POTENTIAL OF SELECTED MACROPHYTES IN REMEDIATING UNIVERSITY OF ELDORET WASTEWATER USING MULTISTAGE TECHNIQUE

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DECLARATION

DECLARATION BY THE CANDIDATE

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To my dear entire family for the inspiration and encouragement you gave me throughout my studies.

ABSTRACT

Treatment of wastewater is a challenge that has afflicted man ever since he discovered that unsafe management and disposal of wastewater into aquatic ecosystems is detrimental to environment. This research was carried out to establish the ability of various macrophytes to remediate wastewater through multistage phytoremediation technique. Water parameters at the influent and effluent of the University of Eldoret wastewater treatment plant were analyzed using standard methods for a period of eight weeks. These included physicochemical and bacteriological parameters, nutrients and heavy metals. Locally available macrophytes were collected from Marura wetland, identified and used for phytoremediation. The macrophytes were; *Azolla pinnata*, *Typha latifolia*, *Nymphaea* spp. and *Ceratophyllum demersum*. Wastewater samples were collected from University of Eldoret wastewater treatment plant. Growth chambers containing wastewater samples were prepared in the laboratory. Macrophytes were established in the growth chambers. Wastewater analysis was carried out initially on setting up the experiment and after every five days for a period of 25 days to determine the changes in the levels of physicochemical and bacteriological parameters, mineral nutrients and heavy metals. After the laboratory experiment, a multistage experiment was established with the macrophytes used earlier in the laboratory and University of Eldoret wastewater. Collection points were established at the end of each growth chamber. Wastewater was sampled from these points and analysed for the above mentioned parameters after every 5 days. The means of the data obtained from analysis of mentioned parameters were calculated and analyzed using one way ANOVA and significant means separated using Tukey's test at 5% level. The results for the influent and effluent were as follows; DO 0.44-1.75 mg/l, 3.03-5.29 mg/l respectively, BOD 432-1396 mg/l, 32-58 mg/l respectively, COD 1204-2654 mg/, 116-156 mg/l respectively, total coliforms 65783-83457 cfu/100ml, 42180 - 62760 cfu/100ml, cadmium 0.044 - 0.109 mg/l and lead 0.06 - 0.153 mg/l. Reduction Efficiency of macrophytes was as follows *Azolla pinnata:* phosphates, nitrates and lead 100%, cadmium 92.19% ($P = 0.00$), feacal coliforms 100%. *Typha latifolia*: lead 100%, phosphates 88.65%, nitrates 89.38% and cadmium 92.19% ($P = 0.00$), feacal coliforms 100%. *Ceratophyllum demersum:* phosphates 90.89%, nitrates 92.12%, cadmium 92.06% and lead 100% $P = 0.00$), feacal coliforms 100%. *Nymphaea* spp.: phosphates, nitrates, feacal coliforms and lead 100%, cadmium 88.96% ($P = 0.00$). In the multistage technique, reduction efficiency was as follows; lead 100%, cadmium 83.4% - 100%, phosphates 93.72 - 100% and nitrates 89.79 - 100% ($P = 0.00$), feacal coliforms 100%. From the results, the University of Eldoret wastewater treatment plant was not efficient in wastewater treatment. The macrophytes investigated were found to be efficient in wastewater treatment. The efficiency of macrophytes was in the following order *Azolla pinnata* > *Nymphaea spp.* > *Typha latifolia* > *Ceratophyllum demersum*. The multi-stage phytoremediation technique was found to be more efficient than individual macrophyte systems. There is need to introduce multistage phytoremediation ponds after the maturation pond in order to upgrade the University of Eldoret wastewater treatment plant hence improve its efficiency.

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LIST OF ABBREVIATIONS/ACRONYMS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
APHA	American Public Health Association
ASAL	Arid and Semi-Arid Land
BOD_5	Five day Biochemical Oxygen Demand
Cfu	Colony Forming Units
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EMCA	Environment Management and Coordination Act
FAU	Formazin Attenuation Units
NEMA	National Environment Management Authority
PAHs	PolyAromatic Hydrocarbons
pH	Potential of Hydrogen ions
PHA	PolyHydroxy Alkanoates
PHB	PolyHydroxyButyrate
TDS	Total Dissolved Solids
UN	United Nations
UNESCO	United Nations Education Scientific and Cultural Organization
UoE	University of Eldoret
VOC	Volatile Organic Compounds
WHO	World Health Organization
WRMA	Water Resources Management Authority

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Water is one of the most cherised natural resource found on earth, without which life would be non-existent. It is an essential component that sustain all life on earth (Rolston *et al.*, 2017). It is the backbone of growth and prosperity for mankind. It is a key determining aspect for economic growth in a country hence its scarcity limits economic, social and political development (Forslund *et al.*, 2009). Water comprises over 70% of the Earth's surface, 97% of this water is found in the marine ecosystem (UNESCO, 2004). The quantity of potable water is limited on earth with accessible fresh water being approximated to be 1% (Corcoran, 2010). Water determines the wellbeing of a country's population therefore concerted efforts are required in its conservation (Hogan, 2010).

There is water deficit and quality crisis in the world. Water has become a scarce resource due to lack of efficient water recycling systems, rapid increase in pollution, lack of urban planning and increase in population. According to Water, (2016) water scarcity is the worldwide risk of greatest concern for humankind and nations. It is on record to meet the 6th goal of Sustainable Development Goals (SDG) that is concerned with clean water and sanitation (Nations, 2017). The demand for water is predicted to increase significantly worldwide. In the nearby future, agricultural sector is likely to abstract more than 70% of the available water globally as nations struggle to feed their escalated populations. In addition, increase in industrialization and urbanization will intensify water demand. Currently, one of the major predicaments experienced as a result of accelerated urbanization is high demand for clean drinking water and high consumption of water.

This has culminated to the extension of portable water supply and sewerage systems in order to meet the increased demand.

In Kenya, about 80 % of the land surface is regarded as Arid and Semi-Arid Land (ASAL). This indicates that Kenya faces a challenge of water scarcity countrywide and hence needs to conserve this indispensable resource (Kenya, 2007). Kenya is among the countries that has the world's lowest water recharge rates (Jacobsen *et al.*, 2012). Currently water that is available per capita is at $650m^3$/year, future projections indicate that this will possibly drop to $359m^3$/year by 2020 and further decline to $235m^3$ by 2025 due to population growth. This is less than the United Nations' recommended minimum value of $1000m^3$/year per capita level (Nations, 2017). In Kenya, water pollution is worsening the problem of water scarcity. Nowadays, most of the surface waters receive large amounts of wastewater which contain various pollutants ranging from eutrophic nutrients to toxic compounds such as heavy metals hence threatening the natural water sources (Pavithra and Kousar, 2016). The degradation of this essential resource can be quantified as the loss of ecosystems, their biodiversity and services that they provide (Hogan, 2010). This problem is ascribed to the concerted efforts of the country to achieve its development goals (Kithiia, 2012).

Sources of water in Kenya are under pressure from urban and industrial wastes, agricultural chemicals such as fertilizers and pesticides in addition to production of hydroelectric power. There is increased demand for water resources as the population tries to acquire water to meet its diversified needs. This has caused major constraints on the scarce resources besides polluting the available water resources. Poor urban planning and weak implementation of environmental policies has escalated this problem. There is also lack of technical knowhow, poor assessment and monitoring of water quality and limited capital which has given rise to serious water pollution and treatment challenges.

Polluted water causes detrimental effects on humans who consume it and on aquatic life. The discharge of toxic effluents from various industries, agriculture and domestic sources adversely affects water quality, aquatic organisms and soil fertility. Toxic chemicals such as heavy metals and eutrophic nutrients are the main water pollutants. The disposal of large amount of heavy metals into water bodies degrades the environment leading to health risks and an upsurge in wastewater treatment cost (Ogoyi *et al.*, 2011). Some pollutants such as heavy metals can be formed during industrial use while others such as eutrophic nutrients may be through concentration and transformation of naturally occurring compounds during their domestic and agricultural uses. Agricultural wastewater contains high level of nitrogen and phosphorus produced from modern intensive agricultural production management due to the excessive use of inorganic fertilizers and large scale livestock farming (Rathore *et al.*, 2016). Discharge of these nutrients into aquatic systems results in eutrophication which is a growing problem in the world leading to unhealthy ecosystems with lack of oxygen and biodiversity (Smith *et al.*, 1999). The discharge of toxic effluents affects the structure, functions and integrity of ecosystems hence mitigation measures need to be taken.

Treatment and reuse of wastewater has become widespread in the world as the source of fresh water diminishes. Wastewater is currently being viewed as an alternative water source that can be treated and reused for various purposes. In this respect, the plight of wastewater is no longer a menace but a solution to the twin problem of wastewater disposal and water scarcity. Globally, treated wastewater reuse is gaining acceptance. In most cases, wastewater reuse is acceptable where other sources of water are unavailable or non-affordable. Reuse of wastewater has not been embraced in Kenya neither has it been recognized by the existing guidelines. National Environment Management Authority (NEMA) does not recognize the re-use of wastewater as a possibility (Kaluli *et al.*, 2011). Kenya's national average rainfall is 400mm, thus there is need to encourage efforts to harvest, store, treat and re-use wastewater (Ashiembi, 2013).

Sustainable Development Goals Target 6.3 states: "by 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally" (Nations, 2017). There is a dire need to increase the levels of wastewater treatment in order to achieve Target 6.3 that is paramount for attaining Agenda 6. The consequences of releasing untreated or inefficiently treated wastewater results to negative effects on public health, adverse ecological impacts as well as increase in poverty.

Heavy metals

Heavy metals are metals of specificied density that is normally higher than 5 g/cm^3 (Sood *et al.*, 2012). The rapid growth of urbanization and industrialization has resulted to heavy metal contamination all over the world. The disposal of untreated or partially treated industrial wastewaters from various domestic and industrial sources has given rise to heavy metal pollution in developing countries. Heavy metals dissolve and accumulate in industrial effluent, sewage and storm water (Megateli *et al.*, 2009). Heavy metals cannot be removed by natural processes because they are not biodegradable. On the contrary, they may be accumulated in aquatic fauna and flora where they can be converted to complex organic compounds which can end up being even more toxic**.** They can undergo bioaccumulation and biomagnification leading to severe detrimental effects (Sa'idi, 2010). Their indiscriminate disposal in wastewater leads to pollution that might have long-term environmental and public health impacts unless immediate action in their remediation is taken.

Heavy metals have adverse impacts on organisms that include; decreased growth and development, nervous system damage, tumors and death (Wu *et al.*, 2010). The effects on plants include reduction in the rate of growth and low levels of chlorophyll production by lead, reduced percentages of seed germination and lipid content by cadmium, retarded growth by chromium and reduction of seed germination by nickel (Akpor *et al.*, 2014).

In order to control aquatic pollution, it's vital to get rid of the heavy metals in wastewater. Currently, chemical precipitation is widely used to treat wastewaters that contain heavy metals in developing countries. These techniques are not cost effective and sometimes they pose operational problem (Akpor *et al.*, 2014). They need supervision and have high maintenance costs (Naidoo *et al.*, 2014). Alternative methods to chemical precipitation are microbial remediation and phytoremediation that can be effectively employed in the removal of heavy metal contamination in wastewater. These technologies are cost-effective and therefore most suitable for developing countries (Jadia *et al.*, 2009).

Eutrophic nutrients

Eutrophic nutrients mainly consist of nitrogen and phosphorus. These nutrients are generally nontoxic at the concentrations typically found in nature but when in excess, they can lead to a degradation referred to as eutrophication (Lu *et al.*, 2019).

Eutrophication is the enrichment of aquatic ecosystems by nutrients, particularly nitrogen and phosphorus, resulting in an increase in phytoplanktons and macrophytes growth hence producing an imbalance in aquatic biota and degrading the water quality (Rathore *et al.*, 2016). Eutrophication of water bodies is caused by over enrichment with mineral nutrients, particularly phosphorus (Waiser *et al.*, 2011).

Eutrophication of water can be increased by anthropogenic activities that accelerate the input of nutrient in aquatic ecosystems due to rapid industrialization, urbanization and intensified agriculture. Human activities in the catchment area can change the structure and functions of aquatic ecosystems (Liu, 2007). Excessive phosphorus, nitrogen and other nutrients when discharged into aquatic ecosystems results in adverse ecological consequences on aquatic ecosystem structures, functions and processes (Rathore *et al.*, 2016).

Currently, eutrophication is a major challenge in most of the developing countries. Cultural eutrophication with phosphorus and nitrogen transforms water bodies from oligotrophic to mesotrophic and then to eutrophic and finally hypertrophic (Khan *et al.*, 2014). It is a persistent problem in aquatic ecosystems and a widespread environmental dilemma. Eutrophication has various consequences such as rapid growth of algae blooms, accelerated growth of macrophytes and creation of anoxia conditions resulting to dead zones (Lu *et al.*, 2019). Eutrophication can also lead to economic losses as it leads to fish kills and raises the costs of water purification for domestic use. Eutrophication has become a worldwide problem that will probably increase due to intensive agriculture, alteration of land use and land cover and over use of inorganic fertilizer (Rathore *et al.*, 2016).

Bioremediation

There are two major categories of biological processes of wastewater cleanup, namely phytoremediation and microbial remediation. Microbial remediation involves the rehabilitation of the ecosystem using microbes such as bacteria and fungi (Leung, 2004). This technique uses biological activities to remove contaminants in the environment and lower their toxicity levels. Some pollutants are souces of carbon that is required for microbial growth before the microbes can help in their breakdown (Wanjohi *et al.*, 2015). In some cases, remediation by microorganisms occurs intrinsicaly without any engineered action to accelerate the remediation process and is referred as natural attenuation (Hamzah *et al.*, 2013). In other cases, there is supplementation of genetically engineered microbes to accelerate the degradation process that is referred to as enhanced microbial remediation. Microorganisms are vital in bioremediation as they produce non-toxic end products and are cost effective (Kumar *et al.*, 2011). In the process of pollutant degradation, microorganisms change the chemistry of the metal through reduction, mobilization or immobilization and accumulation (Orji *et al.*, 2012).

Phytoremediation

Phytoremediation is a relatively modern green technology that is still being researched on (Mitton *et al.*, 2016). Phytoremediation technology uses plants to assimilate, degrade, metabolize or detoxify pollutants (EPA, 2000). It is a natural, eco-friendly and the simplest technique for water treatment(Fellet *et al.*, 2007; Marrugo-Negrete *et al.*, 2015). This technology uses plants and its microbial consortium to minimize the toxic effects of pollutants in the ecosystem hence employed in the removal of pollutants from wastewater (Greipsson, 2011).

Currently, several research are being carried out in the field of phytoremediation due to its nature of being environmental friendly and the option of extracting accumulated pollutants via the harvesting of the plant biomass (Grobelak *et al.*, 2010). Use of phytoremediation has aesthetic value to the public hence easily accepted. It can be employed in field sites of various sizes where other clean up technologies maybe unsuitable, unviable and expensive (Ali *et al.*, 2013).

Among the several plant groups, macrophytes have attained the highest interest in phytoremediation (Greipsson, 2011). To clean up wastewater, effective macrophytes needs to be selected that should have high absorption rate of contaminants, easy to control in propagated dispersion and vigorous growth in wastewater (Roongtanakiat *et al.*, 2007).

Aquatic macrophytes

Macrophytes have ability to reduce heavy metal contaminants in polluted water bodies. They are preferred over other organisms because they are easy to handle and harvest, require minimal capital and are abundant in aquatic ecosystems. Macrophytes have the potential to accumulate heavy metals in their plant cells to about 100,000 times higher than the concentrations in wastewater thus used in heavy metals removal (Mishra and Tripathi, 2008).

Many macrophytes are used as tools in restoration and remediation of degraded environments. They have the capacity of remediating environmental contamination since they are tolerant to various pollutants (Varsha *et al.*, 2010). Researchers have found that hydrophytes can accumulate cadmium, lead, zinc, and copper (Krems *et al.*, 2013). Some hydrophytes have been used for accumalation and recovery of lead and cadmium from industrial effluent (Baharudin, 2008).

1.2 Statement of the Problem

There is a growing concern for the diminishing supply of clean and potable water. The amount of usable water is scarce on earth and is further threatened by climate change that is predicted to alter the occurence, intensity and predictability of rainfall with negative effects on the availability of water. Currently, water pollution is a prominent environmental problem affecting the quantity and quality of utilizable water. Water pollution gravely affects water availability hence there is need for its proper management in order to averse the repercursions of increasing water scarcity. To curb the problem of water shortage, various strategies have been employed to minimize water consumption, but the most sustainable solution is to recycle wastewater into high quality water. Wastewater is treated or recycled to get rid of toxic substances or reduce them to the minimum permissible limit acceptable for human consumption or suitable for the intended general domestic, agricultural or industrial uses. Efficiently treated wastewater is the solution to the twin problem of water shortage and wastewater disposal.

Wastewater treatment is a menace that has plagued developing countries affecting their surface waters as the discharge of inadequately treated wastewater has resulted to degradation of these ecosystems. Unsafe management and discharge of wastewater has become a major threat to public and environment. Disposal of inefficiently treated wastewater is common particularly in developing countries, owing to lack of human resource, finances, infrastructure and institutional capacity.

There is a major concern with the effluent released from University of Eldoret (UoE) wastewater treatment plant to the adjacent Marura wetland and river Chepkoilel. There is high probability that the effluent contains eutrophic nutrients and heavy metals which are not efficiently removed. The treatment plant has a design capacity of 16210m^3/day as per University of Eldoret public health department records. University of Eldoret sewage undergoes biological treatment in a series of four stabilization ponds. University of Eldoret treatment plant was designed to serve a population of 600 persons but the current population is over 15,000 persons. According to UoE public health department reports, this increase in population has affected the treatment process negatively as witnessed by the periodic sample results from the government chemist. According to the UoE estates department report, sludge removal is long overdue and this may affect the operational and design efficiency of the wastewater treatment plant. The sludge in anaerobic pond should be removed every 2-3 years when it has reached half depth of the pond. According to UoE estate department reports, the sludge in the UoE anaerobic pond has not been removed for over twenty years and its more than half depth. As a result, the sewage treatment plant is overwhelmed by the high volume of influent and excessive sludge. This can lead to overflow of untreated or partially treated sewage especially during the rainy seasons resulting to the pollution of Marura wetland and river Chepkoilel.

There is a dearth of information on water quality indicator parameters of UoE treatment plant especially heavy metals probably due to the high costs of analysis of these elements. This presents a gap in vital scientific information, considering that UoE wastewater treatment plant discharges its effluent in Marura wetland where River Chepkoilel passes. Marura is an important wetland to the community and the waters of river Chepkoilel are used to grow crops which are consumed far and wide. Also, the waters are used for domestic activities downstream. The realese of partially treated wastewater to these aquatic ecosystem can have adverse impacts on the public. This inefficient treatment of wastewater exposes a dire need for innovation and development of low cost treatment technologies that will improve the efficiency of UoE treatment plant. Such technologies include the multi-stage phytoremediation which have good purification effect, low instalation cost, convenient operation and management, good landscape and ecological benefits. This type of research (multistage) is less attempted, thus require in depth investigation.

1.3 Justification

Efficient treatment of wastewaters before discharging them into aquatic ecosystems is a requirement to averse the detrimental effects of contaminants on the environment and to safeguard public health. Disposal of untreated wastewater degrades aquatic ecosystems causing biodiversity loss and outbreak of waterborne diseases thus the need for treatment prior to discharge. Wastewater treatment is paramount in order to recycle the large amount of effluents that are released by the current high human population and the growing urbanization and industrialization. Wastewater can be remediated using variable traditional technologies such as chemical, physical, biological treatments or by using artificial membranes to remove undesirable materials from wastewater. However, these methods are inefficient, expensive and may generate a large amount of sludge which is hard to discard. Also these treatment systems require localized well-trained technical staffs with hands-on-training as operational and management activities need to be carried out regularly. Moreover, use of non-biological wastewater treatment methods have at times resulted in generation of additional contaminants or to the formation of toxic sludge due to the many chemicals involved in these processes. There is a definite need for an alternative, non-traditional, efficient, environmentally friendly and cost-effective water treatment technology.

Globally, various technologies have been employed in wastewater treatment but they are ussually expensive and produce adverse impacts to aquatic ecosystems. In Kenya, such treatment technologies are capital intensive and often require Environmental and Social Impact Assessment/Strategic Environmental Assessment during planning and implementation stages. Most local institutions and municipalities have limited resources and therefore are unable to adopt these technologies. As a result efficient management of wastewater has become a big challenge resulting in environmental deterioration and subsequently health hazard.

The financial aspects and repercussion of conventional treatment technologies on water bodies has paved way to phytoremediation where plants are utilized in alleviation of several contaminants from the ecosystems. Phytoremediation technology emphasizes plants significance in abating environmental pollution including heavy metals majority of which are toxic to organisms. It is an alternative approach where plants are employed in removal, stabilization or detoxification of pollutants found in wastewater through phytostabilization, phytodegradation, phytovolatization and phytoaccumulation mechanisms. This method has ecological benefits such as improvement of biodiversity and also sequestration of carbon dioxide. The process of phytoremediation produces environmentally friendly products that not only lessen aquatic pollution but also enhance ecosystem productivity. This method is an innovative green technology since plants are solar-driven and therefore make phytoremediation an economical method that has high capability of attaining sustainable environment.

Finally, there is an extensive call for the adoption of green technology whereby the use of flora has been considered the best method of removing contaminants. This has prompted a search for the best plants to be used for cleaning wastewater. Macrophytes were chosen because they have faster growth rate and are locally available within Marura wetland. The use of macrophytes has also been considered one of the safest method of wastewater purification hence the need to adopt it in this particular study.

1.4 Objectives

Broad objective

To assess the potential of selected macrophytes in remediating University of Eldoret wastewater using the multistage technique.

Specific objective

1. To determine the efficiency of University of Eldoret wastewater treatment plant.
2. To determine the efficiency of macrophytes in remediating wastewater from University of Eldoret wastewater treatment plant.
3. To determine the efficiency of multistage technique in remediating wastewater from University of Eldoret wastewater treatment plant.

Research Questions

1. Is the University of Eldoret wastewater treatment plant efficient?
2. Are macrophytes efficient in remediation of wastewater from the University of Eldoret wastewater treatment plant?
3. Is multistage technique efficient in remediation of wastewater from University of Eldoret wastewater treatment plant?

1.5 Significance of the study

The data generated from the study provided useful information on the state of University of Eldoret wastewater treatment plant. It was established whether the effluent met the Kenyan (NEMA) treatment discharge standards. The data from the multistage phytoremediation technique provided critical and timely information on an economical and affordable technique in the management of wastewater. It provided a cost effective alternative technology for various institutions that are willing to adopt the technology to treat their wastewaters. If the method is adopted by the University of Eldoret, the people living downstream of the treatment plant who use water from river Chepkoilel and Marura wetland will be free from the threats posed by partially treated wastewater.

The study is vital to all the stakeholders in the study area because improved management of wastewater is valuable to the society as it minimises the detrimental impacts on human beings and the entire environment. Development of efficient wastewater treatment systems results to well-maintained ecosystem, protection and conservation of biodiversity and the problem of water pollution is eradicated. This can decrease the existing problem of water scarcity in the area as the efficiently treated wastewater may be re-used for several purposes for example irrigation that is currently conducted using water pumped directly rom river Chepkoilel.

1.6 Scope of the study

Macrophytes sample collection was confined to Marura wetland. The wastewater samples were confined to the inlet of the anaerobic pond and outlet of the maturation pond of the University of Eldoret wastewater treatment plant. The experimental work was carried out in the University of Eldoret. The study was centered on the physicochemical parameters, bacteriological parameters, mineral nutrients (nitrates and phosphates) and heavy metals in the University of Eldoret wastewater. The study determined the potential of macrophytes to phytoremediate waste water.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wastewater

Wastewater is regarded as a mixture of domestic effluent, industrial effluent, water from institutions and commercial establishments, agricultural, horticultural and aquaculture runoff, urban runoff and storm water. Domestic effluent comprises of black water which includes urine, excreta and faecal sludge. Grey water is wastewater from washing clothes and house floor, utensils and bathing (Raschid-Sally *et al.*, 2009).

Wastewater is characterized in terms of its physical, chemical and biological composition. In terms of physical characteristics, wastewater is grey in color, have a stale odor and some solid content. Chemically, wastewater consists of organic compounds such as carbohydrates, fats and proteins. Also, it has inorganic compounds such as phosphates, nitrates, acids, bases, chlorides and heavy metals. Gaseous components include ammonia, hydrogen sulfide, methane, carbon dioxide and nitrogen. Biologically, wastewater is characterized by microorganisms some that are pathogens and others normal flora from domestic sewage. These microbes include bacteria, fungi, viruses, protozoa and algae (FAO, 1999).

Wastewater can be recycled and reused to address the predicament of water scarcity worldwide (Sato *et al.*, 2013). The demand for freshwater have increased while its availability is decreasing due to over-abstraction, pollution and climate change. Treated wastewater is being used as an alternative source of water for irrigation and in hydroponics for fish production. Resources such as nutrients, organic matter and metals can be recovered from wastewater. Supplementary processes can be used to recover biogas from sludge or biofuels from microalgae. Hence, communities are nowadays viewing wastewater as a resource that can provide solutions to their day to day challenges (Water, 2016).

Wastewater is a vital part of the water cycle that requires adequate management in the entire water management processes. These include: water abstraction, purification, distribution, usage, collection after use and clean up to its recycle and recharge of the surface water and underground sources for subsequent water abstractions. In Kenya, management of wastewater have not been adequately addressed by the government, political and social leaders. This neglect can have several adverse consequencies on the sustainability of water supplies, economic growth and on the ecosystem.

Adequate management of wastewater include safe reuse of water and the recovery of other by products (Water, 2016). Proper management of wastewater will aid in mitigating the perilous impacts of disposing untreated wastewater to the environment. Improved wastewater management will aid in reduction of water abstractions and promote sustainable use of resources (WHO, 2016). In developing countries, wastewater management and sanitation are regarded as capital-intensive thus a challenge in wastewater management (Jacobsen *et al.*, 2012). Consequently, much of the wastewater is untreated or partially treated and discharged in aquatic ecosystems. There is need for development of economically viable technologies such as phytoremediation in developing countries.

2.1.1 Wastewater treatment

Wastewater is treated to enable domestic and industrial effluents to be discharged without risk to public health or degradation of the ecosystem. Nowadays, there is evolvement of several concerted efforts to treat wastewater after realizing that release of untreated wastewater into aquatic ecosystems is a great environmental hazard (Malik *et al.*, 2015). The remedy to this problem is through treatment of the raw wastewater before discharge into the ecosystem. Two major wastewater treatment methods that has been developed include conventional and non-conventional wastewater treatment methods.

Conventional wastewater treatment

This technique uses physical, chemical and biological processes in wastewater treatment. It has various levels of wastewater clean up that include preliminary, primary, secondary and tertiary (EPA, 2000). This method is capital intensive and requires skilled operation to achieve consistent results. This method is not popular in developing countries because it uses sophisticated equipment that require electricity and skilled labor which are not easily available in these countries.

Preliminary treatment gets rid of coarse solids and large objects in raw effluent to make the operation and maintenance of consequent treatment levels better (Abu-Orf *et al.*, 2014)). Operations at this level include coarse screening, grit removal and comminution of large objects (Cheremisinff, 2002). Primary treatment remove solids by sedimentation and floating materials (scum) by skimming. About 50% of the total suspended solids, and upto 50% of the biochemical oxygen demand (BOD_5), phosphorous and nitrogen are eliminated. The sludge and the scum are removed and taken to the sludge processing units via pumping (Topare *et al.*, 2011).

Secondary treatment further treats the effluent from primary treatment to get rid of the organics and suspended solids. It utilizes biological processes to remove biodegradable organic matter releasing carbon dioxide, ammonia and water. Common high-rate processes that are used for aeration include rotating biological contactors (RBC), trickling filters or biofilters and oxidation ditches. Tertiary treatment removes pollutants in wastewater that are not removed by secondary treatment such as some eutrophic nutrients and heavy metals (Ta *et al.*, 2016). In some treatment plants, disinfection follows the tertiary treatment. It involves the addition of a chlorine solution to remove bacteria due to its bactericidal effects. The strength of the wastewater determines the chlorine dosage. Ultra violet (UV) irradiation and ozone are also used for disinfection in some treatment plants (Das, 2001).

Natural biological treatment systems

They are also known as non-conventional treatment plants. They include wastewater stabilization ponds and constructed wetlands. They are easy to operate and maintain, require low capital and are eco-friendly. They are usually effective in removal of pathogens when not overloaded (FAO, 2007).

Wastewater stabilization ponds

Wastewater stabilization ponds uses natural wastewater treatment technology. Wastewater is treated naturally by use of anaerobic and aerobic bacteria in the ponds. The concept behind stabilization ponds is that wastewater contain organic matter that acts as food for microbes. These microorganisms convert the organic matter to carbon dioxide and water via respiration process. The energy obtained is used for growth and development. The byproducts of respiration are stable components that do degrade water quality hence stabilization of the organic waste.

These systems are simple, easy to construct, operate and maintain and have good pathogens removal (Bitton, 2005). The efficiency of the ponds depends on the quality of the wastewater (Awuah, 2006). Anaerobic ponds are used as pretreatment for BOD_5, suspended solids and COD removal. This is accomplished by the anaerobic bacteria that decompose the organic matter in the wastewater releasing carbon dioxide and methane (Szabo, 2010). These ponds are 2-5 metres deep (Mara *et al.*, 2007).

The biochemical reactions in anaerobic ponds are in two phases. The first phase is acidogenesis while the second phase is methanogenesis which is a slower rate. Solids in the influent normally settles as sludge in anaerobic pond through the sedimentation process (Coggins *et al.*, 2018). The sludge should be removed when it has reached half depth in the pond and this commonly occurs after two years of operation according to design flow (Alexiou *et al.*, 2003; Mara *et al.*, 2004).

Facultative ponds are usually distinguished as either primary or secondary ponds (Gawasiri, 2003). Facultative ponds have an aerobic zone that is on the upper part and anaerobic zone that is on the lower part (Mara *et al.*, 1992). They are designed for removal of BOD_5 using algae that help oxidize pond. Anaerobic processes takes place in the benthic layer breaking down organic matter and releasing soluble byproducts (Gray, 2004).

Heterotrophic bacteria metabolize suspended organic matter in the wastewater with consumption of oxygen. Algae replenish the dissolved oxygen utilized by the bacteria through the process of photosynthetic. Adequate sunlight and high temperature create conditions which enhance algae growth. Algae also utilize nutrient principally phosphorus and nitrogen presence in the wastewater. The algae and bacteria takes part in BOD removal (Williard *et al.*, 2013).

Wind mitigates short circuiting by mixing the contents in facultative ponds. It also creates turbulence on the surface of the water increasing the amount of dissolved oxygen through water-air interface. Wind mixing is also essential in preventing thermal stratification that can cause anaerobiosis. The longest dimension of the facultative pond should be oriented in the direction of the prevailing wind (Williard *et al.*, 2013).

Maturation ponds are the last ponds in the series with their size and number dependent on bacteria quality that is released in the effluent (Hassan, 2011). They are normally shallow and well oxygenated due to the large population of algae. The purpose of this type of pond is to remove the pathogens and feacal coliforms by oxidation process (Dias *et al.*, 2017). Sedimentation process removes helminth ova and protozoan cysts in stabilization ponds (Verbyla *et al.*, 2017). They also achieve a small removal of BOD_5 but remove more nitrogen and phosphorous than the other ponds (Al-Hashimi *et al.*, 2013).

Wastewater stabilization ponds are suitable for tropical and subtropical regions where there is high sun intensity and consequently high temperatures (Mara *et al.*, 2004). The ponds work well in warm climate and can achieve BOD_5 removal of 60-85% in a short retention time (Alexiou and Mara, 2003). Sludge removal and control of odours through the recirculation process of pond effluent from final ponds are the main operational measures that wastewater stabilization ponds require. These systems has minimal establishment, operation and maintenance cost and are efficient when well designed and maintained. However, they require more land than conventional methods (Al-Hashimi *et al.*, 2013).

Constructed wetland systems

These systems are designed to use aquatic plants to help in wastewater treatment in a regulated environment (Kayombo *et al.*, 2004). They remove most of the pollutants satisfactory for example suspended solids and pathogens (Ratnapriya *et al.*, 2009). Constructed wetlands are ecologically beneficial as they provide habitats for wild animals. They also create good sceneries for recreation and also acts as education centres. They require large sizes of land and can also breed pests such as mosquitoes and flies if not properly designed (Kayombo *et al.*, 2004). They are commonly used for upgrading effluent from stabilization ponds.

Constructed wetlands are capable of removing nutrients from pond effluent and filtering out algae. The utilization of the harvested biomass depends on the type of the accummalated substances in the biomass. Harvested biomass arising from the remediation of nutrients can be used as feedstock for livestock, converted into biogas, composted aerobically to produce fertilizer or used for production of green manure. Harvested biomass arising from the remediation of heavy metals should be dried and then incinerated. The heavy metals in the ash can be recovered if they are economically viable. The ash should be disposed appropriately subject to the type and amount of the heavy metals in the ash, for instance in a well constructed and maintained landfill (Ratnapriya *et al.*, 2009).

Wastewater treatment at University of Eldoret

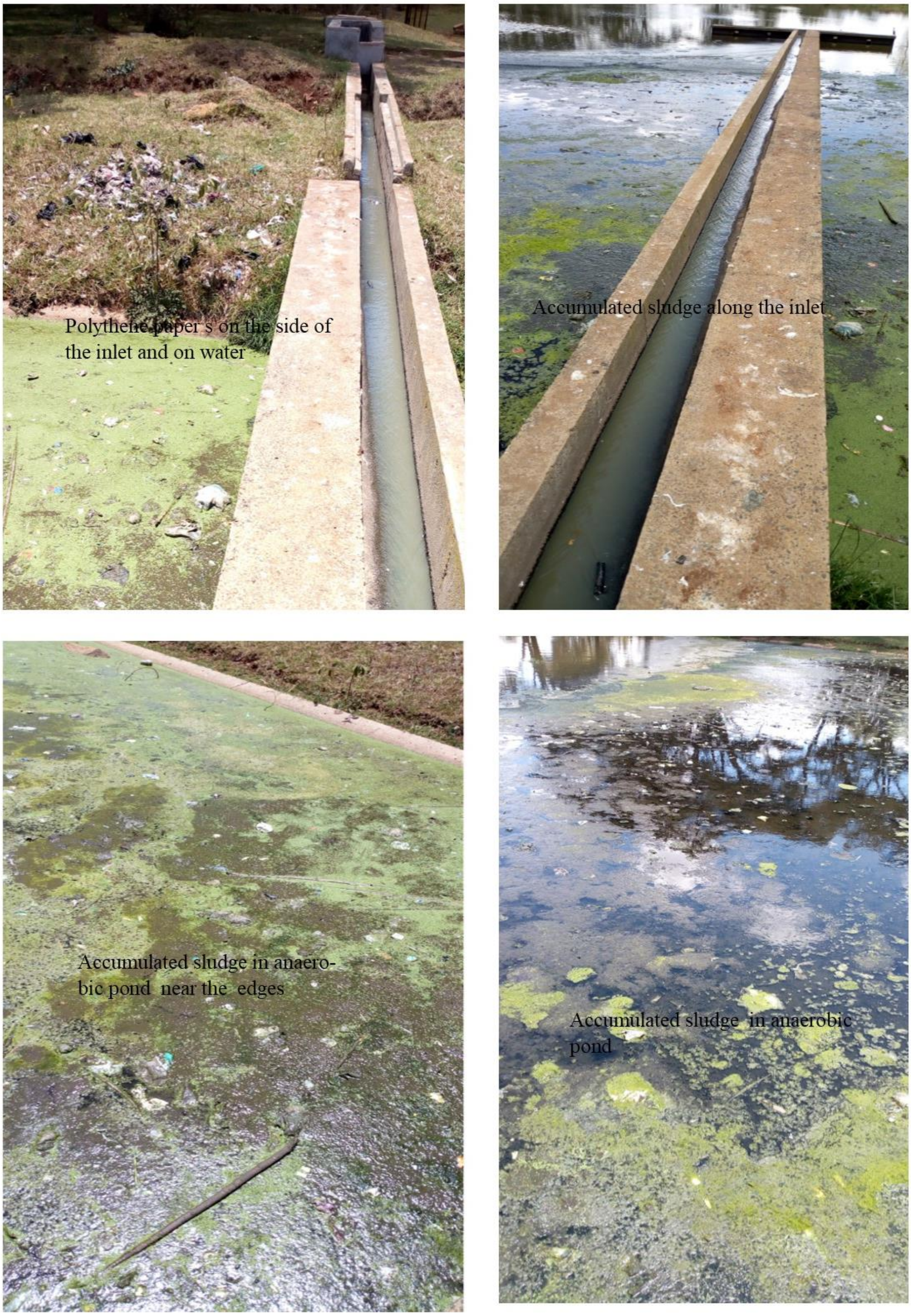
University of Eldoret wastewater treatment plant is situated near University arboretum. Wastewater treatment in UoE is achieved through use of wastewater stabilization ponds. University of Eldoret wastewater undergoes biological treatment in a series of four stabilization ponds. This wastewater originates from various sources such as hostels, lecture room, offices and laboratories found in the University.

The first pond is anaerobic pond whose dimensions are 100 m x 55 m x 1.5 m. Pretreatment takes place in this pond and serves the purpose of removing suspended solids, BOD_5 and some settleable matter by sedimentation process. The raw wastewater is channeled to the first facultative pond (primary pond) whose dimensions are 50 m x 50 m x 1.5 m. This pond drains its effluent to the secondary facultative pond whose dimensions are 50 m x 50 m x 1.5 m. From the second pond, the effluent are channeled to the maturation pond and finally released to the Marura wetland and river Chepkoilel.

Currently, the anaerobic pond of the UoE treatment plant has a lot of sludge that has reduced its depth (Plate 2.1). There is a lot of floating polyethylene bags and plastic bottles on wastewater and also on the sides of the ponds (Plate 2.1). The ponds also lack control valves which are essential for controlling the quantity of effluent being discharge from one pond to the next and also control retention time of influent in the ponds. As a result, there is no predetermined retention time and hence the flow depends on the volume. The pond also produces a rotten egg odor due to increased volumetric load which contains high concentration of organic and inorganic matter. According to Mara *et al.*, (2004) excessive load increases the levels of sulfates and odour. Sulfates are reduced to hydrogen sulfide under anaerobic conditions. A persistence odor that lasts more than ten days indicates that the anaerobic pond has received too much organic load or has an excess amount of sludge accumulation. A properly functioning pond system usually have no odor and when present it is a slightly musty odour (Williard *et al.,* 2013).

Facultative and maturation ponds are covered by algae blooms and duckweed species, a sign of eutrophication (Plate 2.2). Such vegetation growth may cause operational problems such as short circuiting, lack of mixing, improper distribution of influent load and insect breeding. Dense mats of floating macrophytes on a pond systems minimises the amount of sunlight penetrating to the water column. This in turn reduces algae population which are responsible for BOD_5 removal by oxidizing the pond. Reduced sunlight also limits the dissolved oxygen for aerobic bacteria. Floating mats also limit the effects of wind action. Reduced wind action limits the mixing of the ponds contents and amount of DO. Properly operating stabilization ponds should have a minimal vegetation growth (Williard *et al.,* 2013).

The treatment plant aims at degrading most of these nutrients so as to be safe for disposal within the aquatic environment. However, not all the elements within the wastewater are broken down by the natural processes within the ponds due to factors such as increased volumetric load, excessive sludge and flooding due to excess precipitation during the rainy season. The threats related to the release of inefficiently treated wastewater include eutrophication in the receiving aquatic ecosystems, waterborne diseases, loss of biodiversity and odors.



Plates 2.1 University of Eldoret anaerobic pond (Source: Author, 2018)

Plates 2.2 University of Eldoret facultative and maturation pond

(Source: Author, 2018)

2.2 Phytoremediation

The term “Phytoremediation” comprises of the Greek word phyto meaning plant, and Latin word remedian (remove an evil) (Prasad, 2004). Phytoremediation is the concept of utilizing plants to alleviate pollutants in environment (Rew, 2007). It is described as a green biotechnology that facilitates the removal of environmental contaminants (Ali *et al.*, 2013). The principle of phytoremediation is to clean up watewaster that involves selection and utilization of effective macrophyte in accumulation of dissolved contaminats by the plant biomass, its harvesting and beneficial use (Mohanty *et al.*, 2010). Phytoremediation is a good alternative or complementary method that can be used together with or replace mechanical conventional technologies that are capital intensive, require skilled labor and use a lot of energy (Varsha *et al.*, 2010).

Phytoremediation has minimal damage to the ecosystem, is non-intrusive and environmentally sound remediation technology thus the most suitable pollutant removal approach for developing countries (Bruce, 2001). It is a safe alternative to conventional cleanup techniques. This method can be applied in large scale remediation operations in cleanup of various heavy metals. Its solar driven with minimal ecological interruptions (Singer *et al.,* 2007). Phytoremediation is beneficial to the environment since it produces ecofriendly end products at extremely low costs (Cook *et al.*, 2013). The organic contaminants are biodegraded to carbon dioxide and water (Kambhampati, 2013). Phytoremediation technology can be used in phytomining where metal-rich plant residue is obtained. If the accumulated heavy metals are deemed to be economically viable, then they can be recovered from the plants.

In aquatic ecosystems, macrophytes are vital tools for abatement of heavy metals and are therefore considered as phytoremediators in natural aquatic ecosystems (Kumar Rai, 2010). Aquatic plants are of special interest, because they have high capacity to accumulate heavy metals and nutrients in high concentrations compared to terrestrial plants. Aquatic macrophytes are superior to terrestrial plants in wastewater treatment because they have a direct contant with the polluted water, in addition to higher rate of accumulation of pollutant (Dhir *et al.*, 2009).

Phytoremediation techniques have been used by many researchers for remediation of physicochemical parameters, nutrient and other contaminants using macrophytes such as *Eichonia crassipes*, *Pistia stratiote* and *Lemna* species. This technology can be used in removal of the above mentioned parameters in addition to metals, pesticides, hydrocarbons and chlorinated solvents from polluted environment (Macek *et al.*, 2000; Nwoko, 2010).

2.2.1 Mechanisms of phytoremediation

Phytoremediation includes six mechanisms, namely; phytofiltration, phytoextraction, phytotransformation, phytovolatilization, phytostimulation and phytostabilization (Singh *et al.*, 2012)

Phytoextraction

In phytoextraction, plants assimilates, concentrate and precipitate the pollutants in their tissues. This mechanism, also referred to as phytoaccumulation involves biological processes such as pollutant acquisition, transportation and accumulation in shoot. Plants absorb and accumulate pollutants in their aerial tissues which are latter harvested (Huang *et al.*, 2004; Li *et al.*, 2012). Substances that are phytoremediated through this process include radionuclides and heavy metals that are resistant to plant metabolism. The accumulated metal can be recycled from the ash and organic pollutants can be composted (Ficko *et al.*, 2010). Phytoextraction is the best mechanism in elimination of contaminants from soil, sediment and sludge (Fässler *et al.*, 2010).

Phytotransformation

It is also referred to as phytodegradation, a mechanism in which contaminants are taken up from the medium which could be water or soil and biodegraded to non-toxic compounds through the action of plant metabolites such as enzymes (McGuinness *et al.*, 2009). The processes that take place in phytodegradation are similar to the metabolisms of xenobiotics chemicals by man (Burken, 2003).

Phytovolatilization

Phytovolatilization involves the absobtion and movement of water soluble pollutants by plants with consequent contaminant release to the atmosphere without the need of harvesting or disposal (Vaněk *et al.*, 2010). After uptake in plant tissues, volatile chemicals are released as a gas via evapotranspiration process. Both water-mobile and air-mobile organic pollutants move up the plant through diffusion process. Volatile Organic Compounds (VOCs), total petroleum hydrocarbon and polychlorinated biphenyls (PCBs) use this mechanism to move in plants (Macek *et al.*, 2000).

Phytostimulation or Rhizodegradation

It is also referred to as rhizosphere bioremediation. In this mechanism, the biodegradation of organic contaminant is transformed by microorganisms for instance bacteria and mycorrhizae in the rhizosphere (Bisht *et al.*, 2015). This mechanism uses microorganisms to assimilate organic substances for energy production and growth. Plant roots provide food for microbes in soil by releasing compounds like alcohols, acids and sugars that contain organic carbon (Cébron *et al.*, 2009). This remediation process takes place fully without plant uptake of the contaminant in the rhizosphere. Hydrophobic organics are not absorbed by plants, however rhizosphere microbes can biodegrade them through the process of phytostimulation. Such substances include polychlorinated biphenyls (PCBs), Poly Aromatic Hydrocarbons (PAHs) and other petroleum hydrocarbons (Singh *et al.*, 2012)

Phytofiltration

This is the process in which plants assimilate, concentrate and precipitate pollutants from contaminated aquatic environment by their roots (Singh *et al.*, 2012). It is also referred to as rhizofiltration, a mechanism that minimizes the mobility of pollutants and controls migration to the ground water (Rawat *et al.*, 2012). Rhizofiltration is exhibited by the accumulation of contaminants in the rhizosphere. It can be utilized for remediation of heavy metals for example nickel, copper, cadmium, lead, chromium, zinc and mercury which are primarily retained within the roots (Ghosh and Singh, 2005; Moreno *et al.*, 2008). Terrestrial plants are a better choice due to their long and fibrous root system that increase the rhizosphere surface area enhancing the removal of the harmful pollutants (Jadia *et al.*, 2009).

Phytostabilization

Phytostabilization also referred to as phytoimmobilisation involves utilization of selected plant to render pollutants in the environment immobile via uptake and concentration in plant biomass, roots adsorption or precipitation within the rhizosphere inhibiting their mobility in soil, in addition to their mobility by erosion(King *et al.*, 2008). It may also involve the reduction of metal valence. Plant roots hinder pollutants movement and bioavailability by barring direct contact with contaminated soil (Schnoor, 2002). Plants play an essential secondary role of mitigating soil erosion and enriching the rhizosphere with nutrients that promote the growth of microoganisms thus enhancing bioremediation mechanisms. Trees and grasses are preferred for phytostabilization because they have long and fibrous roots that bind and hold the soil (King *et al.*, 2008).

2.2.2 Factors affecting phytoremediation of pollutants

Various factors influence the absortion mechanism.These include pollutant bioavailability, medium properties (soil or water), plant type, nutrient supply for plants and rhizosphere microbes.

Pollutant Bioavailability

Bioavailability determines pollutant toxicity and accessibility to both plants and the rhizosphere microorganisms (Abbaslou *et al.*, 2017). For pollutants to be remediated effectively, they should be in contact with the plant and its associated microrganisms. Pollutant bioavailability depends on biological activity, soil properties, pollutant chemical properties and the abiotic factors for example temperature. Hydrophobicity and volatility affects the mobility of contaminats in soil(Abbaslou *et al.*, 2017). Soil properties that influence the bioavailability of contaminants include: texture, structure, humus, water content and pH (Parrish *et al.*, 2005).

Medium properties

The medium (soil or water) influence the rhizosphere communities that have varying physicochemical characteristics such as pH and aeration resulting in distinct microbial communities. The properties of the medium affect the efficiency of phytoremediation process through the availability of nutrients and oxygen (Abbaslou *et al.*, 2017). The amount of organic matter in the medium has a positive correlation with cation exchange and binding capacity to hydrophobic organic pollutants. The reason being that organic matter usually have high content of decaying plant biomass (Burken, 2003).

Plant type

Plant types differ significantly in regard to the rhizosphere microbial consortium, root exudation, root parameters such as morphology, fine root turnover and root decay. Generally, there are several properties of plant that affect phytoremediation of pollutants. They include; tolerance to pollution, high rate of growth, photosynthetic activity, hardy, high biomass and competitive. The age of the plant influence its physiology for example root activities. More often, young plant roots have higher potential to sorb ions than old plant roots of equal size. Young plants are used for effective contaminant removal (Cofield *et al.*, 2008).

Supply of nutrients

Availability of nutrients is vital in phytoremediation and can be enhanced though addition of fertilizer. Growth and survival of both plants and microorganisms in polluted medium is important as it governs the success of phytoremediation (Álvarez-López *et al.*, 2016). There are three groups of nutrients in relation to the quantity and their critical requirement by microbes in the rhizosphere: macro, micro and trace nutrients. Macronutrients comprise of carbon, phosphorus and nitrogen, the micronutients include calcium, sulfur and magnesium while the trace elements consist of iron, copper, manganese, zinc and cobalt (Abbaslou *et al.*, 2017). Generally, the amount of nutrients that the microorganisms require is proportional to the composition of their cells (Sessitsch *et al.*, 2013). Normally phosphorous and nitrogen are the limiting inorganic nutrients in bioremediation and phytoremediation processes.

Environmental factors

Temperature and moisture affect the remediation of contaminants. Various studies have reported that increase in temperature leads to increase on plant growth which has a positive influence on phytoremediation (Parrish *et al.*, 2005).

2.3. Metabolism of pollutants during phytoremediation process

The particular interactions of a contaminant with the medium and plants is determined by the contaminated medium, the physiological properties of the plant species and the chemical properties of the pollutant. These factors define the mechanism to be used in degradation of a pollutant. In all the mechanisms, phytoremediation starts with the transportation of the pollutant to the plant. Organic substances can be transported to plant tissues and consequently volatilized, they may be fully or partially degraded, changed to less harmful substances and bound in plant tissues (Salt *et al.*, 1998). Usually, a variation exists in the absorption of organics and inorganics contaminants by plant roots. Mobility of organic contaminants within plant cells is facilitated by the process of diffusion, based on their chemical properties (Greipsson, 2011).

2.4 Aquatic macrophytes used in phytoremediation

Aquatic macrophytes consist of a diversified group of photosynthetic plant that are macroscopic. They include bryophytes, pteridophytes (ferns) and aquatic spermatophyte. Macrophytes are classified into four categories depending on their growth forms: The first category includes plants which are rooted in the sediments and emerging above the water column to significant heights. These are referred to as emergent plants, for example *Typha* Spp. The second category comprises of floating leaved macrophytes. These grow on sediments and within a water depth in the range of 0.5-3.0m, for example *Nymphaea* spp., *Potamogeton pectinatus* and *Potamogeton schweinfurthii* (Chambers *et al.*, 2007). The third category includes submerged macrophytes. These are plants that grow completely below the water surface, examples include; *Myriophyllum spicatum*, *Hydrilla* sp., *Ceratophyllum* spp. and Charophytes. The fourth category consists of free-floating macrophytes. These are a diverse group of plants which are not rooted to substratum. Examples include, *Azolla* spp., *Salvinia* spp., and *Lemna* spp. (Chambers *et al.*, 2007).

Macrophytes improve water quality, promotes biodiversity, mitigate climate change by sequestering carbon dioxide, reduce biomethylation and improve hydrological functions (Priya *et al.*, 2012). Some macrophytes species show impressive abilities to assimilate pollutants from the environment. Various macrophytes have been utilized in wastewater treatment (Prasad, 2007). Several of them have been reported to be adequate phytoremediators such as Azolla, *Pistia, Lemna, Wolffia, Hydrilla, Salvinia, Typha, Ceratophyllum, Potamogeton* and *Eichornia crassipes.* The efficiency of the macrophyte is subject to the nature of pollutants and the type of wastewater (Priya *et al.*, 2012).

Macrophytes are efficient in phytoremediation due to their vigorous growth rate, rapid reproduction rate, adaptability to a wide ecological range, simple nutrients requirements and ability to accumulate contaminants (Prasad, 2007). Macrophytes enhance sedimentation along with nutrients retention in their enormous biomass. Their uncontrolled growth is due to their extraordinary potential in uptake of large amounts of nutrients (Mufarrege *et al.*, 2010). This makes them ideal biological filters of water thus can be used to improve the quality of effluent from stabilization ponds (Priya *et al.*, 2012). Some plant species especially hyper accumulators are employed in the evaluation of persistent and acute toxicity of metals and as indicators of aquatic pollution. Research has indicated that during the pollutant stress period, these macrophytes release phytochelatins which form complexes with heavy metals and detoxify them (Mishra *et al.*, 2009).

Currently, there is increased global use of macrophytes in contaminant removal from polluted aquatic ecosystem. Macrophytes are fantastic candidates in research due to their potential as heavy metal accumulators without exhibiting impaired physiological abnormalities (Marques *et al.*, 2009). Several macrophytes have been reported to absorb heavy metals like cadmium, lead, nickel and zinc and could also be used to concentrate and reclaim valuable metal (Chotpantarat *et al.*, 2011). Macrophytes have higher potential to remove higher levels of heavy metals contaminants than terrestrial plants. This is because in water, metals are already present in aqueous form thus sorption by the plants can be achieved easily, while in soils, metals have to be soluble before plants can absorb them (Dhote *et al.*, 2009). Appropriate selection of the plant may make phytoremediation more efficient and reliable in wastewater treatment (EPA, 2000). Some of the macrophytes used in wastewater cleanup are cattail, water fern, coontail, water lily, duck weed, water lettuce and water hyacinth.

2.4.1 Water Fern (*Azola pinnata)*

Azolla pinnata (Plate 2.3**)** is also reffered to as water fern, a free-floating macrophyte (Parikh *et al.*, 2015). It grows naturally in most parts of Africa, Asia and parts of Australia in calm and quiet aquatic ecosystems. The frond consists of many rounded leaves that are green or dark red in color with a velvet appearance. Reproduction is through vegetative propagation (Wagner, 1997). Leaves contain the cyanobacterium *Anabaena azollae* (*Nostocaceae*) that fixes atmospheric nitrogen utilized by the fern (Banach *et al.*, 2012). *Azolla pinnata* has capacity to absorb heavy metals such as lead (Arora *et al.*, 2004) and nutrients (Forni *et al.,* 2001) from polluted water.

Plates 2.3 *Azolla pinnata* (Source: Useful tropical plants database 2014)

Azolla sp. has a short doubling time (2-3 days), is easy to propagate and manage and tolerant to several pollutants. These factors make it an excellent candidate for phytoremediation (Sood *et al.*, 2012).

2.4.2 Water lily (*Nymphaea* spp.)

Nymphaea (plate 2.4) is a genus of the family Nymphaeaceae. The family has eight genera with about 70 known species (Slocum, 2005). It grows in various parts of East Africa and Southeast Asia. Leaves blades can be rounded and pointed (Group, 2009).

Plates 2.4 *Nymphaea* spp. (Source: Useful tropical plants database 2014)

Most *Nymphaea* species exhibit protogynous flowering (Les *et al.*, 1999). Shades of water lily flower range from white, purple, blue, yellow and pink with several species alternating from a light to darker color as the flowering season comes to the end (Povilus *et al.*, 2014). Water lily has the potential to absorb and concentrate heavy metals like chromium and nickel in its biomass (Ziarati *et al.*, 2015; Khan *et al.*, 2016). Water lilies biomass form mats covering the water surface and thus reducing evaporation. Therefore, these plants may also play a role in water conservation.

2.4.3 Cattail (*Typha latifolia*)

Typha latifolia (Plate 2.5) is commonly known as common cattail. Plants are typically unbranched and grow to a height of 1.5-3 meters (Species, 2019). The optimum condition for its growth are flooded area, temperatures range of 10-25°C, rainfall range of 500 - 1500mm and pH range of 4.5-7.5 (Group, 2016). It does not tolerate shading during the growth period. Reproduction is by seeds or vegetatively by rhizomes (Species, 2019). Sexual reproduction is by seeds and invasion of new areas is accomplished through seedling establishment. *Typha latifolia* stands are habitat for wildlife and birds (Lansdown *et al.*, 2014).

Plates 2.5 *Typha latifolia* (Source: Useful tropical plants database 2014)

Typha sp. are vital in the process of phytoremediation due to their high growth rate, absorbing excess mineral nutrients, especially phosphates and nitrates and other pollutants from naturally occurring water bodies, constructed wetlands and wastewater stabilization ponds. It can also be used for bioremediation of toxic spill. For these reasons, they are referred to as water purifiers of untapped value (Anning *et al.*, 2013).

2.4.4 Coontail (*Ceratophyllum demersum*)

Ceratophyllum is a group of submerged free floating, flowering plants of the Ceratophyllaceae family, order Ceratophyllales. Its common name is hornwort or coontail due to its feathery leaves that are arranged in whorls on the stem resembling a raccoon’s tail. *Ceratophyllum* grows completely submerged in quiet and calm waters. It can grow in temperatures of 15-30°C (Christenhusz *et al.*, 2016).

Plates 2.6 *Ceratophyllum demersum* (Source: Useful tropical plants database 2014)

Stems of *Ceratophyllum demersum* can reach 1-3m in length. They produce fluffy, filamentous, bright-green leaves that are narrow and much branched. The leaves are forked, brittle and stiff to the touch. *Ceratophyllum dermesum* are monoecious with tiny and inconspicuous flowers (Group, 2009). The plants have no roots at all, but sometimes they produce modified leaves with a root like appearance, which anchor the plant to the bottom. The plant is vegetatively propagated. *Ceratophyllum demersum* has allelopathic effects that hinder cyanobacteria and phytoplanktons growth.

2.5 Heavy metals

Heavy metals are grouped into two; essential and non-essential. The essential metals are important in the growth and development of organisms for example, iron, manganese, copper, cobalt, molybdenum, vanadium and zinc that play key role in metabolic activities. The non-essential metals are not required by organisms and are normally toxic at low concentrations (Kabata-Pendias, 2011). They incude mercury, arsenic, lead, cadmium and chromium. Some of the metals that are encountered as contaminats in the environment include cadmium, lead, copper and zinc (Sánchez-Chardi *et al.*, 2009).

2.5.1 Sources of heavy metals

Two main sources of heavy metals in the ecosystem are natural and anthropogenic. The natural sources may be due to soil erosion and volcanic activities (Kaizer *et al.*, 2010). The human sources include mining operations, electroplating processes, metal finishing, nuclear power and textile industries. Volcanic eruptions release several heavy metals for instance zinc, lead, mercury, rubidium, gold, arsenic, magnesium, aluminum and copper (Amer *et al.*, 2010). Anthropogenic activities such as combustion of fossil fuel, discharge of municipal wastes and overuse of fertilisers and pesticide increases the levels of heavy metals in the ecosystem (Kabata-Pendias, 2011).Transportation of leachates contaminates non polluted ecosystems (Kamran *et al.*, 2014).

2.5.2 Effects of heavy metals

Heavy metals pollution has adverse effect on biota as it does not undergo biodegradation. They have longterm residence in soil thus poses several health risks to the organisms in the higher trophic levels as a result of bioaccumulation and bio magnification (Uqab *et al.*, 2016). Heavy metals has negative impacts on soil microbes, decrease the rate of plant growth and contaminate ground water. Bioaccumulation of heavy metals in the ecosystem from water to soil, to plant and finally to animals leads to undesirable consequences in organisms (Sardar *et al.*, 2013).

High concentrations of heavy metals in animal's body result in poor health, reproduction problems, compromised immunity and occurrence of tumours (Tunegová *et al.*, 2016). Human's exposure to heavy metals occurs via various routes, which include ingestion through food and drink, inhalation as dust or fume and vaporization. The nature of heavy metals poisoning on human beings may be toxic, teratogenic, carcinogenic, mutagenic or neurotoxic. Metal toxicity is correlated to age, sex, route of exposure, duration of exposure, level of intake, absorption rate, metal oxidation state, solubility, frequency of intake and mechanisms of extraction (Duruibe *et al.*, 2007). These harmful effects are cumulative, thus regular intake of low concentrations of heavy metals for instance lead, can result in major effects on growth and development of children, for example mental development retardation and hearing impairment (Tunegová *et al.*, 2016).

2.6 Mechanism used to remove heavy metals

Physicochemical methods are normally employed in removal of heavy metals from wastewater, however they are very costly and at times produces a toxic sludge which requires further treatment. Some of the conventional techniques employed in heavy metals removal from waste water include the following; reverse osmosis (Sudilovskiy *et al.*, 2008), ion exchange (Hubicki *et al.*, 2012), chemical precipitation and electrodyalis (Barakat, 2011).

Due to the drawbacks of the conventional methods, the search for a novel, efficient, simple and ecofriendly technology in heavy metal removal from wastewater has pointed towards phytoremediaton. Phytoremediation is an efficient and affordable technology providing a solution that is applicable in the extraction of metal contaminants from contaminated environment (Chandra *et al.*, 2009). The application of aquatic plants especially hyper accumulators in metal removal is a step towards achieving environmental sustainability (Shaharuddin *et al.*, 2012).

2.7 Heavy metals of interest in water pollution

Heavy metals of major concern in respect to water pollution are lead, nickel, cadmium, mercury, zinc, copper, chromium and cobalt. These metals gradually reduce organism abundance and this may modify important ecosystem functions for example, decomposition rates, oxygen dynamics and nutrient cycling (Si, 2014). High levels of cadmium and lead in the environment rises alarm based on their adverse effects on human health. They are unimportant to organisms and are readily transferred via food chains (Sigel *et al.*, 2013; Kang *et al.*, 2014).

2.7.1 Cadmium

It is a silver-white, odorless, grayish or blue tinged white powder, having an atomic weight of 112.4 and atomic number 48. It has a density of 8.69g/cm^3, with a melting point of 321.07°C and a boiling point of 767°C. All cadmium compounds have an oxidation state of +2, although they also exist in the +1 state (Al-Ubaidy *et al.*, 2015). The earth's crust contain 0.16 ppm and the soils contain 0.1 to 0.5 ppm (APHA *et al.*, 2005).

Contamination by cadmium mostly arises from mining, electroplating, paint pigments, plastics manufacturing, alloy preparation and batteries (Singh *et al.*, 2010). Cadmium is used in industrial tools and fasteners such as nuts, bolts, screw and nails. It is an impurity in detergents, phosphate fertilizers and refined petroleum products (Morrow, 2000). Anthropogenic activities such as mining and fertilizer application on farm increases soil contamination with cadmium (Monachese *et al.*, 2012).

Many toxicological studies indicates that cadmium causes structural and functional changes in the lungs, kidneys, ovaries, liver and bones (Castro-González *et al.*, 2008). Cadmium is responsible for kidney tubular impairment and osteomalacia. Moreover, it is associated with prostate cancer, high blood pressure, mutations and fetal death (Nagajyoti *et al.*, 2010). In high-exposure areas, chronic poisoning of the population can lead to Itai-itai disease. This diseases was reported in Toyama, Japan, where a river contaminated by cadmium led to the onset of chronic poisoning of the community resulting to Itai-itai disease (Kobayashi *et al.*, 2009).

2.7.2 Copper

Copper is a reddish metal which is extremely ductile, malleable and a good conductor of heat and electricity. It has an atomic weight of 63.546 and atomic number 29. It has a density of 8.96g/cm^3 at 20°C with a melting point of 1,083°C and a boiling point of 2,567°C. It has an oxidation states of +1 and +2 (Meija *et al.*, 2016). Copper compounds are used in electroplating and in preservation of wood. It is used in roofing, wiring, in the laboratories and in cooking utensils. Copper sulfate pentahydrate is used as algaecides on surface waters (NRC, 2000). It is also used in fungicides and insecticides. Dissolved copper can sometimes impart a bitter taste to drinking-water (Zacarías *et al.*, 2001).

Low concentration of copper is important for plant growth and development, where it is vital for numerous metabolic activities but is toxic at higher concentrations (Zacarías *et al.*, 2001). The rate of copper accumulation in the bodies of fish and mollusks is very high compared to the rate at which it is excreted out (Mishra *et al.*, 2008). In humans, copper is required as a trace element for various physiological processes (Barceloux *et al.*, 1999a;). Excessive copper may reach biota due to pollution of the environment caused by man (NRC, 2000).

2.7.3 Nickel

Nickel is a silvery-white lustrous transitional metal with a golden tinge. It is a chemical element with symbol Ni, atomic number 28 and atomic mass of 58.69. It has a melting point of 1455°C and a boiling point of 2913°C. Nickel has a density of 8.91g/cm^3 and an atomic volume of 6.59cm^3/mol (Meija *et al.*, 2016). Nickel is malleable and ductile. It has an oxidation state of +2 and five stable isotopes (Carnes *et al.*, 2009). Nickel mainly occurs in ores but can be occasionally found free in nature (Stixrude *et al.*, 1997; IARC, 2012). It is used in microphone capsules, coinage, stainless steel, magnets and alnico and in making alloys with copper and silver (Davis, 2000). Nickel is essential in the biological functions of some plants, archaebacteria, fungi and eubacteria (Sydor *et al.*, 2013; Zamble *et al.*, 2017).

Nickel compounds are classified as human carcinogens (Colditz, 2015). Human exposure to nickel commonly results to allergic reaction resulting to skin reaction contact dermatitis and sometimes asthma to more sensitive person. Moreover, nickel also can cause lung problems such as bronchitis and cancer. Nickel may function as a prebiotic for the bacteria found in large intestines (Das *et al.*, 2008). The major source of nickel exposure is oral consumption (Haber *et al.*, 2017). Nickel is hazardous at 10 mg/m^3 and hence a risk to public health (Barceloux *et al.*, 1999b).

2.7.4 Cobalt

It is a silver-gray, lustrous, hard ferromagnetic metal that has atomic weight of 58.933 and atomic number 27. It has a specific gravity of 8.9 g/cm^3. It is a chemical element with symbol Co. Its oxidation states include +2 and +3, however compounds with oxidation states that range from −3 to +5 exists. Cobalt has only one stable isotope ^{59}Co, that exists naturally on earth (Meija *et al.*, 2016). Cobalt is utilized in the production of high-strength alloys that are corrosion resistant hence useful for making orthopedic implants and in external beam radiotherapy. Cobalt is also used for food sterilization by radiation treatment. Cobalt has been used in jewelry, sculputure, batteries for mobile device and in rechargeable batteries for electric cars (Biggs *et al.*, 2005).

Cobalt is an important element for organism wellbeing at low concentrations. It is a key constituent of coenzymes called cobalamins (vitamin B_{12}) (Yamada, 2013). Cobalt is also a micronutrient of microorganisms in inorganic form (Cracan *et al.*, 2013). Inhalation of cobalt causes respiratory problems. It also causes contact dermatitis, a skin problems to the people who work in cobalt mining industries. Chronic cobalt intake may lead to serious health problems at low doses (Basketter *et al.*, 2003).

2.7.5 Lead

It is a silvery-grey or bluish metal with atomic number 82 and atomic weight of 207.19. It has specific gravity of 11.34, with a melting point of 327.5°C and a boiling point of 1740.0°C. The oxidation state of lead is +2 (Meija *et al.*, 2016). Primary sources of lead includes; mining operation, smelting, industrial production processes and their emissions, solid waste incinerators and combustion sources. Other sources include lead piping, lead batteries, paint and combustion of coal. Lead gets to the water system through storm water or discharges from domestic and industrial wastewater treatment plants. Industrial areas are likely to have high levels of Lead (Mielke *et al.*, 2011).

Lead is one of the most hazardous contaminant which show adverse effects on organisms. Lead pollution in the environment is normally limited to contaminated areas where it exists as an insoluble form. It causes serious public health impacts such as acute toxicity when present in high concentrations. Corrosion of household plumbing system that are made of lead and erosion of natural lead deposits usually pollutes water (El-Khatib *et al.*, 2014). When lead is ingested by human beings, it is transported through the blood stream and stored in bones, teeth and soft tissues. Lead also affects the nervous system, the kidney and the brain. In aquatic ecosystem, lead affects invertebrate species that are less tolerance to pollution. It reduces the ability of aquatic biota to adapt to anoxia conditions (Waranusantigul *et al.*, 2011).

2.7.6 Chromium

Chromium is steely-grey, lustrous, hard and brittle metal that has atomic weight of 51.996 and atomic number 24. It has a density of 7.15 g/cm^3. It is a chemical element with symbol Cr. It has several oxidation states which include −4, −2, −1, +1, +2, +3, +4, +5 and +6.

Naturally occurring chromium has three stable isotopes although other isotopes can also occur (Meija *et al.*, 2016). Chromium is found in solid state at standard temperature and pressure. It has a melting point of 1907°C and a boiling point of 2671°C. It is highly resistance to corrosion.

More often, chromium is added to steel to make stainless steel that is resistance to corrosion and discolouration (Zhao *et al.*, 2001). Chromium salts are used in industrial processes for example in metal ceramics, chrome plating and as a catalyst in dyes (Dennis *et al.*, 1993). Chromium (III) salts, especially chromium (III) sulfate and chrome alum are used in leather tanning. It is also used as a mordant for fabric dyes (Sreeram *et al.*, 2003). Chromium is applied in metal surface refinery, in electroplating, in car decorations and furniture parts and in plumbing fixtures (Dennis *et al.*, 1993).

Hexavalent Chromium is toxic and carcinogenic (Program, 2016). Health issues associated with chromium (VI) include; ulcers, stomach upset, kidney failure, ulcerations, dermatitis, irritation, edema and death (Doisy *et al.*, 2013). Chromium does not accumulate in the bodies of fish, however high levels of chromium in aquatic ecosystems can damage their gills (Amoikon *et al.*, 1995). Both hexavalent and trivalent states of chromium may exist in water supplies in trace amounts as chromium does not occur in nature. Abandoned chromium mining sites often require environmental cleanup and remediation (Baselt, 2008).

2.7.7 Manganese

Manganese is a hard, brittle, gray-white or silver-gray metal with atomic number 25 and atomic mass of 54.938. It is a chemical element with symbol Mn that is difficult to fuse, but easy to oxidize. It has specific gravity of 11.34, with a melting point of 1245°C and a boiling point of 1862°C. Manganese has one key isotope ^{55}Mn. It has at least 4 stable oxidation states with distinctive colors, these are +2, +3, +4 and +7. It has a density of 7470kg/m^3 and normally exists as a solid at 20°C (Meija *et al.*, 2016).

Manganese is important in industrial application where it is used to form alloy particularly in stainless steels resulting in better properties such as toughness, stiffness, wear resistance, hardness and most importantly strength (Elliott *et al.*, 2018). It is used in glass industry for colouration and also in industries as tinctures of several colors (Chen *et al.*, 2016; Jansen *et al.*, 2017).

In biology, manganese helps in formation of connective tissue in human beings, lack of manganese or decreased supply results in less flexible ligaments and muscles (Takeda, 2003). A number of polypeptides contain manganese, these include manganese containing superoxide dismutase, arginase and diphtheria toxin (Erikson *et al.*, 2019). Manganese (II) ions is vital in metabolism of macronutrients and bone formation. Manganese enzymes are essential in free radical defense systems (Gallicchio, 2014). Exposure of school-age children to excessive manganese is linked to increased intellectual impairments and reduced intelligent quotient (Bouchard *et al.*, 2010).

2.7.8 Zinc

Zinc is a bluish-white, lustrous, transition, diamagnetic metal. It is a chemical element with symbol Zn and atomic number 30. Its normal oxidation state is +2. It is hard and brittle at most temperatures but becomes malleable between 100°C and 150°C. Zinc is a fair conductor of electricity. It has a melting point of 419.5°C, a boiling point of 907°C and a density of 7.133 g/cm^3 at 25°C. Zinc has five stable isotopes (Meija *et al.*, 2016). Zinc compounds have various uses. Several zinc compounds are mostly used, such as zinc sulfide that is used in television screens, clocks, in x-ray equipment and in paints. Antifouling paints contain zinc pyrithione (Konstantinou *et al.*, 2004). Natural processes such as weathering add zinc to the environment however, human activities has contributed the highest zinc levels in the environment through mine waste, sewage sludge, combustion of fossil fuel, pesticide, limestone, particles from galvanized surfaces and phosphate fertilizers (Broadley *et al.*, 2007). Zinc oxide is also used to make various products including make-up, rubber and prescription drugs (Roldán *et al.*, 2003).

Zinc is an essential trace element for good eyesight, taste, smell and memory and its deficiency cause malfunctions of the organs concerned (Hambidge *et al.*, 2007). Although zinc is vital for good health, excess zinc can be harmful. Concentrations greater than 5 mg/l lead to bitter taste in addition to opalescence in alkaline water (Hambidge *et al.*, 2007). Negative impacts of excessive long-term zinc consumption include, hypochromic microcytic anaemia, copper deficiency, nausea, diarrhea, abdominal cramping and genitourinary complications (Saper *et al.*, 2009). Zinc usually finds its way into the household water distribution from deteriorated galvanized iron sheets, dezincification of brass and industrial effluent. Soluble zinc is toxic to fish, invertebrates and plants (Eisler, 1993). Micromolar amounts of the free ion kills some organisms such as Daphinia in water. (Muyssen *et al.*, 2006). Zinc concentrations of 2 ppm can have adverse effects on the amount of oxygen circulated in the body of fish (Heath, 2018).

2.7.9 Iron

Iron is a silvery white transitional metal which is extremely malleable. Iron is found in free metallic state in nature. It has an atomic weight of 55.847 and atomic number 26. It has a density of 7.86g/cm^3 at 20°C with a melting point of 1538°C and a boiling point of 3000°C. Iron has oxidation states of +2, +3, +4 and +6. Iron is a mixture of four stable isotopes (Meija *et al.*, 2016). Iron is essential in the synthesis of hemoglobin. In average, the quantity of iron in the human body is about 4.5gms of which approximately 65% is in the form of hemoglobin, which is responsible for oxygen transportation from the lungs to the other parts of the body (Okam *et al.*, 2017).

2.8 Physicochemical parameters

2.8.1 Temperature

Temperature is important as it affects other properties of wastewater. Many of the physical, chemical and biological characteristics of wastewater are affected by temperature. Respiration rate of organisms is temperature-related, it can increase by 10% or more per 1^{o}C rise in temperature. Thermal pollution leads to significant decline in aquatic life (Andere *et al.*, 2018). Temperature is affected by time of the day, air temperature, sunlight, intensity of clouds, season and depth at which water is sampled. Wastewater influent can also affect temperature. In most cases, wastewater temperature is higher than the local water sources due to high content of warm water from industrial sources or domestic sources.

Biological wastewater treatment works best at a temperature range of 25 to 35°C. Increase in temperature increases the reaction rate in anaerobic ponds up to the optimum temperature. Microbial reactions are reduced at lower temperatures while nitrification stops at very high temperatures. Very low or very high temperatures decreases the metabolism activities of bacteria and algae hence diminishing the efficiency of treatment plants. The optimum temperature for methane forming bacteria is above 20°C and methane production rate increases twice as much for each 10°C to 15°C rise in temperature in the atmosphere (Williard *et al.*, 2013).

2.8.2 Dissolved Oxygen

Dissolved oxygen refers to microscopic bubbles of gaseous oxygen that are dissolved in the water column thus available to aquatic organisms for respiration. There are various factors that determine the amount of dissolved oxygen in a water body. These includes; water temperature, light, the number of photosynthesizing plants, the rate of respiration and decomposition. In stabilization ponds, the amount of dissolved oxygen is vital for bacterial respiration. The DO levels should be assessed before and after wastewater treatment in order to determine the rate of biological activities within the treatment system. Discharge of organic waste alters oxygen balance of the receiving aquatic ecosystems because their breakdown utilizes oxygen (Omoto, 2006).

2.8.3 Potential of hydrogen

Potential of hydrogen (pH) is the measure of the acidity or alkalinity of an aqueous solution on a scale from 1–14. It can also be defined as the measure of the number of H^+ hence a measure of acidity. The balance of positive hydrogen ions and negative hydrogen ions determine the acidity or basicity of an aqueous solution (Gray, 2004). Water pH affects the solubility of many chemicals consequently affecting their availability to aquatic organisms in the effluent receiving aquatic ecosystems. Water pH is affected by the chemicals discharged into the wastewater from industrial and domestic sources (Gray, 2004).

2.8.4 Total Dissolved Solids

Total Dissolved Solids refers to the portion of particles in water which are capable of passing from end to end of a filter of 2μm or smaller standard aperture dimension satisfying particular condition. TDS normally measures the amount of salts dissolved in water. Total dissolved solids consists of inorganic salts mainly chlorides, sulfates, nitrates, sodium, phosphate and other ions. High levels of TDS reduce the rate of photosynthesis in plants (Gray, 2004).

2.8.5 Conductivity

It is the measure of the potential of an aqueous solution to conduct electric current and is denoted by the letter, *k*. This capacity is determined by the presence of ions, their total concentration, their valence and mobility in solution. It also depends on the temperature of measurement (Shoemaker *et al.*, 1989). Conductivity measurements are carried out in order to determine the degree of mineralization and assess the effect of the total concentration of ions on chemical equilibrium and physiological effects on plants and animals. It is measured with the help of EC meter. The SI unit for conductivity is Siemen per centimeter (S/cm) or micro Siemen per centimeter (μS/cm) (Weinner 2013).

Generally, solutions of molecules of organic compounds are usually poor conductors in nature while inorganic compounds normally form solutions which are excellent conductors (Chapman, 1996). Distilled water has a conductivity which ranges from 0.5-3 μS/cm. Most lotic ecosystems have a conductivity ranging from 50 to 1500 μS/cm.

Fresh water stream should have a conductivity ranging from 150 -500 μS/cm to be able to support aquatic biota. High conductivity increases corrosive nature of water (APHA *et al.*, 2005).

2.8.6 Turbidity

Turbidity is the computation of the murkiness of the water, resulting from the existence of suspended matter as well as fine colloidal material like clay and microorganism. Turbidity is caused by suspended particles and dissolved salts in water that scatter light making the water to appear murky thus affecting water clarity (De Godos *et al.*, 2010). It indicates the levels of suspended sediments and hence erosion levels. Increase in turbidity increases the water temperature (Williard *et al.*, 2013).

2.8.7 Biological Oxygen Demand for Five Days

Biological Oxygen Demand is the amount of milligrams of oxygen necessary to oxidize organic carbon in one litre of water. High BOD indicates that the water contains high amount of biodegradable matter (Osibanjo *et al.*, 2007). BOD can also be described as the quantity of oxygen needed to biochemically oxidize a given sewage sample. The BOD is comparative to the sum of organic material available in a sample. In most cases, BOD is calculated as the amount of dissolved oxygen that has been utilized in a water sample after five days incubation (BOD_5).

2.8.8 Chemical Oxygen Demand

Chemical oxygen demand is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. It measures the overall magnitude of the oxidisable materials present in a sample. A strong oxidant (potassium dichromate) is used and the degree to which the oxidant has been consumed is determined. The dichromate ion is reduced to the chromic ion. COD is usually used as a measure of pollutants in wastewater and natural waters (APHA *et al.*, 2005). High levels of BOD and COD are pinned on high amounts of degradable matters in wastewater (Aisien *et al.*, 2010a).

2.9 Bacteriological parameters

Bacteriological pollution is of primary importance in respect to treatment requirements and recycling of wastewater. Bacteriological parameters are used as indicators of water quality. Wastewater contains different types of microorganisms some that are pathogens causing diseases to human beings while others are normal flora found in all mammals including man. It also contains other form of microbes that inhabit any water body. The presence of pathogens in untreated effluent poses a high risk to public health. Pathogenic micro-organisms like bacteria, viruses, fungi, protozoa, rotifers and worms (or their eggs) such as ascaris, round worms and hookworms which occur in human excreta may cause fatal illnesses such as cholera, giardiasis, paratyphoid, leprosy, yellow fever and skin infections (Girones *et al.*, 2010).

To detect the presence of pathogenic bacteria in wastewater, indicator bacteria are mainly used. Their presence indicates high chances of pathogenic bacteria being expected in the same sample water. The most common indicator bacteria that are used are total coliforms, feacal coliforms, feacal streptococcus and *Escherichia coli* (WHO, 2003). Indicator bacteria are normally present in the faeces of warm blooded animals in large quantities and therefore enter into the sewerage system the same time with faeces. These bacteria are easier to detect using simple procedures hence making the process faster and economical. The indicator bacteria are mostly not found in unpolluted natural water bodies.

In wastewater treatment plants, indicator bacteria are usually used to give information on the effectiveness with which specific bacteria have been removed or inactivated by treatment processes with their presence after treatment indicating that pathogens may still be present. Methods of detecting waterborne pathogens are often difficulty to implement, relatively expensive and time consuming. It is also impossible to monitor all known pathogens and also the pathogenic agents yet unrecognized. Use of indicator organisms allows recognition of the potential for pathogen to be present without the need for their actual presence. The main thrust of using indicator organisms is to minimize feacal oral disease transmission (Sanders *et al.*, 2013).

2.9.1 Heterotrophic plate counts/Total bacteria counts

Heterotrophic bacteria utilize organic compounds as their source of carbon. The population of heterotrophs in water depends on the quality of influent, water temperature, detention time and nutrients availability (Wagner *et al.*, 2002). Heterotrophic plate counts can be determined using pour plate method, spread plate method or membrane filtration methods. The method relies on the number of colonies formed on a nutrient media such as plate count agar. The number of bacteria in a given sample is usually too high to be counted therefore serial dilution is carried out to reduce the numbers to countable colonies. One millilitre of the diluted sample is cultured on an appropriate media and cultured at optimum temperature. The total number of colonies that grow on the petri dish are enumerated and multiplied by the dilution factor to establish the number of colony forming units (Cfu) per 1ml of the cultured sample. More often, plates with colonies ranging from 30 -300 are chosen for colony counting because this range is normally considered to be statistically significant. To determine the number of colonies in 100ml of the sample, the number of colonies counted are divide with the milliliters of sample used and multiplied by 100. This gives the percentage bacteria in the wastewater (Sanders, 2012).

2.9.2 Total coliforms

Coliforms are gram negative, rod shaped, facultative anaerobes, non-spore forming bacteria that ferment lactose with gas production and acid formation when incubated at 35° C within 48 hours. They belong to the entrobacteriaceae family. The genera in this family include the *Escherichia*, *Citrobacter*, *Enterobactor,* and *Klebsiela.* They are normal flora in the intestines of warm blooded animals and hence present in large numbers in faeces (Madigan *et al.*, 2008).

Coliforms originate from human and animal waste although they can also be found in surface water, vegetation and soil where they live freely. Detection of these bacteria in drinking water is important because it is an indication of possible feacal contamination although it does not mean that disease causing organisms are present. Presence of total coliforms is determined by growing them in lactose media at about 35°C.

2.9.3 Feacal coliforms

Feacal coliforms is a subgroup of total coliform which have capacity to grow at an elevated temperature of 44° C to 45°C. They are a good indicator of feacal pollution (Doyle *et al.*, 2006). They include only the species that predominantly live in the guts of warm blooded animals and are normally present in large numbers in human feacal material. Therefore if feacal contamination is present, these bacteria would be present. They are composed of both pathogenic and nonpathogenic bacteria. Since waterborne pathogens are associated with feacal contamination, a feacal coliform examination rather than the total coliforms tests gives more direct evidence of the possible presence of pathogens (Sanders *et al.*, 2013).

Feacal coliforms can be detected by simple methods and do not grow in natural waters. The colonies of feacal coliforms are reported per 100ml or Cfu/100 ml. In the wastewater treatment plant, feacal coliforms are removed by die offs due to unfavorable environment, ultra violet light from the sun, algae toxins and predation by other microorganisms (Williard *et al.,* 2013).

2.9.4 Feacal streptococcus

Feacal streptococcus are gram positive spherical bacteria found in the gut of mammals. They ferment glucose without gas production and are catalase negative. These bacteria has enzyme peroxidase that does not use oxygen to break hydrogen peroxide. They do not form spores. They are tolerant to a wide range of temperature such as within 10 - 45°C (Fisher and Phillips, 2009). The manifestation of feacal streptococci, also reffered to as intestinal enterococci is an indication of faecal contamination, particularly of a contamination that occurred a while ago and the less tolerant coliform bacteria, including *E. coli*, may have died by the time the analysis is done. Feacal streptococcus are more resistant to stress and chlorination than *E. coli* and other coliform bacteria. Bile esculine azide agar is normally used for the culturing and enumeration of feacal streptococcus (Sanders, 2012). Tolerance to bile and the potential to hydrolyze esculine is a dependable presumptive test for the identification of enterococci.

2.10 Nutrients

2.10.1 Phosphates

Phosphorous is found in natural water and wastewater mostly as phosphates. Phosphates are salts of phosphoric acid that can condense to form pyrophosphates at elevated temperatures. Inorganic phosphate is found as a free phosphate in biological systems. It is normally a limiting nutrient in ecosystems thus its availability in optimal concentrations promotes the rate of primary productivity. The quantities of phosphorus in soil are generally small, therefore, humans often apply phosphate fertilizers on farmland and when in excess, these fertilizers end up in the surface water through runoffs where they result to eutrophication (Yanamadala, 2005). Increased levels of phosphates and nitrates in water bodies leads to eutrophication adversely impacting ecosystem health (Waiser *et al.*, 2011). Household detergents are one of the main sources of phosphorus input into aquatic ecosystems. These detergents lower the surface tension of wastewater (Ansari *et al.*, 2010a).

2.10.2 Nitrates

Nitrogen is a macronutrient which is vital for the growth of flora and fauna. Inorganic nitrogen may exist in its free state as di-nitrogen gas, as ammonia or as nitrate or nitrite when combined with oxygen. Nitrites and nitrates are oxidized forms of nitrogenous compounds that are produced naturally in the process known as nitrification (Kurosi, 2001). During this process, ammonia is converted into nitrates. This is accomplished by the action of two genus of nitrifying bacteria; *Nitrosomonas* and *Nitrobacter*. Nitrites are normally converted to nitrates by bacteria within a short span of time and therefore are rarely detected in water and when detected, they are normally in low concentrations. Nitrogen occurs naturally in the environment due to biological nitrogen fixation, atmospheric deposition, geological deposits and biodegradation of organic matter. In plants, nitrates deficiency results in chlorophyll reduction. Ammonium and nitrate are the frequently encountered forms of dissolved inorganic nitrogen in water bodies. Excessive amount of nitrates causes illness in infants referred to as methemoglobinemia or blue baby sydrome. Severe methemoglobinemia can result in brain damage and death (APHA *et al.*, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out at the University of Eldoret located in Uasin Gishu county, Moiben sub county about 9 km north east of Eldoret town (Figure 3.1). Uasin Gishu County is located in mid-western Kenya, between 34°55’33” and 36°38’58”E and between 0°2’44”S and 0°55’56”N. University of Eldoret is at an altitude of 2180 metres above the sea level. The mean annual rainfall ranges between 1100 and 1500 mm and mean annual temperature is 23°C.

3.1.1 Study site

Wastewater sampling site

Sampling of wastewater was carried out at the University of Eldoret sewage treatment plant. The micro locations were the inlet of the first stabilization pond (anaerobic pond) and outlet of the last stabilization pond (maturation pond).

Macrophytes sampling site

Macrophytes were collected from Marura wetland (Figure 3.1). This is a permanent riverine wetland located near the University of Eldoret. It lies at an elevation between 2110 and 2140 m above sea level. The wetland has a rich terrestrial and aquatic flora and fauna. It has rich species diversity of over 20 resident and migrant bird species and over 40 different plant species. The dominant plant species is *Cyperus papyrus* that provides materials for roofing, fencing and house costruction, manufacture of chairs, mats and baskets**.** Other plant species include *Typha latifolia*, *Ceratophyllum demersum, Nymphaea* spp., *Azolla pinnata*, *Potamogeton spp.*, and *Pycrus nitinda*. Marura swamp is characterized by agricultural activities, human settlement and herding. The swamp performs several ecological functions and provides numerous social and economic benefits to local communities (Maithya *et al.*, 2015).

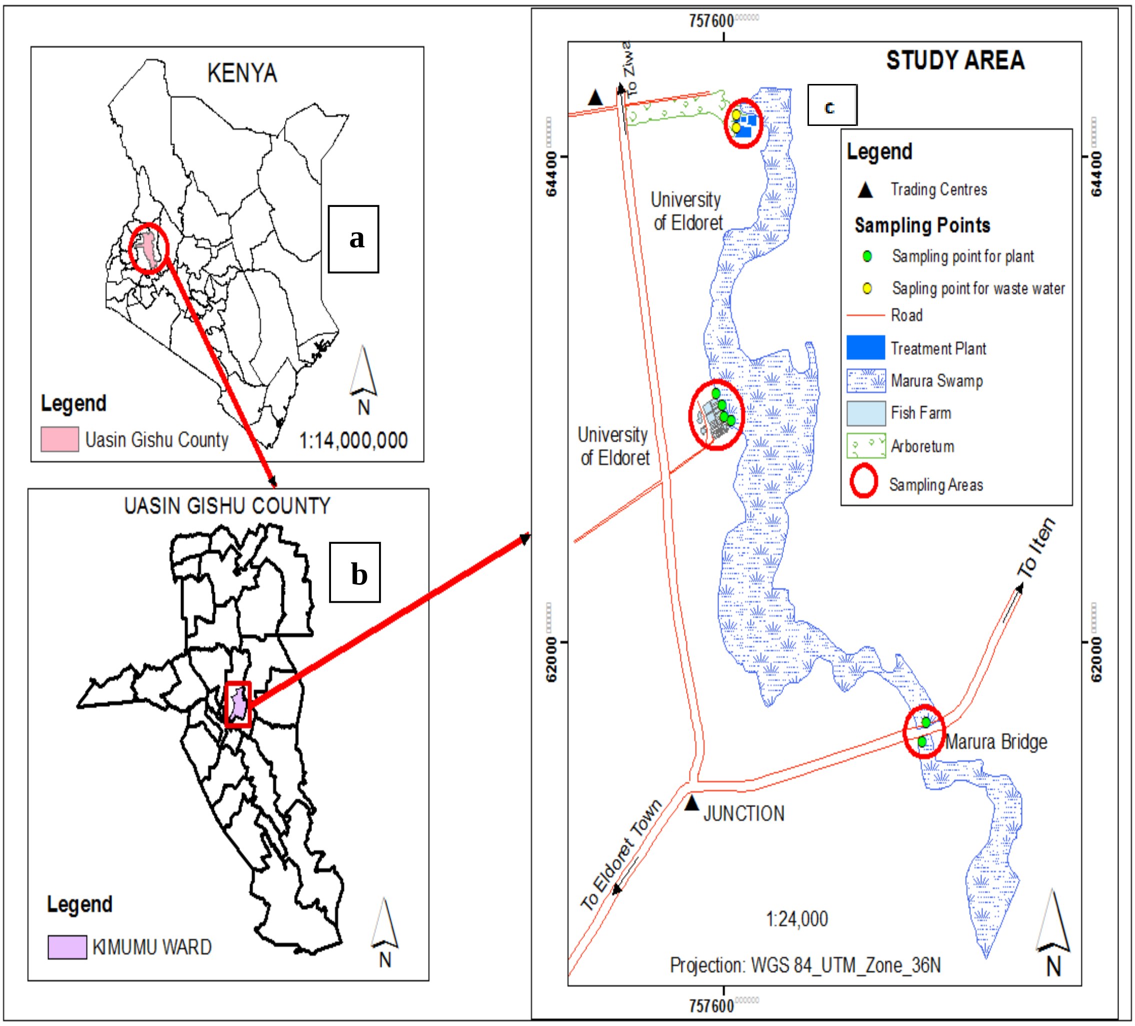


Figure 3.1 Location of study area. (a) General location (b) Uasin Gishu County (c) sampling sites (Source: Samson Odhiambo, Geographic Information System Laboratory, UoE 2019).

3.2 Materials and Methods

3.2.1 Equipments

Table 3.1 shows the equipments that were used.

Table 3.1 List of equipments

Serial No.	Equipment	Model	Manufacturer
1	Atomic Absorption spectrometer	Varian spectra 200	Varian ltd
2	UV-Vis spectrophotometer	DU 720	Wagtech Ltd
3	Autoclave	LS 50 LJ	
4	Laminar flow hood	Class A2	Labconco Ltd
5	Incubators		ELE international
6	pH meter	H19811-5	HANNA instruments Ltd
7	Digital weighing Balance	CY 220	Citizen
8	Portable weighing balance		
9	Digital Hot plate/ magnetic stirrer	ZXC- 2	Beijing zhongxingweiye Co. ltd
10	Turbidity meter	820	HACH Ltd
11	DO meter	SX716	HANNA instruments Ltd
12	Digital colony counter	551C	

3.2.2 Reagents

Table 3.2 shows the reagents that were used.

Table 3.2 List of reagents

S. No.	Reagent	Concentration/Amount	Manufacturer
1	Nitric acid	69-72 % AR	Research lab fine chem industries, India
2	Sulphuric acid	98.08 % AR	Research lab fine chem industries, India
3	Stanneous chloride	2.5 g	Lobachemie Ltd
4	Ammonium molybdite	25 g	Lab Consultants Co.
5	MacConkey agar	51.53g/l	Himedia laboratories PVT Ltd
6	Plate count Agar	23.5g/l	Himedia laboratories PVT Ltd
7	Bile Esculine Azide Agar	56.65g/l	Himedia laboratories PVT Ltd
8	Glycerine	GPR 92.09%	Unilab Ltd
9	Potassium phosphate	0.286 g	Unilab Ltd
10	Brucine	1 g	BDH Ltd poole England
11	Sodium nitrate	0.3427g	Ranbaxy fine chemicals Ltd

3.3 Determination of the efficiency of University of Eldoret wastewater treatment plant

3.3.1 Research design

The study adopted a descriptive design. The descriptive design is a scientific method of research that involves observing, analyzing and describing the behaviour of a subject without influencing it in any way. It was a fact-finding study that involved adequate and accurate interpretation of findings that described the condition of the UoE wastewater treatment plant during the study period. This method was appropriate for the study because it aimed at determining the existing condition of the wastewater effluent from the UoE wastewater treatment plant.

3.3.2 Sample collection of wastewater

Two sampling points at University of Eldoret wastewater treatment plant were selected. Wastewater was sampled using simple random technique. These points were at the inlet of the anaerobic pond and at the outlet of the maturation pond. This generated wastewater samples that were used to determine whether UoE wastewater treatment plant was efficient.

Samples were collected by hand by dipping the sampling bottle beneath the wastewater surface at the two sampling points. Samples were collected weekly between 1000hrs and 1100hrs from October to December in 2018. Samples were collected in triplicate at each sampling point for a period of eight weeks giving a total of 48 samples. During sampling, care was taken to ensure that no floating debris or large organic materials was collected. *In situ* measurements of temperature, pH, total dissolved solids, conductivity, dissolved oxygen and turbidity were carried out to avoid changes during transport and storage.

Sampling bottles were capped before withdrawing them from the water. The samples were then labeled and transferred into a cooler box kept at 4.0 ± 1.0° C using icepacks to stop biochemical reactions. They were then transported to UoE biotechnology laboratory for further analysis.

3.3.3 Determination of levels of water indicator parameters

Physicochemical parameters

Dissolved oxygen

Dissolved oxygen for each sample was measured using a digital multitester dissolved oxygen meter that was calibrated prior to use with the appropriate calibration solution in accordance with the manufacturer's specification. 100 ml of the sample was placed in a beaker, probe electrode inserted, readings taken from the meter when the sensor was stable and recordings made. The readings were taken in triplicate and the probe was washed with distilled water after every reading.

Temperature

Wastewater temperature was measured using a digital multitester dissolved oxygen meter for each sample. 100 ml of the sample was put in a beaker, the probe electrode inserted, readings taken directly from the meter when the sensor was stable and recordings made. The readings were taken in triplicate and the probe was washed after every reading.

Potential of Hydrogen

The pH readings were taken using a digital multitester pH-meter which was calibrated prior to use with standard pH buffers of pH 4, 7 and 10. 100 ml of wastewater sample was put in a beaker and the pH meter electrode inserted. The readings were taken directly from the meter when the sensor was stable and recordings made. The readings were taken in triplicate and the probe was washed using distilled water after every reading.

Conductivity

Conductivity readings were taken using a digital multitester pH-meter that was calibrated prior to use with commercial standards provided by the manufacturer. 100 ml of wastewater sample was placed in a beaker and the electrode dipped in. On the pH meter, conductivity key was pressed to measure conductivity. Readings were taken directly from the meter when the sensor was stable and recordings made. The readings were taken in triplicate and the probe was washed using distilled water after every reading.

Total dissolved solids

Total dissolved solids readings were taken using a digital multitester pH-meter that was calibrated prior to use with commercial standards provided by the manufacturer. 100 mL of wastewater sample was placed in a beaker and the electrode dipped in. On the pH meter, TDS key was pressed in order to enable measurement of TDS. The readings were taken directly from the meter when the sensor was stable and recordings made. The readings were taken in triplicate and the probe was washed using distilled water after every reading.

Turbidity

Turbidity was measured using a portable turbidity meter. This meter (colorimeter) has stored program for various parameters. Colorimeter was calibrated using distilled water prior to use. The distilled water (blank) was put in the cell holder and the colorimeter was capped tightly. Zero icon was displayed. The sample was then put in the cell and its turbidity measured. To achieve this, a stored program number for turbidity was keyed in the colorimeter and turbidity of the sample was displayed in Formazin Attenuation Units (FAU)

Biological Oxygen Demand

Biological oxygen demand (BOD_5) was determined as the difference between the oxygen concentrations of an appropriately diluted sample of 300 ml, before and after incubation for 5 days at 20 ± 1^{o}C. The DO for each sample was measured using a DO meter, before incubation in a BOD incubator for 5 days at 20 ± 1°C. The DO values were again measured 5 days after incubation(APHA *et al.*, 2005). Calculation for BOD_5 (mg/l) was determined using the following equation;

$$\text{BOD5 (mg/l)} = \frac{(D1 - D2)}{P}$$

Where,

D1 = DO (mg/l) value in initial sample

D2 = DO (mg/l) value in final sample

P = decimal volumetric fraction of sample used (APHA *et al.*, 2005).

Chemical oxygen demand

Chemical oxygen demand was determined by micro digestion method. Five millilitres of sulfuric acid reagent, 0.5g of mercuric sulfate and 10 ml of dichromate solution were added to 20 ml of a sample that was put in the glass digestion tube. The tube was put in the well of the microwave system and then connected to a condenser. The tap for cooling water was turned on and as cooling continued, 25 ml of sulfuric acid reagent was added from the top of the condenser. The mixture was digested in the microwave at 150°C for 8 minutes. The solution was transferred into a conical flask after digestion and the excess dichromate in the digested solution titrated with ferrous ammonium sulfate standard solution using ferroin as an indicator (APHA *et al.*, 2005).

Determination of bacteriological parameters

Aseptic techniques were employed in all bacterial analysis. Serial dilution was carried out by transferring 1 ml of the sample water to 9ml of sterile water using a sterile micropipette tip to make a dilution of up to 10^3. One milliliter of the diluted sample water from the three dilutions were inoculated on sterile media using pour plate method.

Pour plate technique is usually used to determine viable plate counts. In this technique, the total number of colony forming units within the agar and on surface of the agar on each single plate are counted. Viable plate counts enables microbiologists to generate growth curves using standardized means and to determine the concentration of cells in the sample from which the sample was plated.

Media preparation

Plate count agar, MacConkey agar and Bile esculine azide agar were used for the cultivation of bacteria. The media were prepared according to the manufacturer's instruction in addition to the amount of media required. They were heated with frequent agitation on a hot plate in order to completely dissolve the powder. They were then sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the media were cooled to 45°C and then dispensed in to sterile petri dishes having the inoculum. The petri dish were swayed smoothly to ensure that the media was equally distributed.

Culturing and counting of bacteria colonies

Total bacteria counts

The standard plate technique was used to determine the colony forming units (Cfu) in the wastewater samples. Plate count agar was used to culture the bacteria for total bacteria counts. This is a general media commonly used for the enumeration of bacteria. One millilitre sample was drawn from each dilution tube and inoculated on a sterile petri dish using a sterile micropipette tip. Each dilution had three replicates. Fifteen millilitres of molten medium was then poured into the petri dish. After solidifying, it was sealed using parafilm to prevent contamination and allow air circulation. It was incubated upside down at 35.0 ± 0.5°C for 48 hrs. The number of colonies formed (Cfu) were counted per sample using digital colony counter to determine bacterial load and the results tabulated. This was done for each sample and for each dilution.

Total Coliforms

Presence of total coliforms was determined using MacConkey Agar. One ml of the sample was drawn from each dilution tube and inoculated on a sterile petri dish using a sterile micropipette tip. Each dilution had three replicates. Fifteen millilitres of molten agar was then poured into the petri dish. After solidifying, it was sealed using parafilm to prevent contamination and allow air circulation. It was incubated upside down at 37.0 ± 0.5°C for 48 hrs. Pink to brick red colonies in the media denoted lactose-fermenting coliforms while colorless or clear colonies denoted non-lactose fermenting (APHA *et al.*, 2005). The number of colonies formed (Cfu) were counted per sample using digital colony counter to determine bacterial load and the results tabulated. This was done for each sample and for each dilution.

Feacal Coliforms

Presence of feacal coliforms was determined using MacConkey Agar. One ml sample was drawn from each dilution tube and inoculated on a sterile petri dish using a sterile micropipette tip. Each dilution had three replicates. Fifteen millilitres of molten agar was then poured into the petri dish. After solidifying, it was sealed using parafilm to prevent contamination and allow air circulation. It was incubated upside down at 44.0 ± 0.5°C for 48 hrs. The number of colonies formed (Cfu) were counted per sample using digital colony counter to determine bacterial load and the results tabulated. This was done for each sample and for each dilution.

Feacal Streptococcus

Presence of Feacal Streptococcus was determined using Bile Esculine Azide Agar. 1 ml sample was drawn from each dilution tube and inoculated on a sterile petri dish using a sterile micropipette. Each dilution had three replicates. Fifteen millilitres of molten agar was then poured into the petri dish. After solidifying, it was sealed using parafilm to prevent contamination and allow air circulation. It was incubated upside down at 37.0 ± 0.5°C for 24 hrs. Dark brown or black colonies in the media denoted the presence of feacal Streptococcus and their absence indicated negative results (APHA *et al.*, 2005). The number of colonies formed (Cfu) were counted per sample using digital colony counter to determine bacterial load and the results tabulated. This was done for each sample and for each dilution.

The number of colonies per 1 ml was calculated as follows

$$\text{Cfu/ml} = \frac{\text{No. of colonies x dilution factor}}{\text{Volume plated (ml)}}$$

Determination of phosphates

Phosphates in wastewater were determined using ammonium molybdate method. This method has good sensitivity and is dependable to concentrations below 0.1 mg of phosphorus per litre. This method was chosen because of the availability of reagents and equipment, its simple, economical, faster and reliable.

Ammonium molybdate reagent, stannous chloride reagent, stock phosphate solution and standard phosphate solutions were prepared as per the procedure outlined in APHA *et al.*, (2005). Twenty five millilitres of purified water was set aside to be treated with the color developing reagent which served as a blank. Twenty five millitres of wastewater sample was measured and put in erlenmayer flask for analysis. 1.00 ml of ammonium molybdate solution was pipetted and added into the Erlenmeyer flask containing the wastewater sample and thoroughly swirled to mix. Two drops of stannous chloride solution was added to the flask and mixed by swirling. Presence of phosphate was indicated by the development of a blue colour in about 5 minutes.

The UV - Vis spectrophotometer was used to measure the absorbance of the samples at a wavelength set at 650 nm. The blank solution was used to set the spectrophotometer to read zero absorbance. The absorbance of the samples was then measured using 650 nanometers wavelength. A calibration curve developed from the standard solutions was used to determine phosphate concentration in the wastewater sample.

Determination of nitrates

Nitrates in wastewater samples were determined by brucine method. The principle of this method involves the reaction of nitrate ions with brucine in the presence of concentrated sulphuric acid to form a yellowish orange nitro complex (Colman, 2010). This method was chosen because of the availability of reagents and equipment, its simple, economical, faster and reliable.

Reagents used for determination of nitrates in wastewater included nitrate stock solution, standard nitrate solution, brucine sulphanic acid solution and sulphuric acid. Twenty five millilitres of purified water was set aside to be treated with the colour developing reagent which served as a blank. Approximately 2 ml of the sample was measured and placed in a test tube, 0.2 ml brucine solution was added and the solution mixed thoroughly. Another 3 ml of concentrated sulphric acid was added into the content and solution mixed thoroughly for 10 seconds. The solution was then left in the dark for 30 minutes for colour development.

The UV - Vis spectrophotometer was used to measure the absorbance of the samples at a wavelength set at 420 nm. The blank solution was used to set the spectrophotometer to read zero absorbance. The absorbance of the samples was then measured using 420 nanometers wavelength. A calibration curve developed from the standard solutions was used to determine nitrates concentration in the wastewater sample.

Determination of heavy metals

Wastewater sample digestion

The wastewater samples were digested using nitric acid digestion method. A 100 ml of a well-mixed, acid preserved sample was measured and transferred to a 250 ml conical flask. Approximately 5 ml concentrated nitric acid was added. The mixture was brought to a slow boiling on a hot plate and evaporated to the lowest volume possible (about 20 ml). The mixture was removed from the hot plate and allowed to cool. After cooling, concentrated nitric acid was added and heating continued until digestion was complete which was indicated by a light coloured clear solution. The solution was allowed to cool after which the walls of the conical flask was washed down with 10 ml distilled water and then filtered. The filtrate was transferred to a 100 ml volumetric flask. The filtrate was topped up to mark using distilled water prior to heavy metal analysis (APHA *et al.,* 2005). This solution was used for the determinations of heavy metals using the atomic absorption spectrometer. Sample blanks were prepared as described above and were analyzed to correct the possible external contributions. Sample blanks and calibration standards were included with every 10 samples for quality control. All the reagents used were of analytical grade and all the vessels were prepared according to procedures outlined in APHA *et al.,* (2005) to avoid external contributions of heavy metals. Wastewater samples were analyzed in the AAS using an air/acetylene flame.

Analysis of the sample using Atomic Absorbtion Spectrophotometer

Instrument Calibration

A hollow cathode lamp was installed for the each heavy metal analyzed in the AAS and the wavelength set according to Table 3.3 below. Determination of the concentration of metals in the samples was done one metal at a time.

Standard solutions of known metal concentrations for each heavy metal were prepared from stock standard solutions of 1000 ppm. The standards were prepared according to the procedures outlined on the varian flame atomic absorption spectrometry analytical methods manual. These standard solutions were used for calibration of the AAS and calibration curves were prepared. The concentrations that were used are indicated on table 3.3 below.

Analysis of Sample

Approximately 1.0 ml of the digested sample was loaded to the AAS. Concentration of the elements were read directly from the instrument readout in mg/l.

Table 3.3 Wavelength, instrument detection limit, sensitivity, metal calibration standards and optimum concentration range for elements

Element	Wavelength (nm)	Flame gas	Instrument detection limit (mg/l)	Slit width (nm)	Calibration standards (mg/l)	Optimum concentration range (mg/l)
Cadmium	228.8	A – Ac	0.0003	0.5	1, 2, 3	0.02 - 3
Lead	217.0	A – Ac	0.0003	1.0	10, 20, 30	0.1 - 30
Copper	324.7	A – Ac	0.0003	0.5	2, 4, 6	0.03 -10
Nickel	232.0	A – Ac	0.0003	0.2	5, 10, 15	0.1 - 20
Zinc	213.9	A – Ac	0.0003	1.0	0.5, 1.0, 1.5	0.01 - 2
Manganese	279.5	A – Ac	0.0003	0.2	2, 4, 6	0.02 - 5
Cobalt	240.7	A – Ac	0.0001	0.2	5, 10, 15	0.05 - 15
Iron	248.3	A – Ac	0.0003	0.2	5, 10, 15	0.06 -15
Chromium	357.6	A – Ac	0.0001	0.2	5,10, 15	0.05 - 15

A - Air; Ac - Acetylene

Table adapted from Varian flame atomic absorption spectrometry analytical methods manual.

3.3.4 Data analyses

The data collected were summarized in tables and subjected to statistical analyses using Minitab statistical package to determine the mean and standard error. Percentage reductions of physicochemical parameters, bacteriological parameters, nutrients and heavy metals was determined by comparison of influent and effluent values. The reduction efficiency for selected parameters was calculated as follows:

$$\text{Reduction efficiency } (E_r) = \frac{\text{Influent concentration} - \text{Effluent concentration}}{\text{Influent concentration}} X\, 100$$

Compliance index for various parameters was calculated to determine whether the effluent discharged from UoE wastewater treatment plant were compliant to the set Kenyan standards for effluent discharge to the environment. This is a statistical tool that shows at a glance the efficiency of a wastewater treatment plant. If the calculated compliance index value is less than 1 (<1) it indicates that the discharged effluent are compliant to the set standards, while a compliance index value of greater than 1 (>1) implies non-compliance. Noncompliance means that the effluent discharged into the environment may have negative impacts in the environment. They may degrade the water quality affecting biodiversity of the surrounding ecosystem.

Compliance index for selected parameters was computed as shown below.

$$\text{Compliance index} = \frac{\text{Effluent concentration}}{\text{Maximum allowable value}}$$

3.4 Determination of the efficiency of macrophytes in remediation of wastewater from University of Eldoret.

3.4.1 Research design

The study adopted experimental design. The experimental design included three basic principles being principle of replication, principle of randomization and principle of local control. The principle of randomization was achieved by random sampling of macrophytes from Marura wetland and wastewater from the University of Eldoret wastewater treatment plant. Control experiments were involved in order to achieve the principle of local control. Principle of replication was achieved by sampling wastewater in replicates and providing replicates for the experimental set ups. The experimental design was considered since the study dealt with quantitative data that included the amount of nutrients and heavy metals absorbed by macrophytes as well as the reduction of bacteria load in waste water. The design was also suitable because of its convenience in the studies that entail biological attribute of a subject.

3.4.2 Selection of macrophytes

The rationale behind the selection of macrophytes that were used in this experiment was based on the ease of availability of the plants, documented plants efficiency in pollutants removal and their primary productivity. From a checklist of several macrophyte species used for phytoremediation, *Azolla pinnata, Nymphaea* spp., *Ceratophyllym demersum* and *Typha latifolia* were selected because of their high reproduction rate, high pollutants removal efficiency and tolerance to pollution and varying environmental factors. They are also locally available hence native to the study area. Native plants were preferred for phytoremediation because they are superior to exotic plants in respect to growth and reproduction in stressful conditions. They mitigate the risk of introducing nonnative species that can end up being invasive. They are also tolerant to local climatic conditions and seasonal changes. Utilization of native species in phytoremediation projects is vital for biological diversity conservation and each natural environment needs a thorough investigation. These plants were identified in the herbarium of the University of Eldoret using taxonomic keys in Agnew *et al.,* (1994), Beentje *et al.,* (1994) and Haines *et al.,* (1983).

3.4.3 Sample collection of the macrophyte

Young and healthy macrophytes samples were sampled randomly by hand from Marura wetland. The plants were put in plastic vessels and transported to the laboratory within few hours of collection where they were cleaned carefully using tap water to remove dirt and dust. They were then subjected to acclimatization in stock tanks containing unchlorinated tap water for one week.

3.4.4 Experimental setup

Randomized design with three replications was used to conduct the experiments. The plants that were maintained in the stock tanks were collected, thoroughly washed with sterile distilled water before being introduced in the experimental troughs. These plants include *Azolla pinnata, Nymphaea* spp*., Typha latifolia* and *Ceratophyllum demersum.* A control comprised of wastewater with no plant, which was necessary for the comparison of the results to establish the potential of the plants in reducing the physicochemical parameters, bacteriological parameters, mineral nutrients (nitrates and phosphates) and heavy metals concentration.

Approximately, 180 litres of wastewater were collected from the outlet of the UoE maturation pond between 800 hrs and 900 hrs using clean sterilized plastic container. They were transported to the University of Eldoret. Ten litrers of wastewater was put in plastic troughs of 15 litre capacity. Approximately 500 g (fresh weight) of each selected plant was inoculated in triplicate in the plastic troughs. A control set up with ten litrers of wastewater without the plants was maintained to assess the role of macrophytes in the removal of pollutants. The experimental set up was kept outdoors in a shed.

Approximately 200 ml of wastewater from the individual treatment sets were collected periodically in triplicate for analyzing the changes in its physicochemical and bacteriological parameters, mineral nutrients (nitrates and phosphates) and heavy metals at initial level and consequently with an interval of 5 days for 30 days. Physicochemical parameters which included pH, oxygen, temparature, conductivity, TDS and turbidity were measured on site.

3.4.5 Determination of levels of water indicator parameters

The levels of physicochemical parameters, nutrients and heavy metals were determined using similar methods as described in section 3.2.3.

3.4.6 Data analyses

The data collected was summarized in tables and subjected to statistical analyses using Minitab statistical package to determine the mean and standard error. Descriptive statistics such as means and percentages were calculated. It was further analyzed using one way ANOVA and means separated using Tukey's test at 5% level. Tables, line graphs and bar graphs were used to present the results.

Percentage reductions of physicochemical parameters, bacteriological parameters, nutrients and heavy metals was determined by comparison of the value before and after treatment. The reduction efficiency for selected parameters was calculated as follows:

$$\text{Reduction efficiency } (E_r) = \frac{\text{Initial concentration} - \text{Reduced concentration}}{\text{Initial concentration}} \; X\ 100$$

3.5 Determination of the efficiency of multistage technique in phytoremediation of wastewater from University of Eldoret.

3.5.1 Research design

The study adopted experimental design. This design is described in section 3.4.1

3.5.2 Selection of macrophytes

The selection of macrophytes was done using similar methods as described in section 3.3.2.

3.5.3 Sample collection of the macrophytes

Sampling of macrophytes was done using similar methods as described in section 3.3.3.

3.5.4 Experimental setup

The experimental set up was a four-stage treatment system. It comprised of four macrophyte columns with different arrangements. The first column of macrophytes was selected randomly followed by systematic arrangement to ensure each macrophyte growth form was included in each column. The macrophytes that were maintained in the stock tanks were collected, thoroughly washed with sterile distilled water before being introduced in the experimental troughs. These macrophytes included *Azolla pinnata (*free floating), *Nymphaea* spp., (floating leaved) *Typha latifolia* (emergent) *and Ceratophyllum demersum* (submerged). A control comprised of wastewater with no plant, which was necessary for the comparison of the results to establish the potential of the macrophye columns in reducing the physicochemical parameters, bacteriological parameters, nutrients (nitrates and phosphates) and heavy metals concentration.

Approximately, 120 litres of wastewater were collected from the outlet of the UoE maturation pond between 800 hrs and 900 hrs using clean sterilized plastic containers. They were transported to the University of Eldoret. Approximately 500 g (fresh weight) of each selected plant was inoculated in triplicate in the plastic troughs. A control set up of wastewater without the plants was maintained to assess the role of macrophytes in the removal of pollutants. The wastewater was put into fifteen, 20 litres pre-sterilized plastic containers and 10 litres allowed to flow to plastic troughs which had a capacity of 15 litres each containing the experimental plants from one stage to the other as shown in Fig. 3.2. They were stacked at different heights next to each other. Each stage had a retention time of 5 days. The flow of wastewater from one stage to another after the 5 days retention time was facilitated by opening a plastic pipe that had a control valve. The experimental set up had three replications.

At the start of the experiment, the wastewater was allowed to flow into the first stage of treatment. This stage contained troughs with the following plants; trough (a_1) *Azolla pinnata,* trough (b_1) *Typha latifolia*, trough (c_1) *Ceratophyllum demersum* and trough (d_1) *Nymphaea* spp. After 5 days in these troughs, the wastewater was allowed to flow into stage 2. This stage contained troughs with the following plants: trough (a_2) *Typha latifolia*, trough (b_2) *Nymphaea* spp., trough (c_2) *Azolla pinnata* and trough (d_2) *Ceratophyllum demersum.* This was attained by opening the tap connecting trough 1 to trough 2 at the end of the 5 days. This procedure was repeated until the treated wastewater flowed into stage 4. The plants in stage 3 were: (a_3) *Ceratophyllum demersum* trough, (b_3) *Azolla pinnata*, trough (c_3) *Nymphaea* spp. and trough (d_3) *Typha latifolia*. The plants in stage four were: (a_4) *Nymphaea* spp., trough (b_4) *Ceratophyllum demersum* trough (c_4) *Typha latifolia* and trough (d_4) *Azolla pinnata.*

Approximately 200 ml of the wastewater was sampled from the fifteen plastic containers at the start of the treatment process and from each trough at the end of the retention time of 5 days. They were analysed in triplicate for the physicochemical and bacteriological parameters, nutrients (nitrates and phosphates) and heavy metals using APHA *et al.,* 2005 standard method of analysis.

3.5.5 Determination of levels of water indicator parameters

The levels of physicochemical parameters, nutrients and heavy metals were determined using similar methods as described in section 3.2.3.

3.5.6 Data analyses

The data collected was summarized in tables and subjected to statistical analyses using Minitab statistical package to determine the mean and standard error. Descriptive statistics such as means and percentages were calculated. It was further analyzed using one way ANOVA and means separated using Tukey's test at 5% level. Tables, line graphs and bar graphs were used to present the results.

Percentage reductions of physicochemical parameters, bacteriological parameters, nutrients and heavy metals was determined by comparison of the value before and after treatment. The reduction efficiency for selected parameters was calculated as follows:

Reduction efficiency (E_r) $= \frac{\text{Initial concentration} - \text{Reduced concentration}}{\text{Initial concentration}} X\ 100$

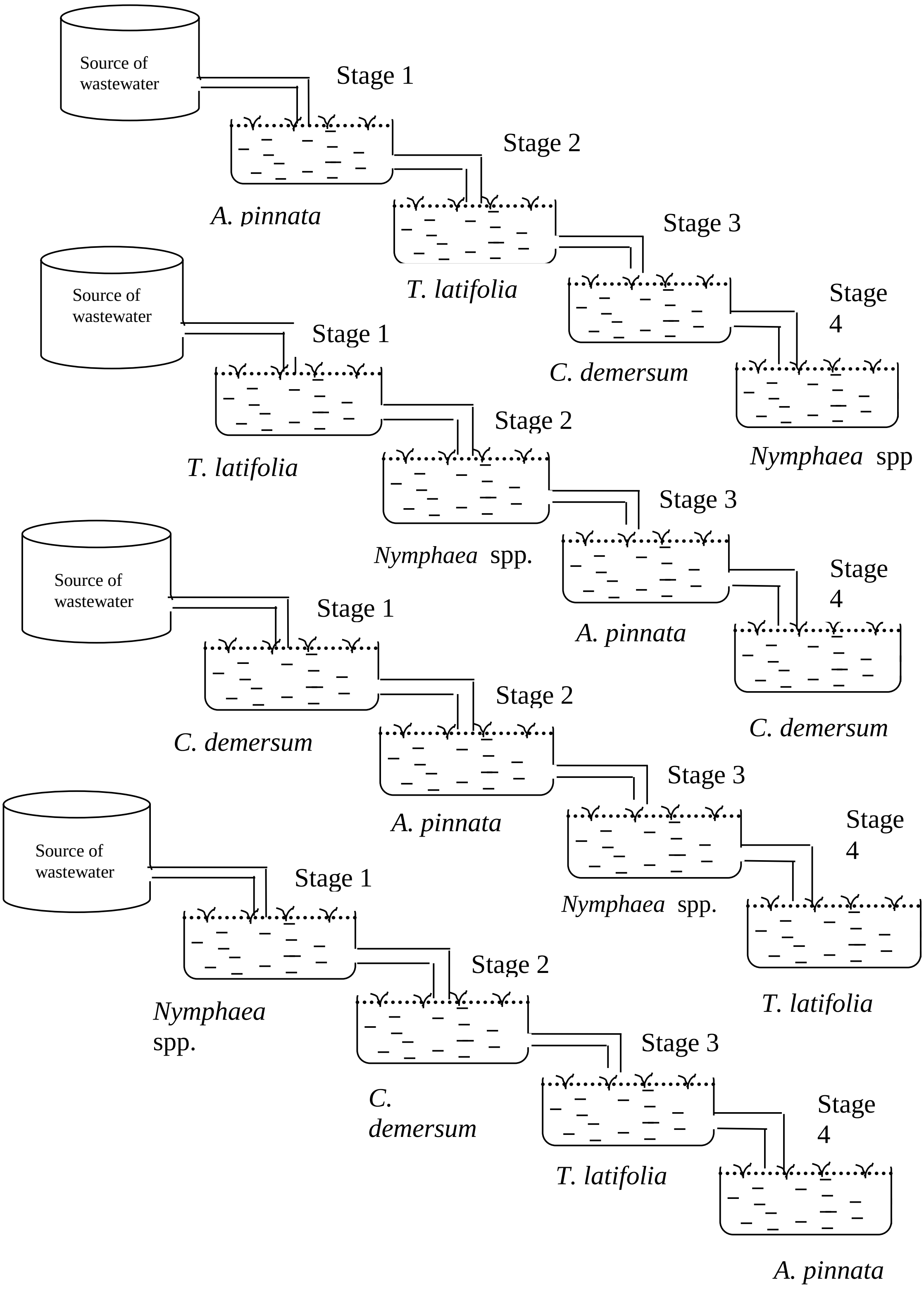


Figure 3.2 The experimental setup for the phytoremediation of University of Eldoret wastewater using multistage technique

CHAPTER FOUR

RESULTS

4.1 Water quality indicator parameters at University of Eldoret wastewater treatment plant

4.1.1 Physicochemical parameters

Temperature

Week one had the lowest temperature throughout the sampling period. The lowest influent temperature was 20.23°C while the lowest effluent temperature was 18.4°C. The highest temperatures were recorded in week five. Highest influent temperature was 25.33°C and the highest effluent temperature was 19.97°C (Table 4.1). The temperature recorded throughout the sampling period was within the NEMA standards.

Table 4.1 Mean temperature recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (°C) Mean ± SE	Effluent (°C) Mean ± SE	Reduction Efficiency (%)	NEMA standards for effluent disposal to environment	Remarks
1	20.23 ± 0.07	18.40 ± 0.27	9.05		Compliant
2	20.50 ± 0.06	18.43 ± 0.07	10.10		Compliant
3	20.70 ± 0.06	18.83 ± 0.29	9.03		Compliant
4	20.73 ± 0.35	19.03 ± 0.07	8.20	± 3 Ambient temperature	Compliant
5	25.33 ± 0.24	19.97 ± 0.12	21.16		Compliant
6	22.47 ± 0.24	19.70 ± 0.06	12.33		Compliant
7	21.30 ± 0.12	18.50 ± 0.06	13.15		Compliant
8	22.43 ± 0.15	19.33 ± 0.23	13.82		Compliant

Dissolved Oxygen

The mean DO for the influent ranged from 0.44 mg/l to 1.75 mg/l. Influent DO was lowest in week seven while the highest recorded DO was in week 2. The effluent mean DO ranged from 3.03 mg/l to 5.29 mg/l. The highest effluent DO was recorded in week

2 while the lowest was recorded in week 5. There was a general increase in DO in the effluent compared to the influent. The highest increase was recorded in the 8th week (Table 4.2).

Table 4.2 Mean DO recoded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	% increase
1	1.45 ± 0.04	4. 33 ± 0.15	66.51
2	1.75 ± 0.06	5.29 ± 0.08	66.92
3	1.56 ± 0.07	4.36 ± 0.10	64.22
4	1.33 ± 0.05	5.16 ± 0.17	74.22
5	0.45 ± 0.01	3.03 ± 0.10	85.15
6	0.52 ± 0.02	3.05 ± 0.15	82.95
7	0.44 ± 0.01	3.09 ± 0.09	85.76
8	0.50 ± 0.01	3. 14 ± 0.11	84.07

pH

The mean pH for the influent ranged from 6.83 to 8.30. Influent pH was lowest during week four while the highest pH was recorded in week 8. The effluent mean pH ranged from 6.87 to 8.5. There was a general increase in pH in the effluent compared to the influent. The highest increase was recorded in the 2nd week (Table 4.2). All the values of pH recorded within the sampling period were within the NEMA standards for effluent disposal to aquatic environment (non-marine) of 6.5 to 8.5.

Table 4.3 Mean pH recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent Mean ± SE	Effluent Mean ± SE	% increase	NEMA standards for effluent disposal to environment	Remarks
1	7.27 ± 0.03	7.37 ± 0.09	1.36		Compliant
2	7.00 ± 0.00	7.40 ± 0.00	5.41		Compliant
3	6.97 ± 0.09	7.20 ± 0.00	3.19	6.5 to 8.5	Compliant
4	6.83 ± 0.07	6.87 ± 0.03	0.58		Compliant
5	8.30 ± 0.00	8.50 ± 0.00	2.35		Compliant
6	7.53 ± 0.15	7.70 ± 0.00	2.26		Compliant
7	7.83 ± 0.09	8.03 ± 0.03	2.49		Compliant
8	7. 93 ± 0.12	8.33 ± 0.12	5.04		Compliant

Total dissolved solids

The highest influent and effluent means were recorded in the 5th week while the lowest means were recorded in the 2nd week. The influent means ranged from 360 mg/l to 780 mg/l while the effluent means ranged from 150 mg/l to 230 mg/l. The levels of TDS recorded throughout the sampling period were within the NEMA standards for effluent disposal to environment which is 1200 mg/l (Table 4.4).

Table 4.4 Mean TDS recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Reduction Efficiency (%)	Compliance index	Remarks
1	403.33 ± 3.33	170.00 ± 0.00	57.85	0.14	Compliant
2	360.00 ± 5.77	150.00 ± 5.77	58.33	0.13	Compliant
3	523.33 ± 6.67	166.67 ± 3.33	68.15	0.14	Compliant
4	423.33 ± 3.33	230.00 ± 0.00	45.67	0.19	Compliant
5	780.00 ± 0.00	230.00 ± 0.00	70.51	0.19	Compliant
6	513.33 ± 3.33	180.00 ± 0.00	64.93	0.15	Compliant
7	456.67 ± 8.82	200.00 ± 5.77	56.21	0.17	Compliant
8	440.00 ± 5.77	213.33 ± 3.33	50.77	0.18	Compliant

Conductivity

The fifth week had the highest recorded levels in the influent and effluent, these were 1576.67 µS/cm and 490 µS/cm respectively. The lowest levels were recorded in the 2nd week where the influent mean level was 750 µS/cm and effluent mean was 320 µS/cm. There was a reduction of the effluent levels compared to the influent. The highest reduction efficiency was 68.92% which was recorded in the fifth week (Table 4.5)

Table 4.5 Mean conductivity recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (µS/cm) Mean ± SE	Effluent (µS/cm) Mean ± SE	Reduction Efficiency (%)
1	826.67 ± 3.33	360.00 ± 5.77	56.49
2	750.00 ± 5.77	320.00 ± 5.77	57.33
3	1070.00 ± 5.77	366.67 ± 3.33	65.73
4	866.67 ± 6.67	380.00 ± 0.00	56.15
5	1576.67 ± 3.33	490.00 ± 0.00	68.92
6	1040.00 ± 5.77	483.33 ± 3.33	53.53
7	953.33 ± 8.82	453.33 ± 3.33	52.45
8	923.33 ± 8.82	420.00 ± 5.77	54.51

Turbidity

The mean turbidity for the influent ranged from 372.33 FAU to 599 FAU. The highest influent mean was recorded in week 6 while the lowest influent mean was recorded in week 2. The mean turbidity for the effluent ranged from 126.33 FAU to 188 FAU. The lowest mean for the effluent was recorded in week1 and 2 while the highest effluent mean was recorded in week 7. The highest reduction efficiency was 77.40% which was recorded in the six week (Table 4.6).

Table 4.6 Mean turbidity recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (FAU) Mean ± SE	Effluent (FAU) Mean ± SE	Reduction Efficiency (%)
1	375.67± 5.70	126.33 ± 2.19	66.37
2	372.33 ± 6.06	126.33 ± 2.03	66.12
3	487.67 ± 9.94	170.33 ± 3.71	65.07
4	507.33 ± 7.26	156.00 ± 6.51	69.25
5	499.33 ± 3.67	170.33 ± 2.03	65.89
6	599.00 ± 4.73	135.33 ± 3.28	77.40
7	395.33 ± 8.69	188.67 ± 7.36	52.28
8	434.00 ± 7.21	140.33 ± 3.84	68.67

Biochemical oxygen demand

The mean BOD for the influent ranged from 432 mg/l to 1396 mg/l. The highest mean for influent was recorded in the 1st week while the lowest was recorded in the 6th week. The mean for the effluent ranged from 32 mg/l to 58 mg/l. The highest mean for effluent was recorded in the 1st week while the lowest was recorded in the 8th week (Table 4.7). The levels of BOD recorded throughout the sampling period were above the NEMA standards for effluent disposal to the environment of 30 mg/l. The levels of BOD recorded during the sampling period were above the NEMA limits for influent disposal to a public sewer of 500 mg/l except for week six.

Table 4.7 Mean BOD recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Reduction Efficiency (%)	Compliance index	Remarks
1	1396 ± 6.43	58 ± 1.02	95.84	1.90	Not compliant
2	1382 ± 5.32	56 ± 1.45	95.94	1.87	Not compliant
3	1296 ± 5.47	44 ± 0.09	96.61	1.47	Not compliant
4	546 ± 3.64	46 ±1.04	91.57	1.53	Not compliant
5	542 ± 3.25	34 ± 0.76	93.92	1.13	Not compliant
6	432 ± 3.73	36 ± 0.89	91.67	1.20	Not compliant
7	600 ± 3.25	35 ± 0.67	94.17	1.67	Not compliant
8	720 ± 3.42	32 ± 0.45	95.56	1.07	Not compliant

Chemical oxygen demand

The highest mean for the effluent was recorded in week 1 which was 2654 mg/l while the lowest mean was recorded in week 7 which was 1204 mg/l. The effluent mean ranged from 116 mg/l to 156 mg/l with the lowest mean recorded in the 3rd week while the highest mean was recorded in the 7th week (Table 4.8). The levels of COD obtained throughout the sampling period were above the NEMA standards for effluent discharge into the environment from the outlet which is 50 mg/l. The levels of COD obtained throughout the sampling period were above the NEMA standards for discharge into public sewer which is 1000 mg/l.

Table 4.8 Mean COD recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Reduction Efficiency (%)	Compliance index	Remarks
1	2654 ± 8.02	140 ±1.27	94.73	2.8	Not compliant
2	2548 ± 7.43	120 ± 1.02	95.29	2.4	Not compliant
3	2436 ± 8.06	116 ± 1.34	95.24	2.3	Not compliant
4	1365 ± 7.56	135 ± 1.62	90.11	2.7	Not compliant
5	1386 ± 6.76	144 ± 2.65	89.61	2.88	Not compliant
6	1255 ± 5.78	144 ± 1.45	88.53	2.88	Not compliant
7	1204 ± 6.01	156 ± 2.54	87.04	3.12	Not compliant
8	1376 ± 5.43	108 ± 1.32	95.15	2.16	Not compliant

4.1.2 Bacteriological parameters

Total bacterial counts

The total bacteria counts (TBC) for the influent ranged from 74917 cfu/100ml to 88710 cfu/100ml. The highest mean for the influent was recorded in week 2 while the lowest mean was recorded in week 5. The mean TBC for the effluent ranged from 62093 cfu/100ml to 84157 cfu/100ml. The lowest mean for the effluent was recorded on week 5 while the highest mean was recorded in week 2. The highest reduction efficiency was 17.11% which was recorded on the 5th week (Table 4.9).

Table 4.9 Total bacteria counts recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (cfu/100ml) Mean ± SE	Effluent (cfu/100ml) Mean ± SE	Reduction Efficiency (%)
1	87103 ± 396	77990 ± 1572	10.46
2	88710 ± 1135	84157 ± 1267	5.132
3	86847 ± 968	81253 ± 1269	6.44
4	81640 ± 503	71757 ± 383	12.11
5	74917 ± 887	62093 ± 936	17.11
6	83633 ± 1697	72863 ± 1109	12.88
7	84347 ± 1299	73110 ± 1154	13.32
8	82930 ± 924	74323 ±1300	10.37

Total coliforms

The highest influent and effluent counts were recorded in the 2nd week while the lowest counts were recorded in the 5th week. The influent counts ranged from 65783 cfu/100ml to 83457 cfu/100ml while the effluent ranged from 42180 cfu/100ml to 62760 cfu/100ml. The levels of total coliforms recorded throughout the sampling period were above the NEMA standards for effluent disposal to environment which is 30 cfu/100ml (Table 4.10).

Table 4.10 Total coliforms counts recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (cfu/100ml) Mean ± SE	Effluent (cfu/100ml) Mean ± SE	Reduction Efficiency (%)	Compliance index	Remarks
1	81950 ± 855	60833 ± 484	25.77	2027.76	Not compliant
2	83457 ± 1332	62760 ± 136	24.79	2092.00	Not compliant
3	80533 ± 1212	60030 ± 492	25.46	2001.00	Not compliant
4	73340 ± 718	57887 ± 547	21.07	1929.57	Not compliant
5	65783 ± 457	42180 ± 493	35.88	1406.00	Not compliant
6	72317 ± 452	50837 ± 232	29.70	1694.57	Not compliant
7	76290 ± 1046	54867 ± 263	28.08	1828.90	Not compliant
8	68937 ± 623	51727 ± 327	24.97	1724.23	Not compliant

Feacal coliforms

Feacal coliforms for the influent ranged from 57083 cfu/100ml to73367 cfu/100ml. Influent cfu were lowest during the 8th week while the highest cfu were recorded in week 2. The effluent cfu ranged from 28337 cfu/100ml to 50043 cfu/100ml. There was a general decrease in cfu in the effluent compared to the influent. The highest decrease was recorded in the 5th week (Table 4.11). All the values of Cfu recorded within the sampling period were above the NEMA standards for effluent disposal to the environment of 30 cfu/100ml.

Table 4.11 Feacal coliforms counts recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (cfu/100ml) Mean ± SE	Effluent (cfu/100ml) Mean ± SE	Reduction Efficiency (%)	Compliance index	Remarks
1	69790 ± 466	45440 ± 341	34.89	1514.67	Not compliant
2	73367 ± 733	50043 ± 339	31.79	1668.10	Not compliant
3	71607 ± 367	43870 ± 624	38.74	1462.33	Not compliant
4	68577 ± 216	38650 ± 514	43.63	1288.33	Not compliant
5	58203 ± 413	28337± 425	51.31	944.57	Not compliant
6	61390 ± 467	40593 ± 206	33.88	1353.11	Not compliant
7	61163 ± 388	39667 ± 387	35.15	1322.23	Not compliant
8	57083 ± 505	35050 ± 516	38.60	1168.33	Not compliant

Feacal streptococcus

Week five had the lowest cfu throughout the sampling period. The lowest influent cfu was 36473 cfu/100 ml while the lowest effluent cfu was 13320 cfu/100 ml. The highest counts were recorded in week two. The highest influent count was 47243 cfu/100 ml and the highest effluent counts was 30733 cfu/100 ml (Table 4.12). The cfu counts recorded throughout the sampling period were above the NEMA standards

Table 4.12 Feacal streptococcus counts recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (cfu/100ml) Mean ± SE	Effluent (cfu/100ml) Mean ± SE	Reduction Efficiency (%)	Compliance index	Remarks
1	46533 ± 429	26653 ± 356	42.72	888.43	Not compliant
2	47243 ± 222	30733 ± 224	34.95	1024.43	Not compliant
3	44473 ± 522	27320 ± 401	38.57	910.67	Not compliant
4	42197 ± 260	20280 ± 191	51.94	676.00	Not compliant
5	36473 ± 442	13320 ± 399	63.48	444.00	Not compliant
6	41357 ± 416	16877 ± 364	59.19	562.57	Not compliant
7	43003 ± 503	21520 ± 312	49.96	717.33	Not compliant
8	37613 ± 397	17467 ± 527	53.56	582.23	Not compliant

4.1.3 Nutrients

Phosphates

The mean for the influent ranged from 4.53 mg/l to 4.98 mg/l. The highest mean was recorded in 4th week while the lowest mean was recorded in 8th week. The mean for the effluent ranged from 3.00 mg/l to 4.50 mg/l. The highest mean was recorded in 7th week while the lowest mean was recorded in 1st week. The levels of phosphates were generally lower in the effluent compared to the influent. The highest reduction efficiency was recorded in the first week (Table 4.13). The levels of phosphates obtained throughout the sampling period were within the NEMA levels for discharge in public sewer which is 30 mg/l. NEMA does not provide specific value for discharge to the environment, it states it as 2 guideline value.

Table 4.13 Mean phosphates recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Reduction Efficiency (%)
1	4.67 ± 0.09	3.00 ± 0.14	35.76
2	4.71 ± 0.03	3.15 ± 0.03	33.12
3	4.67 ± 0.07	4.02 ± 0.08	13.92
4	4.53 ± 0.04	4.20 ± 0.03	7.29
5	4.59 ± 0.08	3.93 ± 0.22	14.37
6	4.76 ± 0.07	3.70 ± 0.28	22.27
7	4.87 ± 0.06	4.50 ± 0.05	7.60
8	4.98 ± 0.14	4.45 ±0.10	10.64

Nitrates

The highest level of nitrates in the influent was recorded in the 6th week which was 17.03 mg/l while the lowest level was recorded in the 8th week which was 3.95 mg/l. The effluent means ranged from 1.47mg/l to 6.17 mg/l. The lowest level was recorded in the

2nd week while the highest level was recorded in the 4th week. The highest reduction efficiency was recorded in the 7th week which was 81.77% (Table 4.14). The levels of nitrates in the influent obtained throughout the sampling period were within the NEMA standards for discharge into public sewer of 20 mg/l. NEMA does not provide specific value for discharge to the environment, it states it as 2 guideline value.

Table 4.14 Mean nitrates recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Reduction Efficiency (%)
1	4.27 ± 0.34	3.83 ± 0.29	10.31
2	4.27 ± 0.18	1.47 ± 0.15	65.57
3	6.23 ± 0.12	2.73 ± 0.27	56.18
4	16.10 ± 0.06	6.17 ± 0.09	61.67
5	12.17 ± 1.69	5.53 ± 0.29	54.56
6	17.03 ± 0.84	13.30 ± 1.11	21.90
7	8.23 ± 0.18	1.50 ± 0.06	81.77
8	3.95 ± 0.03	1.65 ± 0.08	58.23

4.1.4 Heavy metals

Cadmium

Means for cadmium in the influent ranged from 0.044 mg/l to 0.097 mg/l. The lowest mean was recorded in week 6 while the highest mean was recorded in the 5th week. The effluent mean ranged from 0.088 mg/l to 0.109 mg/l. The highest mean was recorded in the 2nd week while the lowest mean was recorded in the 5th week (Table 4.15). All the means recorded throughout the sampling period were above the NEMA standards for effluent discharge to the environment that is 0.01 mg/l.

Table 4.15 Mean cadmium recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Increase (%)	Compliance index	Remarks
1	0.087 ± 0.00	0.095 ± 0.00	8.42	9.5	Not compliant
2	0.080 ± 0.00	0.088 ± 0.00	9.09	8.8	Not compliant
3	0.073 ± 0.00	0.107 ± 0.01	31.78	10.7	Not compliant
4	0.083 ± 0.00	0.107 ± 0.01	22.43	10.7	Not compliant
5	0.097 ± 0.01	0.109 ± 0.01	11.01	10.9	Not compliant
6	0.044 ± 0.01	0.102 ± 0.01	56.86	10.2	Not compliant
7	0.087 ± 0.00	0.104 ± 0.00	16.35	10.4	Not compliant
8	0.092 ± 0.00	0.107 ± 0.01	14.01	10.7	Not compliant

Copper

The highest mean for copper in the influent was recorded in the 8th week which was 0.728 mg/l while the lowest mean was recorded in the 1st week which was 0.026 mg/l. The effluent mean ranged from 0.029 mg/l to 0.741 mg/l. The highest mean was recorded in the 8th week while the lowest mean was recorded in the 1st week (Table 4.16). All the means recorded throughout the sampling period were within the NEMA standards for effluent discharge to the environment which is 1 mg/l.

Table 4.16 Mean copper recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Increase (%)	Compliance index	Remarks
1	0.026 ± 0.00	0.029 ± 0.00	10.34	0.03	Compliant
2	0.053 ± 0.00	0.054 ± 0.00	1.85	0.05	Compliant
3	0.074 ± 0.00	0.077 ± 0.00	4.05	0.08	Compliant
4	0.086 ± 0.00	0.103 ± 0.00	19.76	0.10	Compliant
5	0.125 ± 0.00	0.129 ± 0.00	3.10	0.13	Compliant
6	0.161 ± 0.00	0.166 ± 0.01	3.01	0.17	Compliant
7	0.680 ± 0.01	0.694 ± 0.01	2.02	0.69	Compliant
8	0.728 ± 0.01	0.741 ± 0.01	1.75	0.74	Compliant

Nickel

The mean levels of nickel in the influent ranged from 0.005 mg/l to 0.170 mg/l. The highest mean was recorded in the 8th week while the lowest was recorded in the 1st week. The mean for the effluent ranged from 0.040 mg/l to 0.208 mg/l. The highest effluent mean was recorded in the 8th week while the lowest was recorded in the 1st week (Table 4.17). The levels of nickel obtained throughout the sampling period in the effluent were within the NEMA standards for discharge into the environment which is 0.3 mg/l. The levels of nickel obtained throughout the sampling period in the influent were within the NEMA standards for influent discharge to the public sewer which is 3 mg/l.

Table 4.17 Mean nickel recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Increase (%)	Compliance index	Remarks
1	0.005 ± 0.00	0.040 ± 0.01	87.50	0.13	Compliant
2	0.034 ± 0.00	0.051 ± 0.00	33.33	0.17	Compliant
3	0.036 ± 0.00	0.053 ± 0.01	32.07	0.18	Compliant
4	0.080 ± 0.01	0.086 ± 0.01	6.98	0.29	Compliant
5	0.110 ± 0.00	0.126 ± 0.01	12.70	0.42	Compliant
6	0.140 ± 0.00	0.167 ± 0.01	16.17	0.56	Compliant
7	0.166 ± 0.01	0.197 ± 0.00	15.74	0.66	Compliant
8	0.170 ± 0.00	0.208 ± 0.01	18.24	0.69	Compliant

Cobalt

Cobalt was not detected in influent in the 1st and the 2nd week. The lowest detected influent mean was 0.016 mg/l which was recorded in the 3rd week. The highest influent mean was recorded in the 8th week which was 0.184 mg/l. Cobalt was not detected in effluent in the 1st week of sampling. The lowest detected effluent mean was 0.006 mg/l which was recorded in the 2nd week. The highest influent mean was recorded in the 8th week which was 0.205 mg/l (Table 4.18). All the means for the influent recorded throughout the sampling period were within the NEMA standards for influent discharge to the public sewer which is 1 mg/l. NEMA has not provided the standards for effluent discharge to the environment.

Table 4.18 Mean cobalt recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Increase (%)
1	N.D	N.D	Undefined
2	N. D	0.006 ± 0.01	Undefined
3	0.016 ± 0.01	0.028 ± 0.01	42.86
4	0.033± 0.01	0.059 ± 0.08	44.07
5	0.049 ± 0.01	0.056 ± 0.00	12.5
6	0.033 ± 0.00	0.042 ± 0.00	21.43
7	0.168 ± 0.01	0.189 ± 0.00	11.11
8	0.184 ± 0.00	0.205 ± 0.00	10.24

N.D = not detected

Lead

Lead was detected once in the influent in the 1st week. The mean amount recorded was 0.057 mg/l. In the effluent, lead was detected twice during the 1st and the 2nd week. In the 1st week, the amount of lead recorded was 0.153 mg/l while in the 2nd week, the amount recorded was 0.060 mg/l (Table 4.19). The amount recorded for the two weeks in the effluent was above the NEMA standards for effluent discharge to the environment which is 0.01 mg/l. The amount recorded in the 1st week in the influent were within the NEMA standards for influent discharge to public sewer which is 1 mg/l.

Table 4.19 Mean lead and Chromium recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Increase (%)	Compliance index	Remarks
	Lead				
1	0.057 ± 0.01	0.153 ± 0.01	62.74	15.3	Not compliant
2	N.D	0.060 ± 0.01	Undefined	6	Not compliant
ND not detectable. Lead was not detected in subsequent samplings					
	Chromium				
1	N.D	0.014± 0.01	Undefined	0.007	Compliant
ND not detectable. Chromium was not detected in subsequent samplings					

Chromium

Chromium was only detected once in the effluent in the 1st week. The mean amount recorded was 0.014 mg/l. This level was below the NEMA standards for effluent discharge to the environment which is 2 mg/l (Table 4.19).

Manganese

The mean levels of influent ranged from 0.485 mg/l to 0.724 mg/l. The highest mean was recorded in the 5th week while the lowest was recorded in the 1st week. The mean for the effluent ranged from 0.556 mg/l to 1.01 mg/l. The highest effluent mean was recorded in the 8th week while the lowest was recorded on the 1st week (Table 4.20). The levels of manganese obtained throughout the sampling period in the effluent were within the NEMA standards for discharge into the environment which is 10 mg/l.

Table 4.20 Mean manganese recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	increase (%)	Compliance index	Remarks
1	0.485 ± 0.06	0.556 ± 0.04	12.77	0.06	Compliant
2	0.544 ± 0.03	0.592 ± 0.01	8.11	0.06	Compliant
3	0.578 ± 0.02	0.783 ± 0.06	26.18	0.08	Compliant
4	0.716 ± 0.02	0.883 ± 0.03	18.91	0.09	Compliant
5	0.724 ± 0.02	0.842 ± 0.03	14.01	0.08	Compliant
6	0.658 ± 0.02	0.936 ± 0.147	29.70	0.09	Compliant
7	0.671 ± 0.04	0.994 ± 0.06	32.49	0.10	Compliant
8	0.718 ± 0.02	1.010 ± 0.01	66.51	0.10	Compliant

Zinc

The mean for the influent ranged from 0.242 mg/l to 0. 661mg/l. The highest influent mean was recorded in the 4th week while the lowest mean was recorded in the 2nd week. The mean for the effluent ranged from 0.207 mg/l to 0.319 mg/l. The highest effluent mean was recorded in the 6th week while the lowest mean was recorded in the 2nd week. The levels of zinc obtained throughout the sampling period were within the NEMA standards for effluent disposal to the environment of 0.5 mg/l. They were also within the NEMA standards for disposal to public sewer of 5 mg/l. The highest reduction efficiency was recorded in week 4 which was 65.51% (Table 4.21).

Iron

The mean for the influent ranged from 0.421 mg/l to 3.0 mg/l. The highest influent mean was recorded in the 4th week while the lowest mean was recorded in the 2nd week. The mean for the effluent ranged from 0.377 mg/l to 0.956 mg/l. The highest effluent mean was recorded in the 6th week while the lowest mean was recorded in the 2nd week. The levels of iron obtained throughout the sampling period were within the NEMA standards for effluent disposal of 10 mg/l (Table 4.22).

Table 4.21 Mean zinc recorded at University of Eldoret wastewater treatment plant for eight weeks

(n = 48)

Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Reduction Efficiency (%)	Compliance index	Remarks
1	0.295 ± 0.04	0.248 ± 0.01	15.93	0.50	Compliant
2	0.242 ± 0.00	0.207 ± 0.01	14.46	0.41	Compliant
3	0.397 ± 0.05	0.292 ± 0.02	26.45	0.58	Compliant
4	0.661 ± 0.05	0.228 ± 0.00	65.51	0.46	Compliant
5	0.648 ± 0.05	0.269 ± 0.01	58.49	0.54	Compliant
6	0.424 ± 0.02	0.319 ± 0.01	24.76	0.64	Compliant
7	0.654 ± 0.06	0.286 ± 0.03	56.26	0.57	Compliant
8	0.578 ± 0.06	0.220 ± 0.01	61.94	0.44	Compliant

Table 4.22 Mean iron recorded at University of Eldoret wastewater treatment plant for eight

(n = 48)

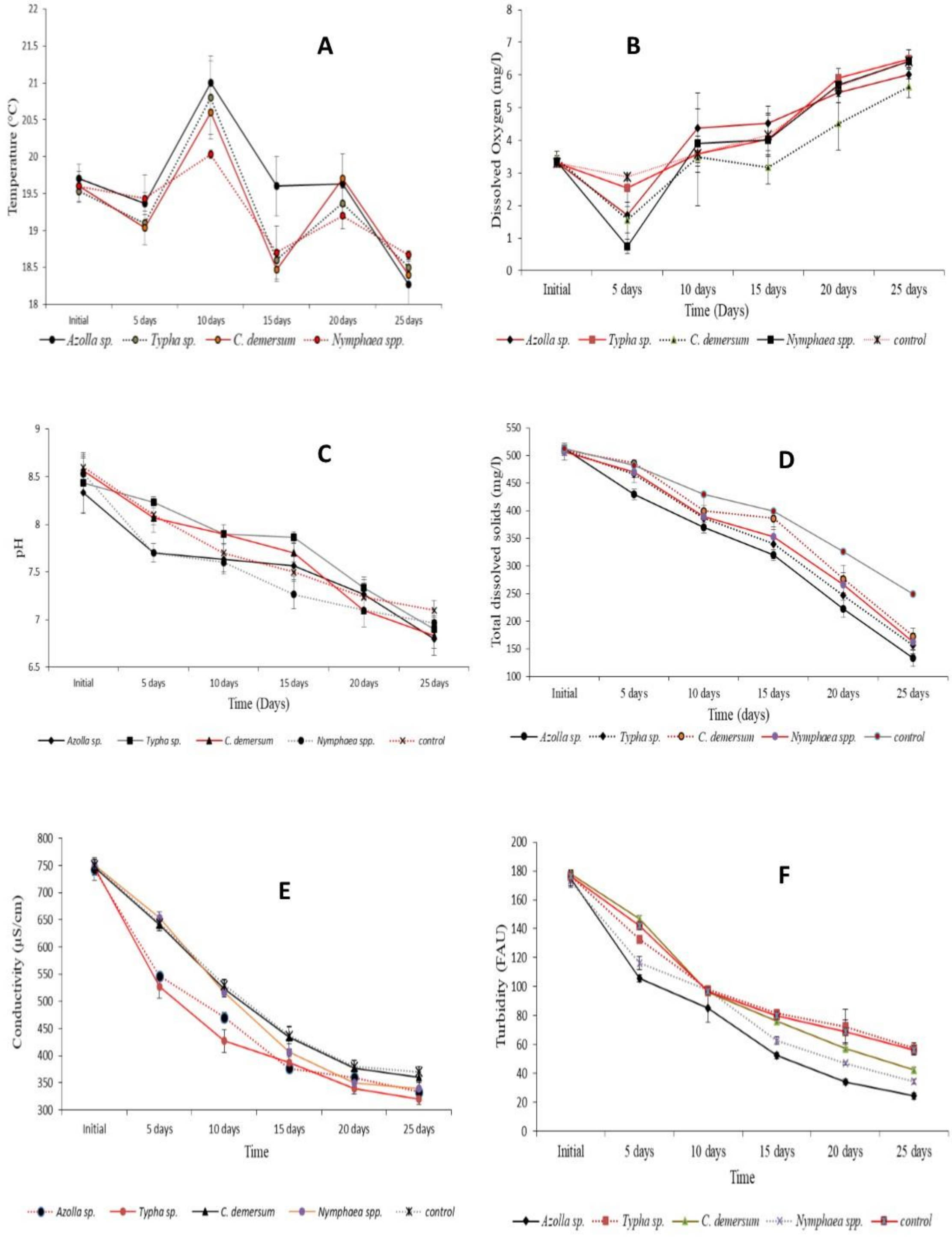
Week	Influent (mg/l) Mean ± SE	Effluent (mg/l) Mean ± SE	Reduction Efficiency (%)	Compliance index	Remarks
1	0.627 ± 0.03	0.486 ± 0.03	22.49	0.49	Compliant
2	0.421 ± 0.04	0.377 ± 0.06	10.45	0.38	Compliant
3	1.028 ± 0.04	0.883 ± 0.02	14.12	0.89	Compliant
4	3.000 ± 0.26	0.920 ± 0.01	68.33	0.92	Compliant
5	2.355 ± 0.02	0.778 ± 0.02	66.96	0.78	Compliant
6	2. 086 ± 0.01	0.956 ± 0.02	54.17	0.96	Compliant
7	1.372 ± 0.01	0.847 ± 0.03	38.27	0.85	Compliant
8	1.128 ± 0.00	0.796 ± 0.02	29.43	0.80	Compliant

4.2 The potential of *A. pinnata, T. latifolia, C. demersum* and *Nymphaea* spp. in remediation of wastewater

4.2.1 Physicochemical parameters

Temperature

There was no significant differences (P = 0.059) in temperatute among the macrophytes and the control during the sampling period, (Figure 4.1a). There was an increase in dissolved oxygen in all experimental set ups (Figure 4.1b). *Ceratophyllum demersum* increased DO by 39.12 %, *Azolla pinnata* by 44.78%, *Nymphaea* spp. by 47.90 % and *Typha latifolia* by 49.15% (Table 4.23). There were no significance differences in the mean DO among the macrophytes (P = 0.651). All macrophytes reduced pH from alkaline to almost neutral (Figure 4.1c). The highest reduction efficiency was achieved by *C. demersum*, 20.30% and the lowest by *T. latifolia* (Table 4.23). There were no significance differences in pH reduction among the macrophytes (P = 0.599).



A = temperature | C = pH | E = conductivity

B = dissolved oxygen | D = total dissolved solids | F = turbidity

Figure 4.1 Potential of *A. pinnata, T. latifolia, C. demersum* and *Nymphaea* spp. to remediate physicochemical parameters in wastewater.

Table 4.23 Reduction efficiency of water quality indicator parameters by *A. pinnata, T. latifolia, C. demersum* and *Nymphaea* spp.

	Plant type				
	Azolla pinnata	***Typha latifolia***	***Ceratophyllum demersum***	***Nymphaea* spp.**	**Control**
	% Er	***% Er***	***% Er***	***% Er***	***% Er***
DO	44.78	49.15	39.12	47.90	48.50
pH	18.37	18.15	20.30	18.29	17.44
Conductivity	55.13	57.11	51.79	54.67	50.67
TDS	74.03	69.28	66.01	67.76	51.30
Turbidity	86.10	67.55	75.98	80.53	68.24
Total coliforms	79.44	88.24	84.95	86	95.49
Faecal coliforms	100	100	100	100	100
Faecal streptococcus	100	100	100	100	100
Phosphates	100	88.65	90.89	100	29.76
Nitrates	100	89.38	92.12	100	60.54

Er = reduction efficiency

Total Dissolved Solids

All the macrophytes reduced the levels of TDS in wastewater (Figure 4.1d). *Azolla pinnata* had the highest reduction efficiency of 74.03% while *Ceratophyllum demersum* had the lowest reduction efficiency of 66.01% (Table 4.23). The mean levels of TDS among the macrophytes were not significantly different (P = 0.505).

Conductivity

Although no significant differences (P = 0.681) were observed among the macrophytes in reduction of conductivity, all the sampled macrophytes reduced conductivity (Figure 4.1e). *Typha latifolia* exhibited the highest efficiency in reduction of conductivity with a reduction efficiency of 57.11% while *C. demersum* had the lowest reduction efficiency of 51.79% (Table 4.23).

Turbidity

All macrophyte species showed a progressive reduction in turbidity levels (Figure 4.1f). *Azolla pinnata* reduced turbidity by 86.1%, *C. demersum* by 75.98%, *Nymphaea* spp. by 80.53% and *Typha latifolia* by 67.55% (Table 4.23). There were no significant differences in the means recorded for the different macrophytes during the sampling period (P = 0.48).

4.2.2 Bacteriological parameter

Total coliforms

Typha latifolia had the highest reduction efficiency of total coliforms which was 88.24% (Figure 4.2). *Nymphaea* spp. reduced total coliforms by 86%, *C. demersum* by 84.95% and A. *pinnata* by 79.44% (Table 4.23). There were no significant differences in the reduction of cfu by the four macrophytes during the sampling period (P = 0.07).

Feacal coliforms and Feacal streptococcus

Although no significant differences were observed among the macrophytes in reduction of feacal coliforms and feacal streptococcus (P = 0.502) and (P = 0.234) respectively, (Figure 4.2), all the sampled macrophytes achieved a reduction efficiency of 100% (Table 4.23).

4.2.3 Nutrients

Nitrates

All macrohytes reduced the nitrates levels in wastewater (Figure 4.3a). *Azolla pinnata* reduced the nitrates by 100%, C. *demersum* by 92.12 %, *Nymphaea* spp. by 100 % and *T.*

latifolia by 89.38 % (Table 4.23). There were significant differences in nitrate reduction among the macrophytes (P = 0.003).

Phosphates

All macrophytes were effective in reduction of phosphates (Figure 4.3b). There were significant differences in the reduction of phosphates among the macrophytes (P = 0.00). *Azolla pinnata* and *Nymphaea* spp. had a 100% reduction efficiency, *Ceratophyllum demersum* had 90.89% while *Typha latifolia* had 88.65 % (Table 4.23).

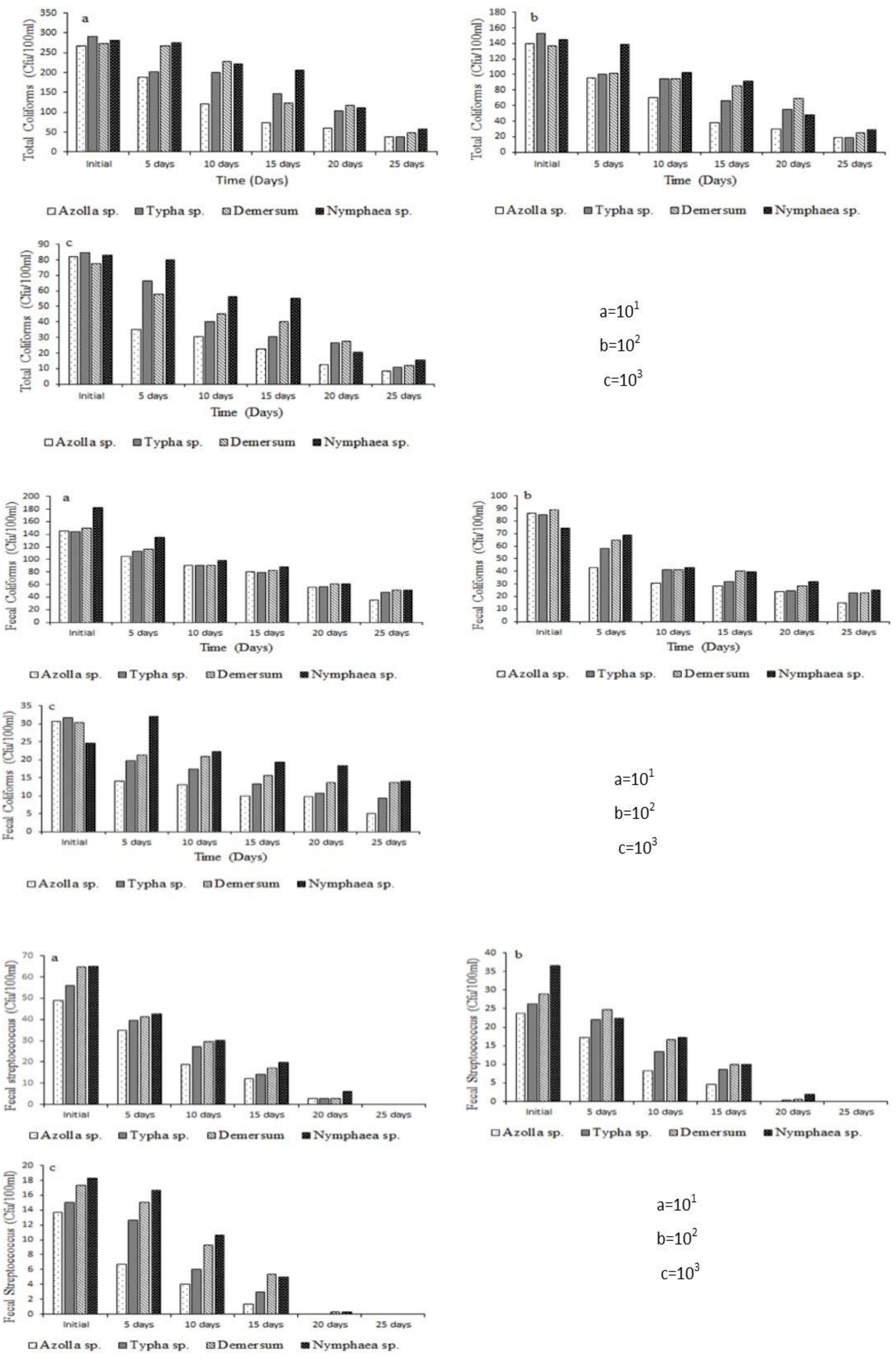


Figure 4.2 Potential of *A. pinnata, T. latifolia, C. demersum* and *Nymphaea* spp. to remove coliforms from wastewater.

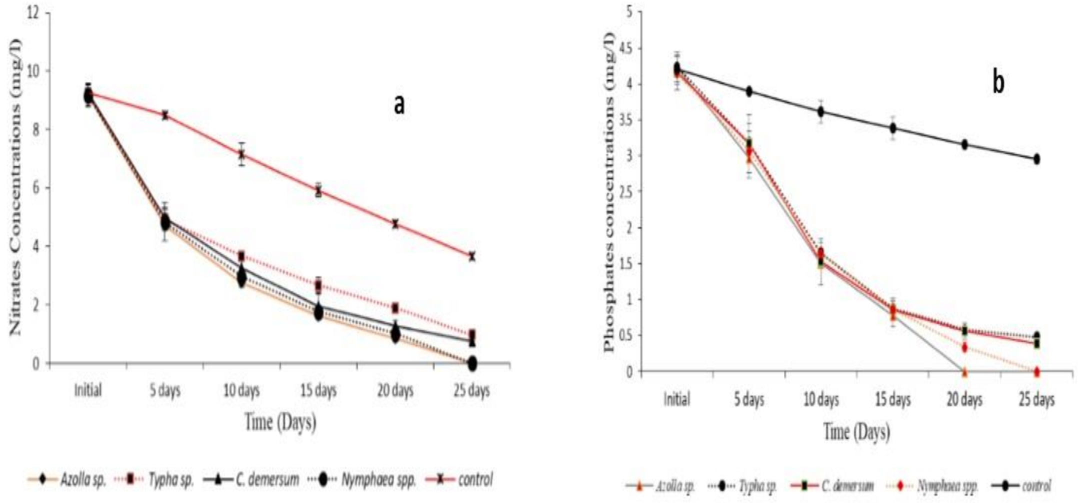


Figure 4.3 Potential of *A. pinnata, T. latifolia, C. demersum* and *Nymphaea* spp. to remediate nitrates and phosphates from wastewater

4.2.4 Heavy metals

Cadmium

The macrophytes reduced the levels of cadmium in the wastewater effluent (Figure 4.4a). *Azolla pinnata* and *T. latifolia* removed cadmium by 92.19%, *Ceratophyllum demersum* by 92.06% and *Nymphaea* spp. by 88.96% (Table 4.24). There were significance differences in cadmium reduction (P = 0.003). Tukey test further established that there were no significant differences in reduction of cadmium among the macrophytes but there were significant differences between the macrophytes and the control.

Copper

All macrophytes exhibited high reduction efficiency (Figure 4.4b). *Azolla pinnata* reduced copper by 85.83%, *T. latifolia* by 85.65%, *Ceratophyllum demersum* by 81.33% while *Nymphaea* spp. reduced copper by 78.87% (Table 4.24). There were no significant differences in reduction of copper among the macrophytes but there were significant differences between the macrophytes and the control, (P = 0.00).

Nickel

All macrophytes were able to reduce nickel by 100% (Figure 4.4c). The control had a reduction efficiency of 31.55% (Table 4.24). There were no significant differences in reduction of nickel among the macrophytes but there were significant differences between the macrophytes and the control, (P = 0.00).

Cobalt

All macrophytes were effective in reduction of cobalt (Figure 4.4d). *Ceratophyllum demersum* reduced cobalt by 95.04%, *Typha latifolia* by 94.98%, *A. pinnata* by 94.72% and *Nymphaea* spp. by 94.67% (Table 4.24). There were no significant difference in reduction of cobalt among the macrophytes but there were significant differences between the macrophytes and the control, (P = 0.00).

Lead

All macrophytes were able to reduce lead by 100% (Figure 4.5e). In the control, a reduction efficiency of 13.50% occurred. (Table 4.24). There were no significant differences in reduction of lead among the macrophytes but there were significant differences between the macrophytes and the control, (P = 0.00).

Manganese

All macrophytes reduced the levels of manganese in wastewater (Figure 4.5f). *Nymphaea* spp. reduced manganese by 88.81%, *T. latifolia* by 86.13%, *C. dermesum* by 85.99% and *Azolla pinnata* by 85.81% (Table 4.24). There were no significant differences in reduction of manganese among the macrophytes but there were significant differences between the macrophytes and the control, (P = 0.00).

Table 4.24 Reduction efficiency of heavy metals by *A. pinnata, T. latifolia, C. demersum* and *Nymphea* Spp.

	% reduction of heavy metals							
Macrophyte	**Cd**	**Cu**	**Ni**	**Co**	**Pb**	**Mn**	**Zn**	**Fe**
Azolla pinnata	92.19	85.83	100	94.72	100	85.81	91.78	94.16
Typha latifolia	92.19	85.65	100	94.98	100	86.13	93.64	94.21
Ceratophyllum demersum	92.06	81.33	100	95.04	100	85.99	92.36	94.85
***Nymphaea* spp.**	88.96	78.87	100	94.67	100	88.81	93.19	95.69
Control	21.88	21.38	31.55	31.15	13.50	32.40	31.11	27.92

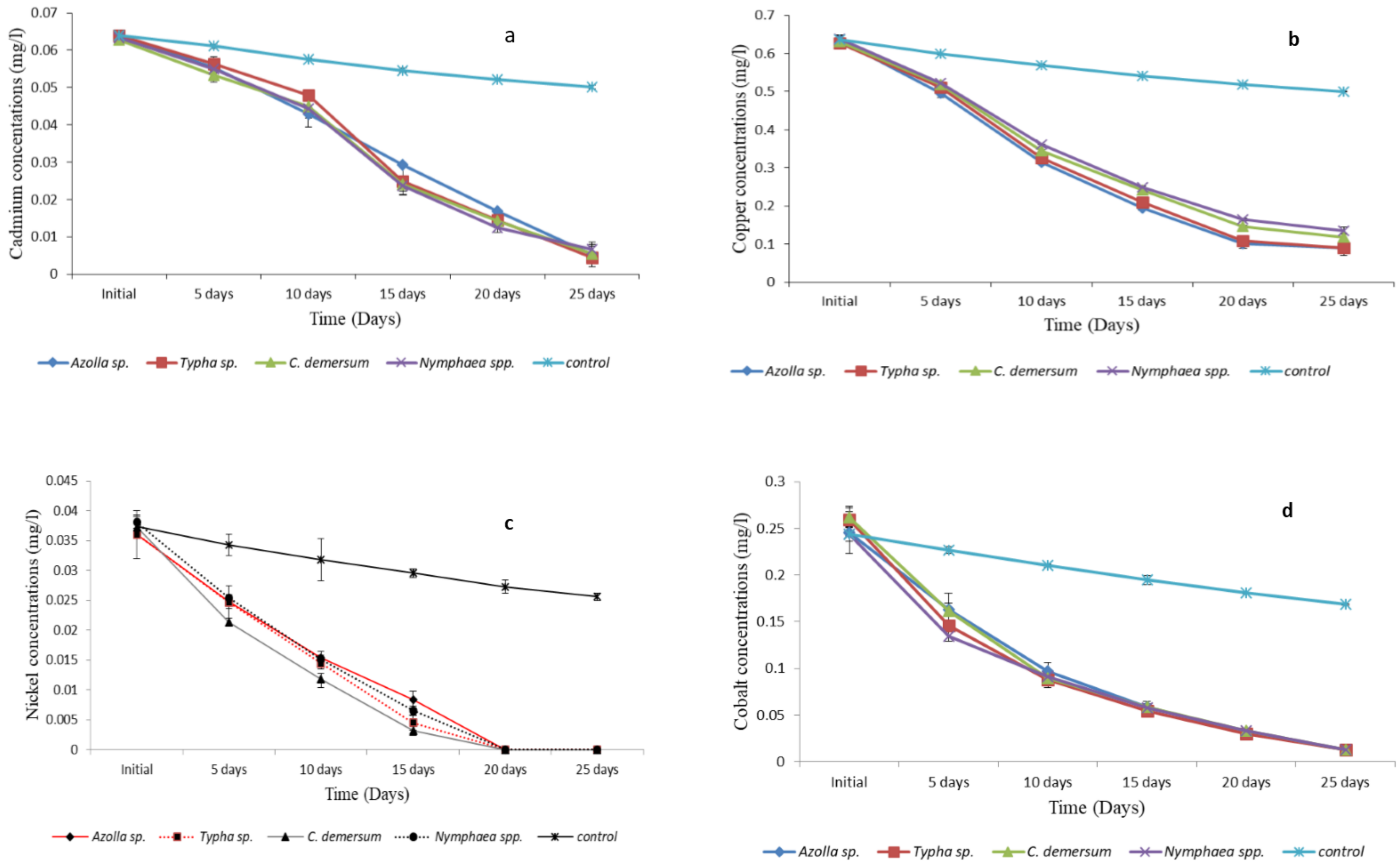


Figure 4.4 Potential of *A. pinnata, T. latifolia, C. demersum* and *Nymphaea* spp. to remove heavy metals from wastewater

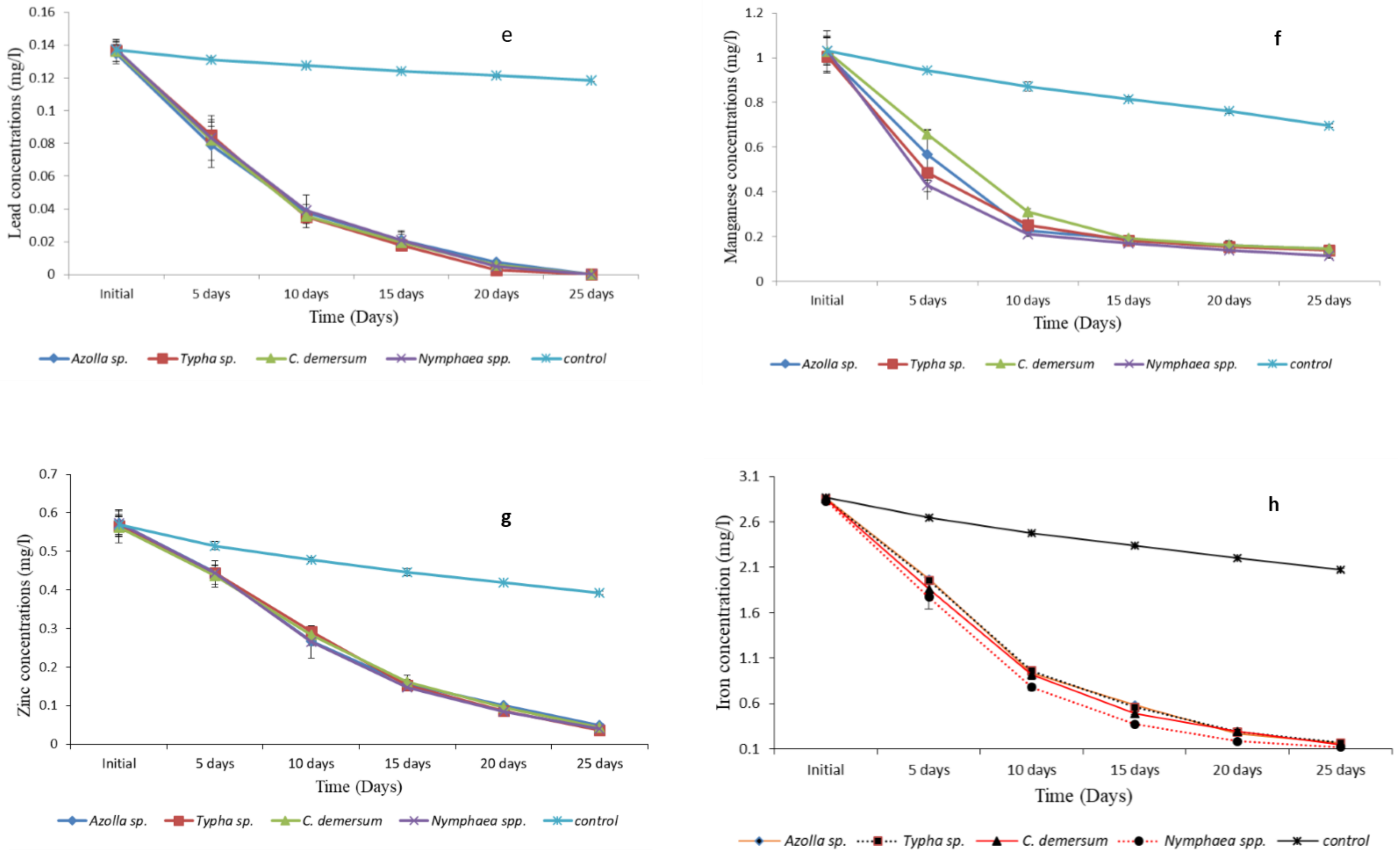


Figure 4.5 Potential of *A. pinnata, T. latifolia, C. demersum* and *Nymphaea* spp. to remove heavy metals from wastewater

Zinc

Zinc was significantly reduced by all macrophytes (Figure 4.5g). *Typha latifolia* reduced zinc by 93.64%, *Nymphaea* spp. by 93.19%, *Ceratophyllum demersum* by 92.36% and A. *pinnata* by 91.78% (Table 4.24). There were no significant differences in reduction of zinc among the macrophytes but there were significant differences between the macrophytes and the control, (P = 0.00).

Iron

Iron was efficiently reduced by all the macrophytes (Figure 4.5h). *Nymphaea* spp. reduced iron by 95.69%, *Ceratophyllum demersum* by 94.85%, *Typha latifolia* by 94.21% and *A. pinnata* by 94.16% (Table 4.24). There were no significant differences in reduction of iron among the macrophytes but there were significant differences between the macrophytes and the control, (P = 0.00).

4.3 The potential of macrophytes in multistage technique in remediation of wastewater

4.3.1 Physicochemical parameters

Temperature

There were no significance differences in the levels of temperature among different columns (P = 0.079). The temperature ranged from 20.5 to 25.5°C (Fig. 4.6 f).

Dissolved Oxygen

All macrophytes columns showed an increase in dissolved oxygen (Figure 4.6e). Column 2 had the highest DO addition of 65.77 %. Column 4 added DO by 33.66%, column 1 by 35.20 %, and column 3 by 33.66% (Table 4.25). There were significance differences in the mean DO obtained for the different macrophytes columns (P = 0.00).

Table 4.25 Reduction efficiency of water quality indicator parameters by macrophytes in multistage technique

	Water quality indicator parameters						
	Dissolved oxygen	**pH**	**Total dissolved solids**	**Conductivity**	**Turbidity**	**Phosphates**	**Nitrates**
Columns	% reduction	% reduction	% reduction	% Reduction	% reduction	% reduction	% reduction
Column 1 *ATCN*	-35.20 ^{ab}	15.48 ^{ab}	79.13 ^{a}	66.92^{b}	70.31^{b}	93.72^{a}	100^{a}
Column 2 *TNAC*	-65.77 ^{d}	16.28 ^{a}	82.27 ^{a}	69.26^{ab}	75.19^{ab}	95.55^{a}	89.79^{b}
Column 3 *CANT*	48.77 ^{c}	14.12 ^{ab}	79.90 ^{a}	68.06^{ab}	67.97^{b}	100^{a}	100^{a}
Column 4 *NCTA*	-33.66 ^{a}	16.67 ^{a}	84.18 ^{a}	71.48^{a}	80.54^{a}	100^{a}	100^{a}
Control	-42.65 ^{bc}	12.94 ^{b}	54.80 ^{b}	51.93^{c}	67.81^{b}	55.35^{b}	73.96^{c}

Means followed by the same letter within the same column are not significantly different at $P \leq 0.05$.
(-) = increase
A = *Azolla* sp. T = *Typha* sp. C = *Ceratophyllum* sp N= *Nymphea* spp.

pH

There was reduction in pH by all macrophyte columns (Figure 4.6d). Column 4 had the highest reduction efficiency of 16.67%. Column 1 reduced pH by 15.48%, column 2 by 16.28% and column 3 by 14.12% (Table 4.25). There were significance differences (P = 0.03) in the reduction of pH with column 2 and 4 attaining higher reduction efficiency than the other columns.

Total dissolved solids

There were significant reductions of TDS by all macrophyte columns (Figure 4.6a). Column 4 reduced TDS by 84.18%, column 2 by 82.27%, column 1 by 79.13% and column 3 by 79.90 % (Table 4.25). There were no significance differences in reduction of TDS among the different macrophytes columns, however there were significance differences between the macrophyte columns and the control, (P = 0.00).

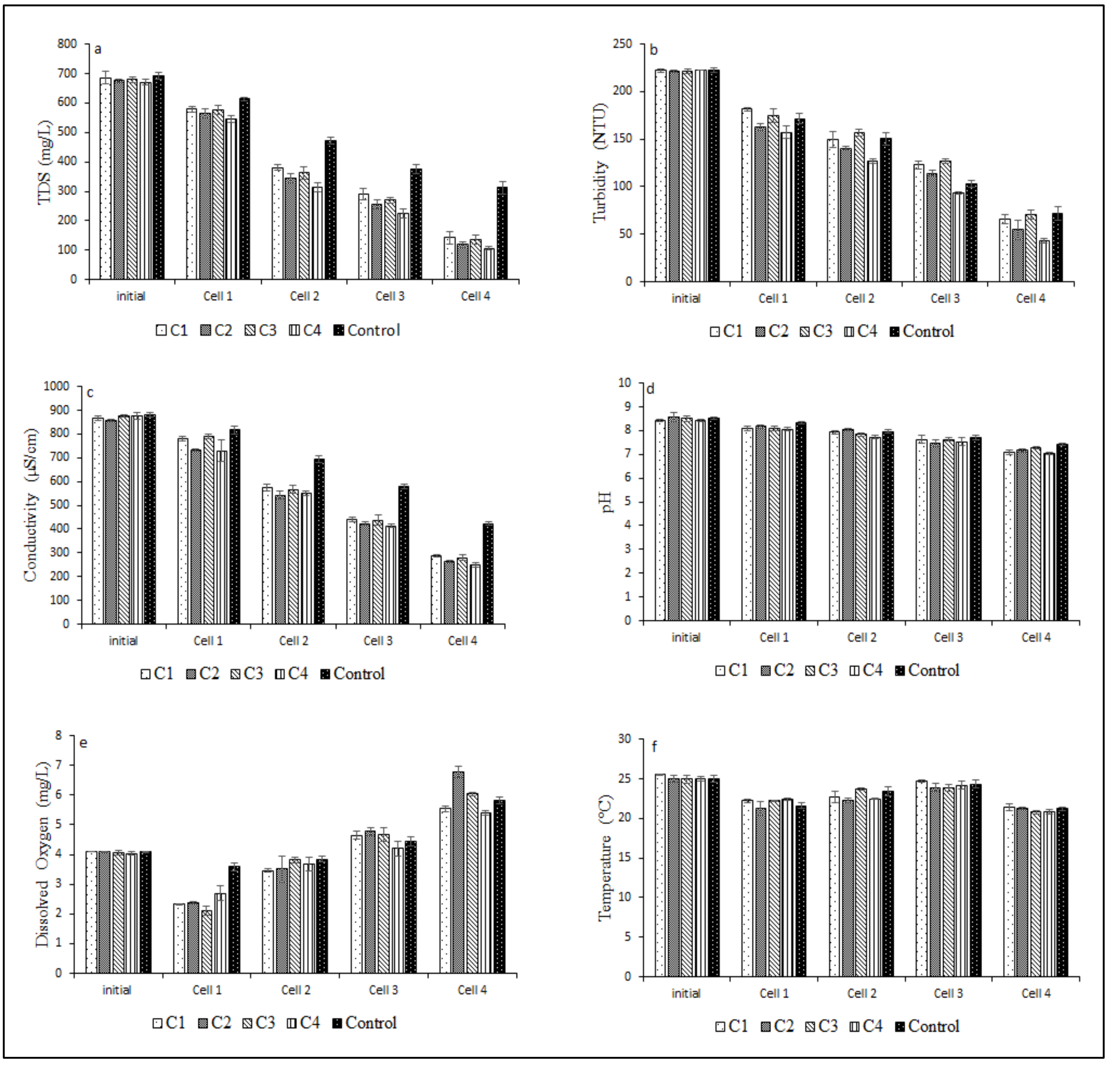


Figure 4.6 Remediation of water quality indicator parameters by macrophytes in multistage technique
(a = TDS, b = turbidity, c = conductivity, d = pH, e = DO and f = temparature).

Conductivity

Column 4 had the highest reduction efficiency of 71.48% (Table 4.25). Column 2 reduced conductivity by 69.26%, column 3 by 68.06% and column 1 by 66.92% (Figure 4.6c). There were significance differences in reduction of conductivity among the macrophyte columns (P = 0.00)

Turbidity

There was reduction of turbidity by all the macrophytes column (Figure 4.6b). Column 4 had the highest reduction efficiency of 80.54% while column 3 had the lowest reduction efficiency of 67.97%. There were asignificance differences in turbidity reduction (P = 0.001). The reduction efficiency attained by column 4 was significantly different from the other macrophyte columns (Table 4.25).

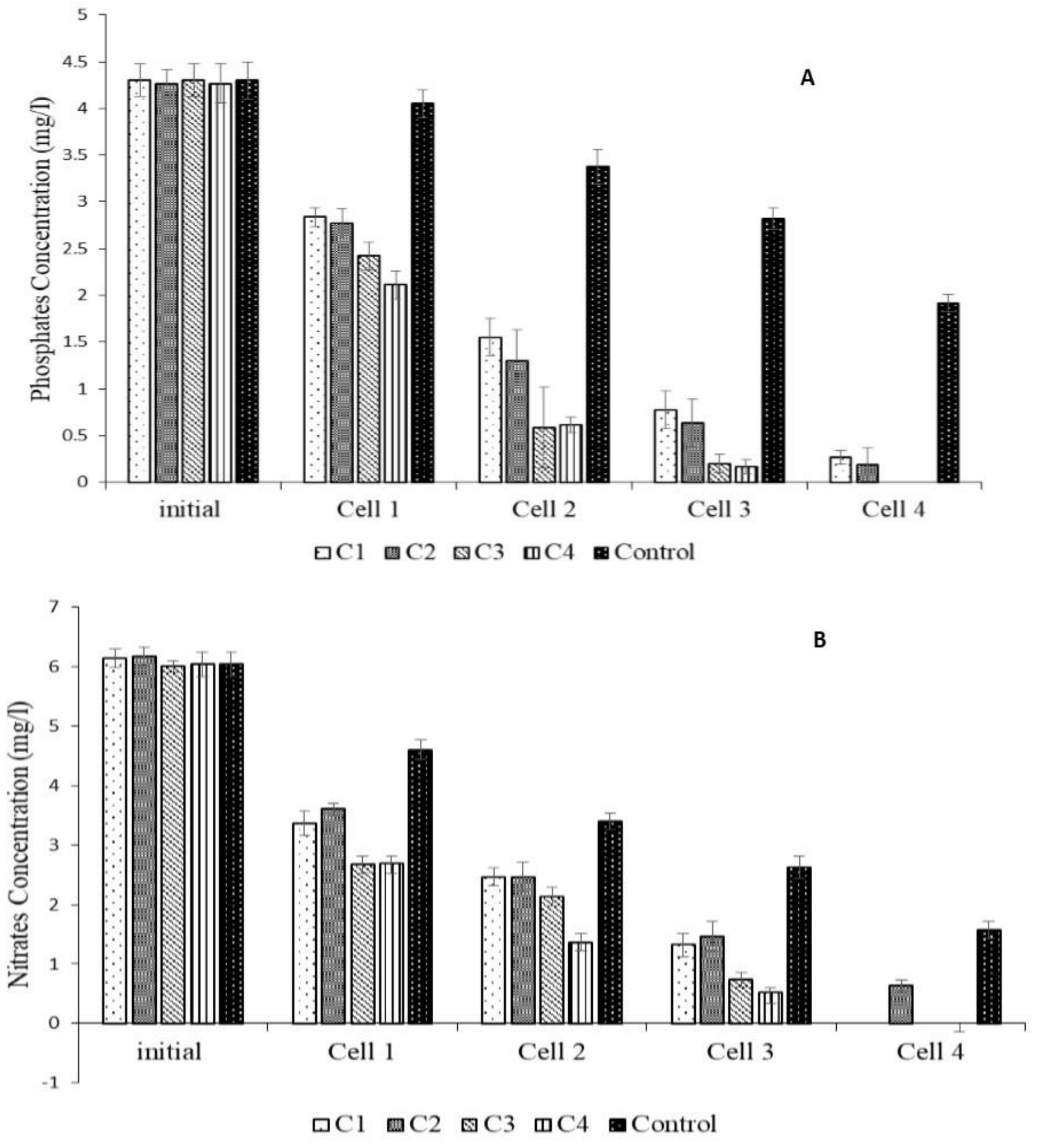
4.3.2 Nutrients

Phosphates

All macrophyte columns reduced the levels of phosphates in the wastewater effluent (Figure 4.7a). Column 3 and 4 reduced nitrates by 100%, column 1 by 93.72% and column 2 by 95.55% (Table 4.25). There were significance differences in the reduction of phosphates between the macrophyte columns and the control but there were no significance differences among the macrophyte columns, (P = 0.00).

Nitrates

There was a general reduction of the levels of nitrates by all macrophyte columns (Figure 4.7b). Column 1, 3 and 4 had a reduction efficiency of 100% while column 2 reduced nitrates by 89.79% (Table 4.25). There were significance differences in the reduction of nitrates among the macrophyte columns. The reduction efficiency of column two was significantly different from the others, (P = 0.00).



A = Phosphates B = Nitrates

Figure 4.7 Reduction of nutrients (phosphates and nitrates) by macrophytes in multistage technique

4.3.3 Bacteriological parameters

Total coliforms

There was a general reduction of the levels of total coliforms in the wastewater effluent (Figure 4.8). Column 2 reduced the total colifoms by 77.82%, column 1 by 68.41%, column 3 by 71.33% and column 4 by 73.74% (Table 4.26). There were no significance differences in reduction of levels of total coliforms by different macrophytes, (P = 0.998).

Feacal coliforms and Feacal streptococcus

There was 100 % reduction efficiency of feacal coliforms and feacal streptococcus in all macrophyte columns and the control (Figure 4.8). There were no significance differences in the reduction of feacal coliforms and feacal streptococcus among the macrophyte columns, (P = 0.948 and P = 0.973) respectively (Table 4.26).

Table 4.26 Reduction efficiency of coliforms by macrophytes in multistage technique

	Total coliforms	**Feacal coliforms**	**Feacal streptococcus**
Columns	% reduction efficiency	%reduction efficiency	% reduction efficiency
Column 1 *ATCN*	68.41[a]	100[a]	100[a]
Column 2 *TNAC*	77.82[a]	100[a]	100[a]
Column 3 *CANT*	71.33[a]	100[a]	100[a]
Column 4 *NCTA*	73.74[a]	100[a]	100[a]
Control	72.15[a]	100[a]	100[a]

Means followed by the same letter within the same column are not significantly different at $P \leq 0.05$.

A = *Azolla* sp. T = *Typha* sp. C = *Ceratophyllum* sp N= *Nymphea* spp.

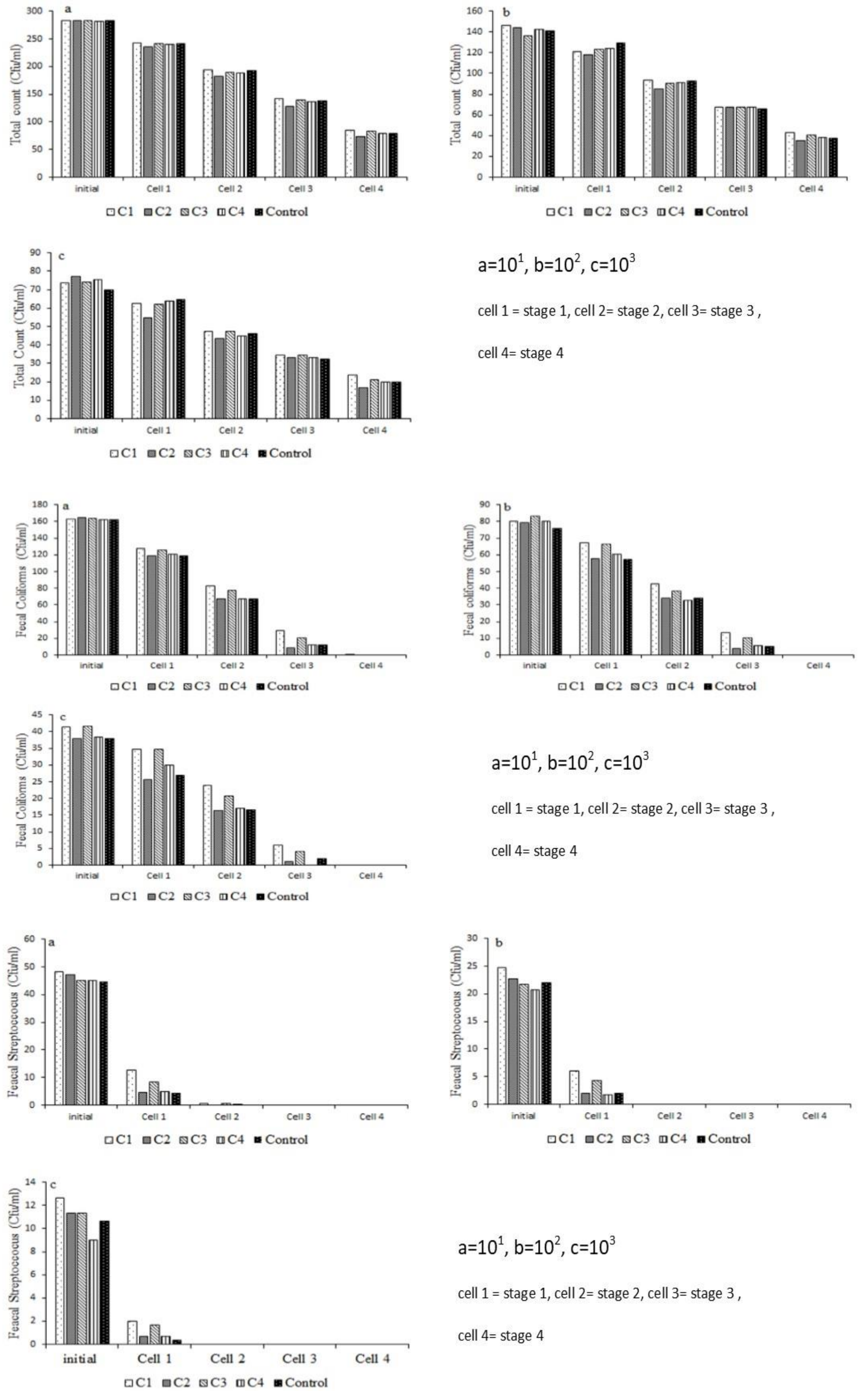


Figure 4.8 Reduction of coliforms under different dilutions by macrophytes in multistage technique.

4.3.4 Heavy metals

Cadmium

All the macrophyte columns reduced the levels of cadmium in the wastewater effluent. Column 3 and 4 reduced cadmium by 100%, column 1 by 83.40% and column 2 by 91.44% (Table 4.27). There were significance differences in the reduction of cadmium between the macrophyte columns and the control but there were no significance differences among the macrophyte columns, ($P = 0.00$).

Copper

Column 4 reduced copper by 81.98%, column 1 by 88.60%, column 2 by 86.40% and columm 3 by 83.39% (Table 4.27). There were significance differences in the reduction of copper between the macrophyte columns and the control but there were no significance differences among the macrophyte columns, ($P = 0.002$).

Iron

Column 1 and 4 reduced iron by 100%, column 2 by 98.24% and column 3 by 95.77% (Table 4.27). There were significance differences in the reduction of iron between the macrophyte columns and the control but there were no significance differences among the macrophyte columns, ($P = 0.001$).

Lead, manganese, zinc, nickel and cobalt

All macrophyte column were efficient in reduction of lead, manganese, zinc, nickel and cobalt as they all attained a reduction efficiency of 100% (Table 4.28 and 4.29). There were no significance difference in reduction of these metals among the macrophyte columns but there were significant differences between the macrophytes and the control ($P < 0.05$).

Table 4.27 Reduction efficiency of heavy metals by macrophytes in multistage technique

	Heavy metals					
	Cadmium		**Copper**		**Iron**	
Columns	Mean ± SE	% E_r	Mean ± SE	% E_r	Mean ± SE	% E_r
Column 1	0.040 ± 0.00[a]	83.40	0.588 ± 0.00[a]	88.60	2.837 ± 0.00[a]	100
Column 2	0.048 ± 0.00[a]	91.44	0.563 ± 0.00[a]	86.40	2.789 ± 0.00[a]	98.24
Column 3	0.053 ± 0.00[a]	100	0.543 ± 0.00[a]	83.39	2.716 ± 0.00[a]	95.77
Column 4	0.053 ± 0.00[a]	100	0.534 ± 0.00[a]	81.98	2.837 ± 0.00[a]	100
Control	0.008 ± 0.00[b]	14.37	0.127 ± 0.00[b]	19.52	0.504 ± 0.00[b]	17.61

% Er = % reduction efficiency

Means followed by the same letter within the same column are not significantly different at $P \leq 0.05$.

Table 4.28 Reduction efficiency of heavy metals by macrophytes in multistage technique

	Heavy metals					
	Lead		**Manganese**		**Zinc**	
Columns	Mean ± SE	% E_r	Mean ± SE	% E_r	Mean ± SE	% E_r
Column 1	0.139 ± 0.00[a]	100	1.128 ± 0.00[a]	100	0.421 ± 0.00[a]	100
Column 2	0.139 ± 0.00[a]	100	1.128 ± 0.00[a]	100	0.421 ± 0.00[a]	100
Column 3	0.139 ± 0.00[a]	100	1.128 ± 0.00[a]	100	0.421 ± 0.00[a]	100
Column 4	0.139 ± 0.00[a]	100	1.128 ± 0.00[a]	100	0.421 ± 0.00[a]	100
Control	0.029 ± 0.00[b]	26.69	0.239 ± 0.01[b]	21.21	0.070 ± 0.00[b]	16.69

% Er = % reduction efficiency

Means followed by the same letter within the same column are not significantly different at $P \leq 0.05$.

Table 4.29 Reduction efficiency of heavy metals by macrophytes in multistage technique

	Heavy metals			
	Nickel		Cobalt	
Columns	Mean ± SE	% E_r	Mean ± SE	% E_r
Column 1	0.039 ± 0.00^a	100	0.272 ± 0.00^a	100
Column 2	0.038 ± 0.00^a	100	0.272 ± 0.00^a	100
Column 3	0.039 ± 0.00^a	100	0.272 ± 0.00^a	100
Column 4	0.038 ± 0.00^a	100	0.272 ± 0.00^a	100
Control	0.012 ± 0.00^b	30.73	0.088 ± 0.00^b	32.61

% Er = % reduction efficiency

Means followed by the same letter within the same column are not significantly different at $P \leq 0.05$.

CHAPTER FIVE

DISCUSSION

5.1 Water quality indicator parameters at University of Eldoret wastewater treatment plant

5.1.1 Physicochemical parameters

The temperature recorded at the inlet was higher than at the outlet. This can be attributed to exothermic reaction that take place in the waste water due to the presence of various dissolved organic and inorganic matter. Temperature is an essential climatic factor that controls the rate of all chemical reactions (Dos Santos, 2018).

High temperature leads to low dissolved oxygen, promotes corrosion and increases solubility of other pollutants. Changes in temperature alter dissolved oxygen. Further, temperature increases oxygen demand that can cause physiological stress on quatic life. The abundance, diversity and distribution of aquatic biota changes in relation to temperature variations in aquatic environments. High water temperature is unsuitable for sensitive species (Andere *et al.*, 2018).

Temperatures recorded at the influent were within the ambient temperatures and were conducive for anaerobic reactions. Gambrill *et al.*, (1986) observed that temperatures of 20^{o}C and above are essential in anaerobic ponds since they facilitate high rates of BOD_5 removal. The temperature recorded at the outlet were generally lower than that of the inlet. This resulted to more dissolved oxygen in the ponds which was essential for the microorganisms and macrophytes.

Generally, dissolved oxygen recorded in the influent was low throughout the sampling period. The DO recorded within the first four sampling was slightly above 1mg/l.

According to Alexander *et al.*, (1988), the DO in raw wastewater in most cases is less than 1mg/l. The DO recorded in the last four week was below 1mg/l. According to Pescod (1996), anaerobic ponds are able to maintain a DO concentration of 0.09 mg/L.

Dissolved oxygen was higher in the effluent compared to the influent. This can be attributed to the degradation of organic matter by the bacteria leading to reduced BOD and COD. Reduction of BOD and COD results in low consumption of dissolved oxygen hence high levels of DO. The results were in agreement with Omoto, (2006) who reported an increase in DO in the effluent. Dissolved oxygen is important for any aerobic biochemical action to take place, its levels are thus indicators of biochemical action. Reduced levels of dissolved oxygen in water impair metabolic reactions in aquatic organism and leads to increased levels of organic materials in water.

Increase in DO in the effluent could also be ascribed to presence of algae and macrophytes in the facultative and maturation pond. These plants are photoautotrophs that utilize carbon dioxide in the presence of sunlight to release oxygen hence could have lead to more dissolved oxygen. Wind effect could have also lead to aeration of the ponds. When wind agitates the surface of the pond, more oxygen dissolves in the wastewater through the water-air interface. The wastewater gets exposed to atmospheric oxygen due to surface turbulence leading to higher dissolved oxygen.

Dissolved Oxygen is indispensable for aquatic life. Organisms present in water require oxygen for metabolism processes. A DO range of 4 -11mg/l is important for the survival of aquatic life (Ronoh, 2017). The amount of oxygen recorded in the effluent for the last four sampling were below 4 mg/l. This may lead to degraded water quality hence affecting the flora and fauna in the Marura ecosystem. A study carried out in river Chepkoilel showed a decrease in dissolved oxygen in Marura wetland at the area immediately after the UoE discharge point (Orwa *et al.*, 2014). Also this area showed a decrease in phytoplankton diversity and abundance compared to other sampled areas along the river (Nyakweba *et al.*, 2014). Decrease in levels of DO in water bodies alters the structure and community composition of these ecosystems where only anoxic tolerant species are able to survive (Nyakweba *et al.*, 2014).

Effluent pH values were higher than influent pH. The results were similar to those of (Ronoh, 2017) who reported an increase in pH in the effluent compared to the influent. This may be ascribed to the amount of carbon dioxide used by algae in photosynthesis. Removing carbon dioxide in water reduces the acidity in water subsequently raising the pH (Williard *et al.*, 2013). The high pH values may be also attributed to biochemical and chemical reactions, for example reactions of bicarbonate and carbonate ions which offer carbon dioxide for the microbes and macrophytes resulting in an excess of hydroxyl ions (Ansari *et al.*, 2010b).

The pH recorded throughout the sampling period was within the NEMA limit hence compliant to the Kenyan standards. The range of pH obtained was optimum pH for methanogenesis process which is usually between pH 6.0 - 8.0. Further, pH influence the performance of a treatment plant since the survival of aquatic organisms depends on narrow pH range. Lettinga *et al.*, (1993) reported that pH of 6.0 is the lowest limit for anaerobic reaction.

The optimum pH level for microbial activities is from 6.0 - 9.0. Outside this range, metabolic activities become impaired and can lead to declines in microorganisms.

Further, pH controls nutrients uptake and biochemical reactions taking place in biota. Low pH increases the rate of release of metals from rocks and sediments in rivers that has an effect on aquatic life like fish's metabolism and ability of water intake through the gills. It irritates fish and reduces the survival of their juvenile stages by affecting their mucous membrane. Low pH also affects amphibians (Ansari *et al.*, 2010b).

Influent TDS values were higher than effluent values. This may be due to the effect of aquatic organisms such as macrophytes that could have absorbed some of the dissolved salts and utilized them for their growth and development. Discharge of effluent that have high levels of TDS can increase the amount of dissolved solids in aquatic ecosystems. Variations in the amounts of TDS can be detrimental to aquatic life as important processes such as osmosis and diffusion can be impaired (Vijay *et al.*, 2010). High levels of TDS in effluent may interfere with extraction of water by flora and fauna in effluent receiving ecosystem. This is likely to change the biodiversity in such an ecosystem where only those organisms that can tolerate high salinity levels will dominate while those that are not salt tolerant will be eliminated (Vijay *et al.*, 2010).

The reduced TDS level in the effluent may be ascribed to the biological utilization of some of the dissolved solids by microbes, algae and macrophytes in the UoE treatment plant. Also some dissolved solids are chemically reactive in wastewater and hence can result in reduction of TDS. The levels of TDS were lower in week one and two which can be attributed to the effect of dilution. During this period, there were heavy rains. TDS can vary significantly with seasons and rainfall events. The effluent TDS obtained throughout the sampling period was compliant to the Kenyan standards.

Influent conductivity values were higher than effluent values. This is attributed to the effect of biotreatment of wastewater at the treatment plant. High conductivity in the influent points out that there is high concentrations of dissolved salt (Aisien *et al.,* 2009). Reduced levels of conductivity in the effluent indicates reduction in the amount of dissolved salts. This may be attributed to the utilization of some essential salts by algae and macrophytes in the facultative and maturation ponds through root absorption (Valipour *et al.*, 2011). The mean levels of effluent obtained throughout the sampling period were above 300 μS/cm. High levels of conductivity could interfere with the process of diffusion and osmosis in organisms affecting their metabolism processes. High levels of conductivity can result to negative physiological effects on plants and animals. It can also increase the corrosion rates (Weinner 2013). Conductivity ranging from 150-500 μS/cm is conducive for the survival of organisms in the water bodies. Conductivity and TDS can influence the pH which in turn can affect the health and survival of aquatic flora and fauna (Sequitur *et al.,* 2003).

The mean levels of effluent were lower than the influent. This could be ascribed to the process of sedimentation which takes place as wastewater moves from anaerobic pond to the subsequent ponds. Suspended mater in the influent such as silt and organic matter are removed principally by physical processes such as sedimentation as wastewater moves from one pond to the other. Suspended and dissolved organic matter in wastewater is assimilated by bacteria. In addition, the natural die offs of some microorganisms and the predation of algae by zooplanktons leads to decreased turbidity (Sequitur *et al.*, 2003). Release of effluent with high turbidity usually impacts negatively to the receiving water body. Turbidity affects the physiological processes of the organisms found in aquatic ecosystems. Increase in turbidity leads to increased water temperature which affects the amount of dissolved oxygen (Shittu *et al.*, 2008).

Biochemical oxygen demand was above the NEMA standards for both inlet and outlet. NEMA standards for inlet is 500 mg/l and for outlet is 30 mg/l. The high BOD_5 in the inlet is attributed to the high organic matter in the raw wastewater. This is an indication of a high degree of organic pollution of UoE wastewater. The high values of BOD_5 in the influent which is twice the allowable limits at the inlet can be imputed to the overloading of the wastewater treatment plant. The high BOD_5 in the outlet indicates that the UoE wastewater treatment plant is not able to efficiently treat the wastewater to a BOD_5 that is within the acceptable limits. This may be due to overloading of the treatment plant. Also the over accumulated sludge might have negatively affected the potential of anaerobic, aerobic and facultative bacteria to degrade the organic matter. The values of BOD_5 for unpolluted waters are normally about 2 mg/l while those of raw domestic sewage are about 500 mg/l. The values of BOD_5 recorded in the UoE wastewater puts it above the values expected for domestic wastewater. These levels are within the industrial effluent which ranges from above 500 mg/l up to 25,000 mg/l.

High BOD_5 leads to high rate of consumption of DO in wastewater which decrease the amount of oxygen that is required by organisms in water. Release of wastewater with high BOD to the natural aquatic environments negatively affects the species biodiversity in these water bodies leading to their death due to anoxia conditions. Decomposition of the additional organic waste due the high BOD necessitates more oxygen further decreasing the limited DO in water (Weinner, 2013). High BOD can lead to creation of anoxia conditions in rivers and streams resulting in death of aquatic species such as fish and macro invertebrates in addition to anaerobiosis and odors. It also reduces species diversity in these ecosystems thus its removal is a principal goal of wastewater clean up. According to Nyakweba *et al.*, (2014), there was reduction in abundance and diversity of phytoplanktons in river Chepkoilel at the UoE discharge point and only the most tolerant species showed an increase.

The reduced levels of BOD_5 in the effluent compared to the influent may be due to the biochemical oxidation brought about by micro-organisms and macrophytes which utilize the polluting organic substances as sources of carbon, while utilizing atmospheric oxygen dissolved in water for respiration. BOD exploits the potential of microbes to oxidize organic matter to carbon dioxide and water using oxygen. Presence of such microbes and macrophytes reduces the BOD_5 due to the utilization of the organic and inorganic matter in the wastewater (Warren and Mark, 2005). Anaerobic digestion involves two processes referred to as acidogenesis and methanogenesis. Acidogenesis is carried out by anaerobes and facultative anaerobes which are present in the anaerobic pond. They degrade organic substances converting them to organic acids. Methanogenesis is carried by strict anaerobes known as acid splitting methane forming bacteria which convert organic acids to methane and carbon dioxide. This stage can also be referred to as gasification. Overloading of the anaerobic pond may impair methanogenesis process leading to inefficient BOD reduction (Mark *et al.*, 2012).

The BOD reduction efficiency recorded throughout the sampling period was above 90%. The highest BOD reduction efficiency was 96.61%. In stabilization ponds, greater than 90% removals of BOD_5 can be attained in well-designed ponds (Mara *et al.*, 2004). Although UoE wastewater treatment plant was able to achieve over 90% reduction efficiency, still it was not able to meet the NEMA standards hence not compliant.

The levels of COD obtained throughout the sampling period for both influent and effluent were above the NEMA standards hence not compliant. The high COD may be ascribed to the overloading of the treatment plant and the effect of over accumulated sludge. According to Alexiou *et al.*, (2003) sludge should be removed when it has reached half depth in the anaerobic pond, otherwise if it over accumulates, it affects the operational and design efficiency of the wastewater treatment plant. The effluent values were lower than the influent values pointing out that the quality of the wastewater had improved although it was not compliant to the Kenya standards. This was similar to Kasima *et al.*, (2014) findings who also reported a decrease in COD in the effluent compared to the influent.

The effluent discharged in Marura wetland are likely to increase the levels of COD concentration in the wetland and in river Chepkoilel. COD is an oxygen-demanding waste hence high levels in effluent can be devastating to receiving aquatic ecosystem. It may upset oxygen balance of these surface waters resulting to hypoxia conditions (Ronoh, 2017). This may have negative impacts on organisms living in this wetland resulting to loss of aquatic life, odors and overall degradation of water quality.

5.1.2 Bacteriological parameters

Total bacteria counts/ heterotrophic counts

Total bacteria counts were fewer in the effluent compared to the influent. This may be ascribed to the natural die off of the bacteria due to exposure to ultra violet light (UV). Ultra Violet radiation is lethal to all types and categories of microbes due to its high energy and short wavelength. This radiation affects the nucleic acid of microorganisms making it unable to synthesize proteins and hence leading to death of the microbe. Bacteria are also destroyed by the formation of singlet oxygen ($^1O_2$) which is formed when bacterial photosynthetic pigments such as bacteriochlorophyll absorb light energy, become excited and act as photosensitizer. The excited photosensitizer transfers its energy to oxygen which then results in singlet oxygen that quickly destroy the cell.

The total bacteria counts were generally high compared to the coliforms. Wastewater has diverse categories of bacteria that originate from various sources. Some of these bacteria are photoautotrophs that utilize the organic matter as a carbon source during the process of photosynthesis. Others are saprophytic bacteria that decompose the dead decaying matter in the wastewater. The decomposers play a vital role in stabilization ponds in relation to the removal of organic matter through mineralization and gasification. Heterotrophic bacteria are important as they metabolise the suspended and dissolved organic matter in the water column. The density and abundance of bacteria in wastewater normally changes with seasons but diversity of species usually decreases with increased loading of a wastewater treatment plant. Occasionally, mobile purple sulphur bacteria occur when there is overloading of facultative ponds and increase in sulphide concentration, with the risk of odour production (EPA, 2000).

The bacteria load during the first three weeks was high in both the influent and the effluent. This could be ascribed to the run offs that ended up in the treatment plant resulting to an increase in bacteria population. The fresh water from precipitation and run offs tend to lower salinity levels hence abundance of bacteria species. Eutrophication might have also lead to an increase in the bacterial load.

Total coliform

Total coliform were fewer in the effluent compared to the influent. This may be ascribed to the natural die off of the bacteria due to exposure to ultra violet light. The total coliforms counts were generally high compared to the feacal coliforms and feacal streptococcus. Wastewater normally has diverse categories of total coliforms that originate from human and animal waste, soil and from run offs. The mean levels of total coliforms were higher in week 1 and 2. This may be ascribed to the addition of total coliforms by runoffs due to the rains. During these two weeks the weather was cloudy and this reduced the light intensity reaching the ponds. This could have led to decline in bacteria die offs (Williard *et al.,* 2013).

Week five had the lowest total coliforms which may be ascribed to the high intensity of sunlight which was accompanied by high temperatures. High sun's intensity may have led to increased die offs of the bacteria. Also increased temperatures favors the growth of algae which leads to increased algae toxins leading to more bacterial deaths (Williard *et al.,* 2013).

Feacal coliforms

The levels of feacal coliforms were higher in the influent compared to the effluent. This may be ascribed to the natural die off of the bacteria due to exposure to ultra violet light. The die offs could be also attributed to unfavourable environmental conditions in the treatment plant that were very different from the conditions in the host. The levels of feacal coliforms were generally high compared to the feacal streptococcus.

The mean level of feacal coliforms in the effluent were above the NEMA allowable limits for discharge to the natural environment. Discharging of effluent with high level of feacal coliforms in the environment may pose high risk to public health. Feacal coliforms are indicator organisms and their presence in high numbers in the effluent points out that pathogenic bacteria might also be present. Disposal of these effluent into the environment may lead to outbreak of waterborne diseases for example typhoid, cholera and dysentery (Girones *et al.*, 2010).

Feacal streptococcus

The levels of feacal streptoccus were higher in the influent compared to the effluent. This may be ascribed to the natural die off of the bacteria due to exposure to ultra violet light. Lower levels of feacal streptococcus in the effluent may be credited to high levels of algae in facultative and maturation ponds. Algae is known for bactericidal capabilities and hence reduces the propagation of pathogenic bacteria. (Ansari *et al.*, 2010b).

The mean level of feacal streptococcus in the effluent were above the NEMA allowable limits for discharge to the natural environment. This may be credited to the shading provided by the macrophytes. High populations of macrophytes in maturation ponds may have reduced the depth of ultra violet light penetration. High populations of duckweed and algae leads to less light penetration and longer survival of these bacteria.

5.1.3 Nutrients

Phosphates

Phosphates mean value in the influent was higher than in the effluent. High phosphate concentration in the influent may be attributed to phosphates in most of the detergent that are used in washing laundry even though there has been some concerted efforts to reduce the practice of adding phosphate to cleaning detergents. Also some phosphates may have come from the laboratories where phosphate salts are sometimes used in various laboratory practicals/experiments. The results were in harmony with Kasima *et al.*, (2014) findings who reported a decrease in phosphates in the outlet.

The reduction of phosphates in the effluent may be attributed to phosphates uptake by various living organisms including bacteria, algae, fungi and macrophytes. Assimilation of phosphates by plants depends on activities of microorganisms that convert insoluble forms of phosphorus to soluble forms. Phosphorus is vital to plants as it is one of the key constituent of several metabolic compounds in their tissues. Phosphates is critical in synthesis of nucleic acids, chlorophylls and energy transfer metabolites. They are normally the limiting factor for most living organisms in the ecosystems. In most aquatic ecosystems, phosphates absorbed by plants are released to the water column through the process of mineralization. The reduction of phosphates in the effluent may also be due to the loss of phosphates through accretion processes within the sediments due to conducive pH range of 6.5 - 8.5 (EPA, 2000).

Discharge of high concentrations of phosphates to water bodies may result to eutrophication (Naeem *et al.*, 2014). The UoE wastewater treatment plant has thick mats of duck weed in the facultative and maturation ponds. The excessive growth of duckweed is an indication of excess nutrients in the wastewater especially nitrates and phosphates.

Nitrates

Nitrates levels were lower in the effluent compared to the influent. The results were in line with Kasima *et al.*, (2014) and Andere *et al.*, (2018) who reported a decrease of nitrates in the outlet of treatment plant, but differed with Ronoh, (2017) findings who reported an increase of nitrates in the effluent. High levels of nitrates in the influent may be attributed to high levels of nitrogenous compounds such as proteins, amino acids and ammonium that could have originated from the feacal materials, urine, from the kitchen and from the laboratories. Low levels of nitrates in the effluent could be ascribed to the nitrates removal by bacteria, algae and macrophytes as they utilized nitrates for their growth and development.

Disposal of high levels of nitrates lead to eutrophication, the nutrient enrichment of aquatic ecosystems causing excessive growth of aquatic flora. In UoE plant, this is evidenced by the excessive growth of duck weed and algae in the facultative and maturation ponds. Orwa *et al.,* (2014) carried out a survey of nitrates along river Chepkoilel and established that the highest nitrates levels were at a sampling point 600m downstream of UoE discharge point. Discharging wastewater with excess nitrates will lead to eutrophication of the receiving ecosystem. Eutrophication alters the environmental characteristics of aquatic ecosystems by altering the trophic food chains. It reduces the species richness and diversity in the ecosystems favouring opportunistic species which flourish and occupy the niche previously occupied by other species (Ansari *et al.,* 2010a). The dissolved oxygen in these aquatic ecosystem becomes depleted when the aquatic plants die and are decomposed by anaerobic bacteria. Depletion of oxygen reduces the biodiversity of aquatic ecosystem and can easily lead to dead zones (APHA *et al.*, 2005). High levels of nitrates in receiving waters that are used for domestic purpose such as drinking has been linked to illness in humans such as methemoglobinemia (Fewtrell *et al.,* 2004) and carcinogenesis (EPA, 2000).

The reduction efficiency obtained throughout the sampling period ranged from 10.31 to 81.77%. According to Alexiou *et al.,* (2003) reduction efficiency in stabilization ponds can range from 70-90%. The reduction efficiency in the UoE stabilization pond was below 70%, only in week 7 that a reduction efficiency of above 70% (81.77%) was achieved.

5.1.4 Heavy metals

Heavy metals are perilous to the ecosystems owing to three key criteria; bioaccumulation, persistence and toxicity (Singh *et al.*, 2012).

Cadmium

The levels of cadmium recorded in the effluent were higher than those recorded in the influent. The percentage increase ranged from 8.42 to 56.86. This was in agreement with Sewe, (2010) and Andere *et al.*, (2018) findings who observed an increase in cadmium level between 19.35% and 22.58 % in series 5 pond which was higher than the influent. The increase in the amount of cadmium in the effluent is attributed to the over accumulated sludge in the stabilization ponds especially in the anaerobic ponds. The source of cadmium may be from paint pigments or from runoffs from the farms where cadmium-containing fertilizers had been applied (Singh *et al.*, 2010).

The cadmium concentrations in the effluent discharged from the UoE plant were above 0.01 mg/l hence were not compliant to the Kenyan standards. Release of high levels of cadmium to the ecosystem can have detrimental effects on the biodiversity. Cadmium is non-degradable and has high stability and toxicity (Singh *et al.*, 2010). In wastewater treatment plant, they can interfere with the health and survival of microorganisms that are responsible for waste degradation (Al-Ubaidy *et al.*, 2015). In human beings, cadmium may cause carcinogenic and non-carcinogenic effects. Cadmium is a reproductive toxicant and an endocrine disruptor (Singh *et al.*, 2010).

Copper

The levels of copper recorded in the effluent were higher than those recorded in the influent. The increase in the amount of copper in the effluent may be ascribed to the delayed de-sludging of the stabilization ponds especially in the anaerobic ponds. The fate of copper in wastewater is influenced by the presence of oxidizing agents, pH, dissolved oxygen and ions. Several studies that investigated the leaching of copper from sludge reported that copper is immobile (ATSDR, 2002).

Dissolved copper ions are removed from solution by sorption to sediments or by precipitation. Adsorption of copper to clay materials depends on the pH and increases with increase of particulate organic materials (Barceloux *et al.*, 1999a). Primary source of copper may be corrosion of copper taps used in plumbing (NRC, 2000). This could have contributed to the concentrations of copper in the wastewater.

The effluent discharged from the UoE treatment plant were compliant to the Kenya standards for effluent discharge to the environment, however, copper concentration of as low as 0.39mg/l can result to death of aquatic invertebrates (Anu *et al.*, 2016). Copper is toxic to algae and this can create a ripple effect in the entire ecosystem inferring that changing one part of an ecosystem has effect on the whole ecosystem (Shahat *et al.*, 2016). High levels of copper in drinking water can cause liver cirrhosis, anaemia, kidney damage, diarrhoea, headache and abdominal pains (Salem *et al.*, 2000).

Nickel

The levels of nickel recorded in the effluent were higher than those recorded in the influent. The increase in the amount of nickel in the effluent is attributed to the over accumulated sludge in the stabilization ponds. The sorption of nickel to sediment increases with increase in pH and organic matter (Mellis *et al.*, 2004). Possible sources of nickel in the wastewater include nickel-plated taps and utensils which may release nickel into water and food (Kamerud *et al.*, 2013). Nickel is also present in shampoos, detergents and coins and hence can be present in grey water (IARC, 2012).

Nickel is important in the growth and development of some plants and microbes (Sydor *et al.*, 2013). It is a vital nutrient for microbes and flora that have metabolites with nickel as an active site. The microbes and macrophytes in the UoE treatment plant could have utilized nickel in the wastewater. The concentration of nickel in the UoE effluent released to Marura wetland were compliant to the Kenyan standards. Discharge of high levels of nickel to the surface water can negatively affect public health. Nickel compounds are classified as human carcinogens. Nickel increases chances of contracting respiratory cancer to the people working in nickel mining industries. Human exposure to nickel commonly results to allergic skin reaction referred to as contact dermatitis and sometimes asthma (Butticè, 2015).

Cobalt

Cobalt was not detected in the influent during the first and second week of sampling. The concentration of cobalt were higher in the effluent in comparison to the influent. This can be ascribed to possible accumulation of cobalt in the over accumulated sludge and in wastewater pathways within the treatment plant. Presence of cobalt in wastewater may be due to the disposal of cobalt containing batteries such as those used in mobile device and in rechargeable batteries for various electronic devices (Biggs *et al.*, 2005). When such batteries are disposed in the environment, runoffs may carry them to the wastewater treatment plant. Kenya limits for cobalt effluent discharge into the environment were not given in the NEMA water quality regulation.

Lead

The lead levels were below detection limit in most of the sampling period. Low concentrations were recorded in the effluent in the 1st and 2nd week. In the influent, lead was detected once during the 1st week of sampling. Possible sources of lead may be from lead piping in water distribution systems, from lead paints and also from lead batteries. Heavy metals such as lead determine the rate of anaerobic digestion even at trace amount (Mielke *et al.*, 2011).

Generally, the levels of lead obtained in the two weeks were low, this may be attributed to minimal use of lead compounds. Nowadays, legislative measures to control use of leaded products such as fuel are being strictly enforced and unleaded petroleum products are encouraged (Thayaparan *et al.*, 2013). Low levels of lead were also reported by Kasima *et al.*, (2014) who carried out a study at Kipevu wastewater treatment plant. In her Study, lead was detected twice out of the five sampling that she carried out.

The levels of effluent were higher compared to the influent, this may be credited to the accumulation of lead in the sludge. The heavy metal ions present in water are precipitated as hydroxides due to the high pH of the wastewater in the stabilization pond, which settles as sludge. The levels of lead that were obtained in the effluent were above the Kenyan standards hence not compliant. Discharging high levels of lead into the environment has adverse impacts on organisms. Lead has lethal effects even at low concentrations and disrupts food chains (Tunegová *et al.*, 2016). Observable effects of lead toxicity in aquatic fauna include blackening in the tail region and spinal deformity (Yamauchi, 2017). In human beings, lead has been reported to be carcinogenic, mutagenic and teratogenic (Kristensen, 2015).

Chromium

The chromium levels were below detection limit in most of the sampling period. Low concentrations were recorded in the effluent in week 1. This indicates that the levels of pollution by chromium in the environment are low. Sewe (2010) carried out a study in Dandora wastewater treatment plant and reported that chromium was below detection limit during her sampling period. In a study carried out by Kasima. *et al.*, (2014), hexavalent chromium was not detected.

The presence of chromium in the effluent and not in the influent may be ascribed to the accumulation of chromium in the sludge and in the wastewater pathways. The results were in contrast with Andere *et al.*, (2018) who reported high levels of chromium in the influent compared to the effluent. Chromium is non-essential to living organisms and hence not utilized in both wastewater and sludge. The possible source of chromium could be from the laboratories where potassium dichromate is used in titration. Exposure to hexavalent chromium over a long period may cause nerve disorder and harm the kidney, circulatory system and liver (Lokeshappa *et al.*, 2012). The levels of chromium recorded in the effluent were within the Kenyan standards hence compliant.

Manganese

The levels of manganese recorded in the effluent were higher than those recorded in the influent. The increase in the amount of manganese in the effluent is attributed to the over accumulated sludge in the stabilization ponds. Possible sources of manganese in the wastewater could be from the laboratory where manganese is commonly encountered as the compound potassium permanganate which is a strong oxidizing agent. Also as manganese oxide that catalyzes the decomposition of hydrogen peroxide and is sometimes used for the small scale production of oxygen gas in the laboratories (Conover, 2009).

Manganese is essential to organisms where it is utilized for growth and development, a trace element for biota (Erickson *et al.,* 2019). Excessive exposure to manganese may result to health problems such as body weakness, drowsiness and even paralysis. Consumption of high amounts of manganese in the human body acts as a neurotoxin (Peres *et al.*, 2016). It can result in an increase in intellectual impairments and reduction in intelligent quotient in children (Bouchard *et al.*, 2010).

Zinc

Zinc mean value in the influent were higher than in the effluent. High zinc value in the influent may be attributed to the use of zinc products by the entire UoE community. Possible sources of zinc in the wastewater may be from shampoos in which zinc has been added as antidandruff (Marks *et al.,* 1985). It could also originate from toothpaste and mouthwash in which chelated zinc has been added to prevent mouth odor (Roldán *et al.*, 2003). Cosmetics, rubber and prescription drugs in which zinc oxide has been added are possible sources of zinc in the wastewater. Zinc usually finds its way into the household water distribution and finally to the wastewater from deteriorated galvanized iron sheets (WHO, 2016). Zinc is also used in manufacture of phosphate fertilizers and pesticide and this could get into the wastewater as a result of storm water (Broadley *et al.*, 2007).

Low zinc levels in the effluent may be as a result of the utilization of zinc by microorganisms and macrophytes. The results of the current study were in line with Andere *et al.*, (2018) who reported a decrease in the concentrations of zinc in the outlet. Zinc is an essential trace element for organisms (Saper *et al.*, 2009). In aquatic ecosystems, zinc concentrations as low as 2µg/ml influences negatively the concentration of oxygen circulated in fish body (Heath, 2018).

Iron

Influent iron levels were higher than effluent levels. High levels of iron in the influent may be due to the use of iron within the University of Eldoret. Iron could have originated from fertilizers, inks and pesticides in which iron has been added. Iron is used in cosmetics and therefore a contaminant in grey water that finally gets in the wastewater treatment plant. Iron is abundance in the rocks and it might have also originated from the weathering process. Reduction of iron in the effluent may be attributed to the utilization by microbes, algae and macrophytes. Some amounts of iron could have also been adsorbed in the sludge and on the wastewater pathways.

5.2 Potential of A. pinnata, T. latifolia, C. demersum and Nymphaea Spp. in phytoremediation.

There was significant reduction in physicochemical parameters, nutrients, bacteria loads and heavy metals in the UoE wastewater by the each macrophyte. All the water quality indicator parameters, nutrients and heavy metals measured at the end of the sampling period were within or below the permissible NEMA standards for the discharge of wastewater into the environment. The uptake and accumulation of pollutants varied among the macrophyte species and also from pollutant to pollutant

5.2.1 Physicochemical parameters

There was no specific trend in temperature. The recorded temperature was influenced by the ambient temperature. All the macrophytes were able to increase DO in the wastewater effluent. Macrophytes carry out photosynthesis where they utilize carbon dioxide and release oxygen. This increases the amount of dissolved oxygen in water. Some addition of oxygen may be ascribed to the surface contact with the atmospheric oxygen. This could have added some oxygen in all the set ups including the control. *Typha latifolia* had the highest increase in DO, 49.15% this may be ascribed to its well-developed roots, rhizomes and rhizoids which oxidize the rhizosphere (Wießner *et al.*, 2005). *Typha latifolia* is an emergent plant, whose most parts are above the water surface. This increases the surface contact of the water with the atmospheric oxygen compared to other macrophytes. *Ceratophyllum demersum* had the lowest DO increase potential 39.12%. This is a submerged plant hence the amount of sunlight reaching it was influenced by physical factors such as turbidity and TDS. The amount of sunlight received reduces the rate of photosynthesis, subsequently reducing the amount of DO released by the plant.

All the macrophytes were able to reduce the level of pH in the wastewater effluent. This may be attributed to the reduction of dissolved compounds and TDS in the wastewater caused by uptake of these substances by plants. Nutrients and metal ions uptake combined with release of hydrogen ion may have lead to pH reduction (Mahmood *et al.*, 2005). The results were in harmony with Snow and Ghaly, (2008) who reported a decrease in pH in their study of cleaning up aquaculture effluent using macrophytes. Biochemical conversions of the various types of organic and inorganic matter could have contributed to change in pH.

All the macrophytes reduced the levels of TDS. This may be as a result o the reduction of the dissolved organic and inorganic substances as a result of absorption and adsorption process of these substances by macrophytes. *Azolla pinnata* was the best in reduction of TDS, with a reduction efficiency of 74.03%. The high efficiency in the removal of organic load by macrophytes may be as a result of better oxygenated environmental conditions created by macrophytes which enabled the microbes associated with the macrophytes to degrade the organic matter at a faster rate (Lee *et al.*, 2008).

Also, some reduction in TDS might be as a result of adsorption of some dissolved substances to the sides of the troughs as the water level decreased. In the control, algae could have utilized some dissolved organic matter for its growth. Koelsch *et al.*, (2006) reported that totals solids can be reduced by 70 - 90% in constructed wetlands due to decrease in flow rate and creation of conducive conditions for infiltration by vegetation.

There was reduction in the levels of conductivity by all macrophytes. Reduction in conductivity could be attributed to decrease in dissolved salts in the wastewater by plants uptake or root adsorption. *Typha latifolia* had the highest reduction efficiency. Snow and Ghaly (2008) reported conductivity reduction by 65.31% in wastewater purified using water hyacinth for four days. Mahmood *et al.*, (2005) reported 55.71% conductivity reduction in water hyacinth based constructed wetland after twelve days of treatment.

In the control, there was a decrease in the levels of conductivity. This may be due to some algae growth in the control credited to high nutrients in terms of phosphates and nitrates. The algae could have utilized some of the dissolved salts in their growth and development leading to reduced conductivity. Generally, there was reduction of turbidity by all macrophyte species. This may be credited to the utilization of some suspended matter by the macrophytes. Also, some amount of the suspended material could have settled as a result of sedimentation process. *Azolla Pinnata* had the highest reduction efficiency (86.10%). This plants has numerous root hairs that could have accelerated the absorption of suspended matter in the wastewater. Rizzo *et al.,* (2012) worked with feedlot effluent and reported suspended matter removal of more than 70% in both treatments with and without plants. Snow and Ghaly (2008) observed turbidy reduction of 59.54% in their study of purification of effluent from engineering industry using *Eichonia crassipes*. Dipu *et al.*, (2011) reported a significant reduction in turbidity after using *Typha* spp. in dairy effluent treatment for 15 days. Kulasekaran *et al.*, (2014) reported turbidity reduction of 93.8 to 98.7% in sewage treatment by *C. demersum.*

5.2.2 Bacteriological parameters

Total coliforms

There was reduction of total coliforms in all experimental set ups. Among the macrophytes, the highest reduction efficiency was attained by *T. latifolia* (88.24%). This plant is emergent in nature and hence did not cover most of the water surface. This increased the surface area of wastewater exposed to the UV radiation hence increasing the rate of die off of the bacteria. *Azolla pinnata* had the lowest reduction efficiency (79.44%). This plant is free floating and hence covered all the available surface area reducing the amount of UV light penetrating the water surface. This reduced the rate of die off of the bacteria.

Azolla pinnata also offers a big surface area for adsorption of microorganisms which increases their survival. According to Kadlec *et al.*, (2009), 90% and above of the coliforms and 80% and above of the feacal streptococci were eliminated in various constructed wetlands systems in their study. The control had the highest reduction efficiency (95.49%). There were no plants in the control hence the intensity of UV light was more than in the other experimental set ups. This may have led to high rates of bacteria die offs. Also, the control lacked the plants, hence bacteria had reduced substances to adsorb to and hence decreased survival.

Feacal coliforms and Feacal streptococcus

High reduction of feacal coliform and feacal streptococcus was vital because the presence of these bacteria in the effluent indicates that pathogenic bacteria might as well be present. All the macrophyte species had a reduction efficiency of 100% for these two types of bacteria at the end of the experiment. The results were in line with the findings of Lim *et al.,* (1998) who observed a 100% reduction efficiency of feacal coliforms in an overland surface treatment used to treat cattle manure. Ikenberry *et al.*, (2000) reported an average feacal coliform removal of 76.6 % in their study of treating animal waste. These types of coliforms are normally associated with warm blooded animals and cannot survive for a long time outside their host species.

5.2.3 Nutrients

Phosphates

All the macrophytes indicated high potential of phosphate removal from eutrophic effluent. *Azolla pinnata* and *Nymphaea* spp. were able to reduce phosphates by 100%. Phosphates are macronutrient thus all the plants utilized the available phosphates. Kulasekaran *et al.*, (2014) reported a reduction of 93.5 - 98.3% in phosphates level by *C. demersum.* It may be inferred that *C. demersum* has high ability to absorb nutrients from wastewater because of its thin and dissected leaves that allows water-plant interaction. *Azolla pinnata* high reduction efficiency could be due to loose and numerous tiny root mats. According to Basilico *et al.,* (2015) major phosphorus removal processes are plant uptake, sorption and precipitation. There was a slight reduction in the levels of phosphates in the control which could be ascribed to algae and microbial uptake, photodegradation, volatilization and sorption to troughs.

Nitrates

Azolla pinnata and *Nymphaea* spp. were able to reduce nitrates by 100%. The results were in harmony with the findings of Simeon and Silhol (1987) and Enduta *et al.*, (2011) who reported 82.9 to 98.1% reduction in nitrate-nitrogen in wastewater using constructed wetlands. *Azolla pinnata* high efficiency in removal of nitrates may be as a result of its thin and loose root mat that promotes water-plant interaction. Vidayanti *et al.*, (2012) reported 70% reduction of phosphates and nitrates by *E. ramosissium* and *Typha sp.* in mono and polyculture techniques.

All macrophytes utilized nitrates since it is an essential macronutrient required for plant growth. Macrophytes accelerated the rate of removal of nutrients from feedlot effluent in a retention time of 10-17 days (Rizzo *et al.,* 2012). The key processes involved in nutrients removal in constructed wetlands include assimilation by plants, nitrification and denitrification (Basilico *et al.*, 2015). The decrease in the levels of nitrates in the control is attributed to the uptake by algae and microbes.

5.2.4 Heavy metals

Cadmium

All macrophytes had high reduction efficiency of cadmium. *Azolla pinnata* and *T. latifolia* performed better in cadmium reduction with reduction efficiency of 92.19%. The results were in harmony with the findings of Rai (2008) who observed 70 - 94% heavy metals removal by *A. pinnata* from ash slurry effluent in India. Yousefi *et al.*, (2013) reported 42 % to 58 % removal rates of lead and cadmium in a constructed wetland used in purification of effluent from a college in 2 and 6 days, respectively. Kumar Rai, (2010) reported 70 to 94 % cadmium reduction by *Azolla pinnata* in thirteen days of treatment. The high growth rate of *Azolla pinnata* along with its free-floating nature could have enabled it to accumulate more heavy metals. Maine *et al.*, (2001) reported that floating macrophytes have potential to accumulate cadmium to significant levels. Also submerged macrophytes such as *C. demersum* has great ability to remove cadmium in environment (Al-Ubaidy *et al.*, 2015).

Copper

All macrophytes reduced the levels of copper significantly. *Azolla pinnata* had the highest reduction efficiency, 85.83%. Elsharawy *et al.*, (2004) reported a reduction efficiency of 59.1% while working with *A. pinnata.* Mokhatar *et al.*, (2011) reported copper reduction efficiency of 97.3% while working with two macrophytes to remove copper in aqueous solution. According to Shafi *et al.*, (2015), *A. pinnata* is a hyper accumulator of copper and zinc and moderate accumulator of lead, chromium and cadmium. Anning *et al.*, (2013), reported 33.84% removal of copper by *Typha latifolia* from wastewater. *Typha angustifolia* can be able to accumulate significant levels of copper from both soil and aqueous medium. *Typha sp.* has fibrous and tap root system that facilitate absorbtion of heavy metals (Sukumaran, 2013).

Nickel

All macrophytes were able to reduce nickel by 100%. The results were in harmony with the findings of Elsharawy *et al.*, (2004) who found out that *A. pinnata* had a reduction efficiency of 73.1%. Mallick *et al.*, (1996) reported that *Azolla pinnata* was able to accumulate nickel, zinc and chromium. Chorom *et al*., (2012) performed an experiment in the laboratory using *C. demersum*, and Nickel concentrations of 6, 4, 2, and 1 mg/dm^3. The results indicated that metal removal efficiency from the medium was 41.7%, 50.0%, 52.5% and 46%, respectively. Parnian *et al.*, (2016) carried out a research with *C. demersum* for 8 days in greenhouse conditions using medium modified with an increasing cadmium and nickel (0, 1, 2, 4 and 6 mg/dm^3). The results indicated that an increase in heavy metals concentration in medium resulted in a decrease in the plant biomass. Efficiency of metal removal by *Ceratophyllum demersum* was 82.0% for cadmium and 52.5% for nickel. Comparative studies of sorption characteristics of various macrophytes with respect to nickel, copper and cadmium accumulation indicated that submerged plants have better sorption characteristics. Among the submerged macrophytes, *Ceratophyllum demersum* was better in sorption (Krems *et al.*, 2013).

Cobalt

All macrophytes were effective in reduction of cobalt. *Ceratophyllum demersum* was the best in cobalt reduction, 95.04%. According to Elsharawy *et al.*, (2004), *A. pinnata* removed 95.0% of cobalt and 90.0% of cadmium from a mixture of wastewater. Phytoremediation efficiency of metals greatly depends on the concentration of these metals in solution, the lower the concentration of the metals in the solution the higher the removal efficiency. The amount of cobalt in the UoE wastewater effluent was low, this could have led to the high reduction efficiency.

Lead

All macrophytes were able to reduce lead by 100%. A minimal change occurred in the control which had a reduction efficiency of 13.50%. Shafi *et al.*, (2015) revealed that *A. pinnata* has an outstanding performance in lead removal since it removed high concentrations of lead in retention time of 10 days and hence concluded that *A. pinnata* is a good accumulator for lead. *Azolla* binds heavy metals in different concentrations with high effectiveness. It is an effective accumulator of cadmium, copper and lead. *Typha latifolia* has potential to absorb large quantities of lead in roots with minimal transportation to the shoots, indicating a potential use for phytostabilization. Nadia *et al.*, (2014) indicated that *C. demersum* has remarkable bioremoval potentialities for lead, it removed 32.2% of lead under high concentration within five days. *Ceratophyllum demersum* was able to accumulate 95.8% of lead from a media that had 0.1 mmol/dm^3 lead concentration in 7 days (Mishra *et al.*, 2006). El-Khatib *et al.*, (2014) reported high accumulation values of lead in *Ceratophyllum demersum*. According to Galadima *et al.*, (2015) adsorption of lead onto *Nymphaea* spp. roots occurs via a monolayer adsorption process, while its adsorption onto the leaves and seeds is via a heterogeneous multilayer process.

Manganese

Nymphaea spp. was the best in reduction of manganese 88.81% followed by *T. latifolia* 86.13%. The results coincided with Hazra *et al.*, (2015) who said that *T. latifolia* was able to accumulate manganese, iron, copper, zinc, and nickel. According to Ahmad *et al.*, (2014), the roots of *Typha sp*. have high retention capacity of trace elements and heavy metals hence can averse bioaccumulation in higher trophic levels. Sasmaz *et al.*, (2008) assessed heavy metals accumulation in *Typha latifolia* growing in a river carrying wastewater and reported that manganese was the most absorbed metal. A reduction efficiency of 65.1% has been reported while working with *A. pinnata* (Elsharawy *et al.*, 2004).

Zinc

Zinc was significantly reduced by all macrophytes. *Typha latifolia* was the best in zinc reduction 93.64% followed by *Nymphaea* spp. 93.19%. According to Rai, (2008), Azolla has great ability to accumulate toxic heavy metals such as nickel, cadmium, mercury, chromium, copper and zinc. In this respect, Arora *et al.,* (2004) reported that *A. pinnata* have potential to absorb chromium, lead, cadmium, zinc and other heavy metals and is tolerant when these heavy metals are present in low concentrations. Keskinkan *et al.*, (2004) reported that *C. demersum* can accumalate copper, zinc and lead.

Iron

All macrophytes were efficient in reduction of iron, they had a reduction efficiency of above 94%. *Nymphaea* spp. was the best in reduction of iron, 95.69%. The macrophytes needed iron for various physiological and biochemical pathways and for chlorophyll production. *Azolla pinnata* has been reported to remove 92.7% of iron and 83.0% of zinc (Elsharawy *et al.*, 2004). Yen and Saiber (2013) reported that *Typha sp*. accumulated high concentration of iron and copper, these two metals are vital for plants growth and development.

5.2.5 Comparison of the remediation potential of *A. pinnata, T. latifolia, C. demersum* and *Nymphaea* spp.

In phytoremediation of wastewater, proper selection of the macrophytes is crucial to ensure efficient treatment of wastewater. Macrophytes that are efficient in phytoremediation are paramount as they enable the discharge of effluent that do not infringe the set standards. Management of wastewater is one of the main goals of sustainable development principles in the conservation of water resources.

Macrophytes varied in their ability to improve physicochemical parameters, absorb nutrient and remove heavy metals. In this study, there was differential accumulation pattern for nutrients and heavy metals indicating that remediation efficacy of studied nutrients and heavy metals was governed by the plant species, the kind of nutrient or heavy metals and their concentration in wastewater. The observed variation could be due to their difference in growth patterns, morphological and genetic characteristic. According to Miller, (1996), plants may uptake toxic substances for various reasons including defense against pathogens, allelopathic effects, sequestration, disposal by leaf abscission and drought resistance.

Azolla pinnata

Azolla pinnata performed better in phytoremediation of wastewater effluent than the other three macrophytes. It was also good in the remediation of other investigated water quality parameters. This was also reported by Rezaie *et al.*, (2014) who proposed that it can be used for restoring polluted aquatic resources. Various studies has been carried out using *Azolla* in heavy metals removal from aquatic environments (Kumar Rai, 2010; Rai, 2008). *Azolla pinnata* has a remarkable potential to hyper accumulate heavy metals from polluted environments (Wagnar, 1997).

Azolla Species have high potential for absorbtion and retention capacities of several heavy metal ions thus utilized in phytoremediate of heavy metal in polluted water reservoirs (Moradi *et al.*, 2013; Sufian *et al.*, 2013). *Azolla pinnata* possesses a remarkable ability to survive in highly polluted waters with wide spectra of pH, temperature and salinity, reflecting its suitability for phytoremediation applications (Thayaparan *et al.*, 2013). A cohesive strategy can be employed using *Azolla* biomass that is produced in the process of phytoremediation as a protein rich feed for livestock, for bioenergy production or can be utilized as green manure if the concentration of accumulated contaminants are below the set standards. *Azolla pinnata* is a phytoremediation agent that can be used for the sanitization of the studied minerals and heavy metals. This technology offers efficient, easy, cost-effective, environmentally-sustainable solution to the challenge of eutrophication and heavy metal contamination in aquatic ecosystems.

***Nymphaea* spp.**

Nymphaea spp. was ranked second position in the phytoremediation of UoE wastewater effluent. It has high potential as an optimal, highly effective phytoremediation tool. *Nymphaea* spp. has a well-developed root system which creates a good medium for filtration and adsorption and also enables the growth of microbial colonies. Floating aquatic macrophytes are capable of growing in vertical and horizontal direction, thus maximizing the photosynthetic and absorption surface area. Moreover, unlike immersed plants, they carry out photosynthesis in an aerial environment where carbon dioxide is not a limiting factor and there is plenty of water.

The present investigation revealed that *Nymphaea* spp. was efficient in nutrients uptake and heavy metals removal. This coincided with the findings of Ziarati *et al.*, (2015) who reported that *Nymphaea* spp can be utilized in the removal of chromium and nickel from polluted water. Galadima *et al.*, (2015) found that *Nymphaea* spp. was able to adsorb cadmium and lead. The high potential of *Nymphaea* spp. may be attributed to a good diversity of rhizosphere bacterial community with potential metal resistance (Kabeer *et al.,* 2014). The current study proposes the use of *Nymphaea* spp. for nutrients and heavy metals removal from contaminated aquatic ecosystems. In addition, *Nymphaea* spp. is highly productive, favourable in respect to machinery use in management and easy to harvest.

Typha latifolia

Typha latifolia was ranked third position in phytoremediation of the UoE wastewater effluent. It is tolerant to many different kinds of contaminants and is widely known to be very resistant to the effects of harsh, heavy metal contaminated environments (Yadav and Chadra, 2011). Its position in comparison with other macrophytes may be due to the number of plant shoots put in each experimental set up. For each experiment, 500g of each plant species was used. For *T. latifolia*, only a few plant shoots achieved this weight.

Typha latifolia was efficient in the nutrients uptake and heavy metal removal from wastewater. This may be attributed to its well-developed roots and rhizomes. These enhance maximum absorption of ions from the water to plant system so that they can be utilized in both growth and development. Roots and rhizomes support rhizospheric microorganisms fostering microbial activity (Wiebner *et al.*, 2005). These microbes assists the macrophyte in rhizodegradation of some of the contaminants and enhance nutrient and metal uptake.

Typha latifolia have high biomass hence they can sequester high levels of nutrients from eutrophic wastewater and absorb various heavy metals (Yadav and Chandra 2011; Mojiri *et al.*, 2013). Anning *et al.*, (2013) indicated that this species has potential to remove cadmium, copper, zinc and lead without exhibiting phytotoxicity signs. According to Parzych *et al.*, (2016), *Typha latifolia* presents diverse accumulation properties in relation to heavy metals present in water. It is able to minimize the toxic effects of these pollutants that would damage physiological processes (Sasmaz *et al.*, 2008). The present study found out that *Typha sp.* have high capacity to sequester nutrients and absorb heavy metals. These traits make *Typha latifolia* an excellent candidate for phytotechnology. This technology is environmentally sound producing no further damage to toxic sites.

The high biomass of *Typha sp*. plays an ancillary role in carbon sequestration hence mitigating global warming.

Ceratophyllum demersum

Ceratophyllum demersum was ranked fourth position in removal of contaminants from wastewater. It is a submerged plant which have a poorly developed root system, however it was able to absorb nutrients and heavy metals significantly. Immersed macrophytes do not have external protective tissues, all the cells of the plants seems to have potential to absorb nutrients and other substances directly from the environment (Guilizzoni, 1991). It is inferred that the sorption mechanism of metals by this species is by ions exchange (Schneider *et al.*, 2001). Submerged plants usually have highly dissected leaves which have an advantage of increasing the surface area for absorption, photosynthesis and reducing water resistance thus avoiding possible damage to the leaves. Denny and Wilkins (1987) reported that use of shoots instead of roots in metal absortion increases in submerged plants. Increased potential in uptake of contaminants is linked to the simplicity of their leaf structure that facilitates accelerated uptake of matter from the ecosystem (Maleva *et al.*, 2004).

Ceratophyllum demersum is a biological indicator of water pollution with cadmium and lead (VahdatiRaad *et al.*, 2012; Dogan *et al.*, 2015). *Ceratophyllum demersum* serves as a hyperaccumulator of zinc, lead, copper, arsenic, chromium, iron and cobalt (Xing *et al.*, 2013). *Ceratophyllum demersum* can be used in bio sorption and bioaccumulation of lead, copper and zinc (Krems *et al.*, 2013) cadmium, nickel (Parnian *et al.*, 2016) chromium, alluminium and zinc (Gałczyńska *et al.*, 2019). Predrag *et al.*, (2005) investigated the concentration of the heavy metal in tissue of *C. demersum* species and found out that this plant can accumulate high levels of lead, cadmium, manganese, copper, iron, cobalt and nickel. This potential may be attributed to the physical traits of the species such as very thin epithelium and use of the whole plant surface in uptake of matter (Predrag *et al.*, 2005).

Jamnická *et al.*, (2006)) reported higher amounts of heavy metals in the submerged plants, compared to free-floating plants in aquatic ecosystems. Their findings differed from the finding of the present study where emergent and floating plants performed better than *C. demersum*. Performance of *C. demersum* in the present study could have been hindered by high turbidity. High turbidity levels blocks light from reaching submerged plants hence hindering photosynthesis. Moreover, low removal rates of nutrients and heavy metals by *C. demersum* may be attributed to its allelopathic characteristic. It produces compounds that prevent the growth of phytoplanktons and blue green algae (Hiscock, 2002). Presence of phytoplanktons in the other macrophytes experimental set up could have contributed to higher removal of the nutrients and heavy metals.

Ceratophyllum demersum has the features for species which are successful in phytoremediation. This hydrophyte demonstrated high potential for biomass production and vegetative propagation even in poor dietary conditions (Aravind and Prasad, 2004). Xing *et al.*, (2013) observed that *C. demersum* demonstrates a positive adaptive strategy in reaction to heavy metals exposure in i*n-situ* studies. The present study nominates this plant for designing of eco-friendly and economical wastewater treatment plants. Additionally, *Ceratophyllum demersum* provides habitat for small organisms and constitutes food source for snails, crustaceans, insects, water birds, fish and rodents (Gałczyńska *et al.*, 2019). This plant is effective in wastewater phytotechnology and can be utilized successfully for removing nutrients and heavy metal pollutants from wastewater.

The study demonstrated that all the four macrophyte species can be used in phytotechnology. They are good in sequestration of mineral nutrients and in accumulatotion of several heavy metals thus candindates for wastewater treatment from various sources. These macrophytes can be used to improve effluent from oxidation ponds.

5.3 Potential of macrophytes in multistage technique in remediation of wastewater

The results indicated that there were significant reductions in physicochemical parameters, nutrients, bacterial loads and heavy metals in the UoE wastewater by the macrophytes in multistage technique. The wastewater from the last stage indicated that the levels of the measured parameters were within or below the permissible NEMA standards for the discharge of wastewater into the environment. The absortion of contaminants varied among macrophyte columns and also from pollutant to pollutant.

5.3.1 Physicochemical parameters

There was no specific trend for temperature, it varied according to the variation of the ambient temperature. However, all the levels obtained throughout the sampling period were within the NEMA permissible levels of effluent discharge to the environment. There was an increase in dissolved oxygen that may be attributed to the reduction of dissolved carbon dioxide and addition of oxygen during the process of photosynthesis (De Godos *et al.,* 2010). Increased DO in the wastewater accelerates the rate of biodegradation by microorganisms. Transfer of oxygen by macrophytes to the rhizosphere is important in promoting the growth of aerobic bacteria (Reddy *et al.*, 1987).

There was a general decrease in the levels of pH obtained from all the macrophyte columns. The reduction efficiency ranged from 12.94 to 16.67%. The results were in harmony with the findings of Aisien *et al.,* (2015) who reported a reduction efficiency of pH ranging from 13.3 to 20% while working with macrophytes to improve the abbaitor effluent. Dipu *et al.*, (2011) observed a significant reduction in pH while working with four macrophytes to improve dairy effluent. Reduction of pH may be ascribed to the uptake of contaminants by macrophytes. Thus pH reduction favored the microbes associated with macrophytes to degrade organic waste in the UoE wastewater (Mahmood *et al.*, 2005). Further, pH is an essential parameter in the process of biosorption affecting metal chemistry and metallic ion competition in solution (Galun *et al.*, 1987).

All the macrophyte columns were able to reduce the levels of TDS in the UoE wastewater effluent. The reduction efficiency ranged from 79.13 to 84.18%. This was in agreement with the finding of Ghaly *et al.*, (2005) who reported TDS reduction range of 54.7 to 91.0% in aquaculture wastewater purified using macrophytes in 12 days. All the TDS levels obtained from all columns at the last stage were within the NEMA permissible levels of effluent discharge to the environment. Reduction of TDS may be attributed to the reduction of organic matter and other dissolved salts as a result of utilization of these substances by macrophytes during their growth and development. The roots of some aquatic macrophytes uptake organic matter present in wastewater to support their growth thus reducing TDS in the wastewater (Krems *et al.*, 2013).

There was a general reduction of conductivity in all macrophyte columns. The reduction efficiency ranged from 66.92 to 71.48%. The results were in line with the findings of Aisien *et al.*, (2015) who reported a reduction efficiency ranging from 63.4 to 89.3 %.

The reduction in conductivity may be attributed to salts removal from the effluent through plants assimilation.

All macrophytes columns reduced the levels of turbidity in the UoE wastewater effluent. The reduction efficiency ranged from 67.97 to 80.54%. The results were in agreement with the findings of Aisien *et al.,* (2015) who reported a reduction efficiency ranging from 71 to 96.8%. Reduction in turbidity may be due to the uptake and biodegradation of organic matter by plants and also the adsorption of some suspended substances to the macrophyte tissues.

5.3.2 Bacteriological parameters

Total coliforms

There was a significant reduction in the levels of total coliforms in all experimental set up. The reduction efficiency ranged from 68.41% to 73.74%. Generally, bacteria decreased in number at each subsequent sampling. Several studies have reported that aquatic plants significantly reduce microbial contaminants in water hence improving water quality. The reduction efficiency depends on associated characteristics of wastewater and plant species (Ottová *et al.,* 1997).

Feacal coliforms and feacal streptococcus

All the macrophyte columns reduced feacal coliforms and feacal streptococcus bacteria significantly. The reduction efficiency in all macrophyte columns was 100%. The results were in harmony with the results of Aisien *et al.,* (2015) who reported a reduction efficiency of 100%. Feacal coliforms and feacal streptococcus have shorter survival period when outside the host's body because their survival is largely dependent on the host. The concentrations of bacteria in the treated UoE wastewater effluent met the NEMA standard for the effluent discharge to the environment.

Constructed wetlands have been reported to have capacity to remove various pathogens such as viruses, fungi, bacteria and protozoan cysts (Greenway 2005). One of the mechanism that leads to reduction of microorganisms in wastewater is sedimentation (Karim *et al.*, 2004). Solar radiation, temperature, filtration, sedimentation and adsorption contribute to the removal of bacteria in tropical regions (Khatiwada and Polprasert 1999).

5.3.3 Nutrients

Phosphates

All macrophyte columns reduced the amounts of phosphates in the UoE wastewater effluent. The reduction efficiency ranged from 93.72% to 100%. The reduction of phosphates is ascribed to the uptake by plants. The results were in agreement with the findings of Ansari and Khan (2010a) who reported more than 75 % total phosphorus and nitrogen removal by macrophytes from eutrophic water.

Removal mechanisms of phosphates include uptake by plants, exchange reactions with sediments, sorption and chemical precipitation (Reddy *et al.*, 1987). Periphyton and microorganisms associated with macrophytes can remove nutrients directly from the wastewater. In the control, phosphates removal may be due to algae uptake and coprecipitation which is brought about by the supersaturation of calcium carbonate as a result of the elevation in pH during the photosynthetic process.

Nitrates

Three macrophyte columns attained a reduction efficiency of 100%, only one column had a reduction efficiency of 89.79%. Ghaly *et al.,* (2005) reported 82.9% nitrate-nitrogen reduction in aquaculture wastewater using constructed wetlands. Fox *et al.,* (2008) reported nitrogen reduction efficiency by aquatic plants that ranged from 60-80 %. Most aquatic plants absorb nitrates directly from water through their roots, shoots and leaves. Moreover, macrophytes carry out a vital role by increasing the surface area and providing a favorable environment in the rhizosphere for microbes to grow (Brix, 1997). These microbes are involved in nitrification processes that contribute to the reduction of nitrates in wastewater. Some nitrates may have been removed though denitrification process (Vymazal, 2013).

5.3.4 Heavy Metals

The macrophyte columns reduced most of the metals by 100%. These include; nickel, zinc, cobalt, manganese and lead. The reduction efficiency for iron ranged from 95.77 to 100%. Some of these heavy metals for instance nickel, zinc, iron, manganese, cobalt and copper are necessary for plant growth in physiological quantities as they carry out significant role in biota. They are essential in maintaining the proper functioning of enzymes and also make up metal-organic compounds such as metalloproteins. However, higher concentrations limit root growth and damage root cells (Krems *et al.*, 2013). A moderate reduction in all heavy metals in the control might be due to biological activities of microorganisms. The final levels of all heavy metals were below the NEMA standard for wastewater discharge to the environment.

In phytoremediation, metals removal efficiency is governed by the concentration of these metals in solution (Ingole and Bhole 2003; Keith *et al.*, 2006). The amount of heavy metals in the effluent were low especially for cobalt, nickel, zinc, manganese and lead. This could have contributed to their high reduction efficiency of 100%. At the final stage, these heavy metals were not detected and hence were assumed to have been completely removed from the treated UoE wastewater effluent. The reduction efficiency of copper ranged from 81.98% to 88.60%. The slower removal of copper in comparison with the other metals may be ascribed to the high initial concentrations of copper compared to the rest of the heavy metals.

All the macrophytes column reduced the levels of cadmium in the UoE wastewater effluent. The reduction efficiency ranged from 83.40 to 100%. Two columns attained 100% reduction efficiency. The slower removal of the cadmium compared to the other metals present in the wastewater effluent may be due to its toxicity. Cadmium is non-essential to plants and hence may be excluded during absorption especially in presence of other essential metals. Most macrophytes have no mechanisms for uptake of non-essential metals like cadmium and absorbtion is via Ca^{2+}, Mn^{2+}, Zn^{2+} and Fe^{2+} transporters in the process of absorbing vital elements (Verbruggen *et al.,* 2009; Marchard *et al.,* 2010). Ferniza-Garcia *et al.,* (2017) researched on the removal of lead, zinc, copper and cadmium in solution using coupled electrocoagulation-phytoremediation treatment and found out that the rate of cadmium removal was slower compared to other metals, which was ascribed to a pH difference that was necessary for removal of cadmium in the technique used.

The macrophytes used can be considered as hyper accumulators of these metals hence their excellent removal efficiency. The results were in harmony with the findings of Aisien *et al.*, (2010b) and Mishra *et al.*, (2008). Roots of various macrophyte have been indicated to produce chelating substances into the rhizosphere that mobilize trace elements, hence minimizing their absorbtion and toxicity (McGrath *et al.*, 1997; Shah and Nongkynrih 2007).

Phytoremediation potential of macrophyte relies on the tolerance level of the macrophyte species. Different macrophytes were able to reduce the heavy metals at different rates. *Azolla pinnata* performed better compared to the other macrophytes in heavy metal reduction. This may be attributed to its high rate of growth and a short doubling time. This plant is also tolerant to varying environmental conditions and to several pollutants (Sood *et al.*, 2012).

The accumulation of heavy metals also relies on the type of heavy metals (VahdatiRaad *et al.*, 2012). The absorbtion of the eight heavy metals was different among the plants. The essential heavy metals were reduced at a higher percentage compared to the non-essential metals. For example, zinc, copper, cobalt and manganese are used in the synthesis of the enzyme that are required in production of other, physiologically active particles (Nyquist *et al.,* 2007). Some heavy metals such as cadmium can cause oxidative stress, even at low concentrations that damage cellular membranes (Harguinteguy *et al.*, 2013). According to Aravind *et al.,* (2005), the existence of metal-metal interactions can influence the accumulation of one metal in the presence of another.

In the occurence of an array of metal contaminants, various metals can be accumulated synergetically by one plant (Brown *et al.,* 1994). All the macrophytes were able to accumulate all the investigated heavy metals resulting to different reduction efficiency. According to Tang *et al.,* (2009) *Arabis paniculata* and *Thlaspi caerulescens* can accumulate zinc and cadmium. More often, there can be varying consequences on the individual uptake of a heavy metal ion, due to complex formation, re-activities, decreased bioavailability, competition and inhibitory effects (Chotpantarat *et al.,* 2011). There was a difference in reduction potential for different heavy metals within a particular plant species. Marbaniang (2013) reported that one of the most critical influences on individual heavy metal absorbtion is the supplementary metal concentrations in the medium. The existing variation may be also attributed to various environmental factors that normally regulate phytoremediation potential such as chemical speciation, metals interaction, redox potential and the initial concentrations of the metal (Garg and Aggarwal, 2011). According to Rofkar *et al.,* (2014), the presence of silicon and copper, decreased the accumulation of arsenic by *Azolla caroliniana.* An investigation of *Elodea Canadensis* potential in sorption of nickel and chromium, established that this plant can accumulate 25 to 40 times more nickel than chromium in an experiment set at similar environmental conditions and within the same duration (Kähkönen *et al.*, 1998). Such scenario occurred in the accumulation of different heavy metals by the same macrophyte species. For example, *Azolla pinnata* was able to accumulate more zinc than copper while *Ceratophyllum demersum* accumulated more nickel than manganese.

The accumulation of heavy metal in macrophytes is subject to the concentration and bio-availability of these metals in the ecosystem, solubility sequence, plant species, plant growth rate, morphological differences and period of vegetation (Gupta *et al.*, 2007; Parzych, 2016). Different macrophytes reduced heavy metals at different percentages. The reduction of the heavy metal was inferred to be subject to the initial concentration of the heavy metal. For instance, lead which is not vital for plants and is a toxicant had a reduction efficiency of 100%. This could be credited to the low concentration of lead in the initial concentrations (effluent). Miretzky *et al.*, (2006) investigated the removal mechanisms of metals by dead macrophytes and found out that sorption efficiency of various metals was higher in the media that had lower initial concentrations. This could be due to filling of the active site by the sorbed metals.

Heavy metals accumulation depends on the species of macrophyte and the potential of their plant tissues to accumalate the pollutants (Hazra *et al.*, 2015). The period of vegetation has impact on the rate and amount of metals sorbed by the plants. The vegetative period is the best period in which plants absorb/adsorb most of the heavy metals. Young plants are preferred in phytotechnology. The morphological difference in plants affect their potential to uptake heavy metals. Plants that have well developed and numerous root systems are good in sorption of heavy metals. Also the plants that have large absorptive surface area that is in contact with the contaminant perform better in heavy metal uptake.

Occurrence of microbial symbionts influence the uptake of metals by plants. The root-colonizing bacteria and mycorrhizal fungi present in the root zone increases metals bioavailability. Plants change the chemical environment of the rhizosphere and provide energy to the microbes hence supporting the transformations of pollutants that are mediated by microbes (Lin *et al.,* 2010). According to Khan *et al.,* (2016) these mycorrhizae immobilize metals in the fungal cells thus inhibiting the assimilation of metals by macrophytes hence carrying out a protective role. The rhizospheric microbes aid the plant to uptake the essential nutrients, for example, manganese, iron and also other elements such as cadmium (Salt *et al.,* 1998). The macrophytes that are known to have a consortium of microorganisms such as *A. Pinnata* and *Nymphaea* spp. performed better in sorption of heavy metals.

Abiotic factors for instance pH, light and temperature has an impact on metal uptake efficiency (Rawat *et al.*, 2012). Temperature affects the ability of macrophytes in metals sorption. According to Fritioff *et al.,* (2005), temperature increase in the range of 278 to 293 K, increases the efficiency of plants in metal sorption. According to Malviya and Rathor (2007) pH influence the amount of metals available for plant's uptake. Hydrogen ions affect heavy metal solubility as they occupy the active sites and affect the ionization degree of the sorbate. González-Acevedo *et al.*, (2012) determined potential of *Lemna minor* to sorb selenium (VI) within a pH range of 2.0 to 8.0 and found out that the highest sorption efficiency was attained at a pH of 6.

Plants remediate contaminants using varying mechanisms such as sorption, transportion, transformation, hyper accumulation and mineralization. According to Veglio *et al.*, (1997), the biosorption processes of metals by plant cells can be either through surface sorption or via extra and intracellular accumulation. Sedimentation processes combined with oxidation and precipitation of metals as insoluble salts are efficient in metal removal from the system (Lesage *et al.*, 2007). When the concentration of the contaminants are in excess, they may have significant negative impacts on the plants' biological and physiological processes. Various processes of phytoremediation are utilized in heavy metals removal from polluted environments. Phytoextraction is responsible for removal of zinc, nickel and copper. Kularatne *et al.,* (2009) reported that removal of manganese is via phytoextraction mechanism.

Multistage phytoremediation technique was found to be more effective than using individual macrophytes. All the mineral nutrients and most of the heavy metals were not detected by the end of the experiment which lasted for a shorter time (20 days) compared to the individual macrophyte set up (25 days). Combining different species of plants in multistage technique may improve tolerance to changing environmental conditions and function in stabilizing the biogeochemical processes (Coleman *et al.,* 2001). Combining plant species may contribute to optimal environmental conditions through improved physical chemical parameters and increase productivity through more efficient use of available resources such as nutrients hence reducing their load in wastewater.

According to Ansari *et al.,* (2010b), combinations of several species of aquatic plants can be employed in developing high-efficient nutrients phytoremediation systems. An ecosystem which is rich and diverse with plant species would be expected to display a wider range of functional traits with increasing opportunities for more efficient resource use due to variation in survival characteristics (Ansari *et al.,* 2010b). Effective resource use enhances productivity resulting to effective performance in reducing pollutants in constructed wetlands.

The multistage technology has a great potential in becoming the basis of the principle of sustainable development which promotes activities that do not affect the environment. Application of this technology in wastewater treatment will provide a chance to restore and rehabilitate aquatic ecosystems for the enhancement of the environmental and also serve other ancillary functions. The use of multistage phytoremediation technique offers a considerable opportunity for reduced cost in wastewater treatment.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The level of some physicochemical and bacteriological parameters and heavy metals at the effluent of University of eldoret wastewater treatment plant exceeded those recommended by NEMA indicating that the wastewater treatment plant was not efficient. These parameters included BOD, COD, coliforms, lead and cadmium.

The four macrophyte investigated were found to be efficient in waste water treatment. The macrophytes include; *Azolla pinnata*, *Typha latifolia*, *Nymphaea* spp and *Ceratophyllum demersum*. They significantly enhanced the UoE effluent quality by improving all the water quality indicator parameters. All the measured water indicator parameters, nutrients and heavy metals were either below or within the NEMA standards for effluent disposal to the environment. The order of reduction efficiency was *Azolla pinnata* > *Nymphaea* spp. > *Typha latifolia* > *Ceratophyllum demersum*. These macrophytes can be used to treat UoE wastewater effluent and other domestic and industrial wastewater.

The multistage phytoremediation technique was found to be more efficient than individual macrophyte systems. This technique lasted for a shorter period than the individual macrophytes but was able to achieve higher reduction efficiencies than the individual macrophytes. The treated effluent from the multistage phytoremediation technique met the wastewater effluent standards for discharge to the environment. All the parameters measured were below or within the NEMA standards by the end of the experiment. Column two that comprised of *Typha latifolia* in stage 1, *Nymphaea* spp. in stage 2, *Azolla pinnata* in stage 3 and *Ceratophyllum demersum* in stage 4 was more efficient in phytoremediation of wastewater compared to the other columns.The order of reduction efficiency was column 2 > column 4 > column 3 > column 1.

6.2 Recommendation

6.2.1 Recommendation from the study

The University of Eldoret wastewater treatment plant needs to be upgraded in order to handle the increased population hence increased volume. The effluent being discharge into Marura swamp should be regularly monitored to ensure that they are compliant to NEMA standards of effluent discharge to the environment. The monitoring programme could raise alarm on failing treatment works thus organize for corrective measures to mitigate pollution of Marura wetland.

There is need to find out the source of the heavy metals in the UoE wastewater treatment plant. If the source is found to be a point source, then measures should be taken to discharge this wastewater into a side stream process. This would entail taking wastewater from the point source discharge and diverting it to its own separate pretreatment process, before being included in the influent of the UoE wastewater treatment plant. This would potentially decrease the high amounts of heavy metals and allow for an easier and much more efficient treatment process. Additionally, conducting such a survey shows that the University is being proactive in its attempts to reduce the parameters that are currently non-compliant to the NEMA standards. This could mitigate the pollution of Marura swamp and also protect the UoE from being fined by the relevant bodies.

Monitoring of the wastewater effluent for indicator bacteria before disposal should be done frequently to avoid the outbreak of waterborne disesases. If instituted after an outbreak, its value diminishes due to the time lag between exposure and development of the disease.

Macrophyte ponds can be added after the maturation pond in order to upgrade the University of Eldoret wastewater treatment plant. The most efficient ponds should be in form of multistage and the best arrangement of macrophytes should be *Typha latifolia* in stage 1, *Nymphaea* spp in stage 2, *Azolla pinnata* in stage three and *Ceratophyllum demersum* in stage 4. These will remediate organic matter, coliforms, nutrients and heavy metals in the wastewater before discharge the effluent into environment. These macrophytes should be harvested at appropriate time and disposed appropriately according to NEMA regulations. The management plan of the treatment plant should include the programmed harvest of the macrophytes and their disposal. This technology is also recommended for other communities willing to adopt it as it is a green technology which is efficient and economical.

6.2.2 Recommendation for further studies

There is need to investigate the remediation potential of other macrophyte species native to the study area.

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APPENDICES

Appendix I: Summary of ANOVA for objective 2

Parameter	F value	P value
Temperature	0.74	0.059
Dissolved oxygen	0.62	0.651
pH	0.69	0.599
Total Dissolved Solids	0.84	0.505
Conductivity	0.58	0.681
Turbidity	0.88	0.480
Phosphates	6.40	0.000
Nitrates	4.29	0.003
Total coliforms	2.38	0.070
Feacal coliforms	0.79	0.502
Feacal streptococcus	1.43	0.234
Cadmium	4.40	0.003
Copper	6.35	0.000
Nickel	7.07	0.000
Cobalt	6.75	0.000
Lead	11.39	0.000
Manganese	9.12	0.000
Zinc	4.92	0.001
Iron	7.97	0.000

Appendix II: **Post hoc test for objective 2**

Tukey test for TDS

Plant type	N	Mean	Grouping
Control	18	400.6	A
Ceratophyllum Sp.	18	372.2	A
Nymphaea Spp.	18	358.3	A
Typha Sp.	18	351.1	A
Azolla Sp.	18	331.7	A

Tukey test for pH

Plant type	N	Mean	Grouping
Typha Sp.	18	7.778	A
Control	18	7.706	A
Ceratophyllum Sp.	18	7.694	A
Azolla Sp.	18	7.550	A
Nymphaea Spp.	18	7.528	A

Tukey test for DO

Plant type	N	Mean	Grouping
Control	18	4.329	A
Typha Sp.	18	4.305	A
Azolla Sp.	18	4.237	A
Nymphaea Spp.	18	4.021	A
Ceratophyllum Sp.	18	3.637	A

Tukey test for phosphates

Plant type	N	Mean	Grouping	
Control	18	3.536	A	
Typha Sp.	18	1.834		B
Ceratophyllum Sp.	18	1.781		B
Nymphaea Spp.	18	1.674		B
Azolla Sp.	18	1.581		B

Tukey test for nitrates

Plant type	N	Mean	Grouping	
Control	18	6.539	A	
Typha Sp.	18	3.894	A	B
Ceratophyllum Sp.	18	3.581		B
Nymphaea Spp.	18	3.294		B
Azolla Sp.	18	3.185		B

Appendix III: **Summary of ANOVA for objective 3**

Parameter	F value	P value
Temperature	2.89	0.079
Dissolved oxygen	44.53	0.000
pH	4.32	0.03
Total Dissolved Solids	55.82	0.000
Conductivity	63.18	0.000
Turbidity	10.74	0.001
Phosphates	64.63	0.000
Nitrates	77.04	0.000
Total coliforms	0.03	0.998
Feacal coliforms	0.18	0.948
Feacal streptococcus	0.13	0.973
Cadmium	5.76	0.000
Copper	4.70	0.002
Nickel	4.16	0.004
Cobalt	2.94	0.026
Lead	5.67	0.001
Manganese	6.07	0.000
Zinc	5.47	0.001
Iron	5.09	0.001

Appendix IV: **Rainfall data during the sampling period**

Rainfall data during the three months of sampling.

Month	No. of days experiencing rainfall	Mean Rainfall (mm)
October	7	55
November	1	3.6
December	5	57.2

Source: UoE metrological center

Appendix V: **Guideline values for discharge into public sewers**

Schedule 6 Guideline values for discharge into public Sewers [The Environmental Management and Co-ordination (Water Quality) Regulations, 2006]

Parameter	Units	Guideline value Maximum allowable (limits)
Biological Oxygen Demand (BOD5 days at 20°C	mgO_2/l	500
Total Suspended Solids	mg/l	250
pH (Hydrogen ion activity, marine)	pH	6.0 - 9.0
Temperature	°C	20 – 35
Chemical Oxygen Demand (COD, mg/l)	mgO_2/l	1000
Phosphates	mg/l	30
Nitrates	mg/l	20
Total Chromium (Cr), max	mg/l	2
Chromium VI (Cr^{+6})	mg/l	0.05
Lead (Pb)	mg/l	1
Cadmium (Cd)	mg/l	0.5
Zinc (Zn)	mg/l	5
Copper (Cu)	mg/l	1
Nickel (Ni)	mg/l	3
Cobalt	mg/l	1

Source: Environmental Management and Co-ordination Act (Water Quality) Regulations, 2006

Appendix VI: **Guideline values for discharge into the environment**

Schedule 7 Guideline values for discharge into the environment [The Environmental Management and Co-ordination (Water Quality) Regulations, 2006]

Parameter	Units	Guideline value Maximum allowable (limits)
Biological Oxygen Demand (BOD5 days at 20°C,	mgO/l	30
Total Suspended Solids	mg/l	30
Total Dissolved Solids	mg/l	1200
Total coliforms	counts/100ml	30
pH (Hydrogen ion activity, non-marine)	pH	6.5 – 8.5
Temperature	°C	Ambient Temperature ±3
Chemical Oxygen Demand (COD)	mg/l	50
Total Phosphorus	mg/l	2 guideline value
Total Nitrogen	mg/l	2 guideline value
Total Chromium	mg/l	2
Chromium VI (Cr^{+6})	mg/l	0.05
Lead	mg/l	0.01
Cadmium	mg/l	0.01
Zinc	mg/l	0.5
Copper	mg/l	1
Dissolved iron	mg/l	10
Dissolved manganese	mg/l	10
Total Nickel	mg/l	0.3

Source: Environmental Management and Co-ordination Act (Water Quality) Regulations, 2006

Appendix VII: Simillarity report

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