of plants in metal polluted waters where the plant absorbs and concentrates the metals in roots and shoots (Zhu *et al*., 1999; Dushenkov *et al*., 1995). Changes in the rhizosphere pH and root exudates also contribute to the precipitation of metals onto the root surface (Prasad and de Oliveira Freitas, 2003). As the plant becomes saturated with the metal contaminants either the roots or the whole plants are harvested for disposal. Rhizofiltration is a cost-competitive technology for the treatment of surface water or groundwater containing low, but significant concentrations of heavy metals such as Cr, Pb, and Zn (Kumar *et al*., 1995). The commercialization of this technology is driven by economics, applicability to many problem metals, ability to treat high volumes, lesser need for toxic chemicals, reduced volume of secondary waste, possibility of recycling, and the likelihood of regulatory and public acceptance (Kumar *et al*., 1995; Dushenkov *et al*., 1995). However, the application of this plant-based technology may be more challenging and susceptible to failure than other methods of similar cost. The production of hydroponically grown transplants and the maintenance of successful hydroponic systems in the field requires the expertise of qualified personnel, and the facilities and specialized equipment required can increase overhead costs (Pravaiz et al., 2011).

2.7 WETLANDS

A wetland is a distinct ecosystem that is inundated by water, either permanently or seasonally, where oxygen-free processes prevail (Keddy, 2010). The primary factor that distinguishes wetland from other land forms or water bodies is the characteristics vegetation of the aquatic plants (Butler, 2010) adapted to the unique hydric soil. Wetlands play a number of roles in the environment, principally water purification, flood control, carbon sink and shoreline stability. Wetlands are also considered the most biologically diverse of all ecosystems, serving as home to a wide range of plants and animal life. Wetlands have unique characteristics: they are generally distinguished from other water bodies or landforms based on their water level and on the types of plants that live within them. Specifically, wetlands are characterized as having a water table that stands at or near the land surface for a long enough period each year to support aquatic plants. Wetlands occur naturally on every continent except Antarctica (Davidson, 2014), the largest including the Amazon River basin, the West Siberian Plain (Fraser and Keddy, 2005), and the Pantanal in South America and the Sundarbans in the Ganges- Brahmaputra delta (Giri *et al.,* 2007). In Nigeria, we have various wetlands such as Hadejia-Nguru wetlands, Jebba wetlands, Lower Kaduna wetlands, Lokoja wetlands and Makurdi wetlands (Elegbede, 2014).

2.7.1 CLASSIFICATION OF WETLANDS

The most common feature of all wetlands is that the water table (the groundwater level) is very near to the soil surface or shallow water covers the surface for at least part of the year (Ramey, 2001). The main characteristics of a wetland are determined by the combination of the salinity of the water in the wetland, the soil type and the plants and animals living in the wetland (Ramey, 2001). Because of the high variability of the conditions, and because of the different needs for distinguishing among different types of wetlands, there is no single wetlands classification system that would account for the manifold aspects of this specific ecosystem type. There are four main kinds of wetlands;

- **Marsh**: This is a type of wetland that is dominated by herbaceous rather than woody plant species (Keddy, 2010). Marshes can often be found at the edges of lakes and streams, where they form a transition between the aquatic and terrestrial ecosystems. They are often dominated by grasses, rushes or reeds (Plate III). If woody plants are present they tend to be low-growing shrubs. This form of vegetation is what differentiates marshes from other types of wetlands such as swamps, which are dominated by trees, and mires, which are wetlands that have accumulated deposits of acidic peat (Rafferty, 2011).

Plate III: Marsh (http://en.m.wikipedia.org>wiki)

- **Swamp**: A swamp is a wetland that is forested (Keddy, 2010). Many swamps occur along large rivers where they are critically dependent upon natural water level fluctuations (Hughes, 2003). Other swamps occur on the shores of large lakes (Wilcox *et al*., 2007). Some swamps have hammocks, or dry-land protrusions, covered by aquatic vegetation, or vegetation that tolerates periodic inundation (Plate 1V). The two main types of swamp are "true or swamp forests and "transitional" or shrub swamps. The water of a swamp may be fresh water, brackish water or seawater. Some of the world's largest swamps are found along major rivers such as the Amazon, the Mississippi, and the Congo (Keddy *et al*., 2009).

Plate 1V: Swamp (http://en.m.wikipedia.org>wiki)

Bog: A type of wetland that accumulates peat, a deposit of dead plant material (often mosses), and in a majority of cases, sphagnum moss (Keddy, 2010). They are frequently covered in ericaceous shrubs rooted in the sphagnum moss and peat. The gradual accumulation of decayed plant material in a bog functions as a carbon sink.

Bogs occur where the water at the ground surface is acidic and low in nutrients. In some cases, the water is derived entirely from precipitation, in which case they are termed ombrotrophic (rain-fed). Water flowing out of bogs has a characteristic brown colour, which comes from dissolved peat tannins (Plate V). In general, the low fertility and cool climate results in relatively slow plant growth, but decay is even slower owing to the saturated soil. Hence, peat accumulates and large areas of landscape can be covered many metres deep in peat (Keddy, 2010; Gorham, 1957).

Plate V: Bog (https://en.m.wikipedia.org>wiki)

iv. **Fen**: Fens are minerotrophic peatlands (Rydin *et al*., 2013) usually fed by mineral-rich surface water or groundwater (Godwin *et al*., 2002). They are characterized by their distinct water chemistry, which is pH neutral or alkaline, with relatively high dissolved mineral levels but few other plant nutrients. Fens frequently have a high diversity of other plant species including carnivorous plants such as Pinguicula (Wheeler and Giller, 1982; Keddy, 2010). They may also occur along large lakes and rivers (Plate VI) where seasonal changes in water level maintain wet soils with few woody plants (Charlton and Hilts, 1989). The distribution of individual species of fen plants is often closely connected to water regimes and nutrient concentrations (Slack *et al*., 1980; Schroder *et al*., 2005).

Plate VI: Fens (http//:en.m.wikipedia.org>wiki)

2.7.2 WETLAND HYDROLOGY

Wetland hydrology is associated with the spatial and temporal dispersion, flow, and physio-chemical attributes of surface and ground water in its reservoirs. Based on hydrology, wetlands can be categorized as riverine (associated with streams), lacustrine (associated with lakes and reservoirs), and palustrine (Ghosh, 2016). Sources of hydrological flows into wetlands are predominantly precipitation, surface water, and ground water. Water flows out of wetlands by evapotranspiration, surface runoff, and sub-surface water outflow. Hydrodynamics (the movement of water through and from a wetland) affects hydro-periods (temporal fluctuations in water levels) by controlling the water balance and water storage within a wetland (Richardson *et al*., 2001). Water chemistry of wetlands varies across landscapes and climatic regions, which is determined by the pH, salinity, nutrients, conductivity, soil composition, hardness, and the sources of water.

2.8 CONSTRUCTED WETLANDS

Constructed wetlands (CWs) are engineered ecosystems designed by humans to remove pollutants from water and wastewater treatment, that resemble hydraulic conditions and habitat occurring in the swamp. Constructed or artificial wetland systems mimic the treatment that occurs in natural wetlands by relying on heterotrophic microorganisms and aquatic plants and a combination of naturally occurring biological, chemical and physical processes.

Constructed wetlands have been used for wastewater treatment for nearly 60 years, the first CW were put in operation in Israel in 1950s (Ignjatovic and Marjanovic, 1985). In Europe first experiments aimed at the possibility of wastewater treatment by wetland plants were undertaken in Germany (Davies *et al*., 2005). CWs are used for treating various wastewater types such as domestic wastewater, acid mine drainage, agricultural wastewaters, landfill leachate, urban storm-water and industrial wastewater including paper and pulp, food processing, petrochemical, chemical, textile and tannery. The efficiency of wetlands to remove the contaminants from the wastewater mainly depends on the root zone interactions between soil, contaminants, plant roots and a variety of microorganisms (Dorota and Krzyszof, 2012).

2.8.1. SURFACE FLOW CONSTRUCTED WETLANDS (SFCW):

A surface flow (SF) wetland consists of a shallow basin, soil or other medium to support the roots of vegetation, and a water control structure that maintains a shallow depth of water. The water surface is above the substrate. SF wetlands look much like natural marshes and can provide wildlife habitat and aesthetic benefits as well as water treatment. In SF wetlands, the near surface layer is aerobic while the deeper water and substrate are usually anaerobic. Storm water wetlands and wetlands built to treat mine drainage and agricultural runoff are usually SF wetlands. SF wetlands are sometimes called free water surface wetlands or, if they are for mine drainage, aerobic wetlands. The advantages of SF wetlands are that their capital and operating costs are low, and that their construction, operation, and maintenance are straightforward. The main disadvantage of SF systems is that they generally require a larger land area than other systems (Davis, 2003).

2.8.2. SUBSURFACE FLOW WETLANDS (SSF):

A subsurface flow (SSF) wetland consists of a sealed basin with a porous substrate of rock or gravel. The water level is designed to remain below the top of the substrate. In most of the systems in the United States, the flow path is horizontal, although some European systems use vertical flow paths. SSF systems are called by several names, including vegetated submerged bed, root zone method, microbial rock reed filter, and plant-rock filter systems. Because of the hydraulic constraints imposed by the substrate, SSF wetlands are best suited to wastewaters with relatively low solids concentrations and under relatively uniform flow conditions. SSF wetlands have most frequently been used to reduce 5-day biochemical oxygen demand (BOD_5) from domestic wastewaters. The advantages cited for SSF wetlands are low temperature, minimization of pest and odor problems, and, possibly, greater assimilation potential per unit of land area than in SF systems. Porous medium provides greater surface area for treatment contact than is found in SF wetlands, so that the treatment responses should be faster for SSF wetlands which can, therefore, be smaller than a SF system designed for the same volume of wastewater. Since the water surface is not exposed, public access problems are minimal (Davis, 2003).

A vertical flow constructed wetland is a planted filter bed that is drained at the bottom. The water flows vertically down through the filter matrix to the bottom of the basin where it is collected in a drainage pipe whereas the horizontal flow is a gravel and sand filled basin that is drained at the side. The water flows horizontally through the basin as the filter materials filters out particles where it is collected in the drainage pipe. The significant advantages of vertical subsurface flow over Horizontal Subsurface flow are the ability to nitrify due to good oxygen transfer (aerobic conditions), high reduction of BOD, suspended solids and pathogens, it requires less space area because of the higher substrate aeration efficiency and less clogging than in a Horizontal Subsurface flow constructed wetland (Davis, 2003).

2.8.3. HYBRID SYSTEMS

The main two types are: Free water surface wetlands (FWS) and Subsurface flow (SF) wetlands. The system requires that all of the removal processes occur in the same space. In hybrid or multistage systems, different cells are designed for different types of reactions. A combined system also produces polished final effluent that are environmental friendly (Adeogun *et al.*, 2014).

2.9 DESIGN OF ENGINEERED WETLANDS

The performance of many systems monitored has varied due to influences of the diverse factors that affect the performance, such as location, type of wastewater, wetland design, climate, weather, disturbance, and daily or seasonal variability. However, Mitsch (1992), suggested guidelines for creating successful constructed wetlands which include keeping the design simple with minimal maintenance, using natural energies, designing the wetland with the landscape and for the extremes of weather and climate, mimicking the natural systems, and giving the system time because wetlands do not necessarily become functional overnight.

2.9.1 WETLAND GEOMETRY

Reed *et al*., (1995) proposed that the flow depth of constructed wetlands should be in the range of 0.1 to 0.6m. Kadlec and Knight (1996) suggested that the aspect ratio L:W should be greater than 2:1 to ensure plug flow conditions. However, very high ratios may result in overflow problems due to resistance increase as a result of the gradual accumulation of vegetation litter.

2.9.2 FIRST-ORDER KINETICS

The principle of the design of Constructed wetlands subsurface flow is based on an assumption of plug flow movement of water through the wetland with first-order reaction kinetics primarily by biological degradation. As an attached biological degradation reactor involving microbes, modeling CWs typically combines biological degradation and system hydraulics. The various design models are principally derived from the basic plug-flow equation. A basic plug flow equation is expressed mathematically in Equation 2.1.

$$\frac{Ce}{Ci} == e^{-KTt} \qquad (2.1)$$

Where,

C_i = influent concentration of BOD concentration (mg/L),

C_e = effluent concentration of BOD concentration (mg/L),

K_T = reaction rate constant (d^{-1}),

t = Hydraulic Retention Time (HRT), (d^{-1}).

The BOD_5 concentration of effluent represented in Table 2.1 is determined by wastewater effluent discharge standards into the surface water bodies and it is dependent on environmental standards and guidelines of a country. These effluent standards of a country influence the design of the wetland systems and some parameters recommended by the World Health Organisation (WHO) are presented in Table 2.2.

Table 2.1 Typical performance of wetlands (representing C_e)

Parameter	Effluent value
BOD	<20mg/L
TN	<10mg/L
TP	<5mg/L
Fecal coliform	1000/100ml

Source: Crites and Tchobanoglous (1998)

Table 2.2 Wastewater Discharge Guidelines

Parameters	Unit	Value
pH	-	6.0 – 9.0
BOD	mg/L	50
COD	mg/L	100
Lead	mg/L	0.05
Chromium	mg/L	0.1
Manganese	mg/L	0.5
Zinc	mg/L	5

Source: WHO (2012)

2.9.3 REACTION RATE CONSTANT

The determination of the BOD rate constant is important for understanding the nature of wastewater. In cases of discharging wastewater into streams, the rate constant would help in predicting the impacts of the wastewater on the life of the stream, the dissolved oxygen values in the stream and the BOD values in the stream (Abdelrasoul, 2001).

The temperature dependence of reaction rate constant in Equation 2.1 is derived from the Van't Hoff- Arrhenius relationship;

$$K_T=K_{20}(\Theta)^{T-20} \quad (2.2)$$

Where,

K_{20} = Rate constant at 20^{o}C (d^{-1}),

Θ = temperature coefficient.

Θ has a value of '1.056' in the temperature range between 20 and 30^{o}C to '1.135' in the temperature range between 4 and 20^{o}C (Tchobanoglous *et al.*, 2003). The value of 1.06 has been used by the US Environmental Protection Agency (EPA) for the design of wetlands. Therefore,

$$K_T=K_{20}(1.06)^{T-20} \quad (2.3)$$

2.9.4 RETENTION TIME (DAYS)

The retention time also known as Hydraulic Retention Time (HRT) can be expressed from Equation 2.1 as;

$$\ln(C_e/C_i)=-K_T t \quad (2.4)$$

t = time (days)

K_T = Rate constant

2.9.5 ORGANIC LOADING RATE

Reed *et al* (1995) also investigated constructed wetlands in the United States and concluded that the organic loading should not exceed $10gm^{-2}d^{-1}$.

2.10 AQUATIC PLANTS USED FOR PHYTOREMEDIATION

The presence of macrophytes is one of the most conspicuous features of constructed wetlands and their presence distinguishes constructed wetlands from unplanted soil filters or lagoons (Greenway, 2007: Vymazal, 2011). Their positive role on the performance of constructed wetlands has been well established in numerous studies measuring treatment with or without plants (Yang *et al*., 2007; Brisson and Chazarenc, 2009; Kadlec and Wallace, 2009). Generally, the performance of wetlands for wastewater treatment depends on the growth potential and ability of macrophytes to develop sufficient root systems for microbial attachment of micro-organisms present in the wastewater and material transformation, while there is a recognition that the improvement of water quality in treatment wetland applications is primarily due to microbial activity (Faulwetter *et al*, 2009; Kadlec and Wallace, 2009). Numerous studies have confirmed that water treatment is improved in vegetated systems compared to systems containing no plants. More so, the uptake and accumulation of pollutants vary from plant to plant and also from species to species within a genus (Singh *et al*., 2003), with the economic success of phytoremediation largely depending on photosynthetic activity and growth rate of plants (Xia and Ma, 2006).

Researchers like Priyanka *et al*., (2017); Tripathi and Shukla, (1991) studied *Eichornia crassipes* (water hyacinth) for the removal of Chromium and BOD respectively. Ugya, (2015) researched on *lemna minor* (duck weed) for the removal of Manganese, Sivastav *et al*., (2007) studied the efficiency of *Spirodela* and *Salvinia* in the removal of Zinc.

Vymazal, (1996) and Crosley, (2012) also studied the behavior of aquatic plants for COD and pH respectively. Also, different plant species like; *Typhalatifolia* (Broadleaf Cattail), *PhragmitesAustralis* (Common reed) and *PistiaStratiotes* (Water cabbage), *Acoruscalamus* (Sweet Flag) have been used for treatment of different types of contaminated water and effluent.

- ***Ludwigiaabyssinica* (Primrose willow)**

*Ludwigiaabyssinica*is an erect or strangling, annual, or occasionally perennial plant that can grow to 60 -300cm tall. The stout stem is well-branched, sometimes becoming more or less woody at the base. Leaves are lanceolate or elliptic, 2-13 cm long, hairless except for some minute hairs on midrib when very young; Petiole 2-20mm long. Flowers in clusters on shoot, axillary, small-leaved shoots, yellow. Capsule 10-20 mm long, light brown, finely pitted when young, becoming smooth, hairless. This plant is not completely aquatic but in river banks, swampl situations and lake sides, widespread in Senegal to North and South Nigeria, South Africa and Madagascar. The cooked leaves and stems provide a black liquid that is used for dyeing straw and fibres to prevent abortions, abdominal pain and for dressing wounds. Products are used for tattoos, ink stains, superstitions and magic (Grubben and Denton, 2004; Akobundu and Agyakwa, 1998; Johnson, D. E., 1997; Oziegbe and Taiwo, 2011).

Table 2.3 Plant Classification of Ludwigia abyssinica

Kingdom	Plantae
Clade	Angiosperms
Order	Myrtales
Family	Onagraceae
Genus	*Ludwigia*
Species	L. abyssinica L.

Source:Ludwigia (https://en.m.wikipedia.org>wiki)

Plate.VII: Picture of*Ludwigiaabyssinica* (https://en.m.wikipedia.org>wiki)

- ***Hydroleaglabra* (False fiddleleaf)**

Description: *Hydroleaglabra* is an erect annual broadleaf herb, about 75 cm high. The stem is cylindrical, thick and spongy, completely hairless, the leaves are alternate simple, narrow and long. They are regularly arranged throughout the stem. The flowers are blue and clustered in the leaves along the stem, they are found in Nigeria, Mali, Ghana, Burkina Faso and Benin. In Sierra-leone, the Mendes grind up the leaves to rub on babies suffering headache (Hutchinson *et al*., 1963; Johnson, D.E., 1997; Akobundu and Agyakwa, 1998).

Table 2.4 Plant Classification of *Hydrolea glabra*

Kingdom	Plantae
Clade	Angiosperms
Order	Solanales
Family	Hydrleaceae
Genus	*Hydrolea*
Species	H. glabra L.

Source:Hydrolea (https://en.m.wikipedia.org>wiki)

Plate VIII: Picture of*Hydrolea* (https://en.m.wikipedia.org>wiki)

- ***Ceratophyllumdemersum* (Hornwort)**

Ceratophyllumdemersum commonly known as hornwort,' is a species of *Ceratophyllum*. It is a submerged, free floating aquatic flowering plant with a cosmopolitan distribution. *Ceratophyllumdemersum* has stems that reaches lengths of 100-300cm with numerous side shoots making a single specimen appear as a large, bushy mass and grows in still or very slow moving water. The leaves are produced in whorls of six to twelve and each leaf is 0.8-4 cm long. It is propagated by cuttings and is frequently used as a model organism for studies of plant physiology (Hiscock, 2003). this species is often used as a floating freshwater plant in both cold water and tropical aquaria.

Table 2.5 Plant Classification of *Ceratophyllum demersum*

Kingdom	Plantae
Clade	Angiosperms
Order	Ceratophyllales
Family	Ceratophyllaceae
Genus	*Ceratophyllum*
Species	C. demersum *L.*

Source: Ceratophyllum (https://en.m.wikipedia.org>wiki)

Plate IX: Picture of *Ceratophyllum*(https://en.m.wikipedia.org>wiki).

2.11 REMOVAL PROCESS BY AQUATIC PLANTS

Most common heavy metals in water are in the form of positive ions. One possible basic way to remove these ions is to place a negatively charged material into the water which would attract the positively charged ions. The roots of many plants have a negative charge, which acts as a magnet to the positively charged ions. Even the dried and dead roots still have the negative charge, and this would be strong enough to attract the positive ions of heavy metals (Beddri and Zubaidah, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

The materials used in this research for the construction of a laboratory–scale Vertical Subsurface Flow (VSSF) wetland system include:

- Glass sheet for construction of wetland of 5mm thick.
- Washed gravel of different sizes (<20mm) for the inlet and outlet zones.
- Gate valves of 20mm for control of wastewater flow through the wetland.
- Fine sand of sizes (<0.5mm) as the substrate.
- Plastic taps of 20 mm size fitted at the outlet zones.
- Polyvinyl chloride (PVC) pipes of size 20mm to connect the various cells of the wetland.
- 2 Plastic bucket of 50 litres storage capacity as storage and sedimentation tank.
- 4 Jerry cans of 25 litres were used to convey the water from the Paper Recycling Industry, Kaduna.
- *Hydroleaglabra* (False fiddleleaf), *Ludwigiaabyssinica* (Primrose willow) and *Ceratophyllumdemersum* (Hornwort).

3.1.1 Apparatus, Equipment and Reagents

Table 3.1 gives a list of Apparatus and Equipment used for this research work and their specifications including manufacturers, while Table 3.2 shows the Reagents used for laboratory analysis.

Table 3.1 Apparatus and Equipment used in research

S/N	APPARATUS	SPECIFICATION	MANUFACTURER
1	Filter paper	Grade 6	Whatman England
2	Beaker	250ml	Labplex England
3	BOD bottles	250ml	Wheaton USA
4	Conical flasks	250ml	Pyrex England
5	Retort stand and clamp	Rectangular	
6	Measuring cylinder	1000cm	Kinax USA
7	Volumetric flask	250ml	Pyrex England
8	Conical flask	250ml	Pyrex England
9	Glass rod	5mm diameter	Pyrex England
10	pH meter	model pHS-25	Rex-China
11	Reflux flasks	25ml	Gallenkamp England
12	Pipettes	10ml	Pyrex England
13	Burette	50ml	Pyrex England
14	Incubator	_	Raven Britain
15	Petri dishes	60mm	Pyrex England

Table 3.2: Reagents used in Laboratory analysis

S/N	REAGENTS	MANUFACTURER/SOURCE
1	Hydrochloric acid	AnalaR, BDH Chemicals Ltd, England
2	Mercuric Sulphate	AnalaR, BDH Chemicals Ltd, England
3	Starch indicator	May and Baker
4	Alkali-iodide azide	AnalaR, BDH Chemicals Ltd, England
5	Sodium thio-sulphate	May and Baker
6	Potassium dichromate	AnalaR, BDH Chemicals Ltd, England
7	Silver sulphate	AnalaR, BDH Chemicals Ltd, England
8	Nitric acid	AnalaR, BDH Chemicals Ltd, England
9	Distilled water	-
10	Manganese sulphate	AnalaR, BDH Chemicals Ltd, England
11	Sulphuric acid	AnalaR, BDH Chemicals Ltd, England
12	Ferroin indicator	May and Baker

3.2 Methods

3.2.1 Sources of data

The main source of data were the results of laboratory tests carried on wastewater samples collected before and after passing through the constructed wetland cells.

3.2.2 Sampling of wastewater

The wastewater samples were collected from the discharge point of the paper recycling industry in Southern Kaduna. Grab samples of the raw wastewater obtained from the outlet point were taken with the aid of four jerry cans of 25litres capacity. The wastewater influent was analysed and then poured into the storage tank and allowed to settle for two weeks before passing it through the various wetlands of both the batch and continuous flow operational process. The experiment was monitored with the acclimatization of the plants throughout the retention period of 7, 14, 21, 28 days and effluent collected via the respective taps containing each aquatic plants namely: *Hydroleaglabra* (False fiddleleaf), *Ludwigiaabyssinica* (Primrose willow) and *Ceratophyllumdemersum* (Hornwort) using clean bottles. The samples were taken to the Multi-User Science Laboratory Research (MUSLR) and Water Resources and Environmental Engineering (WREE) Laboratory for analysis.

3.2.3 Design of the Constructed Wetland

Wetland geometry

In calculating the size of the constructed wetland cell, the following parameters were determined:

- The flow depth of the wetland cell = 0.40m (Reed et al., 1995)
- The aspect ratio of L:W used for the geometry design equals 2:2, avoidance of high ratio to prevent overflow problem.

  L X W =Area; 0.20m x 0.20m = 0.04m^2

Calculation of reaction rate constant for BOD at appropriate temperature

The reaction rate constant K_T(day^{-1}) for BOD at appropriate temperature was calculated from the Van't Hoff- Arrhenius relationship (Equation 2.2)

For $K_T = k_{20} (1.06)^{23-20}$

$= 0.678 (1.06)^{23-20}$

$= 0.808$/day

Calculation of reaction rate constant (K_{BOD})

$K_{BOD} = K_T d\eta$

(3.1)

Where Kt = Reaction rate constant

d = Depth of water column (m)

η = Porosity of the substrate medium (Bousfield, 2001)

K_{BOD} = 0.808 x 0.40 x 0.35

K_{BOD} = 0.113m/day

Calculation of retention time (HRT)

Reed *et al*., (1995) stated that BOD retention time can be computed using Equation 2.1

Ce= 20mg/L (Crites and Tchobanoglous (1998)

Ci = 67mg/L (BOD sample)

$\frac{Ce}{Ci} = e^{-0.808t}$

$\ln\frac{Ce}{Ci} = -0.808t$

t = 1.50 days

Organic loading rate

$Lorg = \frac{Cd_w\eta}{t}$

Where C = influent BOD concentration, mg/L

d_w= wetland depth, m

η = porosity (EPA, 1993)

$L_{org} \leq 10gm^{-2}d^{-1}$ (Reed et al., 1995)

$L_{org} = 67 X 0.40 X 0.35/1.50$

$L_{org} = 6.25 gm^{-2}d^{-1}$

$6.25 \leq 10gm^{-2}d^{-1}$(design is ok)

3.2.4 Experimental Set Up of Vertical Sub-Surface Constructed Wetlands(VSSF)

Thelaboratory- scale vertical flow constructed wetlands were constructed behind the Water Resources and Environmental Engineering Department to mimic natural weather conditions, using four identical cells (glass tubs) with dimensions of 0.40m x 0.20m x 0.20m. The base of the cells was filled with gravels of <10mm size to a depth of 0.10m, followed by substrate of <0.5mm to a depth of 0.20m and then covered with gravels of <10mm size. The gravels were placed at the top and base of the constructed wetland to prevent clogging throughout the glass cells to ease collection of treated effluent at the outlet. Three healthy plants with similar state of growth namely; *Hydroleaglabra* (False fiddleleaf), *Ludwigia abyssinica* (Primrose willow) and *Ceratophyllum demersum* (Hornwort) were sourced within Zaria environs and carefully uprooted then hand-planted in the various wetlands. The cells (constructed wetlands) were kept moist with tap water for two weeks before introducing a mixture of tap water and wastewater into the cell weekly for three weeks to reduce shock to the plants until the plants became well established and developed new shoots (Plate X and XI) before wastewater was fully introduced into the cells (Plate XII and XIII). The wastewater was regulated by a plastic gate valve from the storage tank and evenly distributed through PVC pipes from the storage tank to the cells.

Plate X: Batch Flow: Experimental set-up showing the various plants moist with tap water prior to untreated effluent recharge.

Plate XI: Continuous Flow: Experimental set-up showing the various plants moist with tap water prior to untreated effluent recharge.

3.2.5 Experimental Scenarios

1 Batch flow

The inlet pipe was opened with the aid of a control valve with no mixing in the storage tank which underwent anaerobic digestion and the influent discharged fully across the constructed wetland until it was saturated and the inlet point closed as shown in Plate XII, until detention times of 7, 14, 21 and 28 days were attained. After each detention time, the effluent was collected through the outlet point and taken to the laboratory for analysis.

Plate XII: Batch flow experimental setup fully recharged with untreated sample.

2 Continuous flow

With no mixing in the storage tank which underwent anaerobic digestion, the inlet pipe was allowed open with a constant flow rate of 1.785L/d as shown in Plate XIII. After each detention time of 7, 14, 21 and 28 days was attained, the valve was closed and the treated effluent of about 750ml was collected through the outlet point and taken to the laboratory for analysis.


Plate XI: Continuous flow experimental setup fully recharged with untreated sample.

3.2.6 Parameter Analysis

Table 3.3 gives the list of parameters analysed in the laboratory using standard method of measurement.

Table 3.3 Parameters analysed in the laboratory

S/N	PARAMETERS	UNITS
1	BOD	mg/l
2	COD	mg/l
3	pH	-
4	Lead (Pb)	mg/l
5	Chromium (Cr)	mg/l
6	Manganese (Mn)	mg/l
7	Zinc (Zn)	mg/l

1pH

pH is a measure of the acidity or alkalinity of an aqueous solution. Specifically, pH is the negative logarithm of the molar concentration of hydrogen ions and because pH is measured on a logarithmic scale, an increase of one unit indicates an increase of ten times the amount of hydrogen ions. A pH of 7 is considered to be neutral. Acidity increases as pH values decrease, and alkalinity increases as pH values increase.pH is a simple parameter but is extremely important, since most of the chemical reactions in aquatic environment are controlled by any change in its value. pH measurement is useful in effluent treatment to determine the efficiency of the wetlands, anything either highly acidic or alkaline would kill marine life. Thus, pH is having primary importance in deciding the quality of wastewater effluent, (Lokhande *et al.*, 2011) and the pH of the wastewater sample was measured using a digital pH meter by placing the electrode in the buffer with a pH value of 7 and begin reading. The calibrate button is pressed to begin reading, allowing the pH reading to stabilize before letting it sit for approximately 1-2 minutes.

2 Biological oxygen demand (BOD) Analysis

Biological Oxygen Demand is the measure of the oxygen required by micro-organisms while breaking down organic matter and it is one of the most common measures of pollutant organic material in water. Increase in BOD can be due to heavy discharge of industrial wastewater effluent from pulp and paper mills, wastewater treatment plants, feedlots, and food-processing plants, failing septic systems, and urban storm water runoff. It is necessary to note that low BOD content is an indicator of good quality water, while a high BOD indicates polluted water (Dasgupta and Yildiz, 2016). The Biological Oxygen Demand was carried out using the following bioassay procedure;

- BOD bottle was filled with the water sample.
- 2ml of Manganese sulphate was added to water sample in the BOD bottle. The bottle was closed and the excess was then discarded and shaken thoroughly.

- 2ml of Alkali-iodide azide was also added to the water sample in the bottle and then closed. The excess was also discarded and also was shaken thoroughly.

- The bottle was then allowed to stand for at least 5 minutes until when the supernatants cleared above the clock.

- 2ml of conc. H_2SO_4 was then added and also the excess was discarded and then the bottle was shaken thoroughly.

- 200ml of the water sample was measured from the BOD bottle and then poured into the conical flask.

- 3 drops of starch indicator were added to the sample in the conical flask were a blue-black colour was obtained.

- The sample in the conical flask was titrated with Sodium thio-sulphate until a colourless colour was achieved which is the end point. The titre values were then recorded. The difference between the final titration value and the initial value give the dissolve oxygen (DO) value.

- The entire procedure was repeated using the same water sample. But this time after adding the conc. H_2SO_4, the water sample in the BOD bottle was then incubated at 20^oC for 5 days.

- After the 5 days, 200ml was measured from the incubated water sample into the conical flask and starch indicator was then added.

- The sample in the conical flask was titrated with Sodium thio-sulphate until the end point was reached.

- The titre values were recorded and the difference between the titre values was also obtained which is the DO at day 5.

- The BOD was obtained by subtracting the value of the dissolve oxygen at first day and the dissolved oxygen at the fifth day.

3. Chemical oxygen demand (COD)

Chemical Oxygen Demand which is the measure of amount of oxygen required to breakdown both organic and inorganic matter was determined. COD is similar in function to BOD, in that both measure the amount of organic compounds in water. High COD levels indicate the toxic state of the wastewater along with the presence of biologically resistant organic substances. It is an important, rapidly measured parameter used to measure the load of organic pollutants in industrial wastewater (Sugasini and Rajagopal, 2015).

The Chemical Oxygen Demand was carried out using the following titration procedure;

- 0.4g of Mercuric sulphate was weighed and put into the refluxing flask.
- 4 pieces of glass beads were introduced into the refluxing flask to avoid overflow during the refluxing process.
- 20ml of the sample was also measured and poured into the refluxing flask.
- 10ml of Potassium dichromate was measured into the refluxing flask using a pipette.
- 30ml of conc. Sulphuric acid with Silver sulphate was also measured into the refluxing flask using a measuring cylinder.

- The refluxing flask with its content was taken to the heating mantle where it was held by a retort stand and clamp, and the setup was connected to a trip through a hose for water circulation process. The refluxing process was done for an hour, after which the solution was allowed to cool down.
- After cooling, the refluxing flask was rinsed with 40ml of distilled water to make up for 100ml of the overall content.
- 3 drops of Ferrion indicator was added and mixed thoroughly.
- The solution was titrated with standardized Ferrous ammonium sulphate until colour changed to green and then to blue-green colour which later changed to brown-green and finally reddish-brown colour. This was called the end point and then the final reading was taken.
- The COD was obtained using the formula

$$COD=(A–B)XNX8000/V \quad (3.3)$$

Where:

A = Titration for blank (mL);

B = Titration for sample(mL);

N= Normality.

V= Volume of the sample (mL)

3.2.7 Heavy metal analysis

1 Digestion procedure for heavy metal extraction

The wastewater samples of about 20ml were digested using 1ml of sample effluent to Nitric acid and Hydrochloric acid (HCl) in the ratio 3:1 and heated in a hot plate to about 120^{o}C for 45 minutes, allowed to cool, filtered with filter paper to remove any remaining particulate then analyzed using Microwave Plasma-Atomic Electron Spectrophotometer as shown in Plate XII.

2 Metal analysis by microwave Plasma atomic emission spectroscopy (MP-AES)

The metals were analyzed using Microwave Plasma Atomic Emission Spectroscopy (Agilent 4200 MP-AES) instead of the more conversant Atomic Absorption Spectroscopy due to its edge such as; low running cost since it operates on air, thus eliminating the use of combustible gases. Higher sensitivity with superior detection limits for difficult samples with improved dynamic range.

Results obtained from MP-AES Analysis were obtained in mg/L and were converted to parts per million (ppm) using the following equation:

$$ppm = mg/l \times V/m \tag{3.4}$$

Where:

mg/l = instrument reading unadjusted concentration;

V = final volume that the sample was made up to after microwave digestion(L);

m = digested sample mass (g).

Plate XII: Microwave Plasma Atomic Emission Spectroscopy (Agilent 4200 MP-AES)

3.2.8 Data Analysis

The removal efficiency of the constructed wetland with respect to the selected parameters was calculated according to a method stated by Ugya *et al*., (2015) that;

Removal efficiency =$(1- \frac{Ce}{Ci})$ X 100 (3.5)

Where Ci = influent concentration, mg/l;

Ce = effluent concentration, mg/l.

The statistical analysis of the collected data was carried out using Microsoft Excel package. The analysis of variance (ANOVA) tool was carried out at 5% significant level ($p < 0.05$) to determine the removal efficiency of each plants and their level of significance (difference between the mean values for each parameter) in the constructed wetlands.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 General Treatment Performance

The results of Laboratory analysis of influent and effluent wastewater samples obtained from paper recycling industry using Vertical Subsurface Flow (VSSF) constructed wetland are discussed in this chapter.

4.1.1 Characteristics of the Raw Wastewater Samples

The initial concentrations of Chromium, Lead, Manganese, Zinc, BOD, COD and pH of the waste water sample were recorded and presented in Table 4.1.

Table 4.1: Initial Characteristics of the wastewater before treatment

S /N	Parameters	Initial characteristics of Influent	Discharge Standards
1	Lead (Pb)	0.412mg/l	0.05mg/l
2	Chromium (Cr)	0.472mg/l	0.10mg/l
3	Manganese (Mn)	1.046mg/l	1.00mg/l
4	Zinc (Zn)	5.464mg/l	5.00mg/l
5	BOD	67mg/l	50mg/l
6	COD	189mg/l	180mg/l
7	pH	5.20	6-9

4.1.2 Removal Efficiency by Individual Aquatic Plants

The effluent was analysed for BOD, COD, pH and some heavy metals which are Lead (Pb), Chromium (Cr), Zinc (Zn), Manganese (Mn).

1. Lead (Pb)

The results for the removal of Lead using the Batch and Continuous flow of Vertical Subsurface Flow with respect to a given aquatic plant within 28 days retention time is shown in Table 4.2.

Table 4.2: Outlet Concentration and Removal Efficiency of Lead (Pb) from VSSF Wetlands using Batch and Continuous Flow.

HRT (days)	Aquatic Plants	Outlet Concentration (mg/l)		Removal Efficiency(%)		W.H.O Effluent Standard (mg/l)
		B F	C F	B F	C F	
7	C.D	0.360	0.336	12.62	18.45	0.05
	H.G	0.320	0.286	22.33	30.58	
	L.A	0.312	0.244	24.27	40.78	
	CONTROL	0.392	0.386	04.85	06.31	
14	C.D	0.304	0.296	26.21	28.16	
	H.G	0.272	0.264	33.98	35.92	
	L.A	0.226	0.232	45.15	43.69	
	CONTROL	0.336	0.360	18.45	12.62	
21	C.D	0.276	0.316	33.01	23.30	
	H.G	0.166	0.256	59.71	37.86	
	L.A	0.132	0.206	67.96	50.00	
	CONTROL	0.324	0.344	21.36	16.50	
28	C.D	0.212	0.252	48.54	38.83	
	H.G	0.152	0.186	63.11	54.85	
	L.A	0.112	0.126	72.81	69.42	
	CONTROL	0.304	0.336	27.48	18.45	

HRT: Hydraulic Retention Time, BF: Batch Flow, CF: Continuous Flow, C.D: Ceratophyllum demersum, H.G: Hydrolea glabra, L.A: Ludwigia abyssinica

Ludwigia abyssinica, had a removal efficiency of 72.81% and 69.42% for the batch and continuous flow respectively, which is in line with the results reported by Hossein *et al*., (2014) of lead having a removal efficiency of 60.05%. *Hydrolea glabra* had a significant removal efficiency of 63.11% and 54.85% for batch and continuous flow. *C. demersum* having the least removal efficiency of 48.54% and 38.85% for batch and continuous flow which is below 50%, This result is in accordance with Schneider and Rubio (1999) who indicated that the result for *C.demersum* had low metal adsorbtion compared to other aquatic plants, but Keskinkan., *et al*., (2004) who compared the values of heavy metals in *C. demersum* with the values in other research stated contrary that *C. demersum* can be very effective in heavy metal removal under dilute metal concentration and this does not agree with the result of this study.

2 Chromium (Cr)

The results for the removal of Chromium using the Batch and Continuous flow of Vertical Subsurface Flow (VSSF) with respect to a given aquatic plant within 28 days retention time is shown in Table 4.3.

Table 4.3: Outlet Concentration and Removal Efficiencies of Chromium (Cr) from VSSF Wetlands using Batch and Continuous Flow.

HRT (days)	Aquatic Plants	Outlet Concentration (mg/l)		Removal Efficiency(%)		W.H.O Effluent Standard (mg/l)
		B F	C F	B F	C F	
7	C.D	0.306	0.372	35.17	21.19	0.1
	H.G	0.232	0.355	50.85	24.79	
	L.A	0.206	0.266	56.36	43.64	
	CONTROL	0.425	0.446	09.96	05.51	
14	C.D	0.246	0.358	47.88	24.15	
	H.G	0.240	0.296	49.92	37.29	
	L.A	0.195	0.204	58.69	56.78	
	CONTROL	0.360	0.412	23.73	12.71	
21	C.D	0.260	0.332	44.92	29.66	
	H.G	0.192	0.252	59.32	46.61	
	L.A	0.166	0.192	64.83	59.32	
	CONTROL	0.337	0.384	28.60	18.64	
28	C.D	0.206	0.304	56.40	35.60	
	H.G	0.100	0.226	78.81	52.10	
	L.A	0.044	0.167	90.72	64.60	
	CONTROL	0.316	0.346	33.10	26.69	

HRT: Hydraulic Retention Time, BF: Batch Flow, CF: Continuous Flow, C.D: Ceratophyllumdemersum, H.G: Hydrolea glabra, L.A: Ludwigia abyssinica

Ludwigiaabyssinica shows higher efficiency for all the heavy metals analysed. The initial concentration of 0.47mg/l was reduced to 0.04mg/l in 28 days with overall percentage removal of 90.72% for the Batch Flow and was reduced to 0.30mg/l in 28 days with overall percentage removal of about 64.60%, for Continuous Flow. *Hydroleaglabra*reduced the concentration of Chromium from an initial concentration of 0.47mg/l to 0.10mg/l in 28 days with an overall efficiency of 78.81% removal for Batch Flow and for Continuous flow, reduced it to 0.22mg/l in 28 days with overall percentage removal of about 52.10%. *Ceratophyllumdemersum* reduced the concentration of Chromium from 0.47mg/l to 0.20mg/l in 28days with an overall efficiency of 56.40% removal for Batch Flow and for Continuous flow, reduced it to 0.30mg/l in 28 days with overall percentage removal of about 35.60% removal.

3 Manganese (Mn)

The results for the removal of Manganese using the Batch and Continuous flow of Vertical Subsurface Flow (VSSF) with respect to a given aquatic plant within 28 days retention time is shown in Table 4.4.

Table 4.4: Outlet Concentration and Removal Efficiencies of Manganese (Mn) from VSSF Wetlands using Batch and Continuous Flow.

HRT (days)	Aquatic Plants	Outlet Concentration (mg/l)		Removal Efficiency(%)		W.H.O Effluent Standard (mg/l)
		B F	C F	B F	C F	
7	C.D	0.090	1.036	91.40	00.96	1.0
	H.G	0.082	0.840	92.16	19.69	
	L.A	0.078	0.784	92.54	25.05	
	CONTROL	0.188	1.086	82.03	03.24	
14	C.D	0.078	0.946	92.54	09.56	
	H.G	0.066	0.736	93.69	29.64	
	L.A	0.057	0.460	94.55	56.02	
	CONTROL	0.180	1.016	82.79	02.68	
21	C.D	0.056	0.872	94.65	16.63	
	H.G	0.040	0.624	96.18	40.34	
	L.A	0.021	0.360	97.99	65.58	
	CONTROL	0.172	0.916	83.56	12.43	
28	C.D	0.052	0.726	81.00	31.20	
	H.G	0.024	0.492	90.00	53.30	
	L.A	0.018	0.206	93.30	80.40	
	CONTROL	0.162	0.784	38.20	25.20	

HRT: Hydraulic Retention Time, BF: Batch Flow, CF: Continuous Flow, C.D: Ceratophyllumdemersum, H.G: Hydrolea glabra, L.A: Ludwigia abyssinica

For Batch flow: Manganese with initial concentration of 0.046mg/l was reduced to a concentration of 0.018mg/l by *Ludwigia abyssinica* in 28 days and a percentage removal of 93.30%, *Hydroleaglabra* reduced the concentration to 0.024mg/l in 28 days with 90.00% removal efficiency, *Ceratophyllumdemersum* reduced the concentration to 0.052mg/l in 28 days with overall percentage removal of 81.0% and Control reduced the concentration to 0.162mg/l in 28 days with removal efficiency of 38.20%. For Continuous flow: Manganese was reduced to a concentration of 0.206mg/l by *Ludwigiaabyssinica* in 28 days and a percentage removal of 80.40%.*Hydroleaglabra* reduced the concentration to 0.492mg/l in 28 days with 53.30% removal efficiency. *Ceratophyllumdemersum* was the least which reduced the concentration to 0.726mg/l in 28 days with overall percentage removal of 31.20% efficiency. A similar study of the removal efficiency of Manganese was recorded by Ugya, (2015), who observed a 94.3% treatment using *Lemna minor* (duckweed).

4 Zinc (Zn)

The results for the removal of Zinc using the Batch and Continuous flow of Vertical Subsurface Flow (VSSF) with respect to a given aquatic plant within 28 days retention time is shown in Table 4.5.

Table 4.5: Outlet Concentration and Removal Efficiencies of Zinc (Zn) from VSSF Wetlands using Batch and Continuous Flow.

HRT (days)	Aquatic Plants	Outlet Concentration (mg/l)		Removal Efficiency(%)		W.H.O Effluent Standard (mg/l)
		B F	C F	B F	C F	
7	C.D	2.912	3.063	35.06	31.38	5.0
	H.G	2.536	2.820	43.64	36.83	
	L.A	1.546	1.772	65.37	60.30	
	CONTROL	3.064	3.584	31.36	19.71	
14	C.D	2.406	2.897	46.10	35.10	
	H.G	2.154	2.045	51.75	54.19	
	L.A	1.020	1.154	77.15	74.15	
	CONTROL	2.840	3.189	36.38	28.56	
21	C.D	2.106	2.440	52.82	45.34	
	H.G	1.820	1.520	59.23	66.09	
	L.A	0.786	1.020	82.39	77.15	
	CONTROL	2.340	2.846	47.58	36.25	
28	C.D	1.846	2.121	58.65	52.49	
	H.G	1.524	2.009	65.86	54.99	
	L.A	0.352	1.898	92.11	57.48	
	CONTROL	1.963	2.284	56.03	48.84	

HRT: Hydraulic Retention Time, BF: Batch Flow, CF: Continuous Flow, C.D: Ceratophyllumdemersum, H.G: Hydrolea glabra, L.A: Ludwigia abyssinica

The Batch flow has 0.352mg/l, 1.524mg/l, 1.846mg/l and 1.963mg/l concentration reduction for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* and Control, respectively. The Continuous flow has 1.898mg/l, 2.009mg/l, 2.121mg/l and 2.284mg/l concentration reduction for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* and Control respectively. The removal efficiency for Batch flow was 92.11%, 65.86%, 58.65% and 56.03% for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* and Control, respectively. The Continuous flow has 57.48%, 54.99%, 52.49% and 48.84% for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* and Control respectively. Although, Srivastav *et al*., (2007) observed that the removal efficiency of Zinc fell within the range of 50 – 95% for both *Spirodela* and *Salvinia* aquatic plants.

5 Biological Oxygen Demand (BOD)

The results for the removal of BOD using the Batch and Continuous flow of Vertical Subsurface Flow (VSSF) with respect to a given aquatic plant within 28 days retention time is shown in Table 4.6.

Table 4.6: Outlet Concentration and Removal Efficiencies of BOD from VSSF Wetlands using Batch and Continuous Flow.

HRT (days)	Aquatic Plants	Outlet Concentration (mg/l)		Removal Efficiency(%)		W.H.O Effluent Standard
		B F	C F	B F	C F	(mg/l)
7	C.D	36	39	46.27	41.79	50
	H.G	32	35	52.24	47.76	
	L.A	28	32	58.21	52.24	
	CONTROL	42	45	37.31	32.84	
14	C.D	26	28	61.19	58.21	
	H.G	28	25	58.21	62.69	
	L.A	22	21	67.16	68.66	
	CONTROL	30	37	55.22	44.78	
21	C.D	22	26	67.16	61.19	
	H.G	18	22	73.13	67.16	
	L.A	16	20	76.12	70.15	
	CONTROL	23	29	65.67	56.72	
28	C.D	17	23	74.63	65.67	
	H.G	13	16	80.60	76.12	
	L.A	10	12	85.07	82.09	
	CONTROL	20	25	70.15	62.69	

HRT: Hydraulic Retention Time, BF: Batch Flow, CF: Continuous Flow, C.D: Ceratophyllumdemersum, H.G: Hydrolea glabra, L.A: Ludwigia abyssinica

Paper mill wastewater which is usually whitish in colour, increases the turbidity of water body. This in turn hampers the photosynthesis process, causing alteration in the habitat thereby amounting for the high value for the BOD (Aslam *et al*., 2004). For the Batch Flow: *Ludwigiaabyssinica* reduced the concentration of the BOD from 67mg/l to 10mg/l in 28 days retention time. *Hydroleaglabra* reduced the concentration to 13mg/l in 28 days: *Ceratophyllumdemersum* has the least concentration reduction of 17mg/ in 28 days retention time. The Control actively reduced the concentration of the BOD to 20mg/l within 28 days. For the Continuous Flow: *Ludwigiaabyssinica* reduced the concentration of the BOD from 67mg/l to 12mg/l in 28 days retention time. *Hydroleaglabra* reduced the concentration to 16mg/l in 28 days: *Ceratophyllumdemersum* has the least concentration reduction of 23mg/l in 28 days retention time. The Control actively reduced the concentration of the BOD to 25mg/l within 28 days. The removal efficiency for Batch flow was 85.07%, 80.60%, 74.63% and 70.15% for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* and Control, respectively. The Continuous flow has 82.09%, 76.12%, 65.67% and 62.69% for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum*and Control respectively. It was observed that the BOD removal efficiency increased as the retention time increases. These results conform with a similar study carried out by Tripathi and Shukla (1991) who reported over 96% reduction in BOD using *Eichornia crassipes* (water hyacinth) and algae for sewage treatment. The Control actively reduced the concentration of the BOD to a 70% and 63% (batch and continuous flow) removal efficiency rate, which might be triggered by settling of suspended solids in the wetlands.

6 Chemical Oxygen Demand (COD)

The results for the removal of COD using the Batch and Continuous flow of Vertical Subsurface Flow (VSSF) with respect to a given aquatic plant within 28 days retention time is shown in Table 4.7.

Table 4.7: Outlet Concentration and Removal Efficiencies of COD from VSSF Wetlands using Batch and Continuous Flow.

HRT (days)	Aquatic Plants	Outlet Concentration (mg/l)		Removal Efficiency(%)		E.P.A Effluent Standard (mg/l)
		B F	C F	B F	C F	
7	C.D	118	124	37.57	34.39	180
	H.G	105	111	44.44	41.27	
	L.A	98	106	48.15	43.92	
	CONTROL	132	142	30.16	24.87	
14	C.D	101	113	46.56	40.21	
	H.G	89	98	52.91	48.15	
	L.A	82	85	56.61	55.03	
	CONTROL	116	123	38.62	34.92	
21	C.D	86	90	54.50	52.38	
	H.G	71	78	62.43	58.73	
	L.A	52	61	72.49	67.72	
	CONTROL	98	114	48.15	39.68	
28	C.D	73	80	61.38	57.67	
	H.G	66	78	65.08	58.73	
	L.A	35	42	81.48	77.77	
	CONTROL	81	89	57.14	52.91	

HRT: Hydraulic Retention Time, BF: Batch Flow, CF: Continuous Flow, C.D: Ceratophyllum demersum, H.G: Hydrolea glabra, L.A: Ludwigia abyssinica

The COD concentration was reduced to 35mg/l, 66mg/l, 73mg/l, and 81mg/l for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* and Control, respectively for Batch flow. The concentration was reduced to 42mg/l, 78mg/l, 119mg/l, and 89mg/l for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* and Control respectively for Continuous flow. The Removal efficiency by the Batch flow was 81.48%, 65.08%, 61.38% and 57.14% for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* and control, respectively. The Continuous flow removal efficiency was 77.77%, 58.73%, 57.67%, and 52.91% for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* and Control respectively. The COD removal efficiency by the constructed wetlands falls within the range of result by Vymazal, (1996), who reported COD maximum efficiency removal of 59 - 91%.

7 pH

The results for the increase in pH using the Batch and Continuous flow of Vertical Subsurface Flow (VSSF) with respect to a given aquatic plant within 28 days retention time is shown in Table 4.8.

Table 4.8: Outlet Concentration of pH from VSSF Wetlands using Batch and Continuous Flow.

HRT (days)	Aquatic Plants	Outlet Reading		W.H.O Effluent Standard
		B F	C F	
7	C.D	5.49	5.45	6.0-9.0
	H.G	5.57	5.62	
	L.A	5.64	5.58	
	CONTROL	5.37	5.26	
14	C.D	5.77	5.60	
	H.G	5.94	5.80	
	L.A	6.03	5.87	
	CONTROL	5.41	5.37	
21	C.D	5.93	5.78	
	H.G	6.22	5.94	
	L.A	6.29	6.18	
	CONTROL	5.89	5.59	
28	C.D	6.20	6.05	
	H.G	6.30	6.30	
	L.A	6.45	6.40	
	CONTROL	6.11	6.03	

HRT: Hydraulic Retention Time, BF: Batch Flow, CF: Continuous Flow, C.D: Ceratophyllumdemersum, H.G: Hydrolea glabra, L.A: Ludwigia abyssinica

All the three aquatic plants and the control increased the pH to WHO Standards, these increase may be caused by temperature, dissolved minerals, rain and low aeration. For Batch flow; *Ludwigiaabyssinica* increased the pH to 6.45, *Hydroleaglabra* increased the pH to 6.30 while *Ceratophyllumdemersum* increased the pH to 6.20 and Control increased the pH to 6.11. For the Continuous flow; *Ludwigiaabyssinica* increased the pH to 6.40, *Hydrolea glabra* increased the pH to 6.30 while *Ceratophyllumdemersum* increased the pH to 6.05 and Control increased the pH to 6.03. The influent and effluent values range between 5 – 7, which agrees with Crossley (2012), He found that most aquatic macrophytes can tolerate a range of pH levels, preferable 5 – 7.5.

4.2 Graphical Representation of Experimental Findings for Batch Flow

The graphs show the concentrations for all the parameters tested which are Lead, Chromium, Manganese, Zinc, BOD, COD and pH from the influent and the performance of the aquatic plants with respect to the retention time which of 7,14, 21 and 28 days.

4.1 Graphical representation of lead removal by individual aquatic plants

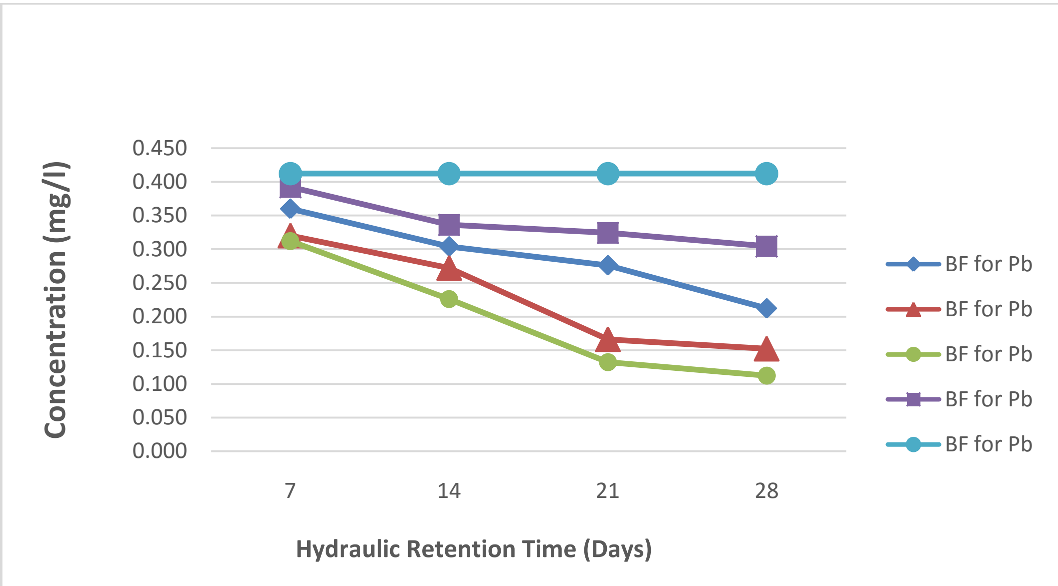

Figure 4.1: Lead removal by individual aquatic plants (Batch flow)

Figure 4.1 shows the extent of lead removal from the wastewater with respect to three individual aquatic plants and the time of retention. The chart depicts that*Ludwigiaabyssinica* was more effective in the removal of lead and as the time of retention increases, the efficiency also increases. *Hydrolea glabra*also was very effective but it doesn't actually withstand adverse pressure as in the case of*Ludwigia abyssinica.* For *Ceratophyllum demersum*, the curve indicates that the plant did not perform well as compared to*Hydrolea glabra*and*Ludwigia abyssinica*. The control also shows a significant reduction in the concentration of lead with respect to the retention time.

4.2 Graphical representation of chromium removal by individual aquatic plants

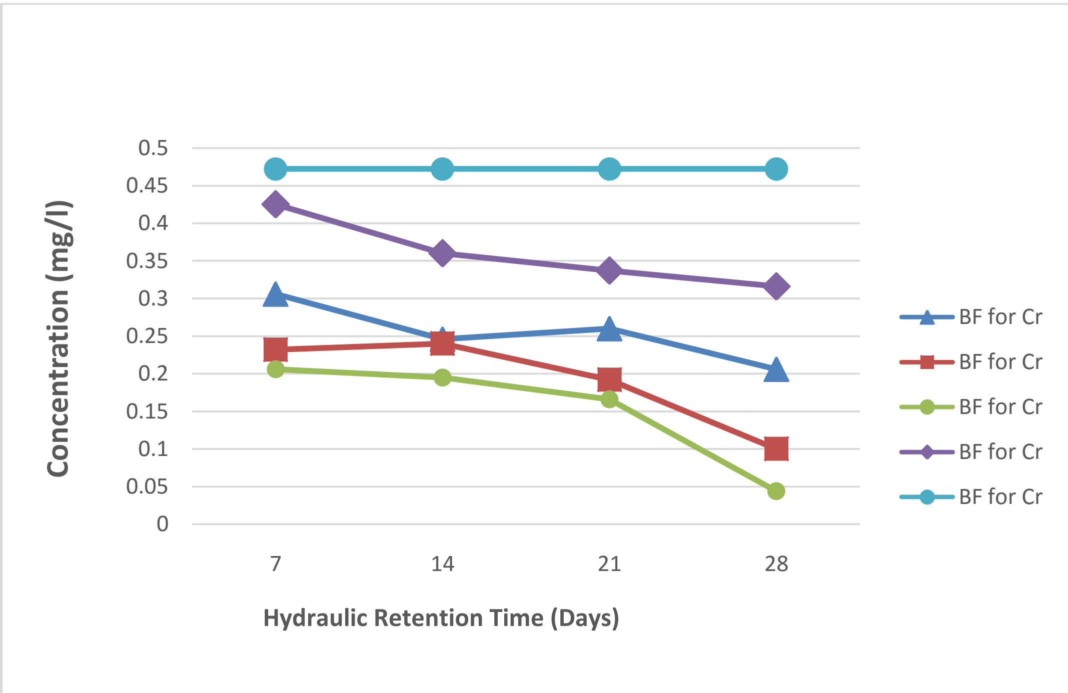

Figure 4.2: Chromium removal by individual aquatic plants (Batch flow)

The chart in Figure 4.2 depicts that *Ludwigiaabyssinica* was more effective in the removal of Chromium between 21days of retention and the 28days of retention. As the time of retention increases, the efficiency also increases.*Hydrolea glabra*also followed the same pattern as*Ludwigia abyssinica*but less effective. For*Ceratophyllum demersum*, Chromium concentration has significantly reduced between the 7-14 days of retention but between the 14-21 days of retention, it was observed that the curve tends to rise indicating increase in concentrations, this is an indication that the plant cannot actually survive longer period in wastewater. The control also shows a significant reduction in the concentration of chromium with respect to the retention time.

4.3 Graphical representation of manganese removal by individual aquatic plants

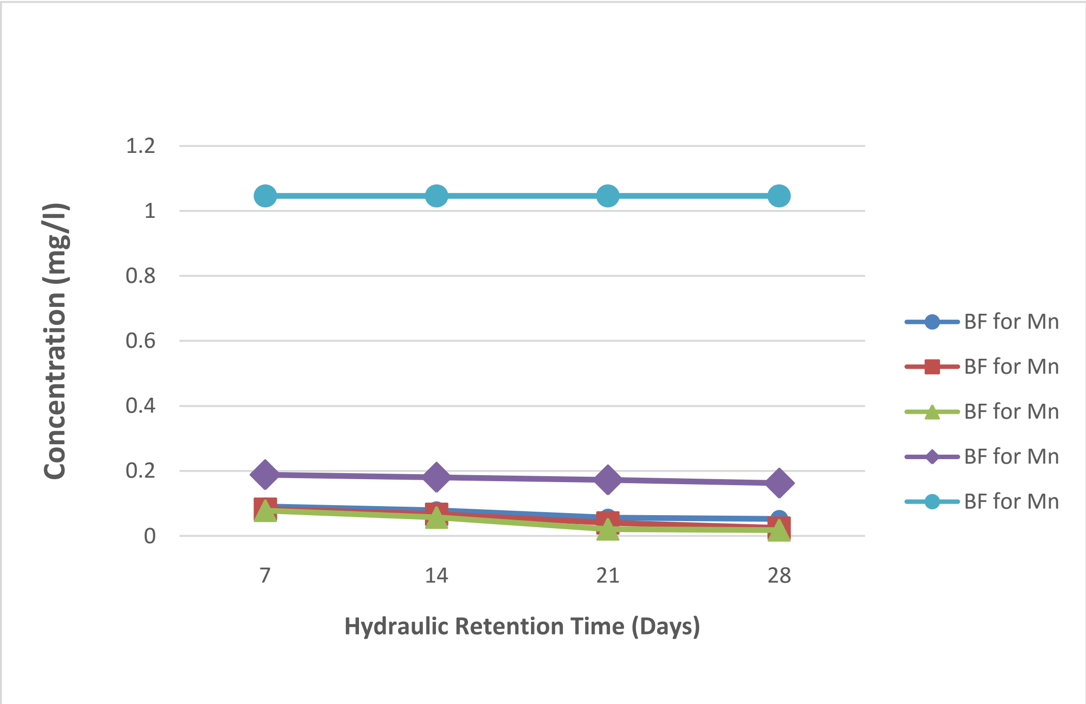

Figure 4.3: Manganese removal by different aquatic plants (Batch flow)

Figure 4.3 above depicts that *Ludwigia abyssinica*has the least concentration of Manganese after the retention period of 28 days. *Hydrolea glabra*and *Ceratophyllum demersum*was considerably effective in the reduction of cadmium with respect to the periods of retention. The curve shows that manganese removal is uniform for the three plants. The control was also significantly effective and its efficiency increased with respect to the retention time.

4.4 Graphical representation of zinc removal by individual aquatic plants

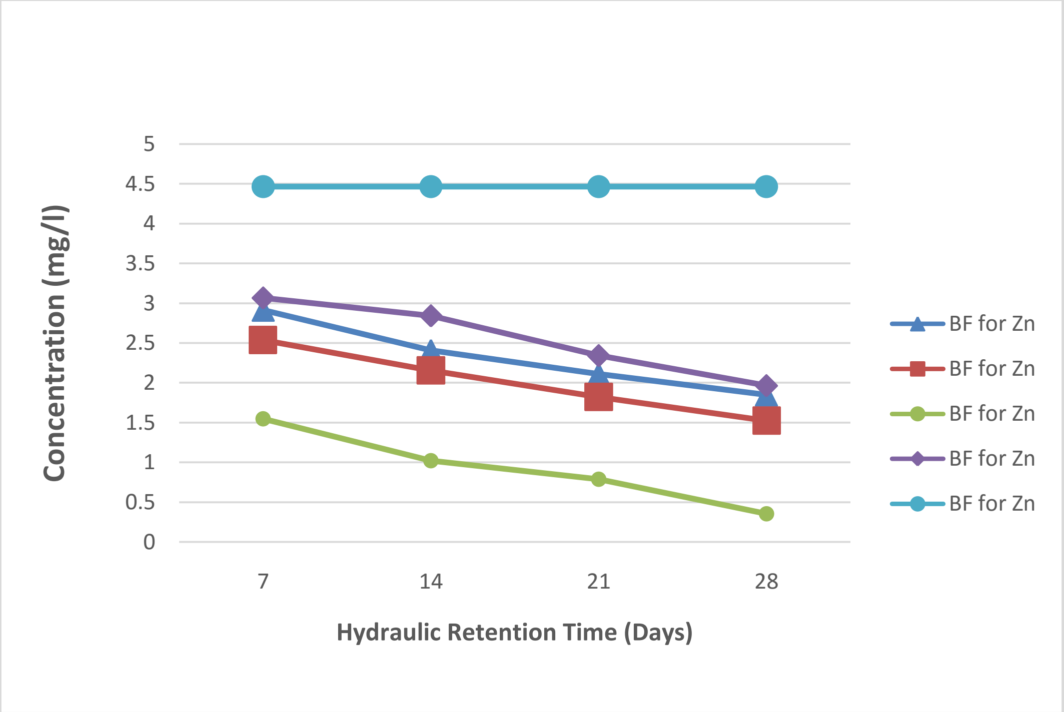

Figure 4.4: Zinc removal by individual aquatic plants (Batch flow)

Figure 4.4 shows the extent of Zinc removal from the effluent with respect to the three aquatic plants and the time of retention. *Ludwigia abyssinica*still indicates a more active aquatic plant compared to *Hydrolea glabra*and *Ceratophyllum demersum* in the removal of Zinc from the wastewater. The control also shows reduction in the concentration of Zinc with respect to the retention time.

4.5 Graphical representation of BOD reduction by individual aquatic plants

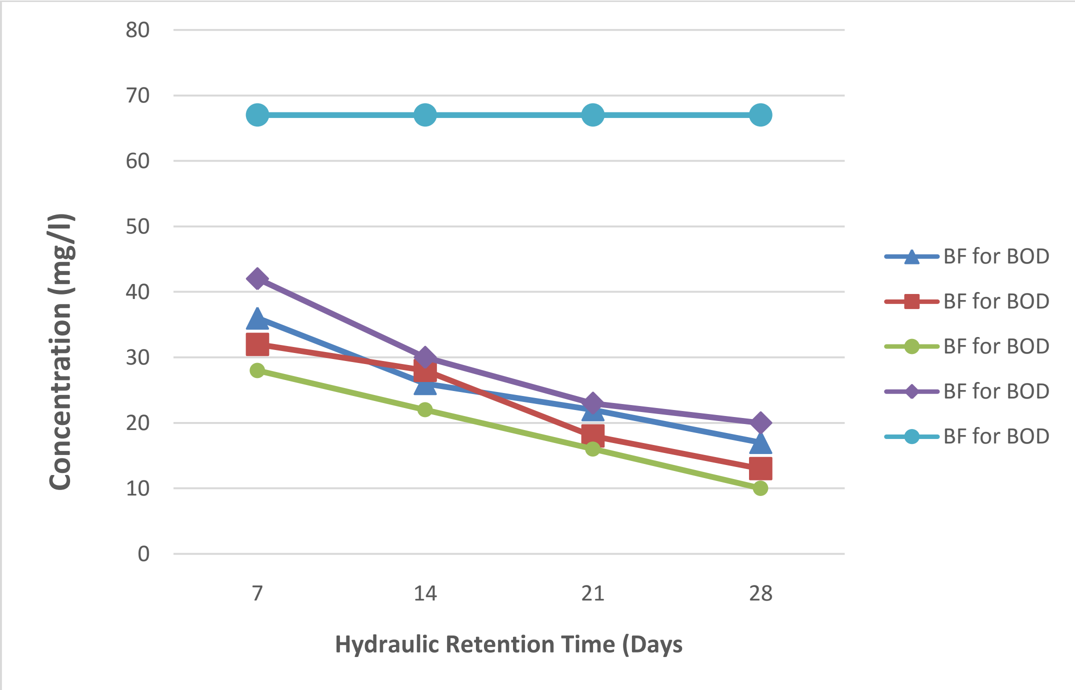

Figure 4.5: BOD reduction by individual aquatic plants (Batch flow)

Figure 4.5 illustrates the relationship between the initial BOD value before passing through the constructed wetland and the value obtained after the retention period of 7, 14, 21 and 28 days with respect to the different aquatic plant. *Ludwigia abyssinica*,*Hydrolea glabra*and*Ceratophyllum demersum* shows a significant removal for the retention period of 7, 14, 21 and 28days. The permissible discharge limit for BOD for industrial wastewater is 50mg/l (WHO, 2012), therefore the values obtained for all the experimental setup were within the permissible discharge limit wastewater and all the three aquatic plants sufficiently reduce the BOD value to a permissible discharge limit of 50mg/l. The control was not too effective in the reduction of BOD because of less biological activities within the media.

4.6 Graphical representation of COD reduction by individual aquatic plants

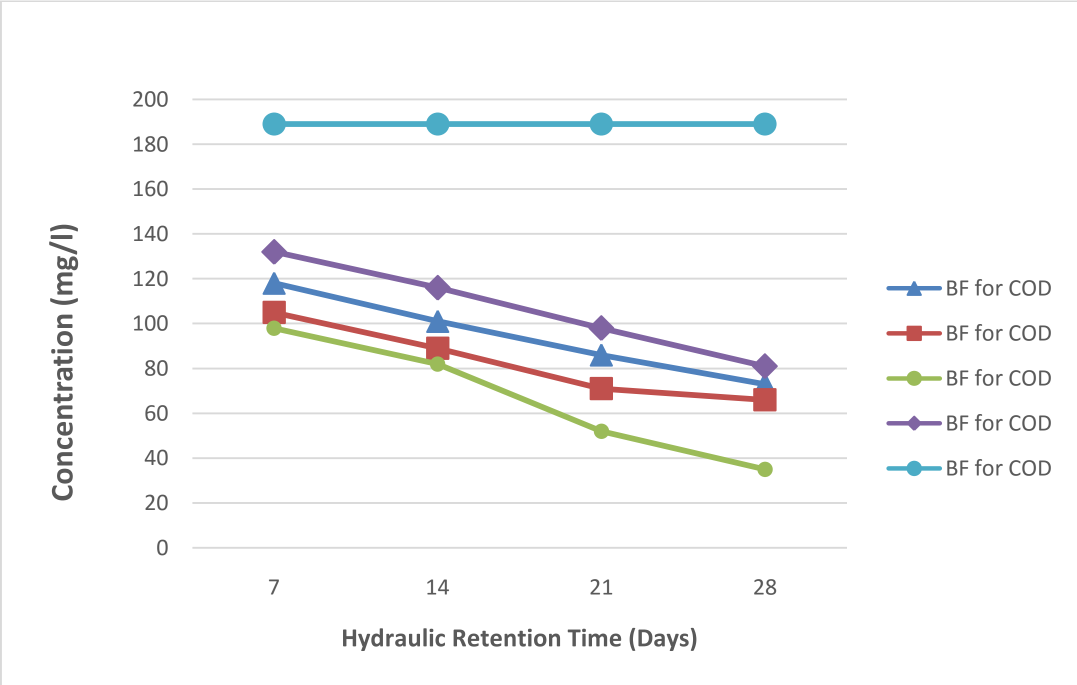

Figure 4.6: COD reduction by individual aquatic plants (Batch flow)

Figure 4.6 above shows the trend of COD was reduced within the duration of 28 days by the individual aquatic plants. *Ludwigia abyssinica* shows more reduction value throughout the retention time from 7 days to 28 days, same trend was observed for *Hydrolea glabra*, *Ceratophyllum demersum* and Control.

4.7 Graphical representation of pH increase by individual aquatic plants

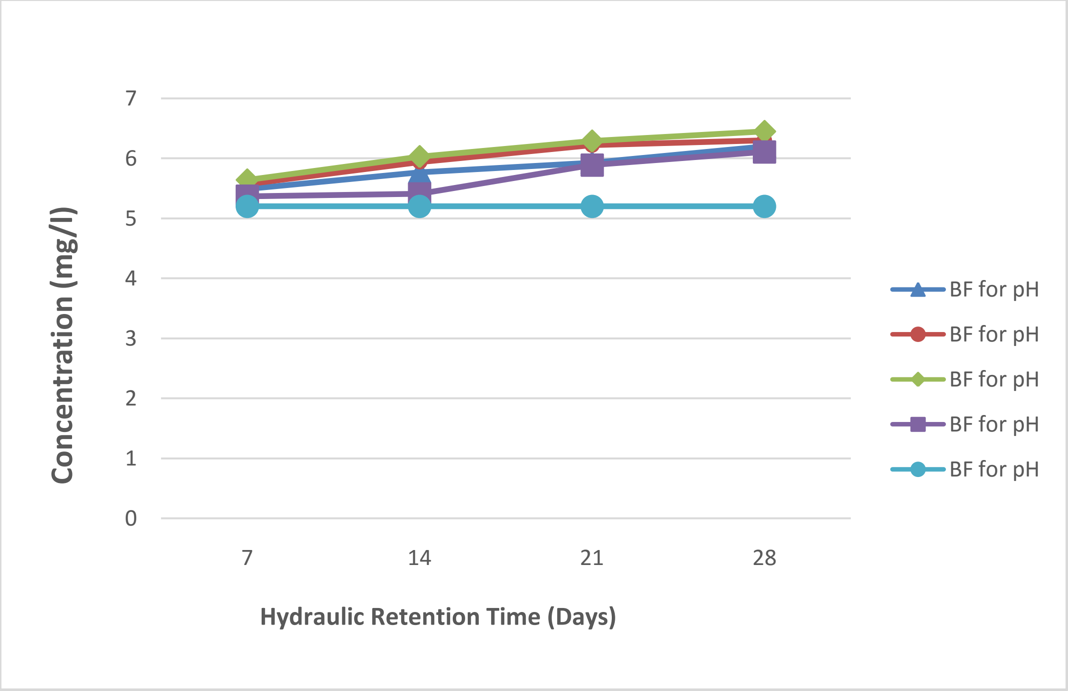

Figure 4.7: pH increase by individual aquatic plants (Batch flow)

The initial pH value for the wastewater was 5.20. The standard pH limit that is permissible for the discharge of industrial effluent is given between the ranges of 6-9. Considering the results obtained from the constructed wetland, Figure 4.7 shows that the three plants and control were effective in increasing and maintaining the pH within the permissible discharge limit.

4.3 Graphical Representation of the Experimental Findings for Continuous flow

4.8 Graphical representation of lead removal by individual aquatic plants

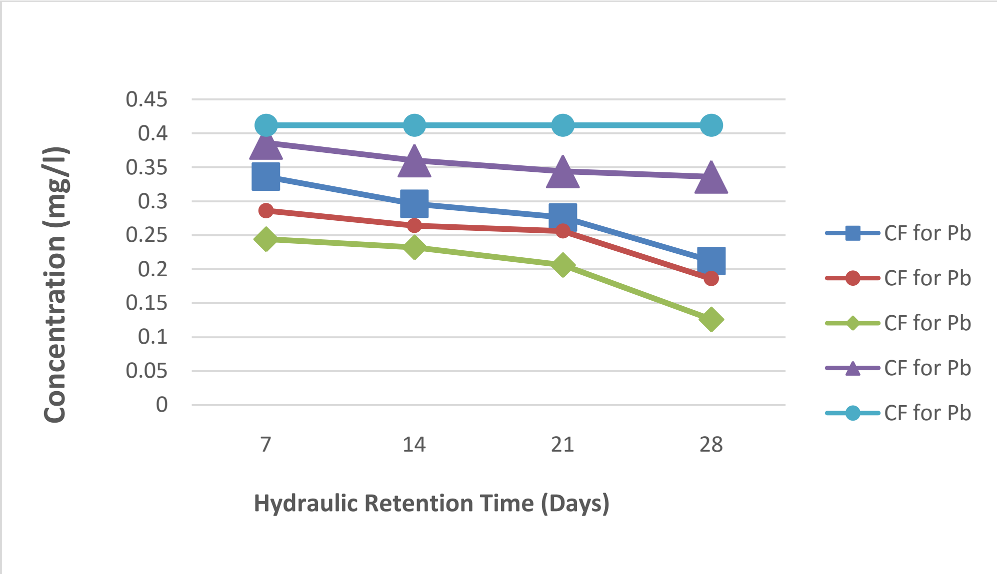

Figure 4.8: Lead removal by individual aquatic plants (Continuous flow)

From Figure 4.8 above, the trend upon which Lead was reduced under continuous flow operating system with respect to three individual aquatic plants and the time of retention. The chart depicts that *Ludwigia abyssinica* was more effective in the removal of lead then followed by *Hydroleaglabra*and then*Ceratophyllum demersum*. The control also shows a significant reduction in the concentration of Lead with respect to the retention time.

4.9 Graphical representation of chromium removal for individual aquatic plants

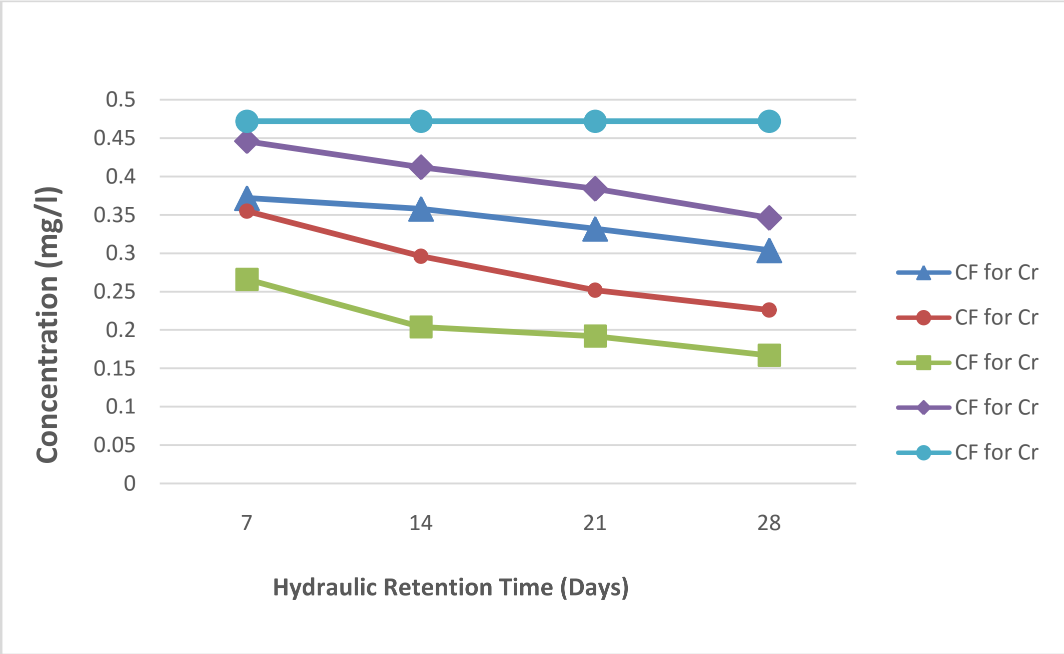

Figure 4.9: Chromium removal by individual aquatic plants (Continuous flow)

Within the period of 28 days with sample collection of 7 day intervals, Figure 4.9 shows the trend on how the chromium in the wastewater was reduced. *Ludwigia abyssinica*was more effective in the removal of lead then followed by *Hydrolea glabra*and*Ceratophyllum demersum*with the least as Control.

4.10 Graphical representation of manganese removal by individual aquatic plants

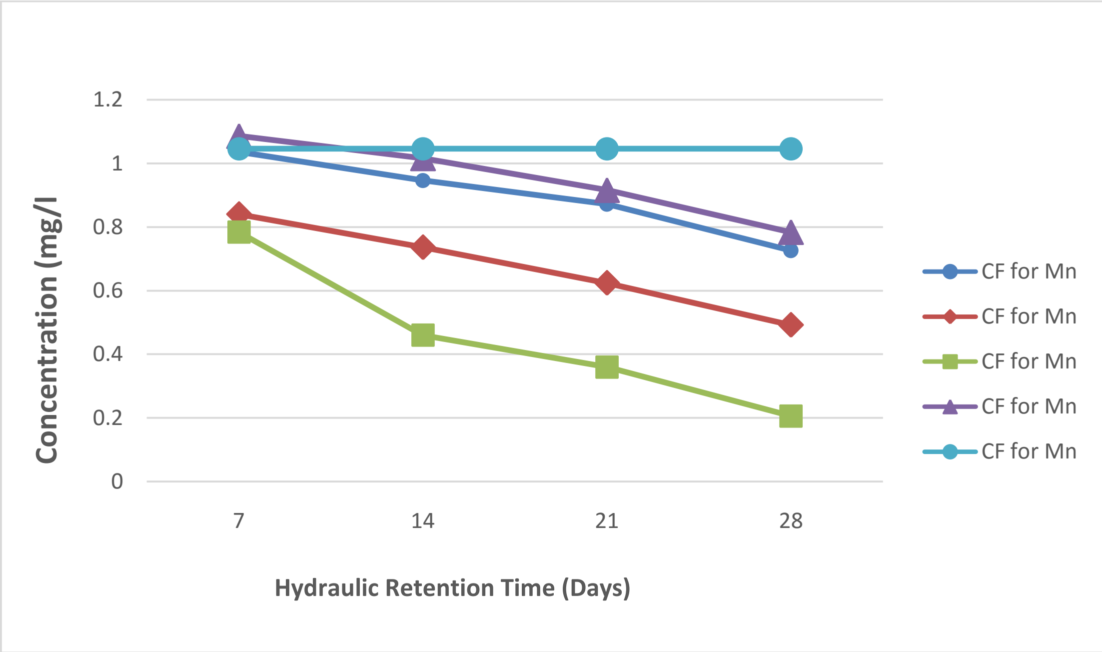

Figure 4.10: Manganese removal by individual aquatic plants (Continuous flow)

From Figure 4.10, *Ludwigia abyssinica* was more effective in the removal of Manganese between 7 days to 14 days. *Hydrolea glabra*was also effective in comparism with *Ceratophyllum demersum*. For *Ceratophyllum demersum* and Control, there was a slight difference with regard to the performance in the reduction of concentration of manganese between 7 days to 14 days and rapid reduction between 21 days to 28 days.

4.11 Graphical representation of zinc removal by individual aquatic plants

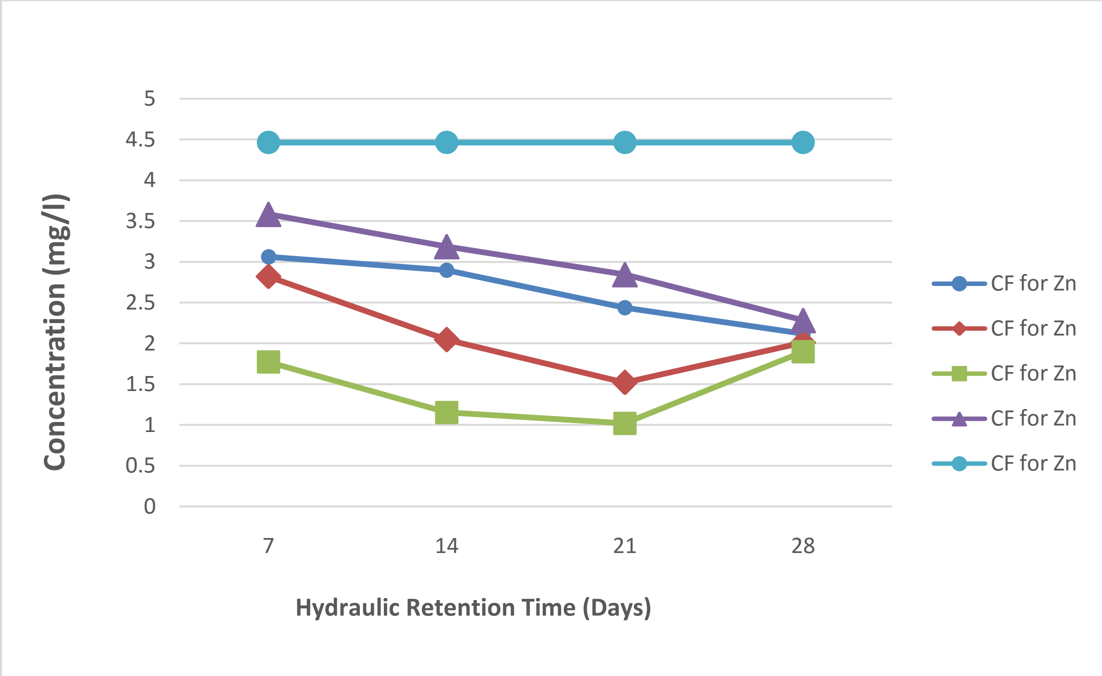

Figure 4.11: Zinc removal by individual aquatic plants (Continuous flow)

Figure 4.11 shows the trend on how Zinc was reduced within the duration of 28 days by the individual aquatic plants. *Ludwigia abyssinica* shows more reduction value from 7 days to 21 days but an increase from 21 days to 28 days, same trend was observed for *Hydrolea glabra*.*Ceratophyllum demersum* and Control reduced significantly from 21 days to 28 days.

4.12 Graphical representation of BOD removal by individual aquatic plants

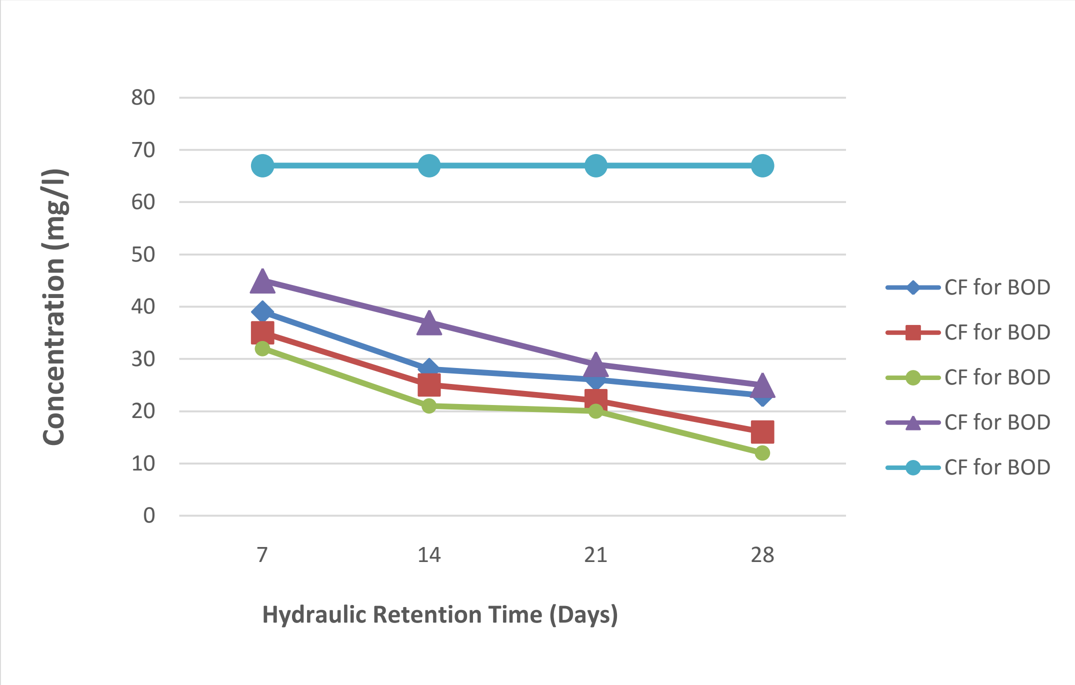

Figure 4.12: BOD removal by individual aquatics plants (Continuous flow)

Figure 4.12 above shows the relationship between the initial BOD value and the three plants *Ludwigia abyssinica*, *Hydrolea glabra and Ceratophyllum demersum*. BOD was significantly reduced by all the three aquatic plants within the duration of the experiment. At the end of the 28 day period, the values obtained for all the three aquatic plants were within the range of the allowable discharge limit given by WHO, (2013).

4.13 Graphical representation of COD removal by individual aquatic plants

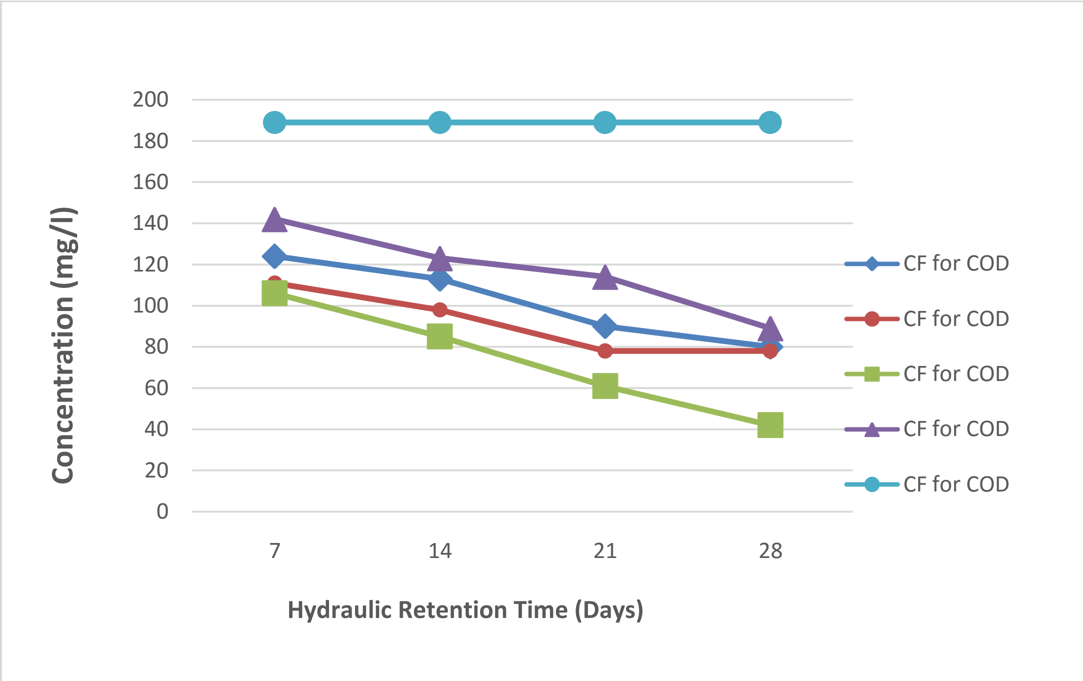

Figure 4.13: COD removal by individual aquatics plants (Continuous flow)

Figure 4.13 illustrates the relationship between the initial COD value and the three plants *Ludwigia abyssinica*, *Hydrolea glabra and Ceratophyllum demersum*. COD was significantly reduced by all the three aquatic plants within the duration of the experiment.

4.14 Graphical representation of pH increase by individual aquatic plants

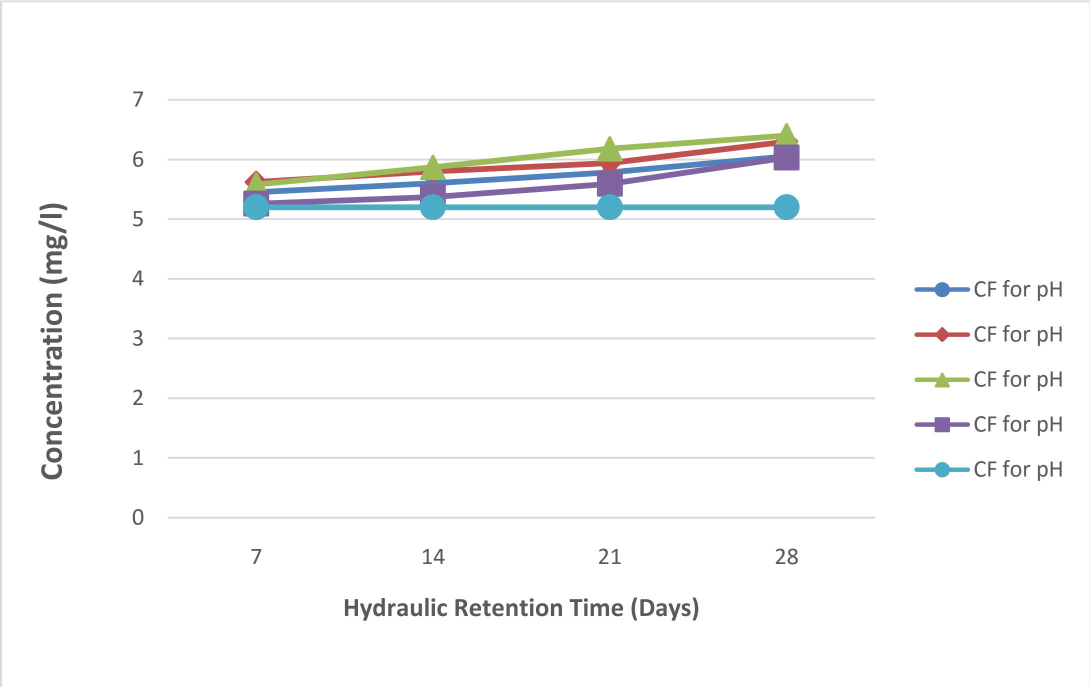

Figure 4.14: pH increase by individual aquatic plants (Continuous flow).

Figure 14 shows that the trend by which pH was increased by the constructed wetland. The pH was gradually increased to a level within the allowable discharge limit sate. From the graph above, the initial pH value was 5.20 and after the duration for the experiment, all the three plants which are *Ludwigia abyssinica*, *Hydrolea glabra*and*Ceratophyllum demersum* increased the pH value to 6.40, 6.30, and 6.05 respectively.

These observations were subjected to statistical assessment using Microsoft excel 'ANOVA' 2016 and the results are shown in Table 4.9.

4.4 Data analysis

4.4.1 Statistical analysis

Table 4.9 Summary of Analysis of Variance for the Batch and Continuous Flow using the three plants and Control.

PLANTS	BF			CF		
	F	P-VALUE	F CRIT	F	P-VALUE	F CRIT
CD	63.09046	2.4E-12	2.572712	70.58377	7.92E-13	2.572712
HG	53.15676	1.29E-11	2.572712	75.33782	4.15E-13	2.572712
LA	22.45745	4.03E-08	2.572712	21.5475	5.8E-08	2.572712
CONTROL	63.42781	2.28E-12	2.572712	78.65603	2.7E-13	2.572712

Table 4.9 shows that the ANOVA result of the Vertical Subsurface flow using the Batch and Continuous flow is statistically determinant. For details of the ANOVA results, see Appendix A.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, the efficiency of Vertical Subsurface Flow Constructed Wetland was evaluated in the reduction of heavy metals and as well as physico-chemical parameters. The following conclusions were derived at the end of the study;

- Paper recycling industrial effluent has considerable amount of heavy metals as well as organic pollutants such as the presence of lead, chromium, zinc, manganese, BOD and COD, which is being discharged to Romi stream.

- The results showed that vertical subsurface flow constructed wetland can be used in the reduction of heavy metals from Paper recycling industrial effluent.

- The control used with no plant interaction reduced the concentration of pollutants due to binding to porous media, which adds up to the efficiency of the system.

- Among the three plant used in the constructed wetland; *Ludwigia abyssinica*was the most effective plant, followed by *Hydrolea glabra*. *Ceratophyllum demersum* which is a submerged plant performed poorly as it decomposed two weeks before the design retention time.

- For batch flow, *Ludwigia abyssinica* has a removal efficiency of Lead 72.8%, Chromium 90.7%, Manganese 93.3% and Zinc 92.11% and for continuous flow it has 69.4% for Lead, 64.6% for Chromium, 80.4% for Manganese and 57.48% for Zinc. For batch flow, *Hydroleaglabra* has removal efficiency of Lead

63.1%, Chromium 78.8%, Manganese 90.0% and Zinc 65.86% while for continuous flow has efficiency for Lead 54.8%, Chromium 52.1%, Manganese 53.3% and Zinc 54.9%. For batch flow, *Ceratophyllumdemersum* has a removal efficiency of Lead 48.5%, Chromium 56.4%, Manganese 81.0% and Zinc 58.65% and for the continuous flow it has Lead (38.8%), Chromium 35.6%, Manganese 31.2% and Zinc 52.49%.

- The values for the BOD and COD for both the batch flow and continuous flow obtained for the aquatic plants shows a very significant percentage removal. For BOD *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* has efficiency of 85.07%, 80.60%, 74.63% respectively while the continuous flow has 82.09%, 76.12%, 65.67% respectively. The COD for the batch flow has 81.48%, 65.08%, and 61.38% for *Ludwigiaabyssinica*, *Hydroleaglabra*, *Ceratophyllumdemersum* respectively while for the continuous flow; it has 77.77%, 58.73% and 57.67% respectively.
- The Batch Control efficiency is 56.03% for Zinc, 38.2% for Manganese, 33.1% for Chromium, 27.5% for Lead, 70.15% for BOD and 57.14% for COD. And Continuous Control has 48.84% for Zinc, 25.2% for Manganese, 26.7% for Chromium, 18.5% for Lead, 62.69% for BOD and 52.91% for COD.
- The pH values for both batch and continuous feeding flow is between 5.45 to 6.11 showing that the constructed wetland has a high buffering capacity (ability to maintain pH within a narrow range).
- The control with no plant interaction, which served as a filter media reduced the concentration of pollutants, probably due to particulates filtered mechanically as

water passed through substrates and gravitational settling of solids, which adds up to the efficiency of the system.

5.2 Recommendations

On the basis of this research and the accompanying analysis, the recommendations for further research are:

1. Constructed wetland should be employed by Paper recycling industry as a means of effluent treatment prior to discharge into Romi Stream.

2. Further research into the utilisation of *Hydroleaglabra* and *Ludwigiaabyssinica* as a means of heavy metal removal from water due to their ability to accumulate heavy metals is necessary.

3. There is need for commercialisation of the enormous amount of biomass produced by converting it into products like pulp, fibre-boards, bioenergy.

4. The results obtained may serve as a base for further research on phytoremediation like Plants translocation of heavy metals from root to shoot in biological monitoring of heavy metals contamination.

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APPENDIX

APPENDIX A

DETAILS OF ANOVA RESULTS

Appendix A1:ANOVA FOR BATCH FLOW USING C.D PLANT

ANOVA FOR BF USING C.D PLANT

Source of Variation	SS	df	MS	F	P-value	F crit.
Between Groups	28289.68	6	4714.947	63.09046	2.4E-12	2.572712
Within Groups	1569.396	21	74.73313			
Total	29859.08	27				

Appendix A2:ANOVA FOR BATCH FLOW USING H.G PLANT

ANOVA FOR BF USING H.G PLANT

Source of Variation	SS	df	MS	F	P-value	F crit.
Between Groups	21704.73	6	3617.454	53.15676	1.29E-11	2.572712
Within Groups	1429.104	21	68.05258			
Total	23133.83	27				

Appendix A3:ANOVA FOR BATCH FLOW USING L.A PLANT

ANOVA FOR BF USING L.A PLANT

Source of Variation	SS	df	MS	F	P-value	F crit.
Between Groups	12258.82	6	2043.137	22.45745	4.03E-08	2.572712
Within Groups	1910.541	21	90.97812			
Total	14169.36	27				

Appendix A4:ANOVA FOR BATCH FLOW USING CONTROL

ANOVA FOR BF USING CONTROL

Source of Variation	SS	df	MS	F	P-value	F crit.
Between Groups	36158.35	6	6026.392	63.42781	2.28E-12	2.572712
Within Groups	1995.248	21	95.01182			
Total	38153.6	27				

Appendix A5:ANOVA FOR CONTINUOUS FLOW USING C.D PLANT

ANOVA FOR CF USING C.D PLANT						
Source of Variation	SS	df	MS	F	P-value	F crit.
Between Groups	32758.17	6	5459.696	70.58377	7.92E-13	2.572712
Within Groups	1624.362	21	77.35058			
Total	34382.54	27				

Appendix A6:ANOVA FOR CONTINUOUS FLOW USING H.G PLANT

ANOVA FOR CF USING H.G PLANT						
Source of Variation	SS	df	MS	F	P-value	F crit.
Between Groups	26297.17	6	4382.861	75.33782	4.15E-13	2.572712
Within Groups	1221.698	21	58.17611			
Total	27518.86	27				

Appendix A7:ANOVA FOR CONTINUOUS FLOW USING L.A PLANT

ANOVA FOR CF WITH L.A PLANT						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	17148.85	6	2858.141	21.5475	5.80E-08	2.572712
Within Groups	2785.519	21	132.6438			
Total	19934.37	27				

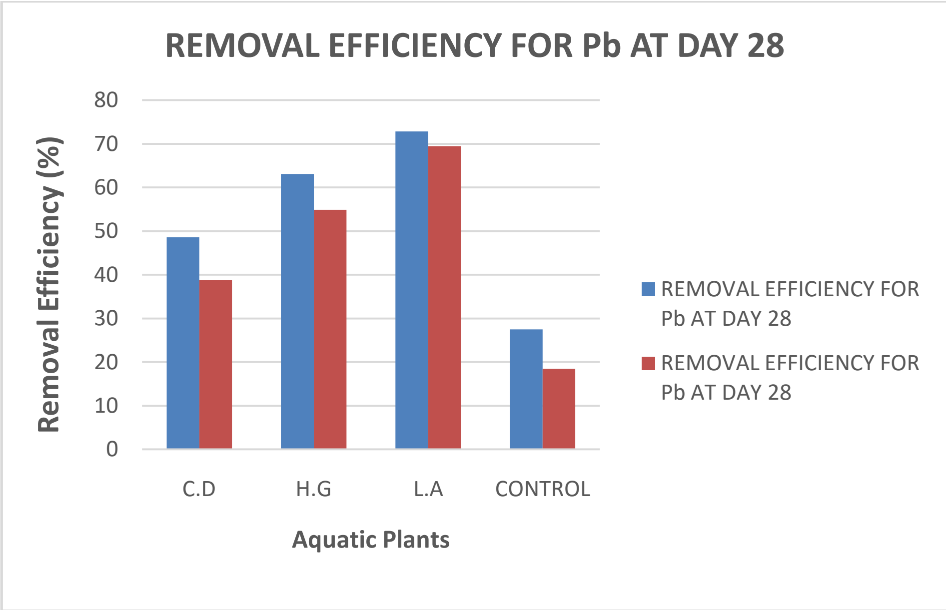
Appendix A8:ANOVA FOR CONTINUOUS FLOW USING CONTROL

ANOVA FOR CF USING CONTROL						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	43507.33	6	7251.222	78.65603	2.70E-13	2.572712
Within Groups	1935.969	21	92.18901			
Total	45443.3	27				

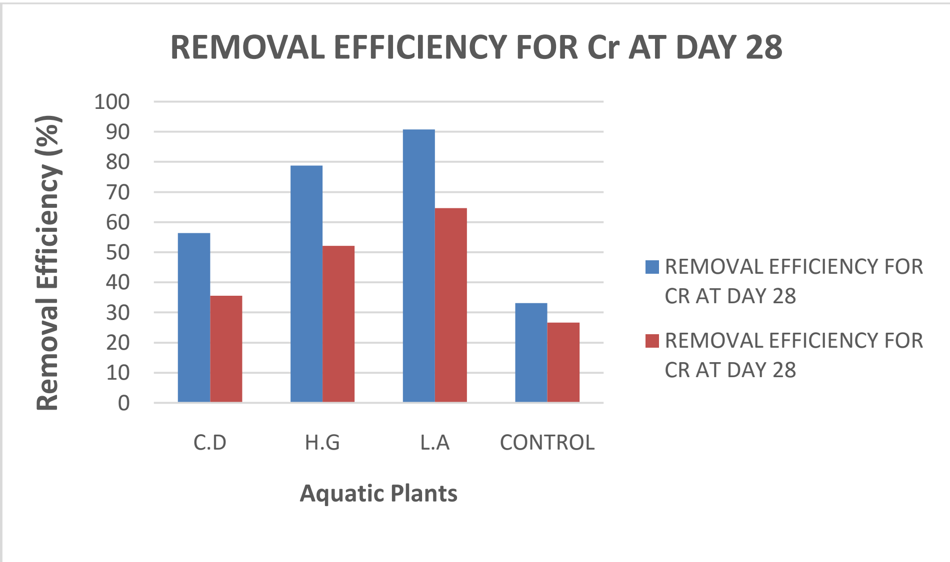
APPENDIX B

GRAPHS OF COMPARISON RESULTS FOR REMOVAL EFFICIENCY

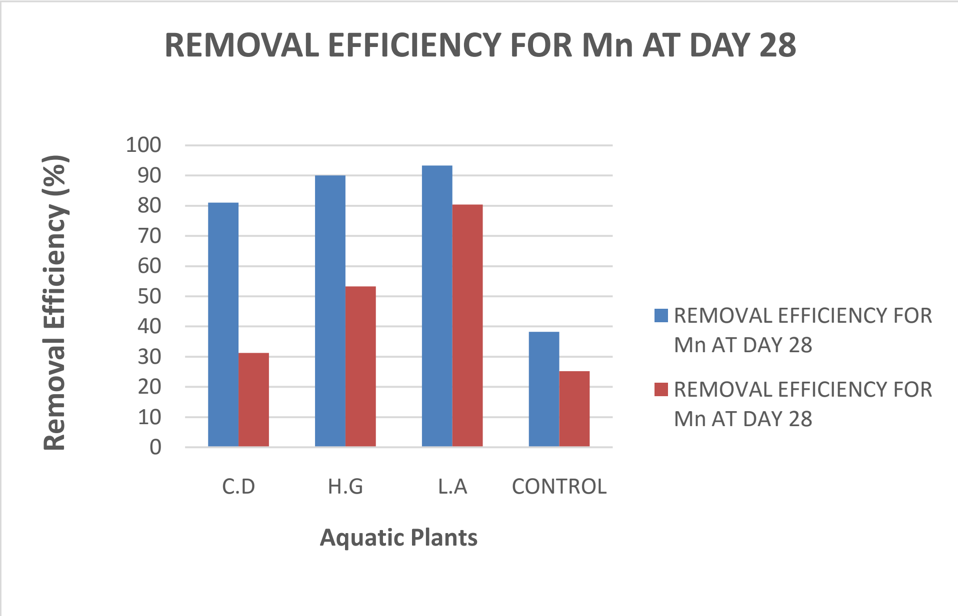
Appendix B1: Comparison of the Removal Efficiency for Lead (Pb) using Batch and Continuous Flow



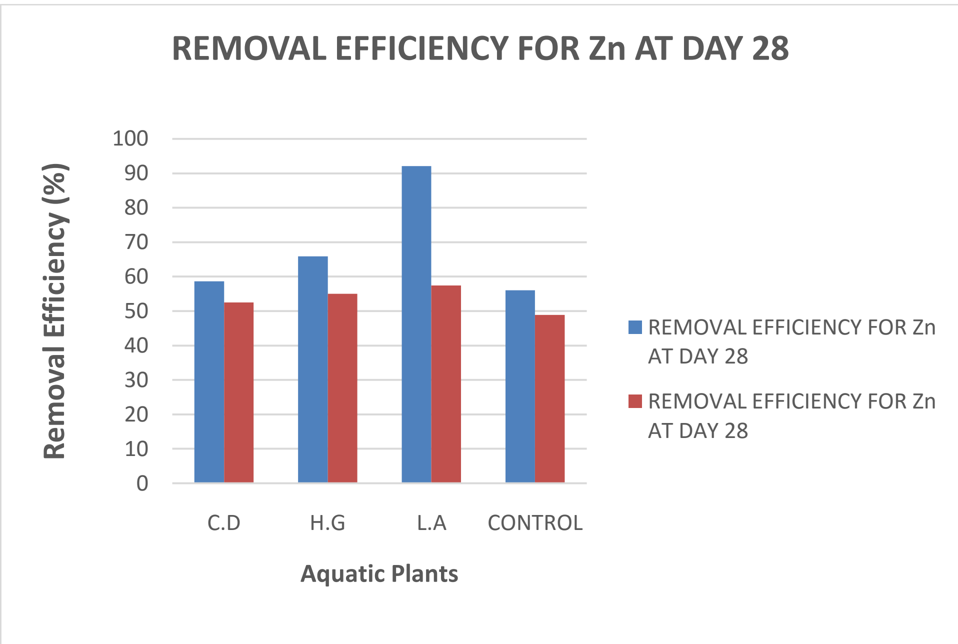
Appendix B2: Comparison of the Removal Efficiency for Chromium (Cr) using Batch and Continuous Flow



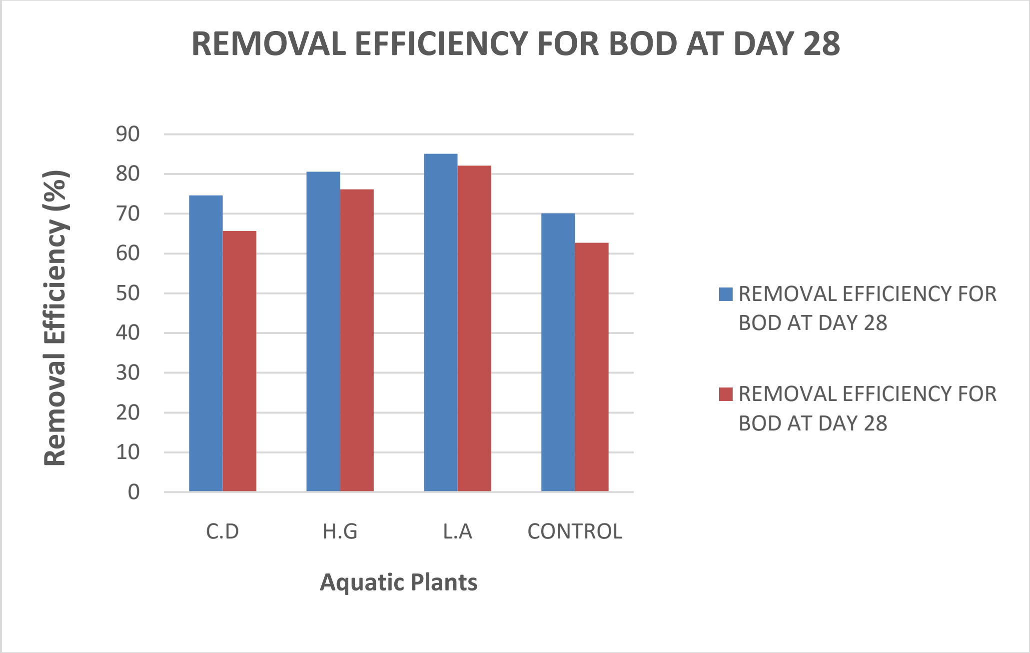
Appendix B3: Comparison of the Removal Efficiency for Manganese (Mn) using Batch and Continuous Flow



Appendix B4: Comparison of the Removal Efficiency for Zinc (Zn) using Batch and Continuous Flow



Appendix B5: Comparison of the Removal Efficiency for BOD using Batch and Continuous Flow



Appendix B6: Comparison of the Removal Efficiency for COD using Batch and Continuous Flow