PHYTOREMEDIATION OF IRRIGATION WATER USING *LIMNOCHARIS FLAVA*, *TYPHA LATIFOLIA* AND *THALIA GENICULATA* IN A CONSTRUCTED WETLAND

by

PERCY ERASMUS KORSAH

BSc. Biological Sciences (Hons.)

A Thesis submitted to the

Department of Theoretical and Applied Biology,

Kwame Nkrumah University of Science and Technology

in partial fulfillment of the requirements for the award of the degree of

MASTER OF PHILOSOPHY

Faculty of Biosciences,

College of Science

JUNE 2011

DECLARATION AND CERTIFICATION

I hereby declare that this submission is my own work towards the Master of Philosophy (MPhil.) Degree, and that, to the best of my knowledge it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

………………………………… ………………………. ……………..

Student's Name Signature Date

………………………….

Student's ID

Certified by:

……………………................ ....……………… ……………..

Supervisor's Name Signature Date

Certified by:

……………………………… …………………………. ……………..

Head of Department's Name Signature Date

DEDICATION

To my late Grandma

Mary Gordon.

To my dad

Etienne Ossinga

To my mother

Baaba Korsah

To my siblings

Francois, Tony, Francis, and Theophile.

And to the Almighty and merciful God for the opportunity, inspiration and strength to come this far and also to my colleagues and senior members of the department of Theoretical and Applied Biology (TAB) who supported me in numerous ways and finally my dearest ones: Mrs. Wendy-Tina Korsah and my Kids.

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to the International Foundation of Science for funding my research and the Kwame Nkrumah University Science and Technology (KNUST) for granting me the opportunity to upgrade my educational qualification.

My sincere thankfulness goes to Mr. Alex Anning and Mr. Patrick Addo-Fordjour of the Department of Theoretical and Applied Biology, who encouraged and supervised me throughout this research. Further appreciation also goes to Prof. Esi Awuah, of the department of Civil Engineering, who despite her busy schedule gave very productive consultations on this project.

I also desire to express my deep thanks to Dr. E.J.D. Belford for his extensive input during this research. My esteemed appreciation goes to all the senior members of the department of Theoretical and Applied Biology, for their advice, support and co-operation. I am also grateful to Prof. Anthony Adimado, immediate past Dean, School of Graduate Studies, for his guidance and advice resulting in the successful completion of this work. May God bless you abundantly.

In addition, I wish to express my thanks to Mr. Anthony Abutiate of the Soil Research Center, Kwadaso for his assistance in my Laboratory work. Noteworthy is the prayer and variable support from my family and friends throughout my study period.

Abstract

Irrigation water quality is a critical public health issue in most developing countries, where farmers continue to rely on wastewater for vegetable production due to limited access to sources of clean water. Phytoremediation, the technique that utilizes a plant's inherent ability to accumulate metals, is fast emerging as a relatively cheap and environmentally friendly alternative to conventional wastewater treatment methods. A corollary of this is an urgent need to identify plant species with the appropriate suite of characteristics for phytoremediation. The purpose of this study was to evaluate and compare the phytoremediation potentials of *Limnocharis flava, Thalia geniculata and Typha latifolia* using a horizontal sub-surface flow (SSF) constructed wetlands. The system comprised a storage tank, sedimentation tank, three parallel treatment columns and an effluent tank. Each column in turn had two rectangular serially arranged cells or ponds connected by inlet and outlet pipes, and were both planted with only one of the three plant species. All cells were supplied with irrigation water from a common source. Duplicate plant and water samples were collected from October 2010 to March 2011, and analyzed for Fe, Cu, Zn, Pb and Hg using the atomic absorption spectrophotometer. Bioaccumulation and translocation factors varied greatly among species for different metals. The results showed substantial accumulation of the trace metals by the plants, with Fe (~1600mg kg^{-1}) and Pb (5.71mg kg^{-1}) as the most and least accumulative metals respectively. *L. flava* and *T. geniculata* hyper-accumulated Hg. Mean removal efficiencies ranged from 40-80%, 48-54%, 44-54%, 18-32% and 8-38% respectively for Fe, Hg, Zn, Pb and Cu. The removal efficiencies of the species differed depending on the metal. *L. flava*, *T. latifolia* and *T.geniculata* were most efficient ($p < 0.001$) at removing Fe, Cu and Pb respectively. Both *T. geniculata* and *T. latifolia* appeared to remove zinc better than *L. flava* ($p < 0.021$), but there was no statistically significant difference in the removal rates of Hg by the plants. Similar trends were observed for the bioaccumulation factor, which increased substantially with time. The plants accumulated most of the metals in their roots. The findings demonstrate the capabilities of the three phytoremediants for improving the quality of irrigation water used for vegetable production.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

APHA	American Public Health Association
BAF	Bioaccumulation Factor
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CW	Constructed Wetland
DO	Dissolved oxygen
EPA	Environmental Protection Agency
FWS	Free water surface systems
KMA	Kumasi Metropolitan Assembly
KNUST	Kwame Nkrumah University of Science and Technology
Lf	*Limnocharis flava*
RE	Removal Efficiency
SSF	Sub-surface flow
SS	Suspended Solids
TDS	Total Dissolved Solids
TF	Translocation Factor
Tg	*Thalia geniculata*
Tl	*Typha latifolia*
UNESCO	United Nations Educational, Scientific and Cultural Organization
VSB	Vegetated Submerged Bed systems

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Rapid increase in urban populations translates into increased demand for resources and a heightened need for improved sanitation and waste treatment facilities. Anthropogenic activities affecting water resources continue to raise concerns on the deteriorating water quality of many water bodies due to the indispensable nature of water. Low-cost and environmentally friendly methods of wastewater treatment have always been a major concern of environmentalists worldwide. The use of phytoremediation to clean up contaminated water is becoming popular as an alternative to conventional wastewater treatment methods. This is far more predominant in the developing countries where extensive research is underway to identify many more species with characteristics suitable for phytoremediation (Willey, 2007). This technology is however yet to be fully explored in Ghana and many other developing countries in tropical Africa.

Urban agriculture does not only reduce the need for food crops to be transported from the rural areas but also serves as a means of income for some urban dwellers. Inocencio *et al.* (2003) reported that about 900,000 hectares of farmland in developing countries are irrigated using wastewater. The use of wastewater as a source of irrigation in the cultivation of vegetables has become a common practice in Ghana due to increased scarcity of water, low capital and lack of access to sewage treatment plants (Obuobie *et al*., 2006; Amoah *et al*., 2007). The limited intervention of the legislative and environmental institutions on regulatory mechanisms for pollution control, coupled with the absence of adequate infrastructure and facilities for wastewater treatment is a major setback for safe waste water use in Ghana. It is therefore very essential that cheaper and more practical options are available for wastewater treatment to support these farmers and eliminate the dangers associated with the ingestion of heavy metals and other pollutants through irrigated crops. Porcella *et al*. (1996) reveal that consumption of heavy metals through food or drinking water cause death, cancers (liver, kidney, bladder, lung and prostate) skin changes, damage to the reproductive system and general development of both man and other animals.

Wastewater is often used by farmers either directly from the wastewater drains or indirectly through wastewater-polluted irrigation water (Keraita *et al*., 2002). This practice poses serious occupational and health risks to the farmers and their family members, the consuming public and the local communities (Blumenthal and Peasey, 2002). Several studies, using mainly microbial indicators, have highlighted the potential risks associated with wastewater re-use for vegetable production in Ghana. A study by Sarpong (2007) found high levels of lead, arsenic, iron, cadmium, mercury, copper (Pb, As, Fe, Cd, Hg, Cu) and other heavy metals above WHO recommended values for most surface waters used for vegetable production in the Kumasi metropolis. Such high levels of heavy metals can lead to serious threats to humans and aquatic ecosystems due to their toxicity, persistence in the environment and bio-accumulative effect on food chain (Forstner and Wittman, 1983). However scanty knowledge exists on the chemical quality (particularly in relation to heavy metals) of wastewater used for irrigation of vegetable crops in Ghana.

Peer *et al*., 2005, define the term 'Phytoremediation' as a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition useable for private or public applications. The technology has the potential for removing complex mixtures of pollutants, combining the effects of removal of unwanted components, biomass production and varied aesthetics (Ensley, 2000). This technology is evident in many wetlands. Natural wetlands serve as nature's filtration centres for the removal of organic materials, suspended solids, nutrients, pathogens, heavy metals and other toxic pollutants. They also provide other ancillary benefits such as supporting primary production and enhancement of wildlife habitat (Denny, 1997). Wetlands processes include microbial breakdown of organic materials, precipitation, sedimentation, adsorption and plant uptake of heavy metals. According to US EPA (1993), constructed wetlands are planned systems designed and constructed to employ wetland vegetation to assist in treating wastewater in a more controlled environment than occurs in natural wetlands. Constructed wetlands present numerous advantages including low cost and maintenance, low energy consumption, robustness and sustainability (US EPA, 1993). To obtain maximum efficiency, it is prudent to ensure consistency in design, construction and operation and the execution of appropriate design tools and methodologies suitable for local conditions (US EPA, 1993). Approximately 90% of pollutant and waste removal is achieved through the processes taking place in wetlands (Liao *et al*., 2004).

Many plant species including *Typha, Phragmites, Scirpus, Leersia,* Juncus and *Spartina* have been commonly exploited (Bareen and Khilji, 2008) for absorption of specific heavy metals and other pollutants often in constructed wetlands, and work is still in progress to identify more species, especially indigenous species for phytoremediation. The identification and use of indigenous species provides a cheaper alternative as well as limits bio invasion. However, little is known about the use of *Limnocharis* and *Thalia* for phytoremediation even though they appear to share similar habits and habitats with *Typha* (Anning and Yeboah-Gyan, 2007). According to Ganjo and Khwakaram (2010), an aquatic macrophyte for phytoremediation must have the following characteristics: (a) fast growth rate, (b) high biomass production, and (c) the ability to accumulate high concentrations of nutrients and heavy metals over a long time exposure with no damage concerns. Success of the wastewater treatment process is therefore highly dependent on finding a suitable plant species.

1.2 Problem statement

Vegetable farmers in Kumasi continue to utilize wastewater to irrigate their crops including fresh-eating vegetables, despite the health implications. The farmers often use wastewater in its untreated form because of lack of functional, cost-effective and efficient wastewater treatment plants in the Kumasi metropolis. The wastewaters which are obtained from many sources, depending on the location of farms, may have substantial amounts of heavy metals. However, while extensive studies have been carried out on the microbial quality of these wastewaters and the associated risks, only few studies have focused on heavy metal compositions of such resources in Ghana. The few studies conducted so far have provided evidence of heavy metal contamination of wastewater from different parts of the Kumasi Metropolis, particularly from the KNUST campus, Suame Magazine Area, Komfo Anokye Teaching Hospital and Kaase. In light of this, identification and evaluation of appropriate methods for wastewater purification prior to irrigation become important research agenda.

1.3 Goal and objectives of study

The purpose of the research was to evaluate and compare the potentials of three wetland plants, *Limnocharis flava*, *Thalia geniculata*, and *Typha latifolia* as phytoremediants of heavy metal contaminated irrigation water. *T. latifolia* has been used for phytoremediation in other countries but was included in the study to serve as a reference plant.

The specific objectives of the study were to:

a) determine the concentrations of Fe, Cu, Pb, Zn and Hg in irrigation water accumulated over time by *Limnocharis flava*, *Thalia geniculata*, and *Typha latifolia* in a sub-surface flow constructed wetland *b)* determine the bioaccumulation factors and translocation factors of *Limnocharis flava*, *Thalia geniculata* and *Typha latifolia* for different heavy metals present in irrigation waters under experimental conditions.

c) determine the removal efficiencies of heavy metals (Pb, Hg, Fe, Zn and Cu) from irrigation water by *Limnocharis flava*, *Thalia geniculata*, and *Typha latifolia* in the constructed wetlands.

d) assess the growth response of the three phytoremediants to accumulation of heavy metals over time.

1.4 Justification of the study

Environmental health is currently one of the most persistent issues in developing countries and Ghana is no exception. Despite the findings of Sarpong (2007) and Obuobie *et al*., (2006) highlighting the pollution levels and the threats posed by many commonly used wastewater sources, many farmers in Kumasi continue to use untreated wastewater for irrigation purposes. This research will help to create awareness about the use of heavy metal-laden irrigation water in urban and peri-urban areas of Kumasi, and the concomitant environmental and health implications.

Anning and Yeboah-Gyan (2007) revealed that *Limnocharis flava* and *Thalia geniculata* are two fast growing macrophytes that often share habitat with *Typha latifolia*, a common phytoremediant (Phillips *et al*., 2010) that dominates many heavy metal contaminated sites in Ghana. However, there is no information on their use in wastewater purification. Successful evaluation of *Limnocharis* and

Thalia for phytoremediation will provide a low-cost and environmentally friendly treatment alternative to conventional methods of waste water purification for irrigation purposes which are currently associated with rapidly escalating costs of construction and operation. Despite the threat posed by irrigating crops with heavy metal polluted wastewater, no studies have been conducted in Ghana on using constructed wetlands to purify such waters. Wetlands are also associated with emerging or renewed application of aesthetic, wildlife, and other incidental environmental benefits.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Composition of wastewater

Wastewater is produced from different sources, and hence made up of variable components. Interactions with the natural environment also influence and contribute to the different components and properties of wastewater. Wastewater is characterized by the presence of organic matter, suspended solids, nitrogen, phosphorus, pathogens, water and toxic compounds such as metals, herbicides, etc. (Denny, 1997). These components occurring different concentrations and together influence and contribute to properties such as turbidity, alkalinity or acidity, conductivity, etc. These properties interact further to influence the components of wastewater. The pH value of wastewater is dependent on the temperature, whiles both properties influence the survival of pathogens and other organisms. Solubility of heavy metals and other components are influenced by the alkalinity/acidity of the wastewater. The degree of solubility of some components directly affects the conductivity and turbidity of the waste water. Nature also has direct influence on the properties of wastewater. Treatment and reuse of wastewater is therefore dependent on its composition and properties.

2.2 Wastewater for Irrigation

Traditionally, domestic wastewater has been used for crop irrigation in many countries since it contains plant nutrients. The practice of re-using treated wastewater in agriculture is encouraged to reduce demand on freshwater resources. However, the danger posed by the bioaccumulation of hazardous wastes, especially heavy metals and pesticides, in food chain is of major concern. Teisseire and Guy (2000) have also elucidated the fact that plant enzymes and the overall yield are greatly influenced by heavy metals.

In many cases, heavy metals occur in natural bodies of water at levels below their toxic thresholds. However, due to their non-degradable nature, such low concentrations may still pose risk of damage via uptake and subsequent bioaccumulation by organisms, which cannot effectively metabolize and excrete the absorbed metals. Several scientific observations have shown that heavy metals are bioconcentrated or bioaccumulated in one or several compartments across food webs (Otitoloju and Don-Pedro, 2006). Metal bioaccumulation can be of importance from the public health point of view, especially when humans consume the accumulators. This phenomenon is now being explored in the assessment of environmental quality, in addition to chemical surveys of water and sediment.

2.3 Wastewater treatment

Different techniques are employed in wastewater treatment procedures. The more expensive conventional methods employ a combination of chemical, biological and physical methods to treat wastewater; while cheaper alternatives being sort today includes the use of less machinery and more natural treatment techniques. Bioremediation is an aspect of biotechnology which employs biological agents such as microbes, plants etc, to degrade contaminants or extract certain pollutants to ameliorate and restore the physical environment (Willey, 2007). It has been demonstrated, for example, that certain varieties of mustard plant can remove metals such as chromium, lead, cadmium and zinc from contaminated soil. Also hydroponic plant cultures have been used to remove toxic metals from aqueous waste streams (Willey, 2007).

Soil microbes at the root-soil interface share a symbiotic relationship with plants, which secrete nutrients and supply oxygen to the rhizophere to accelerate bioremediation in surface soils. There are relatively higher numbers of metabolically active micro-organisms in the rhizosphere as compared to an unplanted soil. The aspect of bioremediation which involves the use of green plants to treat contaminated soil and water samples is known as phytoremediation.

2.3.1 Phytoremediation

Phytoremediation involves the use of photosynthetic plants to rid soils and water of contaminants such as toxic heavy metals and metalloids, which are transformed to inert forms or bound into harmless compounds (Willey, 2007).

The biological treatments that collectively connote phytoremediation actually consist of several specific processes:

a) Phytoextraction - uptake of substances from the environment, with storage in the plant (phytoaccumulation).

b) Phytostabilisation - reducing the movement or transfer of substances in the environment, such as, limiting the leaching of substances contaminating the soil.

c) Phytostimulation - enhancement of microbial activity for the degradation of contaminants typically associated with the rhizosphere.

d) Phytotransformation - uptake of substances from the environment, with degradation occurring within the plant (phytodegradation).

e) Phytovolatilisation - removal of substances from the soil or water with release into the air, possibly after degradation.

f) Rhizofiltration - removal of toxic metals from groundwater. The process involves the adsorption or precipitation onto plant roots or absorption into roots of contaminants that are in solution surrounding the root zone, due to biotic processes. Plant uptake, concentration, and translocation might occur, depending on the contaminant. Rhizofiltration first results in the contaminants being immobilized or accumulated on or within the plant. Contaminants are therefore removed when plants are harvested.

g) Rhizodegradation – refers to the breakdown of an organic contaminant in soil through microbial activity that is enhanced by the presence of the root zone. Rhizodegradation is also known as plant assisted degradation, plant aided *in-situ* biodegradation, and enhanced rhizosphere biodegradation. Root exudates are compounds produced by plants and released from plant roots. They include sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavanones, enzymes and other compounds.

The microbial populations and activity in the rhizosphere are directly influenced by these exudates, and can result in increased organic contaminant biodegradation in the soil. Additionally, the rhizosphere substantially increases the surface area where active microbial degradation can be stimulated. Degradation of the exudates can lead to co-metabolism of contaminants in the rhizosphere. Plant roots can affect soil conditions by increasing soil aeration and moderating soil moisture content, thereby creating conditions more favourable for biodegradation by indigenous microorganisms.

Phytoremediation takes advantage of the nutrient utilisation processes of the plant to take in water and nutrients through roots, transpire water through leaves, and act as a transformation system to metabolise organic compounds, such as oil and pesticides. Alternatively, they may absorb and bio-accumulate toxic trace elements, including heavy metals such as lead, cadmium and selenium. Heavy metals are closely related to the elements plants use for growth.

2.3.1.1 Advantages and disadvantages of phytoremediation

The biggest advantage of using plants for cleaning the environment is the utilization of their inherent agronomic traits and benefits of plants like high biomass, extensive root systems, ability to withstand environmental stress, etc. (Bizily *et al.*,1999). Plant-facilitated bioremediation is aesthetically pleasing and makes the environment green and clean. As the entire process is solar energy driven, no artificial source of energy is required to drive the bioremediation process, making it cost-effective and environmentally friendly (Bizily *et al.*, 1999). Plants offer a permanent, *in situ*, non-intrusive, self-sustaining method of removal of soil contaminants. Planting vegetation on a contaminated site also reduces erosion by wind and water. Phytoextraction enables one to reclaim and recycle precious metals and other useful materials from the soil making the process economically beneficial for investors (Moffat, 1995). In addition, plants used in bioremediation do not disturb the topsoil, thus conserving its utility (Sykes *et al.*, 1999).

Despite the many obvious advantages, a few concerns regarding the phytoremediation technology have been expressed. Firstly, the process is slow compared to conventional treatment techniques. Plants can take many growing seasons to clean up a site due to slow growth pertaining to climatic restrictions and species variations. Secondly, hyper-accumulator plants with short roots can clean up soil or groundwater near the surface *in situ*, but cannot remediate deep aquifers without further design work (Sykes *et al.*, 1999). Thirdly, plants that absorb toxic materials may contaminate the food chain as animals inhabiting the contaminated area might consume these plants (Moffat, 1995). Fourthly, phytoremediation technique is less efficient for hydrophobic contaminants, which bind tightly to soil (Bizily *et al.*, 1999). Fifthly, volatilization of compounds can transform a groundwater pollution problem to an air pollution problem (Raskin, 1996).

The greatest problem, however, concerns the fate of the plants used for phytoremediation, which would be rich in certain contaminants after remediation.

Biodegradation or recycling of the plants returns the contaminants either fully or partially into the soil (Gratao *et al*., 2005). The public opposition to developing genetically modified plant species or crops also presents a challenge to the advancement of phytoremediation technique and development of transgenic hyperaccumulator plant species (Shah, 2007).

2.4 Wetlands

Wetlands are defined as land where the water table is near the ground surface long enough each year to maintain saturated soil conditions. A wetland can be an area of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed six metres" (UNESCO-IHE, 2010). Denny (1997) also defined a wetland as a shallow, seasonally or permanently waterlogged or flooded area which normally supports hydrophytic vegetation. Phytoremediation is an affordable technology that is most useful when contaminants are within the root zone of the plants (top three to six feet of the soil). For sites with contamination spread over a large area, phytoremediation may be the only economically feasible technology.

For the purposes of phytoremediation, wetlands can be described as shallow waters with at least 50% aerial cover of submerged or emergent macrophytes or attached algae (Denny, 1997). Natural wetlands have long been used for the disposal of waste. Wastewater treatment in natural wetlands occurs spontaneously and purification is mostly confined to some reduction in the biological oxygen demand (BOD). All three biodegradation processes, namely aerobic, anoxic and anaerobic, are expected in wetlands and thus are applicable in heavy metal polluted waste water treatment. Aquatic plants remove pollutants by directly assimilating them into their tissue and by providing a suitable environment for micro-organisms to transform pollutants and reduce their concentrations.

2.4.1 Constructed wetlands

A 'constructed' wetland is defined as a wetland specifically constructed for the purpose of pollution control and waste management at a location other than existing natural wetlands (Ganjo and Khwakaram, 2010). Although natural wetlands have been used for wastewater treatment for years, the purposeful construction of wetlands is a relatively new technology. Traditionally, wetlands have been engineered and constructed for three principal uses (1) to compensate for the loss of natural wetlands from agriculture or urban development (2) to provide flood control, and (3) for the production of food and fibre. Constructed wetlands are also used for polishing already partially oxidised industrial or domestic waste, and the removal of specific pollutants such as nitrogen, phosphorus, copper, lead, organic compounds and pesticides from all wastes including agricultural or urban storm run-off.

Constructed wetlands are a cost-effective alternative to conventional treatment systems, simple to both install and operate. Furthermore, constructed wetlands are low-cost technologies which are able to control environmental pollution. These treatment wetlands utilise plant-based enzymatic biochemical processes, which work in conjunction with indigenous microbial activity to optimise rhizospheric biodegradation and plant tissue phytodegradation.

Constructed wetland technology is currently evolving into an acceptable and an economically competitive alternative for many wastewater treatment applications. They can effectively remove nitrogen and phosphorus better than secondary treatment at a conventional wastewater treatment plant (Denny, 1997).

There are many benefits in using constructed wetlands in phytoremediation. First, wetlands are one of the least expensive treatment systems to operate and maintain. Wetlands possess highly efficient biological systems that effectively remove contaminants without the addition of expensive chemicals or extra energy requirements. Secondly, wetlands are aesthetically pleasing. Constructed wetlands offer a more natural choice to traditional treatments that may be offensive or unappealing in design. Thirdly, constructed wetlands can provide habitat for many aquatic and terrestrial species. Depending on the nature of the treatment and the contaminant, wetlands can provide a long-term habitat alternative for many species to thrive.

A number of studies have revealed that the use of constructed wetlands can be an effective method in treating and removing many chemicals and heavy metals from water (US EPA, 1993; Denny, 1997). Constructed wetlands can be used to treat acid mine drainage, municipal wastewater, as well as runoff and wastewater from agricultural areas, petroleum processing, industrial activities and military bases. Wetland treatment systems use rooted, water tolerant plant species and shallow, flooded, or saturated soil conditions to provide various types of wastewater treatment. Only those wetlands with plant species adapted to continuous flooding are suitable for receiving continuous flow of wastewaters. Constructed wetlands are designed to mimic natural wetlands, but optimize the biological and physical properties in order to maximize efficiency.

Effective wetland performance depends on adequate pre-treatment, water composition and required treatment level, hydraulic loading rates, and knowledge of successful operation strategies. The most common difficulties experienced in wetland treatment systems have been related to maintaining proper soil conditions. When a wetland is overloaded, highly reduced conditions result, causing plant stress and a reduction in waste removal efficiency (Denny, 1997).

The technique employed in constructed wetlands is to drain pre-treated wastewater into appropriately engineered gardens or forests of phreatophytes – plants known for fast growth and high water usage rates from the phreatic zone or zone of saturation. These plants and their microbial active rhizosphere will transform pollutants, including the nutrient nitrogen, into valuable biomass and use up the remaining water via evaporation and transpiration (US EPA, 1993).

2.4.1.1 Types and functions of constructed wetlands

Two major types of constructed wetlands have been identified. The first is free water surface systems (FWS) and the second is sub-surface flows systems (SSFS), also called root zone, rock-reed filters or vegetated submerged bed systems (VSB). The free water surface wetlands are the most common and they possess the following features (US EPA, 1993):

- basins or channels with natural or constructed subsurface barriers of clay or impervious material to avoid seepage
- soil or another available medium to support the emerging vegetation, and
- wastewater flowing slowly over the soil surface at a shallow water depth.

The subsurface flow system consists of a trench or bed at the bottom of which is an impermeable layer of clay or a plastic liner. The bed contains rocks or other material that can support the growth of new vegetation. Water flows about 6 to 12 inches below the bed surface. The local geology and soil conditions must be investigated before developing a design (US EPA, 1993).

2.4.1.2 Compartments and features of ideal purifier wetlands

Wetlands can be compartmentalised into these zones: sediment, root zone/pore water, litter/detritus, water, air, plants then the roots. These zones are dependent upon each other and the constructed wetland must be operated under certain conditions to achieve efficiency. It is essential that there is maximum contact between sediments and the root zone. The system should be free of channelization, dead zones and short circuiting. A high hydraulic resistance and a long detention time are very essential in the operation of the wetland.

2.4.2 Economics of wetland- Water reuse

Wetlands are useful for recreational purposes such as hunting, fishing and working. They can also serve as study sites in education and photography. The effluent of wetlands, based on the quality of water produced can be used for irrigation of crops, watering of gardens and golf courses. They can be applied at domestic or commercial levels for cleaning purposes and flushing toilets, as well as, cooling water for engines and fire fighting.

2.4.3 Plants for wetlands

Wetland plants are autotrophic component of the ecosystem. They use light energy from the sun, CO_2 from the air and nutrients from the water and soil to produce organic matter. There are two main groups: (a) phytoplankton – microscopic plants in the water column; (2) hydrophytes– plants that grow in water or on a substrate that is periodically or permanently deficient in oxygen. Hydrophytes have special adaptations; tissue to transport oxygen from leaves to roots, special roots. Based on sessility, there are three main aquatic macrophyte types: Emergent, submerged and floating-leaved.

There are reports on wetland plants like *Typha, Phragmites, Scirpus, Leersia, Juncus* and *Spartina* in reducing the levels of heavy metals in polluted waters (UNESCO-IHE, 2010; Denny, 1997). Such hyperaccumulator plants can be exploited for treatment of metals-containing wastes (Ensley, 2000). To protect themselves from metal poisoning, plants must have developed a mechanism by which the heavy metal entering the cytosol of the cell is either immediately excluded or complexed and inactivated, thus preventing the metal from inactivating catalytically active or structural proteins.

Wetland plants have been shown to have nutrient conservation strategies involving internal cycling. Nutrients absorbed during growth are translocated to the below-ground storage organs during senescence of above-ground parts. Later, these nutrients are mobilised upwards for use by the young shoots (stems and leaves) in the next growing period. Reallocation of biomass between compartments is essential for surviving water level changes. Species that can maintain allocation to shoots without any adverse effect on total or below- ground mass are at a distinct advantage.

Plant based treatment systems are now accepted throughout the world as an appropriate solution for wastewater treatment, and constructed wetlands are not only used for treating domestic sewage, but for treating abattoir wastewater, landfill leachate, highway run-off, contaminated groundwater, and agricultural and animal wastes (Denny, 1997). Plant based systems have many applications in low-income countries, because of the low operating and maintenance costs; maintenance which local people could be trained to do.

Suitable plants for wastewater treatment include members of the families Cyperaceae, Juncaceae and Typhaceae. The selected species should have a high production rate and show a high standing crop throughout the year. Other criteria include: high oxygen transport capability, tolerance to adverse concentrations of pollutants, tolerance to adverse climatic conditions, resistance to pests and disease and ease of management. Aquatic plant species should also be selected based on the following criteria: ease of propagation, capacity for adsorption of pollutants, tolerance of hyper-eutrophic conditions, ease of harvesting, and potential usefulness of harvested material. Emergent macrophytes selected for growth in artificial systems should be robust in habit and be readily available in the local area.

2.4.3.1 *Typha latifolia* (Family Typhaceae)

Typha latifolia (cattail/Reedmace), is an important component of marsh ecosystems and is often dominant among emergent wetland vegetation. Cattail is propagated by seeds or cuttings; are considered invasive due to its ability to take over marshes and freshwater environment and form dense, nearly monotypic, stands of vegetation. Plants can reach 2-3 meters high and can be distinguished by its distinctive fruiting spike and tall sword-shaped leaves. Distribution of *Typha* spp is nearly worldwide; in Africa, North and Central America, Great Britain, Eurasia, New Zealand, Australia, and Japan (Lan *et al.*, 1992).

T. latifolia has shown a tolerance to high concentrations of lead, zinc, copper, and nickel, and has been employed in secondary waste water treatment schemes. It has also in recent years been proposed as a biomass crop for renewable energy (Ghosh, 2005). Other uses for *Typha latifolia* include thatch roofing, or woven into mats, chairs and hats; a source of fibre for rayon and a crude, greenish brown paper; torches and tinder; pollen used in making fireworks; stuffing pillows, insulation, crude floatation devices, wound dressing, and lining for diapers. The soft tender shoots are also edible and used in delicacies (Ghosh, 2005).

2.4.3.2 *Limnocharis flava* (Family Limnocharitaceae)

Common names include sawah-flower rush, sawah-lettuce, velvetleaf, yellow bur-head. *L. flava* is a perennial aquatic herb propagated by seed and vegetative shoots, and is dispersed by water. Plants grow to 1m height and are distinguished by succulent angled stems and triangular-shaped leaf and flower stalks and produces 'octopus-like' inflorescences consisting of up to 15 three-lobed yellow flowers. Its fruits are spherical and made up of crescent shaped segments that eventually split off, carried by water currents to disperse seeds to new locations (CRC, 2003). *L. flava* has a short stout rhizome (about 3 cm long and 3 cm in diameter) and numerous fibrous roots. Distribution was originally associated with North and Central America and parts of Africa. Plants occur in shallow swamps, ditches, pools and wet rice fields, and may become a very invasive environmental weed of streams and wetlands if left to grow unchecked (Abhilash, 2004). *L. flava* is cultivated in some countries as an ornamental plant in homes and gardens. It is also cultivated in rice fields and eaten as a vegetable in South-East Asian countries. Abhilash (2004), reports its use in pig and cattle fodder, as well as green manure for application in rice paddies.

2.4.3.3 *Thalia geniculata* (Family Marantaceae)

This perennial aquatic weed, 1-2.5meters tall, is also known by other names such as arrowroot, bent alligator-flag, and fire-flag. Propagation is by seeds or dividing rhizomes, tubers, corms or bulbs (including offsets) and planting. This large broad leafed aquatic plant has small, delicate purple flowers, fibrous roots and produces rhizomes. The Plant grows in ponds, roadside ditches, swamps and the edges of lakes, dams and other water courses in the Americas and tropical West Africa.

2.5 Heavy metals in irrigation water

Heavy metals, metallic chemical elements with a high density, are naturally components of the earth's crust. Although at trace levels some heavy metals (e.g. selenium and zinc) are essential for the human body, most of them are toxic or poisonous even at low concentrations. Heavy metals include the elements arsenic, cadmium, chromium, mercury, lead and thallium. Typically, they enter the body via the food chain, air or drinking water. Likewise, contaminants have been introduced into the soil, with negative consequences not only for the food chain, but also for drinking water. Ultimately human health is at risk. As a consequence, contamination levels in urban and industrial waste water need to be controlled, as do levels in soil and sludge from treatment plants. The physicochemical and chemical characterisation of waste water at the inlet (influent) and the outlet (effluent) of the treatment plant is an effective way to control treatment process efficiency and to verify that the final quality of the effluent complies with the regulations. Table 1 shows the recommended limits for heavy metal constituents in reclaimed water for irrigation.

Table 1 : Recommended limits for heavy metal constituents in reclaimed water for irrigation

Constituent	*Long-term use (mg/L)	*Short-term use (mg/L)	FAO** (mg/L)	Remarks
Copper (Cu)	0.2	5	0.1	Toxic to a number of plants at 0.1 to 1.0 mg/L in nutrient solution.
Zinc (Zn)	2	10	2	Toxic to many plants at widely varying concentrations; reduced toxicity at increased pH (6 or above) and in fine-textured or organic soils.
Lead (Pb)	5	10	2	Can inhibit plant cell growth at very high concentrations.
Iron (Fe)	5	20	5	Not toxic to plants in aerated soils, but can contribute to soil acidification and loss of essential phosphorus and molybdenum.
Mercury (Hg)				

*Source: APHA, 1992

**Source: USEPA, 1993.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location and construction of the treatment wetland

A subsurface flow wetland system was constructed at the premises of the Department of Theoretical and Applied Biology, KNUST for this study. The mean annual rainfall for the study area is 1300mm and average daily temperature of 26 ºC respectively. Rainfall distribution is bimodal with peaks between March and June, and September and October. The evapo-transpiration is between 86 mm in October to 157 mm in January while the mean annual evapo-transpiration is 1412 mm. The geology presents a sandy loam soil nature and the absence of rocks. The rivers which are present in the KNUST vicinity are Wiwi and Sisa. Effluents from drains connected to several laboratories and halls of residence in the University contribute to pollution of these water bodies, which serve as irrigation water source for farmers in the surrounding communities who cultivate various kinds of fresh-eating vegetables.

3.1.1 Design parameters and construction of S.S.F wetland

Using both laboratory and field test methods, wastewater samples were collected and analysed prior to the determination of the total surface area of the wetland cells and hydraulic loading rate appropriate for the system to function effectively. The heavy metals to be analysed for included the toxic ones such as

Lead (Pb), Mercury (Hg), Zinc (Zn), Iron (Fe) and Copper (Cu). Dimensions for the wetland unit were obtained by using relationship for plug flow reactor and first-order model (Appendix 7). The calculated influent BOD loading was 70 mg/L, COD of 136 mg/L and SS of 74 mg/L. It is required that the effluent BOD and SS should be respectively 35mg/L and 15 mg/L. These effluent values were taken based on the requirements of Ghana EPA guidelines where final effluent BOD must be less than 50 mg/L and SS, less than 35 mg/L.

3.1.2 Compartments and components of S.S.F C.W.

The experimental wetland had six treatment cells each with dimensions 2.1 m long, 1 m wide and 0.8 m deep. After excavation works, the compartments of the wetland were constructed from 5 inch concrete blocks and plastered with mortar. The storage tank consisted of a one thousand litre (1000 L) capacity water tank, mounted on a one metre raised concrete platform from the ground level. The elevated tank provided the added advantage of gravity aided flow of water to the treatment wetland. The system comprised a storage tank, sedimentation tank, three parallel treatment lines or columns and an effluent tank or collector. Each column in turn had two rectangular serially arranged cells or ponds which were connected by inlet and outlet pipes (see Figure 1, Plate 1). The main pipeline (2 inches diameter) was connected from the storage tank, for the distribution of irrigation water through three sub-main pipelines (1½ inches diameter) provided with valves to ensure equal flow to all of the three initial cells in the constructed wetland. Baffles were inserted under each of the treatment cells to help prevent short-circuiting. The wetland cells were rendered impermeable by placing 50 mil high density polyethylene (HDPE) film at the bottom. This was carried out in order to avoid groundwater infiltration and soil contamination by percolation.

Washed and sieved sand from Afisiyaso in Kumasi was used in the constructed wetland due to its good hydraulic conductivity of 368.87 cm^3/ d. The cells were then filled with coarse sand (0.6-2 mm diameter) to a depth of about 0.2 m and an additional 0.1 m layer of washed gravels of sizes 6.25 mm as the filter medium. Both inlet and outlet points of the cells were then filled with washed stones of sizes 30-40 mm. The gravels aided to obtain even flow of water and also helped to keep plants sturdy in the cells. A free board of 0.2 m was created to accommodate expected increase in water volume, occasional flooding as well as build up of sediments and litter over time. Steel sieves of pore size 4 mm were used for retaining large particles and/or debris at the outlet points of all the cells including the sedimentation tank. These units were periodically cleaned to achieve maximum filtration. The sieves also helped to prevent the gravels from getting washed into the collecting effluent pipes and causing an interruption in the flow of water.

3.1.3 Flow rate and retention time

The inflow discharge was 1 m^3 per day and the approximate hydraulic residence time was 5-7 days. The influent entered the wetland through a PVC pipe of diameter 1½ inches (3.81 cm) which was fitted along the width of each cell and at a height of 70 mm from the bottom. A perpendicular drip dispersion tube of diameter 2.54 cm with aligned holes at 50 mm regular distances to produce a laminar flow was attached to the inlet pipe. A swivel-elbow PVC pipe was attached to the outlet of each treatment cell, to facilitate easy control of the water level in the cells. The effluent pipe had a diameter of 2 inches to enhance maximum calculated outflow and was attached 15cm from the floor of the cells to allow for complete draining when necessary. Before the introduction of the plant species being studied, the constructed wetland was filled with water to check for leakages and seepage. The sedimentation tank required extra fortification to handle the pressure introduced by the large volume of waste water.

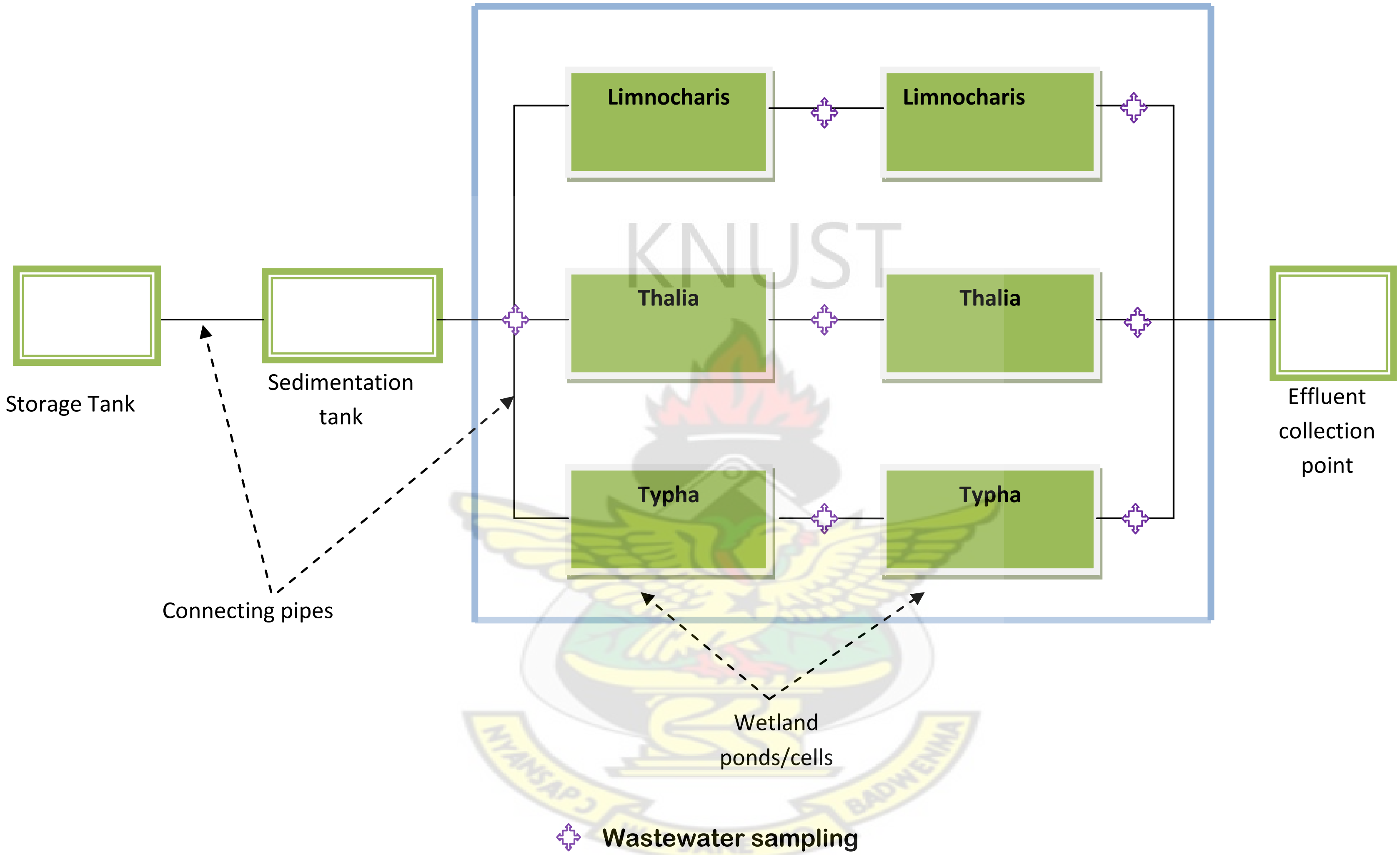


Figure 1: Schematic presentation of the constructed wetlands and experimental design for the study

3.2 Stocking of plants

Healthy young plants of the three different species were collected from natural wetlands at Atonsu Agogo in Kumasi, and transplanted immediately into the constructed wetlands. This was carried out very early in the morning to help reduce the mortality of rhizomes and root stocks. Collection was however, more difficult at areas with high water levels as prevalent in most wetlands. Seed trials in the green house proved less successful for use in the constructed wetlands, since they had low germination rates and required very long periods of dormancy. The use of rhizomes had a lower efficiency as some died after transplanting. The most efficient option for this study, thus, involved the use of cuttings and young plants. The entire root system was taken along with some soil. Transplanting was carried out with the attached soil to help inoculate the constructed wetland with microbes from the donor wetland, which was very important in the reduction of parameters such as BOD, COD and TSS (Denny, 1997). Locally available plants to be used were separated into individual root and rhizome units, with the mature stem cut back to <1 ft (< 0.3 m) before planting. The roots were placed in the filter medium in the wetland cells, with the growing shoot projecting above the surface of the media. To encourage deeper root penetration, the water levels in the cells were lowered after planting until transplants were fully established. It was necessary to replace dead plants in cells after two weeks of planting. The first column was planted with *Thalia geniculata*, the second with *Limnocharis flava* while the last column was planted with the commonly used phytoremediant, *Typha latifolia*, as the reference plant. Plants were grown to cover at least 50% of the surface of each cell. The planting density for all the three different species was 5-6 shoots per square metre, this allowed for open spaces within the cells allowing aeration and enhancement of UV treatment of pathogens. The sun served as energy source in the constructed wetlands; hence the total area exposed was of great priority.

Plate 1 : Constructed wetland with the three phytoremediants beginning to establish themselves.

3.3 Stabilization and operation of constructed wetland

The wetland was allowed a period of two months for plants to grow and adapt to their new environment. Water from river Wiwi was gradually added on a weekly basis, after the first 2 weeks of planting, to enable the plants acclimatise.

During the stabilization period, changes in the physicochemical parameters of the treatment wetlands were continuously monitored and recorded. Daily readings on pH, conductivity and temperature were recorded using a Thermo scientific, model Orion 4 Star Plus portable multi-parameter tool kit. Measurements of growth parameters such as height, leaf-area, and stem diameter were also recorded on a fortnightly basis from one month after planting (June 2010) until the end of the study period (March 2011). This was done using a tape measure and a grid for leaf area calculations. The plants were tagged with ribbons serving as labels for identification, to allow for accurate data collection on individual plants.

When plants were well established, the process for wastewater treatment in the constructed wetland was initiated. The operational water level of the wetland was 0.4 m. The typical operation sequence consisted of pumping water from the point of the river where the effluent from the anatomy laboratories were emptied, using a Honda WB20 model water pump, into the water tank. The modified tap was then opened to deliver $1m^3$ of irrigation water into the cells via the sedimentation tank on a daily basis. Water from the sedimentation tank flowed into the first set of parallel treatment cells, then through the second set of treatment cells and finally into the effluent tank for collection. The water was typically retained in the treatment cells for about 4-7 days, to allow for remediation processes to occur.

3.4 Monitoring and Analyses of Heavy Metal Concentrations

3.4.1 Atomic Absorption Spectroscopy

In the twelfth week of planting, at least two separate plants of each species were collected from each of the six treatment cells into sterilized plastic bags, and sent to the laboratory for analysis. In the laboratory, the plants were thoroughly washed in distilled water to remove all adhered soil particles, and sorted out into roots and shoots. After chopping into smaller pieces, the plant samples were weighed to determine their fresh weights and dried in an oven at 80 ^{o}C for 72 hours to determine their dry weights. The dry samples were crushed in a mortar to pass through a 1mm sieve. Well-mixed subsamples (2 g each) were taken in a 250 ml glass beaker and digested with 10 ml aqua regia on a water bath for 2 hours. After evaporation to near dryness, the samples were dissolved in 10 ml nitric acid and hydrochloric acid, filtered and then diluted to 50 ml with distilled water. The heavy metal concentrations in the plant samples were then determined using a BUCK Scientific atomic absorption spectrophotometer (AAS), model 210VGP. The accuracy or the detection limit of the instrument was evaluated separately for each heavy metal by preparing standards and analysing these along with the samples. Readings were taken three times for each sample to obtain the average. Root and shoot samples were analysed separately.

Similarly, duplicate water samples were collected in sampling bottles from each of the treatment cell as well as the effluent tank and transported immediately to the laboratory for determination of dissolved heavy metals in the water. The samples were filtered using Whatman No. 41 (0.45 micro meter pore size) filter paper. The samples were then concentrated on a water bath and digested using 10 ml nitric acid and hydrochloric acid. The concentration of heavy metal contaminants in the wastewater were analysed using the atomic absorption spectrophotometer. The heavy metals measured included Lead (Pb), Mercury (Hg), Zinc (Zn), Iron (Fe) and Copper (Cu). The levels of the metals in the samples were compared with both locally and internationally accepted standards for irrigation. The determination of metal concentrations in the water and plant samples was repeated six times on a monthly basis, from October 2010 to March 2011.

3.4.2 Bioaccumulation Factors, Translocation Ability and Removal Efficiency of plants.

From the results of the water and plant samples analyses, the bioaccumulation factors (BAF), translocation factor (TF), as well as the removal efficiency (RE), were obtained. The BAF is defined as the ratio of heavy metal concentration in a plant tissue to concentration of the same metal in the surrounding water (Yoon *et al*., 2006) and is derived by the following formulae: BAF = (P/E) I, where *I* is the heavy metal, *p* represents the metal concentration in plant tissue (mg/kg dry weight) and *E* represents metal concentration in the water (mg/L). The TF was determined as the ratio of heavy metal concentration in the shoot to the concentration of the same metal in the root (Massa e*t al*., 2010),TA = (Ar/As)i, where *Ar* represents the metal concentration accumulated in the roots, As represents the metal concentration in shoots and *i* is the heavy metal. Removal efficiencies of heavy metals (Fe, Cu, Zn, Pb, Hg) by the macrophytes, were calculated using the formula: *Removal efficiency (%) = [(inlet pollutants-outlet pollutants)/ inlet pollutants] x 100* (Ganjo and Khwakaram, 2010).

3.4.3 Data analyses

The R statistical software (R Development Core Team, 2011) was used for all the analyses and plotting. Analysis of variance (ANOVA) was performed to statistically compare the species' accumulation of heavy metals, their bioaccumulation factors and translocation abilities. Similarly, differences in the removal efficiencies and leaf area increments of plants were evaluated statistically using ANOVA. All statistical analyses were preceded by normality tests using the Shapiro-Wilk normality test. Multiple comparison tests, where necessary, were performed using the *kruskalmc* command in the R package "pgirmess". Where assumptions of ANOVA were not met, the non-parametric alternative, Kruskal analyses was used. Correlations of the removal efficiencies of the plants with their bioaccumulation of heavy metals and leaf area increments were tested using the Pearson correlation test. All analyses were performed at α-level of 0.05 ($P \leq 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Characteristics of irrigation water, soil and plant samples used for the study

The initial characteristics of the irrigation water, soil and plant samples used for the study are presented in Table 2. After analyses of the initial heavy metal contents of river Wiwi, at the point of sampling and pumping for treatment, concentrations of all heavy metals (with the exception of Cu) in the water samples were within the safe limits set for irrigation water. Iron (Fe) was the most abundant element with a mean concentration of 1.77 mg L^{-1} of wastewater while Hg (0.05 mg L^{-1}) was least abundant. Analyses of the soil for both total and extractable heavy metal contents again revealed Fe (258.4 mg kg^{-1} of soil) having the highest concentration followed by Cu with total mean concentration of 34.5 mg kg^{-1} of soil. Lead (Pb) had the lowest concentration (1.75 mg kg^{-1}) of the five metals in the soil. The total heavy metal content of the soil used for the study was more than that in the water sample.

Total mean concentration of Fe in the three plants was about fivefold greater than that of all four remaining metals combined. Lead had the lowest concentration of all the metals in the plants, with a grand mean estimating 14 mg kg^{-1}. Among the three plant species, *T. latifolia* had the highest amounts of Fe (585.6 mg kg^{-1}), Cu (28.4 mg kg^{-1}) and Pb (5.6 mg kg^{-1}), whereas the highest concentrations of Zn (86.0 mg kg^{-1}) and Hg (5.16 mg kg^{-1}) were recorded in *T. geniculata*. It is interesting to note that the concentration of Hg in all the three plant species and that of Cu in *T. latifolia*, were within the toxic range observed for most plants (Table 2). However, none of the plants had accumulated sufficient quantities of heavy metals to qualify it as a hyperaccumulator, based on the limits shown in Table 2.

The values of pH and electrical conductivity in waste water samples averaged 7.34 and 248.45 µS cm^{-1} in *T. latifolia* planted cells, 7.46 and 190.18 µS cm^{-1} in *L. flava* cells, whiles *T. geniculata* treatment cells had 7.27 and 239.93 µS cm^{-1}. Mean Temperature readings in the treatment cells during the study period presented a minimum value of 29.44 ^{o}C (*T. geniculata*) and a maximum value of 30.63 ^{o}C (*L. flava*). There was no significant difference between the temperature of influent and effluent water samples (Appendix 10).

Table 2: Initial heavy metal concentrations of irrigation water, soil and plant samples used for the study

Sample	Concentration of heavy metal				
	Fe	**Cu**	**Zn**	**Pb**	**Hg**
Plant					
Limnocharis flava (mg/kg)	468.0 (10.2)	19.38(2.9)	72.4(6.0)	4.2(0.8)	4.06(0.1)
Thalia geniculata (mg/kg)	570.8 (11.6)	17.88(1.8)	86.0(7.9)	4.2(1.1)	5.16(0.0)
Typha latifolia (mg/kg)	585.6 (9.3)	28.4 (2.7)	82.4(6.5)	5.6 (0.5)	5.14(0.5)
Soil (mg/kg)	258.4 (0.1)	34.5 (0.00)	6.65 (0.05)	1.75 (0.05)	6.35(0.02)
Water (mg/l)	1.77 (0.52)	0.31 (0.01)	0.42 (0.00)	0.19 (0.01)	0.05 (0.00)
Safe limit(mg/L)[a]	5.0	0.2	2.0	5.0	-
Toxic concentration (mg/kg)	40-500[b]	20-100[c]	100-400[c]	30-300[c]	1-3[c]
Hyper-accumulation Limit (mg/kg)[d]	10000	1000	10000	1000	10

Means were calculated from two duplicate samples. [a] Safe limits of heavy metal concentration in irrigation water (Pescod, 1992); [b] Toxic threshold of Fe for most plants (Ganjo and Khwakaram, 2010); [c]Toxicity threshold of metals for plants (Massa et al., 2010). [d]Criterion for designating a plant as a hyperaccumulator (Massa et al. 2010); in parenthesis are standard errors of the means.

4.2 Bioaccumulation of heavy metals by the test plants over time in the constructed wetlands

The concentrations of heavy metals absorbed by the three macrophytes over the entire study period (October 2010 to March 2011) are presented in Figure 2, while Figure 3 shows the bioaccumulation factors of the three phytoremediants over a six month period. Heavy metal accumulation in all three phytoremediants generally increased with time, but the accumulation rates of the plant species varied for different metals (Figures 2 and 3). Iron had the highest accumulation, increasing from an initial concentration of about 600 mg kg^{-1} to approximately 1600 mg kg^{-1} at the end (March) (i.e. about 160% increment). Statistical analysis showed no significant differences (P = 0.667) in the accumulation rates of Fe among the three plant species. On the contrary, the BAF varied significantly ($P < 0.001$) among the species, with the highest value found in *L. flava*. The amounts of Fe accumulated by the plants were far below their hyperaccumulation limits (Table 2; Figures 2 and 3).

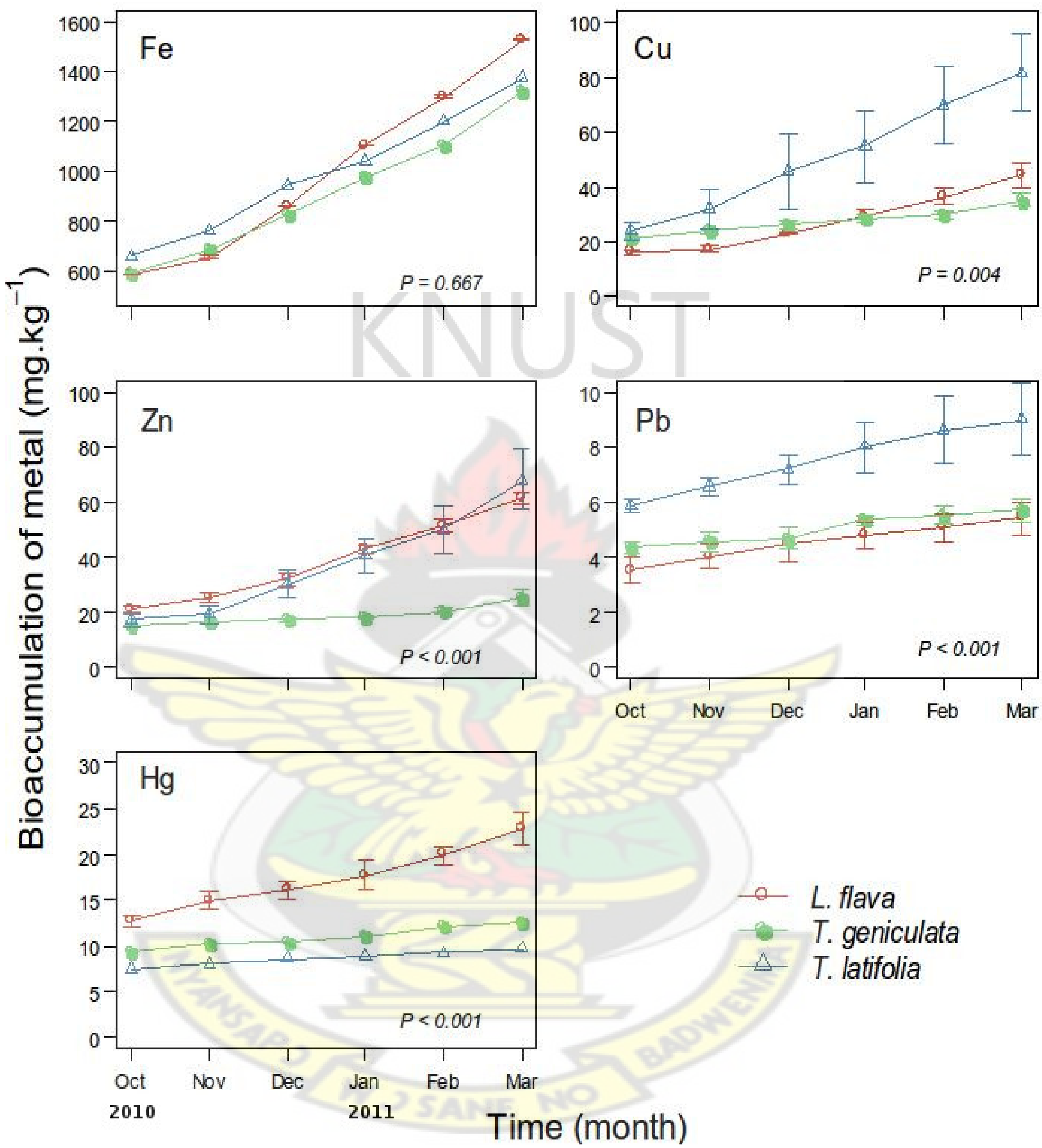


Figure 2: Mean concentrations of heavy metals accumulated by *Limnocharis flava*, *Thalia geniculata* and *Typha latifolia* in the constructed wetlands over the six month study period. *P*-values indicate the statistical difference among the plants. Error bars are standard errors of the means.

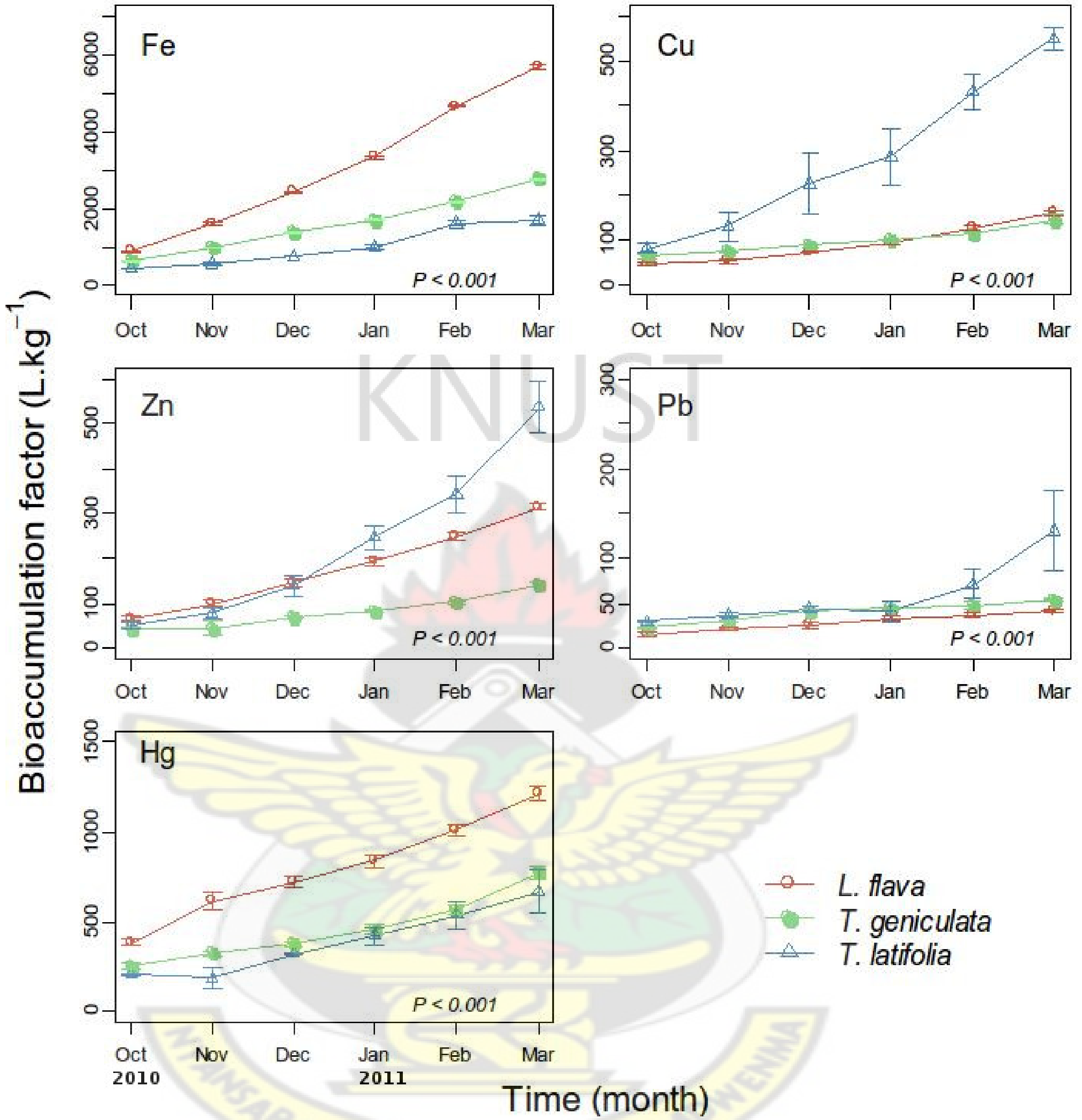


Figure 3: Bioaccumulation factors of various heavy metals by *Limnocharis flava*, *Thalia geniculata* and *Typha latifolia* compared over a six-month period (October - March). P-values indicate the statistical difference among the three plant species. Error bars are standard errors of the means.

Copper was the next most accumulative metal after Fe, although its concentration (grand mean: 35.33mg kg^{-1}) was one order of magnitude lower than that of the latter. While the concentration of Cu in *T. latifolia* increased steadily from an initial value of 23 kg^{-1} to 81.43 mg kg^{-1} after six months, only marginal increases were observed in *L. flava and T. geniculata*, resulting in a significant statistical difference among the three species ($P = 0.004$). Multiple comparison tests showed no significant difference between the accumulation rates of Cu by *L. flava* and *T. geniculata* ($P > 0.05$). Similar trends were obtained for the BAFs (Figure 2)

The third most accumulative metal in the plants was Zn with an overall mean of 31.71 mg kg^{-1}. *L. flava* and *T. latifolia* accumulated significantly higher concentrations of Zn compared to *T. geniculata* ($P < 0.001$). By October, at least 20 mg kg^{-1} of Zn had been taken up by each of of the plants (*L. flava* and *T. latifolia*). This value increased more than threefold by March. However, the level of the metal in *T. geniculata* barely changed after the initial value of approximately 20 mg kg^{-1}. Despite the increases in the concentration of Zn in *L. flava* and *T. latifolia* over time, the values were still below both the toxic and hyperaccumulation thresholds.

Mercury (Hg), with a grand mean of 12.31 mg kg^{-1}, was only higher than Pb in terms of the concentration accumulated by the plants. In spite of this, Hg concentration in all three species (> 10 mg kg^{-1}) exceeded the toxicity limits of the metal reported for other plants (Table 1; Figure 1). More interestingly, the levels of Hg sequestered by *L. flava* and *T. geniculata* were beyond the hyperaccumulation limit observed for most plants. The concentration of Hg differed significantly ($P < 0.001$) among the three species. In *L. flava*, mean concentration of Hg increased from 12.73 in October to

22.82 mg kg^{-1} in March while those of *T. geniculata* and *T. latifolia* only changed slightly over time.

The least accumulative metal among the five heavy metals evaluated was Pb, which had a grand mean of 5.71mg kg^{-1}. The amount of Pb accumulated by *T. latifolia* was significantly higher than the other two plant species ($P < 0.001$). Multiple comparisons showed no statistical difference between the bioaccumulation of the metal by *L. flava* and *T. geniculata* ($P > 0.05$). The quantities of Pb accumulated were below the toxic and hyperaccumulation cut-offs (Table 2).

4.3 Translocation factors of plants

The translocation factors of the three macrophytes are presented in Figure 4. The translocation factors (TFs) varied widely among species for the various heavy metals but were generally stable over the duration of the study. The TF for Fe was less than 0.4 in all species; this also varied significantly ($P < 0.001$) among the species, with highest value found in *T. geniculata*. The TF for Cu was greater than unity in all species but differed significantly among them ($P = 0.001$). For Zn, TF of *T. geniculata* almost remained constant and above one while those of *L. flava* and *T. latifolia* decreased to values below one over time, resulting in the statistical difference ($P = 0.017$) observed among the three species.

For Pb, TF of *L. flava* decreased slightly over time but did not deviate much from one. On the contrary, TF of *T. latifolia* was less than unity indicating accumulation of the metal in roots. While the TFs of *T. geniculata* and *T. latifolia* fluctuated around one throughout the study period that of *L. flava* consistently stayed above one, indicating the effectiveness of the latter to translocate Pb to its shoots. However, there were no statistical differences among the TFs of the species (P = 0.667). The Translocation factor for Hg differed significantly ($P < 0.001$) among the species. It averaged about 1.5 for *T. geniculata,* indicating the effectiveness of the species to accumulate the metal in its shoots. *L. flava* and *T. latifolia* had average TF values of 1.0 and 0.3 respectively.

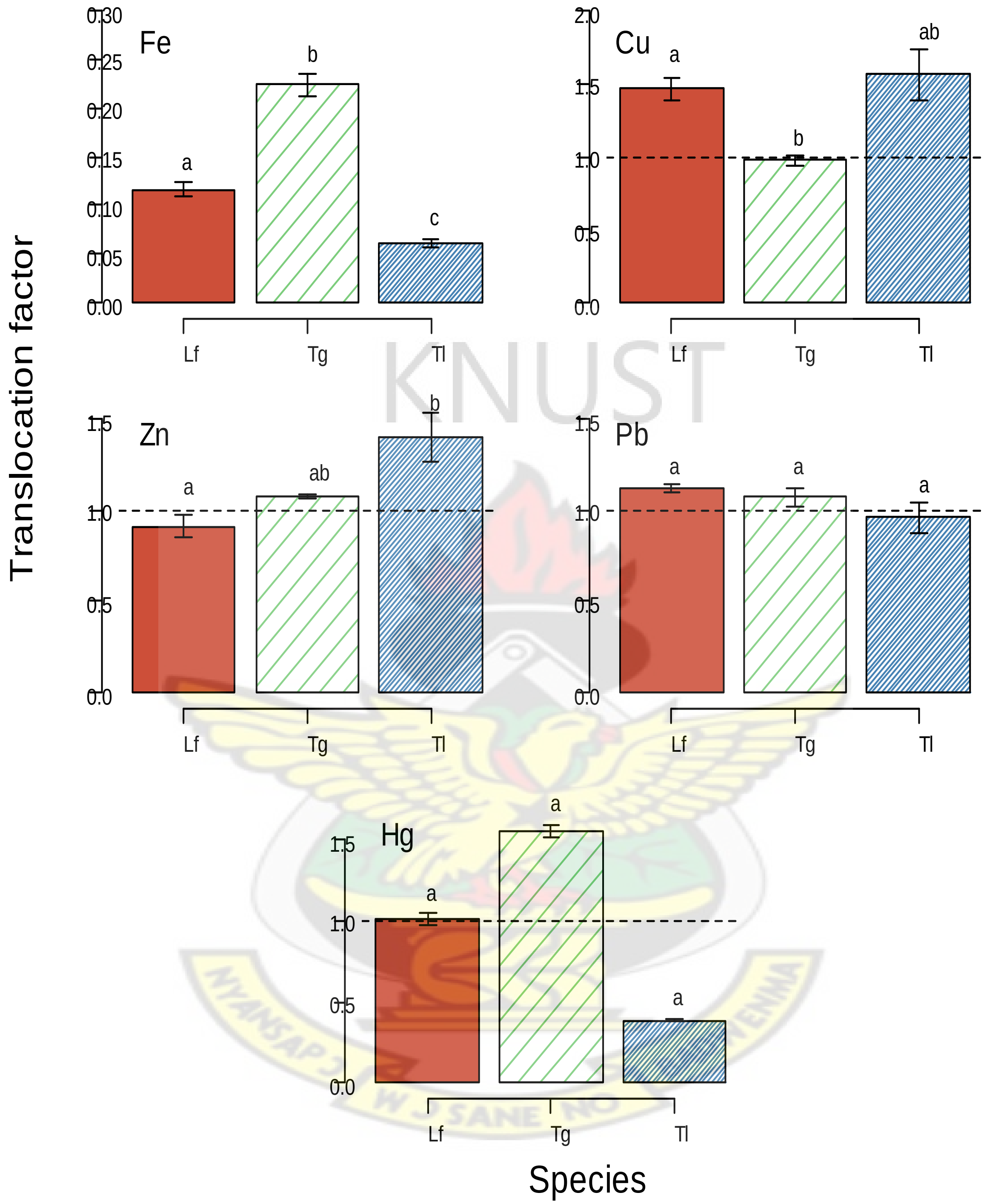


Figure 4: Translocation factors of heavy metals compared for the three candidate phytoremediants (*Limnocharis flava* (Lf), *Thalia geniculata* (Tg) and *Typha latifolia* (Tl) Error bars represent the standard errors of the means. For each metal, bars with different letters are statistically different ($P< 0.05$). Bars above the horizontal dashed line indicate translocation of metal from root to shoot of plants.

4.4 Removal efficiency of heavy metals

Figure 5 below represents the removal efficiencies of heavy metals (Fe, Cu, Zn, Pb, and Hg) by the three candidate phytoremediants after two stages of treatment. The different elements showed a variation amongst their percentage reduction in the concentration of heavy metals in the constructed wetland. However, no significant differences ($P > 0.05$) in removal efficiency were found between the first and second stages of phytoremediation, despite slight increases in per cent and absolute amounts of metals after the second stage of removal. Removal efficiency of Fe, which ranged from a mean of 34.28% in *T. latifolia* to 77.09% in *L. flava*, was the highest among the five metals studied. The removal efficiencies of Fe by *L. flava and T. latifolia* were significantly different ($P < 0.05$). The second highest percentage reduction was observed in Hg with *L. flava* (51.61 %) again performing better than the other two species. *T. latifolia* reduced the amount of Hg in the wastewater by as much as 46.63 % while *T. geniculata* recorded 45.04 %. These differences were, however, statistically insignificant ($P > 0.05$). The third largest percentage reduction was observed in Zn, but in this case, *T. latifolia* (51.88%) was somewhat more effective at doing this compared to the other plant species. *L. flava* and *T. geniculata*, respectively, managed 44.45% and 41.58% removal of Zn. Again, these differences were not statistically significant ($P > 0.05$). Lead (Pb) and Cu recorded the lowest percentage reduction by the plant species in the constructed wetland. *T. geniculata*, *T. latifolia* and *L. flava* removed 30.26%, 20.29 % and 11.62 % of Pb respectively. In the case of Cu, 33.84, 9.39 % and 4.21 %, respectively, were removed from the wastewater by *T. latifolia*, *T. geniculata* and *L. flava*.

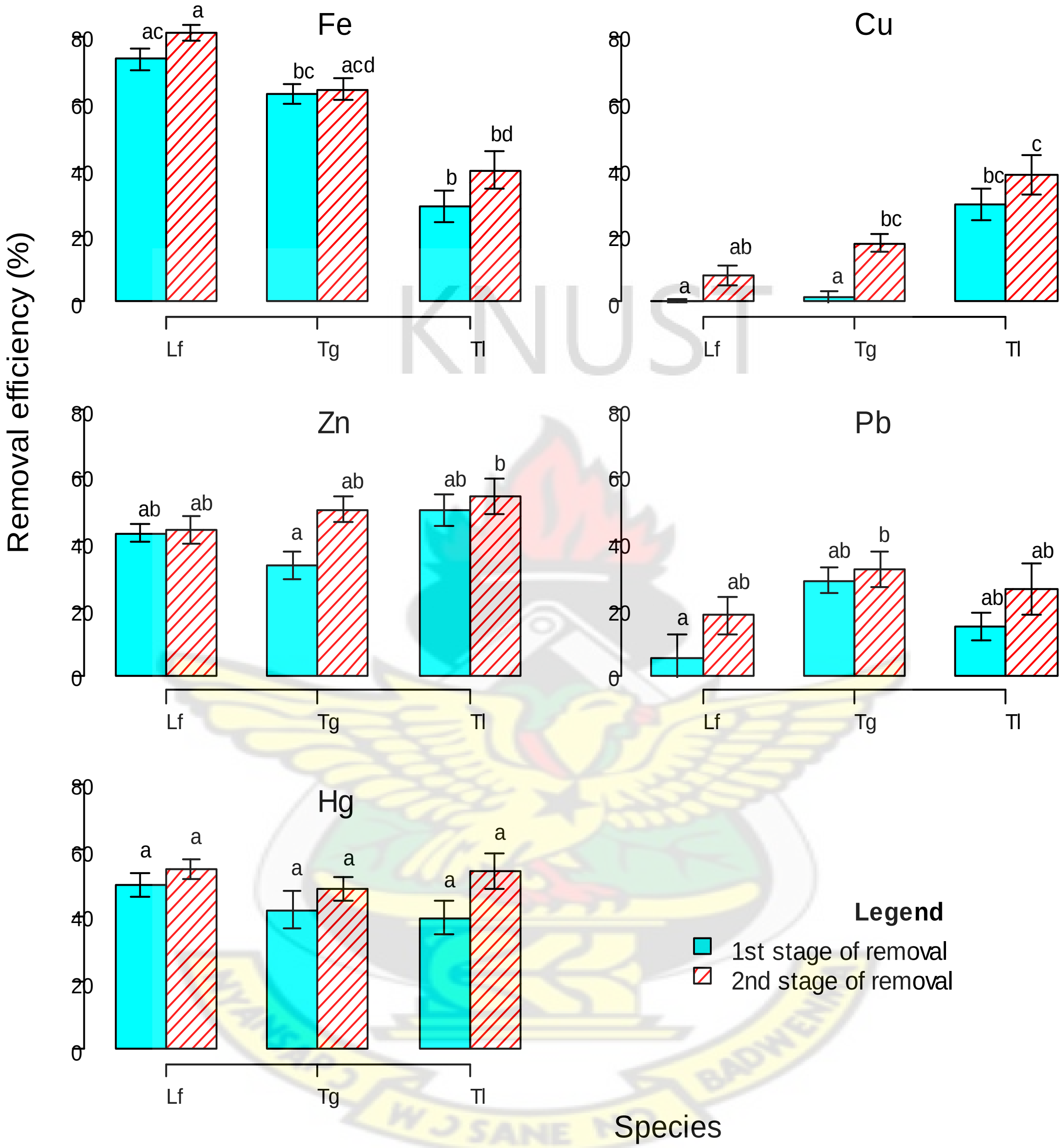


Figure 5: Removal efficiencies of heavy metals (Fe, Cu, Zn, Pb, Hg) by the three candidate phytoremediants, *Limnocharis flava* (Lf), *Thalia geniculata* (Tg) and *Typha latifolia* (Tl) after two stages of treatment. Error bars represent the standard errors of the means. For each metal, bars with different letters are statistically different ($P < 0.05$).

4.5 Correlations of removal efficiencies with bioaccumulation factor of the phytoremediants

Correlations of removal efficiencies with bioaccumulation rate of the macrophytes measured over a six month period can be seen in Figure 6. There was an overall very high significant correlation of removal efficiencies with bioaccumulation factors for all of the analyses conducted here. All the elements and macrophytes exhibited a high level of signifance ($P < 0.05$) in their correlation values. *T. geniculata* recorded the highest r-values, closely followed by *T. latifolia* and finally *L. flava*.

Correlation coefficients for Fe ranged from 0.86 for *T. latifolia* to 0.76 (*L. flava*). The r value obtained for the Fe removal efficiency correlation with bioaccumulation rates for *T. geniculata* was also very high (0.85). All these correlation coefficients had very significant p-values. Cu had very significant p-values and very strong correlation for *T. geniculata* and *T. latifolia*, and a relatively weak correlation for *L. flava* with a coefficient of 0.47. Correlation coefficients obtained for Zn ranged from 0.7 to 0.78 and also had very significant p-values. Coefficients of zinc correlations for all the macrophytes were very similar. Correlation coefficients obtained for Pb were also very strong ranging from 0.89 (*T. geniculata*) to 0.74 (*L. flava*). Hg recordered the highest r-values for all the macrophytes. Both *T. latifolia* and *T. geniculata* recorded r- values of 0.91, and *L. flava* (0.81). Apart from the very strong correlation exhibited by the removal efficiencies with the bioaccumulation factors of the different phytoremediants, the associated p-values exhibited very high significance.

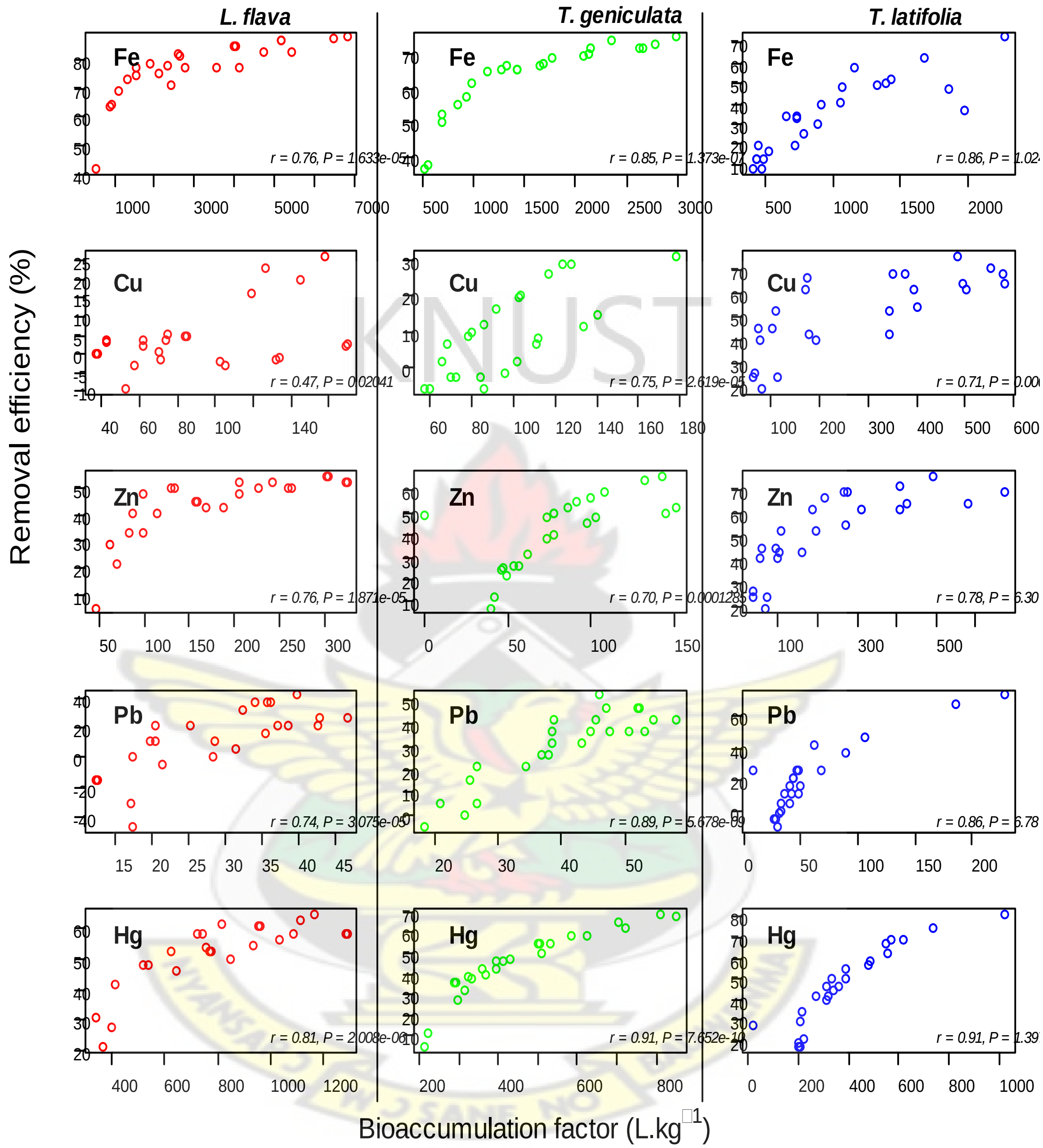


Figure 6: Correlations of removal efficiencies with bioaccumulation factor of the phytoremediants (*Limnocharis flava*, *Thalia geniculata* and *Typha latifolia*) measured over a six month period. *P*- and *r*-values indicate the strength of the correlation between the two variables.

4.6 Analysis of leaf area

Increments in leaf area for the three candidate phytoremediants over the entire study period are presented in Figure 7. There was a general increase in leaf area of *L. flava*, *T. geniculata* and *T. latifolia* with respect to time. Specifically, *T. latifolia* and *T. geniculata* showed particularly gradual and progressive increases in leaf area during the whole period of the research with *T. latifolia* having higher increments in leaf area than *T. geniculata* most of the time. *T. latifolia* showed a slight retardation in leaf area increment from October to January, during which period, *T. geniculata* exhibited the highest leaf area with reference to all the macrophytes cultivated in the treatment plant. *L. flava* exhibited the least increase in leaf area from August to March, with respect to all the macrophytes present. The results obtained show a very high level of significance between the leaf area increments of all the plants at a p-value of 1.085 $x10^{-13}$.

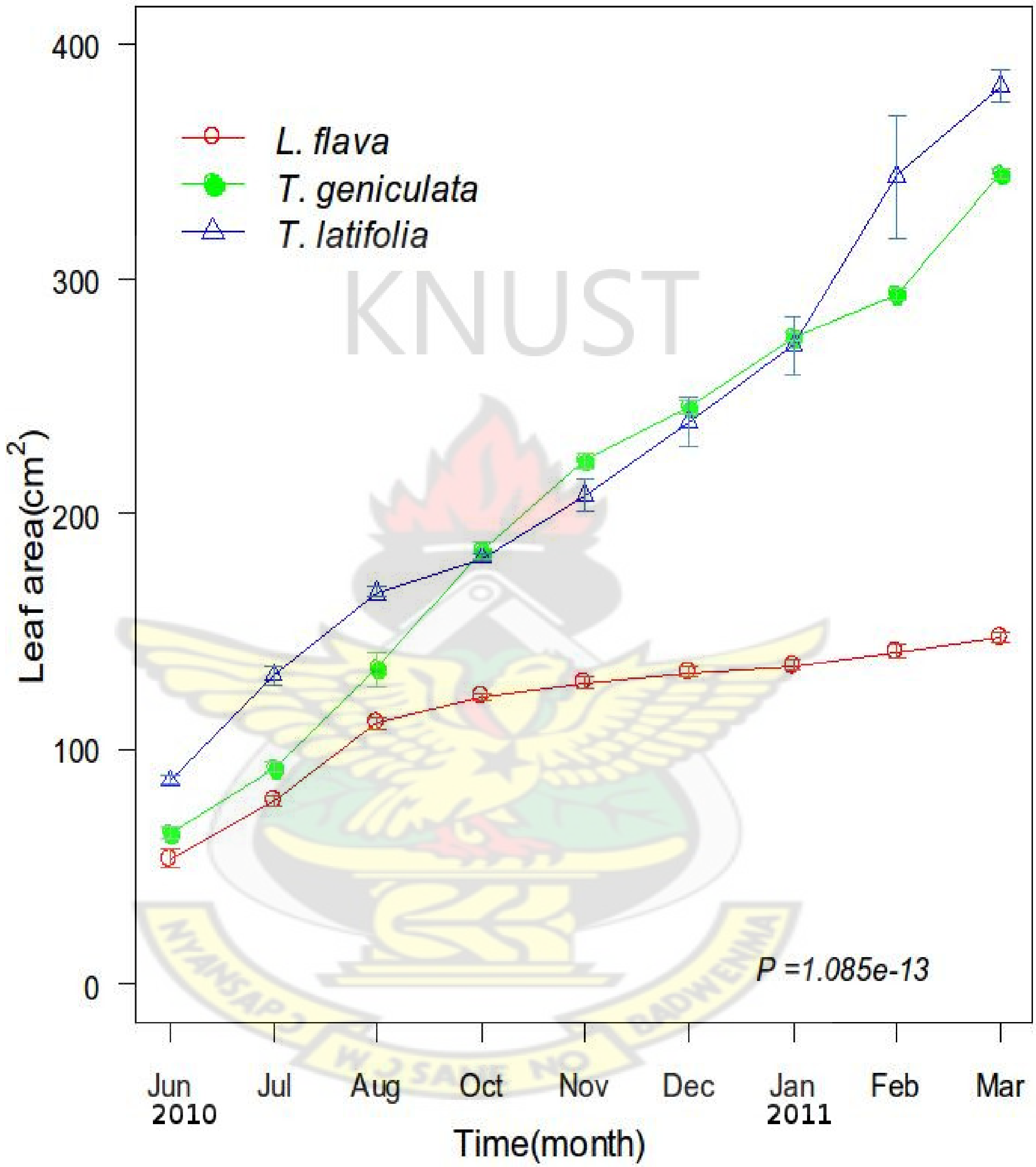


Figure 7: Leaf area increment *of Limnocharis flava*, *Thalia geniculata* and *Typha latifolia* over the period of phytoremediation in a constructed wetland.

4.7 Correlations of leaf area increments with bioaccumulation factor of macrophytes

Figure 8 represents the results of the correlation analysis of leaf area increments with bioaccumulation rates of the three macrophytes. For *L. flava*, there was high correlation between the bioaccumulation factor and the leaf area increment for the heavy metals Fe, Cu and Zn (r = 0.49, 0.48 and 0.62 respectively). Additionally, all those results were statistically significant with p-values of 0.015, 0.0012 and 0.001 respectively. On the other hand, there was an inverse correlation between the bioaccumulation factor and the leaf area increment for Pb (r = -0.05) and Hg (r = 0.43) although the relation was only significant in Hg (p = 0.036 and p = 0.0799 in Hg and Pb respectively).

For *L. geniculata*, most of the relations were not significant. (p = 0.103, 0.881, 0.086 and 0.491 for Fe, Cu, Pb and Hg respectively). . However, there was significant relationship between leaf increment of *L. geniculata* and bioaccumulation factor of Zn (r = 0.75; p = 0.0000275). All the relations were positive except for Cu (r = -00.03) and Pb (r = -0.36). For *T. latifolia,* most of the relations were statistically significant namely those for the heavy metals (p = 0.03, 4.25×10^{-5} and 0.005 for respectively Cu, Zn and Pb) There was also strong correlation between the bioaccumulation and leaf area increment of the following metals: Cu, Zn and Pb (r = 0.59, 0.74 and 0.55 respectively).

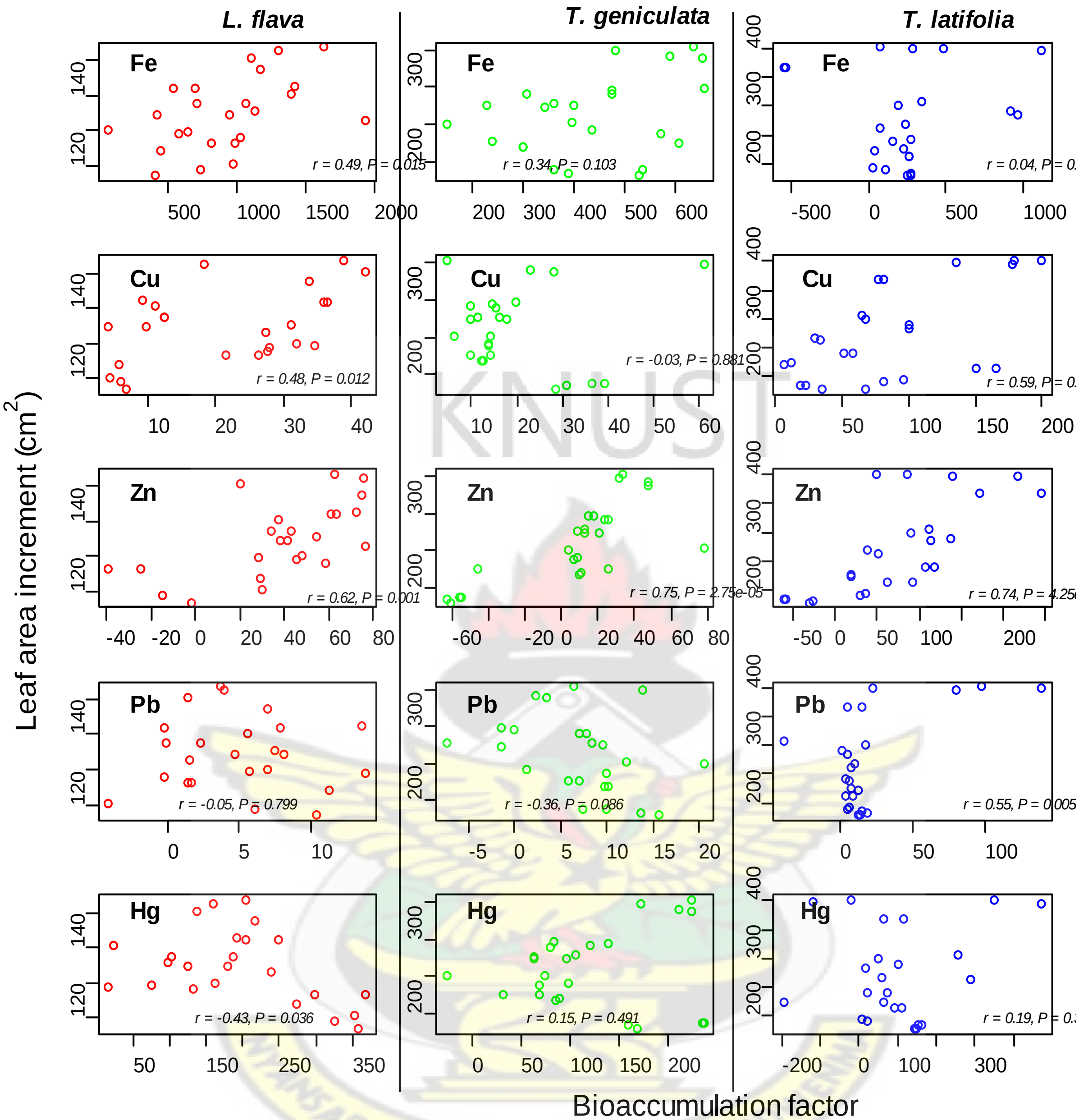


Figure 8: Correlations of leaf area increments with bioaccumulation factor of *Limnocharis flava*, *Thalia geniculata* and *Typha latifolia*.

CHAPTER FIVE

5.0 DISCUSSION

Repeated irrigation with applications of wastewater effluent tends to accumulate trace elements in the soil surface and become part of the soil matrix. They could also accumulate in crops to a level that is detrimental to the health of humans, domestic animals, and wildlife that consume the crops. Irrigation water used for this study was collected from only one source which happened to have low heavy metal loading. The concentrations of zinc, lead and iron were below the accepted irrigation standards. Despite the fact that these concentrations are low, they could be potentially active when bioaccumulation and amplification sets in with continuous use of such polluted irrigation waters (Sawidis *et al*., 1985; USEPA, 2000). This point is corroborated by the high heavy metal loading of the soil samples used for the present study. Poor quality irrigation waters such as these clearly pose environmental and public health hazards (Gupta *et al*., 2008; Chen *et al*., 2010); hence the need for wastewater treatment prior to agricultural use. Heavy metal contamination of water bodies is thought to be less problematic in Ghana because of the scarcity of industries (Keraita *et al*., 2002; Cofie and Drechsel, 2006). Nonetheless, large volumes of untreated wastewater of domestic, hospital and industrial origins, fraught with heavy metals, are often released into water bodies in peri-urban Kumasi and other parts of the country (McGregor *et al.*, 2002). For example, Sarpong (2007) found high levels of Fe, Cu, Pb, Cd, As and Hg above WHO recommended values for irrigation water in the Kumasi metropolis. Akoto *et al.*, (2008) also observed high levels of Pb in the Owabi stream in Kumasi.

The sub-surface flow (SSF) constructed wetland was selected due to its high efficiency in pollutant removal compared with the surface flow or sub-surface vertical flow constructed wetland as well as its efficiency in odour control and the absence of mosquitoes (USEPA, 1993). Less land area is also required for the construction of the SSF wetlands (Rousseau, 2005). A sedimentation tank was added to the constructed wetland to help reduce the concentration of easily degradable organic solids, which would have otherwise proceeded to accumulate and clog the inlet points of the first set of treatment cells. Preliminary treatment also helped to reduce odours and protect the plants at the entry zone of the constructed wetland from adverse impacts of high pollutant loading. The shallow operating depth (0.4m) enhanced the transfer of oxygen and hence desirable root formation and penetration (Rousseau, 2005). The average Retention Time or Hydraulic Retention Time (HRT) was 5 days. Fluctuations in the concentrations of contaminants in the influent irrigation water increased the HRT with time. The relatively higher precipitation rates due to persistent rains, on the other hand reduced the HRT. Longer retention time allows for UV treatment of pathogens (Rousseau, 2005). The high HLR of 1 m^3 was to compensate for the high evapotranspiration (1412 mm), since high evapotranspiration is known to reduce flow rate in wetland systems (Rousseau, 2005). The addition or loss of water (precipitation, evapotranspiration) in constructed wetland have impact on the inflow and outflow concentrations of the pollutants, resulting in water quality parameter fluctuations (US EPA, 2000).

The ability of a plant to tolerate unusually high levels of phytotoxins in its tissues, besides rapid biomass production and accumulation rates, is one commonly used criterion for selecting potential plants for bioremediation (Prasad and Freitas, 2003; Shah and Nongkynrih, 2007; Krämer, 2010). Tolerance of the plant species to phytotoxic levels of Fe and Hg (and Cu in *L. flava*) in their natural habitats, thus, shows their potential for phytoremediation of heavy metal contaminated wastewater. As a matter of fact, *T. latifolia* is widely recognized and used for phytoremediation in other countries (Ganjo and Khwakaram, 2010; Phillips et al., 2010); its inclusion in the present study was to serve, in part, as a reference plant. A much lower toxicity limit reported by Krämer (2010) for Pb (0.6-28mg kg^{-1}) puts all three plants beyond the toxicity threshold of Pb and further increases the potential of these plants for phytoremediation. However, none of the plants would be qualified as a hyperaccumulator based on the initial levels of heavy metals accumulated from the natural environment alone (Shah and Nongkynrih, 2007; Krämer, 2010).

Absorbed elements were compartmentalized into the shoot and root regions of the macrophytes. Absorption of heavy metals by plant species was dependent on both the type of plant as well as the element concerned. The degree of upward translocation was dependent on the species of plant and the particular metal. Most phytoremediants retain more of their metal burden in belowground structures, while others redistribute a greater proportion of metals into their shoots. These characteristics influence the phytoextraction abilities of a particular plant species. Storage in roots is most beneficial for phytostabilization of the metal contaminants, which are least available when concentrated below ground. Roots of macrophytes can accumulate greater amounts of heavy metals due to its cortex parenchyma with large intercellular air spaces (Massa *et al*., 2010). Translocation of biomass between compartments is essential for surviving water level changes. Species that can maintain allocation to shoots without an adverse effect on total or below- ground mass are at a distinct advantage. Information about translocation is important for the siting and use of wetlands for phytoremediation. A lower metal concentration in shoot is usually preferred, in order to prevent accumulated metals from entering the ecosystem through food chain (Yang *et al.*, 2003).

Typically, plant species with high bioaccumulation factors (BAFs) and translocation factors (TFs) above unity are favoured for phytoremediation (Krämer, 2010). Hyperaccumulator plants with the capacity to take up extremely high amounts of heavy metals (usually 0.1% to 3% of dry weight) (Table 1) are even more desirable. On these bases, *L. flava*, *T. geniculata* and *T. latifolia* have considerable potential for decontamination of heavy metal polluted irrigation water as evidenced by their relatively high BAFs (or accumulation rates) and translocation abilities in the constructed wetland. Increases in the accumulation rates and BAFs in all plants during the course of the experiment underscore the importance of time in phytoremediation, and agree with the observation by Lai *et al*. (2010) that increasing the growth time promotes accumulation of heavy metals. Besides the time lag required for plant establishment, metal accumulation involves several physiological and biological processes (Peer *et al*., 2005), which may take considerable time to develop and become functional.

Variations in the bioaccumulation rates, BAFs and TFs among the three plant species most likely reflect their intrinsic abilities to sequester different trace metals. Iron (Fe) is one of the most abundant and commonly used metals on earth, and several previous studies have provided evidence of its predominance in water bodies in Ghana (e.g., Sarpong, 2007; Akoto *et al*., 2008; Balfors *et al*., 2007). As expected, Fe not only occurred in high concentrations in the water and soil samples used for this experiment, but was also accumulated in almost equal amounts by the three plant species. Although, the plants accumulated more Fe than the rest of the metals, most of it remained in the roots. These results agree strongly with previous findings that *T. latifolia* is good at extracting Fe from surrounding waters and generally into the roots (Ganjo and Khwakaram, 2010). The retention of Fe in the plant, as quoted by Ganjo and Khwakaram (2010), is accomplished through immobilization in the rhizosphere. Similarly, the plant species appeared to be tolerant to phytotoxic concentrations of Cu, Pb and Zn and none of them could also attain the critical hyperaccumulation threshold. These results are not surprising given the fact that only a few plants have been reported to actually hyperaccumulate these heavy metals despite reports of their tolerance among many species (Peer *et al*., 2005; Shilev *et al*., 2008). *T. latifolia* is clearly a better accumulator of Cu and Pb than the other plant species, but with respect to Zn extraction, both *T. latifolia* and *L. flava* are comparatively better than *T. geniculata*. In a similar constructed wetland experiment, Ganjo and Khwakaram (2010) found the highest levels of Zn and Cu in a congeneric species of *T. latifolia* (*T. angustifolia*). How these plants respond to extreme concentrations of the heavy metals would be interesting to know given the observation by Kapourchal *et al*. (2009) that increasing Pb concentration in soils promoted its accumulation in raddish (*Raphanus sativus*).

A major highlight of this study is the observation of the high prospect of *L. flava* and *T. geniculata* as hyperaccumulators of Hg. Mercury is a highly toxic metal that can accumulate in the environment and in animals through the food chain. This, coupled with the little progress made so far in the search for hyperaccumulators of Hg (Su *et al*., 2009), makes the present finding quite interesting. Although the BAF of *L. flava* was higher than that of *T. geniculata*, the relatively large size of the latter suggests it could be equally or even more effective Hg hyperaccumulator compared to the former. The high TFs of the two plants (particularly *T. geniculata* which was consistently more than unity) further indicate their feasibility for Hg hyperaccumulation (Krämer, 2010). Su *et al*. (2009) reported comparatively higher concentrations of Hg for the Chinese brake fern (*Pteris vittata*) and Indian mustard (*Brassica juncea*) grown in hydroponic solutions. However, as observed by these researchers, the Hg levels in the plants are dependent on the concentration in the growth media. In this regard, the Hg levels observed in this study (averaging 0.17% and 0.11% respectively for *L. flava* and *T. geniculata*) are quite significant considering the low levels of the metal in the irrigation water used. This point is given further credence by the high BAFs of the two plant species. In their study, Su *et al*. (2009) also observed very high root/shoot ratios of Hg concentrations in their plants, which contrast the observed TFs of the plants (except *T. latifolia*) tested in the present study.

In order to establish a significant relationship between the bioaccumulation of elements by species and their increments in leaf area, increments in the leaf area was correlated with bioaccumulation rates of *Limnocharis flava, Thalia geniculata* and *Typha latifolia.* Significant relationships were found between the bioaccumulation of

Cu, Fe, Zn and Hg by *L. flava* and its increments in leaf area after correlation analyses. Increments in the leaf area were correlated with bioaccumulation rates of *T. latifolia* revealing a significant difference for Cu, Zn and Pb. A similar correlation for *T. geniculata* produced significant relationships for Pb only.

The removal efficiencies, however, did not differ greatly among the plant species unlike the BAFs or TFs. For instance, with reference to Hg, *L. flava* differed significantly from *T. geniculata* and *T. latifolia* in terms of BAFs and TFs but no statistical differences were found in terms of their removal efficiencies. Apparently, the high rate of biomass production in *T. geniculata* and *T. latifolia* compensated for their relatively low rates of Hg accumulation. These results are consistent with the fact that plant species with rapid growth and high accumulative ability tend to be most successful as phytoremediants (Prasad and Freitas, 2003; Shah and Nongkynrih, 2007; Krämer, 2010). The high efficiency of Fe removal (up to ~ 34, 60 and 80% respectively by *T. latifolia, T. geniculata* and *L. flava*) from the irrigation water is plausible, given its high accumulation in the plants. Similar studies by Ganjo and Khwakaram, (2010) and other researchers using *Typha* species yielded between 33 and 91% reduction in Fe, and thus agree with the results obtained in the present study. The generally low concentration of Hg in the wastewater samples and its high rates of accumulation in the plants may be reasonable explanation for the high removal efficiency of this metal. Why the plants could not efficiently reduce the concentration of Pb and Cu in the irrigation water is not immediately clear. However, as Peer *et al*. (2005) pointed out, Pb has extremely low solubility which poses a major challenge for phytoremediation.

The available leaf area usually has a very strong correlation with photosynthesis, and was therefore used as a measure of growth performance in this study. The increment in leaf area of the different plant species in the six different treatment cells was calculated from the difference between the means of the leaf areas measured for successive growing months. Figure 6 shows that in correlations where strong relationships exist, any change in leaf area directly affects the rate of bioaccumulation of the related element in a particular plant species. Appendix 9 provides data on the growth performance indicators measured during the operation of the constructed wetland.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study demonstrated the feasibility of the use of a sub-surface flow constructed wetland for decontaminating wastewater prior to its use in irrigated-vegetable production in urban and peri-urban Kumasi and possibly Ghana at large. The effluent water quality met international standards for irrigation water as well as Ghana Environmental Protection Agency standards for discharge into the environment. Operation and maintenance of the treatment wetland did not involve any complex procedures and as such can serve as one of the best alternatives for treating waste water in a developing country like Ghana.

In terms of accumulation, translocation and removal of heavy metals, both *L. flava* and *T. geniculata* compared fairly well with *T. latifolia*, a commonly used phytoremediant. Correlation analysis indicated significant congruence between the removal efficiencies and BAFs or TFs, suggesting that much of the reduction in the heavy metal content of the irrigation water is attributable to their accumulation by the plants. The removal of substantial quantities of heavy metals (particularly Fe, Zn and Hg) from the irrigation water by the three macrophyte species provide support for the use of phytoremediation as a wastewater treatment method. Mean removal efficiencies ranged from 40-80%, 8-38%, 44-54%, 18-32% and 48-54% respectively for Fe, Cu, Zn, Pb and Hg. Similar to the bioaccumulation rates and translocation factors, the removal efficiencies of the three species depended on the metal. However, selection of plants for remediation should be done carefully in the light of the variability in accumulation rates, BAFs and TFs of species with different metals. The plant species upon harvesting can serve as raw material for floor tiles and roofing materials. Metals absorbed into plants can be recuperated after drying and ashing. Special off gas treatment can be used to prevent air pollution during burning.

6.2 Recommendations

i. Since this was the first study involving the use of constructed wetland for phytoremediation in Ghana, the results and the pilot plant can be used as a model for teaching students involved in remediation biology and Civil engineering.

ii. Further monitoring of the treatment plant should be carried out over a longer period to determine its peak performance as well as considerations made for the implementation of large scale constructed wetlands for remediation of contaminated water.

iii. Managers and researchers seeking to use any of these plants in phytoremediation should consider continuous measurements of biomass production during the remediation period.

iv. Concurrent assessment of the growth performance and the accumulation of heavy metals in vegetable crops irrigated with the decontaminated water should be considered.

v. Extensive research should be done to identify other potential phytoremediants, particularly indigenous species.

vi. Finally, visitors of the constructed wetlands should be mindful of snakes which are attracted by tadpoles in the sedimentation tank.

REFERENCES

Abhilash, P.C. (2004), *Invasive Species Specialist Group*, School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala, India & IUCN/SSC.

Akoto, O., Bruce, T.N., Darko, G. "Heavy metals pollution profiles in streams serving the Owabi Reservoir", *African Journal of Environment Science and Technology 2008*, Volume 2: 354-359

American Public Health Association (APHA), (1992), *Standard methods for the examination of water and waste water*, 18th Edition, Washington D.C.

Amoah, P., Drechsel, P., Abaidoo, R.C., Henseler, M., "Irrigated urban vegetable production in Ghana: microbiological contamination in farms and markets and consumer risk group", *Journal of* Water Health 2007, Volume 5 (3): 455-466

Anning, A. K. and Yeboah-Gyan, K., "Diversity and distribution of invasive weeds from various habitats in Ashanti Region, Ghana", *African Journal of Ecology, 2007,* Volume 45: 355 - 360

Balfors B., Jacks G., Singh N., Bhattacharya P, Koku JE., "Contamination of water resources in Tarkwa mining area of Ghana: linking technical, social-economic and gender dimensions", *ISRN KTH/LWR Reports* 2007, 3016 SE

Bareen, F. and Khilji, S., "Bioaccumulation of metals from tannery sludge by *Typha angustifolia*", *African Journal of Biotechnology*, 2008. Volume 7 (18): 3314 - 3320

Bizily, S., Rugh, C, L., Summers, A. O., Meagher, R. B., "Phytoremediation of methylmercury pollution: merB expression in *Arabidopsis thaliana* confers resistance to organomercurials", *Proceedings of the National Academy of Sciences of the United States of America*, 1999, Volume 96(12), 6808-6813

Blumenthal, U. J. and Peasey, A., "Critical Review of Epidemiological Evidence of Health Effects of Wastewater and Excreta in Agriculture", *Background paper for WHO guidelines for the safe use of wastewater and excreta in agriculture*, 2002 WHO, Geneva.

Chen, Z.F., Zhao, Y., Zhu, Y., Yang X., Qiao J., Tian Q., Zhang, Q., "Health risks of heavy metals in sewage-irrigated soils and edible seeds in Langfang of Hebei province, China", *Journal of Science, Food and Agriculture*, 2010, 90: 314-320

Cofie, O.O, Drechsel, P., "Water for food in the cities: the growing paradigm of irrigated (peri)-urban agriculture and its struggle in Sub-Saharan Africa", *African Water Journal*, 2006, 1: 23-32

CRC (Weeds of CRC). 2003. *Weed of the Month: Limnocharis Flava.* CRC Organisation, Australia.

Denny, P., "Implementation of constructed wetlands in developing countries", *Water Science and Technology*, 1997, Volume 35, pp. 22-34

Ensley, B.D., (2000), *Rationale for use of phytoremediation*. In: Phyto-remediation of toxic metals using plants to clean up the environment, (Raskin I, Ensley BD Eds.) John Wiley & Sons. New York, pp. 3-12.

Forstner, U and Wittmann, G.T.W., (1983), *Metal pollution in the aquatic environment*, Springer-Verlag, Berlin.

Ganjo D.G.A., Khwakaram A.I., “Phytoremediation of wastewater using some aquatic macrophytes as biological purifiers for irrigation purposes: removal efficiencies and heavy metals Fe, Mn, Zn and Cu”, *World Academy of Science and Engineering Technology*, 2010, Volume 66: 565-588

Ghosh, M., Singh, S.P., “A review of phytoremediation of heavy metals and utilization of its byproducts”, *Applied Ecology and Environmental Research*, 2005, Volume 3 (1): 1-18

Gratão, P.L., Prasad, M.N.V., Cardoso, P.F., Lea, P.J., Azevedo, R.A., “Phytoremediation: green technology for the cleanup of toxic metals in the environment”, *Brazilian Journal of Plant Physiology*, 2005, Volume 17, 53-64.

Gupta, N., Khan, D.K., Santra, S.C., “An assessment of heavy metal contamination of vegetables grown in wastewater irrigated areas of Titagarh, West Bengal, India”, *Bulletting of Environment, Contaminants and Toxicology*, 2008, Volume 80: 115-118.

Inocencio, A., Sally, H., Merrey, D., Jong, I., “Irrigation Capital Investment Costs in sub-Saharan Africa: An Overview of Issues and Evidence”, *Review of literature report submitted to the World Bank and the Collaborative Program Partners*, 2003. Pretoria: IWMI.

Kadlec, R.H., Knight, R.L., (1996), *Treatment Wetlands*, CRC Press, Boca Raton, Los Angeles.

Kapourchal, S.A., Pazira E., Homaee M., “Assessing raddish (*Raphanus sativus* L.) potential for phytoremediation of lead-polluted soils resulting from air pollution”, *Plant, Soil and Environment*, 2009, volume 55 (5): 202-206

Keraita, B., Drechsel P., Huibers, F., Raschid-Sally, L., “Wastewater use in informal irrigation in urban and peri-urban areas of Kumasi, Ghana”, *Urban Agriculture Magazine*, 2002, Volume 8: 11-13

Krämer, U., “Metal hyperaccumulation in plants”, *Annual Review: Plant Biology*, 2010, Volume 61: 517-534

Lai, H.Y., Juang, K.W., Chen, Z.S., “Large area experiment on uptake of metals by twelve plants growing in soils contaminated with multiple metals”, *International Journal of Phytoremediation*, 2010, Volume 12(8): 785-797

Liao, S. and Chang, W., “Heavy metal phytoremediation by water hyacinth at constructed wetlands in Tawain”, *Journal of Aquatic Plant Management*, 2010, Volume 42: 60 - 68

Lan, C., Chen, G, Li. L, Wong, M.H., “Use of cattails in treating wastewater from a Pb/Zn mine”, *Environmental Management*, 1992, Volume 16 (1): 75-80

Massa, N., Andreucci, F., Poli, M., Aceto, M., Barbato, R., Berta G., “Screening for heavy metal accumulators amongst autochtonous plants in a polluted site in Italy”, *Ecotoxicology and Environmental Safety*, 2010, Volume 73: 1988-1997

McGregor, D.F.M., Thompson, D.A., Simon, D., "Water quality and management in peri-urban Kumasi, Ghana. In: Land-Water Linkagesin Rural Watersheds", *FAO Land and Water Bulletin*, 2002, Volume 9, pp 5

Moffat, A.S., "Plants proving their worth in toxic metal cleanup", *Science*, 1995, Volume 269 (5222), 302-303

Obuobie, E., Keraita, B., Danso, G., Amoah, P., Cofie, O., Raschid-Sally, L., Drechsel, P., "Irrigated urban vegetable production in Ghana: characteristics, benefits and risks", *IWMI-RUAF-IDRC-CPWF*, Accra, Ghana: IWMI 2006, 150 pp.

Otitoloju, A.A., Don-Pedro, K.N., "Influence of joint application of heavy metals on level of each metal accumulated in the periwinkle *Tympanotonus fuscatus* (Gastropoda: Potamididae)", *Tropical Biology*, September 2006; 54 (3).

Peer, W.A., Baxter, I.R., Richards, E.L., Freeman, J.L., Murphy, A.S., (2005), *Phytoremediation and hyperaccumulator plants*. In: Clomp LWJ, Martini E, and Tames MJ (Ends). Molecular biology of metal homeostasis and detoxification: from microbes to man. Topical Current Genetics. 14. New York, NY: Springer-Verlag. Pp. 299-340.

Pescod, M.B., (1992), *Wastewater treatment and use in agriculture*. Food and Agriculture Organization (FAO) Irrigation and Drainage Paper 47. Rome, Italy. 169pp.

Phillips, L.A., Armstrong, S.A., Headley, J.V., Greer, C.W., Germida, J.J., "Shifts in roots-associated microbial communities of *Typha latifolia* growing in naphthenic acids and relationship to plant health", *International Journal of Phytoremediation*, 2010, Volume 12: 745-760

Porcella, D.B., Chu, P., Allan, M.A. (1996), *Inventory of North American Hg emissions to the atmosphere*, NATO ASI Series, Kluwer Academic Publishers, Dordrecht, The Netherlands

Prasad M.N.V., Freitas H.M., "Metal hyperaccumulation in plants – biodiversity prospecting for phytoremediation technology", *Electronic Journal of Biotechnology*, 2003, Volume 6 (3): 285-321

R Development Core Team (2011). R: a language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.r-project.org/.

Raskin, I. (1996). *Plant genetic engineering may help with environmental cleanup*. Proceedings of the National Academy of Sciences of the United States of America 93(8), 3164-3166

Rousseau D. (2005). *Performance of constructed treatment wetlands: model-based evaluation and impact of operation and maintenance. PhD thesis*, Ghent University, Ghent, Belgium, pp. 300

Sarpong, Y.A., 2007. *Spatial distribution of heavy metal accumulation in water and sediment samples in River Subin, an urban river in Kumasi*. MSc. Thesis. Department of Theoretical and Applied Biology, KNUST, Kumasi. 131pp.

Sawidis, T., Chettri, M.K., Zachariadis, G.A., Stratis, J.A., "Heavy metals in aquatic plants and sediments from water systems in Macedonia, Greece", *Journal of Ecotoxicology and Environmental Safety*, 1995, Volume 32. pp. 73–80

Shah, K., Nongkynrih, J.M., "Metal hyperaccumulation and bioremediation", *Biology of Plants*, 2007, Volume 51 (4): 618-634

Shilev, S., Naydenov, M., Tahsin, N., Vancheva, V., Draganova, D., "Phytoextraction of Pb and Cd by maize plants in hydroponic conditions", *Journal of International Research Publications on Ecological Safety*, 2008, Volume 3: 132-139

Su, Y., Han, F.X., Chen. J., Shiyab, S., Monts, D.L., "Phytotoxicity and phytoremediation potential of mercury in Indian mustard and two ferns with mercury contaminated water and oak ridge soil – 9241", Proceedings of WM 2009 Conference, March 1-5, 2009, 12pp

Sykes, M., Yang, V., Blankenburg, J., and AbuBakr, S., (1999), *Biotechnology: working with nature to improve forest resources and products*. International Environmental Conference, 631-637

Teisseire, H., and Guy, V., (2000), "Copper induced changes in antioxidant enzymes activities in fronds of duckweed (*Lemna* minor)" *Plant Sciences*, 2000 Volume 153: 65-72

UNESCO – IHE, (2010), *Wetlands for Water quality*. Institute for Water Education.

US EPA (1993), *Constructed wetlands for wastewater: treatment and wildlife habit*, US EPA 832-R-93-005, North Bainbridge Island, Washington. pp 9.

USEPA., (2000), *Bioaccumulation Testing and Interpretation for the Purpose of Sediment QualityAssessment*. U.S. Environmental Protection Agency. EPA-823-R-00-001. February.

Willey, N., (2007), *Phytoremediation: methods and review*, Humana Press Incorporated, Totowa New Jersey.

Yang, B., Shu, W., Ye, Z., Lan, C., Wong, M., "Growth and metal accumulation in Vetiver and two Sesbania species on Lead/Zinc mine tailings", *Chemosphere publications*, 2003,Volume 53:1593-1600

Yoon, J., Cao, X., Zhao, Q., Ma, L.Q., "Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site", *Science and Total Environment*, 2006, Volume 368: 456-464

APPENDICES

APPENDIX 1: Results from initial analysis of irrigation water

PARAMETER	SAMPLE
pH	7.10
COD (mg/L)	136
BOD(mg/L)	70
T.S.S(mg/L)	74
SALINITY(mg/L)	0.1
DO(mg/L)	1.80
CONDUCTIVITY(µs/cm)	721

APPENDIX 2: General Effluent Quality Guidelines for Discharges into Natural Water Bodies.

Parameter	**Maximum Permissible Level**
Temperature (°C)	< 3 °C above ambient
Turbidity (FTU)	75
Conductivity (µs/cm)	750
Suspended Solids (mg/l)	50
TDS (mg/l)	50
pH value	6 - 9
Biochemical Oxygen Demand (mg/l)	50
Chemical Oxygen Demand (mg/l)	250
Nitrate – Nitrogen (mg/l)	0.1
Nitrate (mg/l)	75
Phosphate – Phosphorus (mg/l)	2.0
Grease (mg/l)	10
Faecal Coliforms (No./100ml)	500
E-Coli (No./100ml)	10

Source: Ghana Environmental Protection Agency, 1997

APPENDIX 3: Guidelines for interpretation of water quality for irrigation

Potential irrigation problem		Units	Degree of restriction on use		
			None	Slight to moderate	Severe
Salinity					
Ec_w[1]		dS/m	< 0.7	0.7 - 3.0	> 3.0
or					
TDS		mg/l	< 450	450 - 2000	> 2000
Infiltration					
SAR[2] = 0 - 3 and EC_w			> 0.7	0.7 - 0.2	< 0.2
	3 -6		> 1.2	1.2 - 0.3	< 0.3
	6-12		> 1.9	1.9 - 0.5	< 0.5
	12-20		> 2.9	2.9 - 1.3	< 1.3
	20-40		> 5.0	5.0 - 2.9	< 2.9
Specific ion toxicity					
Sodium (Na)					
	Surface irrigation	SAR	< 3	3 - 9	> 9
	Sprinkler irrigation	me/l	< 3	> 3	
Chloride (Cl)					
	Surface irrigation	me/l	< 4	4 - 10	> 10
	Sprinkler irrigation	m^3/l	< 3	> 3	
Boron (B)		mg/l	< 0.7	0.7 - 3.0	> 3.0
Trace Elements					
Miscellaneous effects					

Nitrogen (NO_3-N)[3]	mg/l	< 5	5 - 30	> 30
Bicarbonate (HCO_3)	me/l	< 1.5	1.5 - 8.5	> 8.5
pH	Normal range 6.5-8			

[1] EC_w means electrical conductivity in deci Siemens per metre at 25°C
[2] SAR means sodium adsorption ratio
[3] NO_3-N means nitrate nitrogen reported in terms of elemental nitrogen

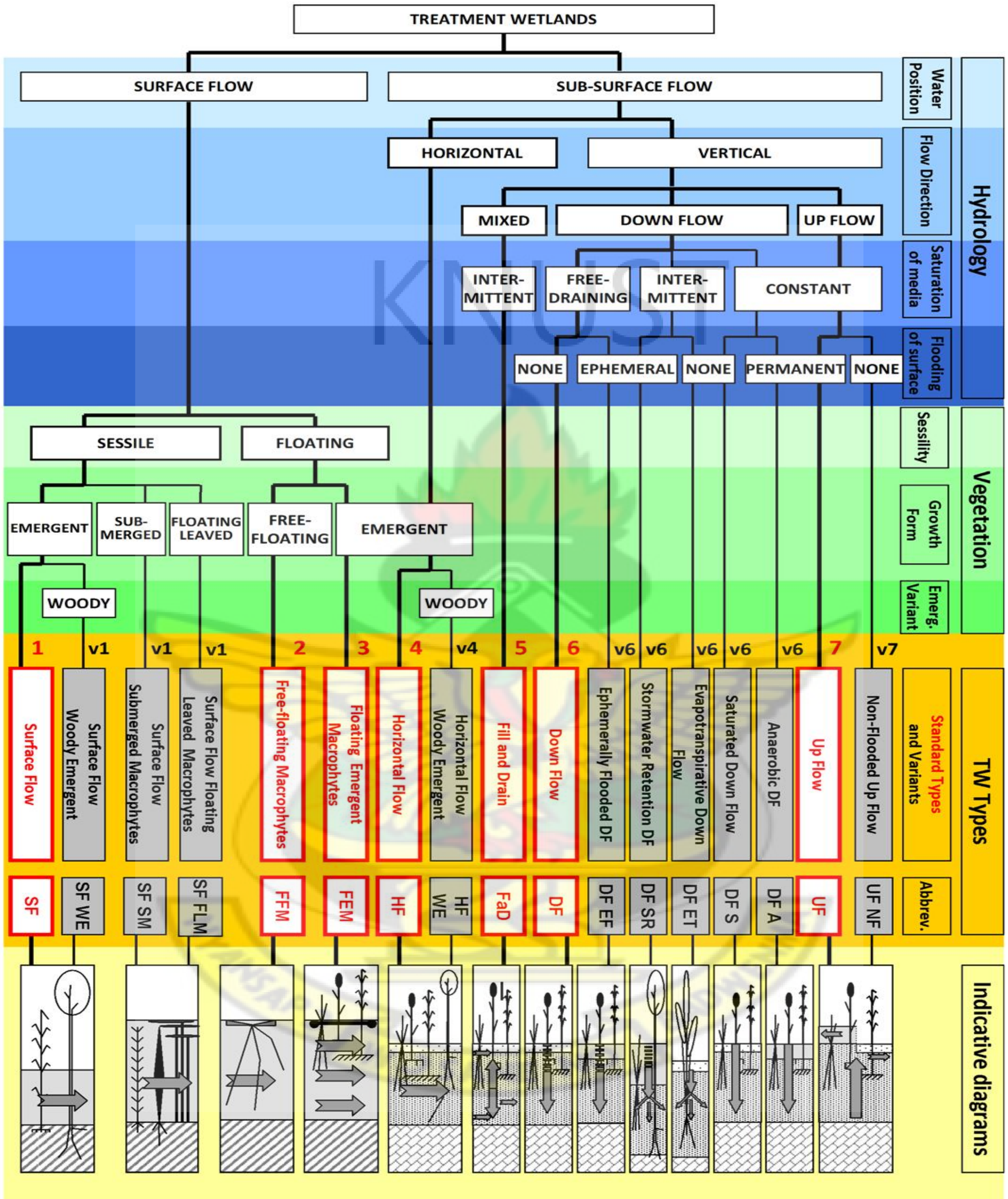
Source: APHA (1992)

APPENDIX 4: Meteorological Data of Kumasi (1990-2003)

Month	Average Rainfall (mm)	Average Temperature (°C)	Max. Daily Temperature (°C)	Min. Daily Temperature (°C)	Mean Evaporation (mm)
January	17	26.1	32.3	19.8	159
February	64	27.0	33.0	21.5	129
March	140	27.4	32.7	22.1	136
April	131	27.2	32.5	21.9	130
May	179	27.0	32.0	21.9	138
June	218	25.0	29.0	21.0	100
July	160	24.7	28.3	21.0	111
August	89	24.1	27.4	20.7	96
September	160	25.7	29.5	22.0	102
October	150	26.5	31.0	22.0	89
November	70	27.0	32.1	22.0	124
December	24	25.7	31.1	20.3	121

Source: Ghana Meteorological Agency, Kumasi

APPENDIX 5: Classification of treatment wetlands



Source: UNESCO-IHE 2010

APPENDIX 6: Irrigation water treatment plant design

Design Calculations

$\frac{dC}{dt} = -k_v C$ ……… (3.1); Where C is organics concentration,

k_v is a reaction constant. Equation (3.1) can be rearranged and integrated as follows:

$$\frac{dC}{C} = -k_v dt \rightarrow \int_{in}^{out} \frac{dC}{C} = -k_v \int_{in}^{out} dt \ ;$$

$$\rightarrow \left[\frac{C_{out} - C^*}{C_{in} - C^*} \right] = e^{(-k_v t)} \xrightarrow{2,3,4} \left[\frac{C_{out} - C^*}{C_{in} - C^*} \right] = e^{(-k_A / HLR)}$$

C_{in} = C(t=0) while as C_{out} = C(t=τ). The following formulae were used to transform the above equation: kA = kV ε d............................ (3.2)

HLR = Q / A..................... (3.3); HLR is the hydraulic loading rate.

V = Q τ = A d ε……………..……. (3.4)

Explanation of symbols:

kv = first-order volumetric removal rate (1/day)

kA = first-order areal removal rate (m/day)

C* = background concentration (mg/L)

τ = hydraulic residence time (days); A = surface area (m2)

d = water depth (m) and ε = porosity (dimensionless)

The final equation is given by Cout = Cin * $e^{(-kBOD/HLR)}$ …………..(3.5)

for the BOD_5 (Rousseau,2005); Ah is the plan area of the wetland unit (m^2); kBOD is the specific removal rate constant at 20 ^oC,

From equation (3.1), the hydraulic retention time (HRT) is calculated as follows:

$$\ln\frac{Cout}{Cin} = -k.HRT \rightarrow HRT = \frac{-\ln(Cout/Cin)}{k}$$ …………..(3.6); where as

$$k_t = k_{20}o^{(T-20)}$$ ..…. (3.7) and the detail of the design is given below.

First-order kinetics simply means that the rate of removal of a particular pollutant is direct proportional to the remaining concentration at any point within the wetland cell. Two idealized mixing theories may be applied: the First order kinetics and the Rule of Thumb (CW design manual, 1995).

BOD of Sample = 70mg/L

COD of Sample = 136 mg/L

TSS of Sample = 74 mg/L

For the sedimentation tank;

Taking L: B = 5:1⇒ $L/_B = {}^5/_1$; L = 5B

Also L: B = 18:1⇒ $L/_D = {}^{18}/_1$; L = 18D

Using a bottom slope of ${}^1/_{10}$;

But volume = L × B × D ⇒ L × $^{L}/_{5}$ × $^{L}/_{18}$ = $^{L^3}/_{90}$ = V

L^3 = 90V

L = $\sqrt[3]{90V}$ .... (1)

But Hydraulic Retention Time = $\tau = {}^{V}/_{Q}$

Using a retention time of one day, that is 24 hrs

⇒24 = $^{V}/_{Q}$; V = 24Q

V = 24 × 60 × (1.667 × 10^{-5}) = 1.44m^3

Substituting, V = 1.44 m^3 into (1);

⇒ L = $\sqrt[3]{90 \times 1.44}$ = 5.06m

B = $^{L}/_{5}$ = $^{5.06}/_{5}$ = 1.012m

D = $^{L}/_{18}$ = $^{5.06}/_{18}$ = 0.218m

∴ The dimensions of the proposed rectangular sedimentation tank = 5.1m × 1m × 0.3m

Using a minimum treatment of 25% treatment before influent enters wetland.

($^{25}/_{100}$ × 70) = 17.5 ⇒ 70 – 17.5 = 52.5mg/L

The settling tank would remove 60% of the suspended solids i.e. ($^{60}/_{100} \times 74$) = 44.4mg/L and the remaining SS is 29.4 mg/L which is less than 35 mg/L which recommended by EPA standards.

Designing the orifice

Q = AV

Q = 1.667×10^{-5} m^3/s or 1.44 m^3/day

From the formula for design of orifice

$$d = \sqrt{4Q / \pi Cd\sqrt{2gH}}$$

Using Cd = 0.68

g = 9.8m/s

H = 5mm = 0.05m

$$d = \sqrt{4 \times 1.668 \times 10^{-5} / \pi \times 0.68\sqrt{2 \times 9.81 \times 0.05}}$$

d = $\sqrt{0.09}$ = 0.3m

d = 0.3m

Design of the constructed wetland (Trapezoidal in shape)

From Cout = Cin $\times e^{(-1<\frac{BOD}{HLR})}$ or KBOD = Kv $\times$ n $\times$ Dw

n = porosity, Dw = water depth.

Using a temperature of 25^{o}C, N = 0.4, Kn = 368.87, K_{20} = 1.6

For coarse sand; n = 0.40, Kv = 1.60

KBOD = 1.6 $\times$ 0.40 $\times$ 0.40 = 0.256

$\Rightarrow$ 35 = 52.5 $\times e^{(\frac{-0.256}{HLR})}$; ln $({}^{35}/_{52.5}) = {}^{-0.256}/_{\mathrm{HLR}}$

$\Rightarrow$ -0.405465 = ${}^{-0.256}/_{\mathrm{HLR}}$; ${}^{-0.405465}/_{-0.256} = \mathrm{HLR} = 1.583\mathrm{m/day}$

HLR = Q/A_h; = Ah = Q/HLR = ${}^{\frac{0.48}{3}}/_{1.583} = 0.303m^2$

Taking a gravel depth of 0.6m from the surface and assuming a slope of 1%,

Q = Kh $\times$ B $\times$ Dw $\times$ (dh/L) $\Rightarrow$ B = $\mathrm{Q}/\left(\mathrm{Kh}\ \times \mathrm{Dw}\ \times \frac{\mathrm{dh}}{\mathrm{L}}\right)$

B = $\mathrm{Q}/(\mathrm{Kh}\ \times \mathrm{Dw}\ \times\ \mathrm{S})$

B = ${}^{1}/_{(368.87\ \times 1.44 \times 0.001)} = 1.9,$

L = ${}^{0.803}/_{0.26} = 1.9\mathrm{m}$

The K_T value at 25°C

$K_T = 1.6(1.1)^{(18.6 - 25)} = 0.86d^{-1}$

$$t = \ln\frac{Ce}{Co}\Big/K_T = -\ln\frac{35}{52.5}\Big/0.86 = 0.47d^{-1}$$

For the tangential wetland,

B = 0.46m, b = 0.59m, L = 1.9m, d = 0.8m (depth of cell)

For a rectangular treatment cell (L/W Ratio should be min 2:1)

B+b=W= 1.05.

2W=2.1

L=2.1, W= 1.05, d=0.8

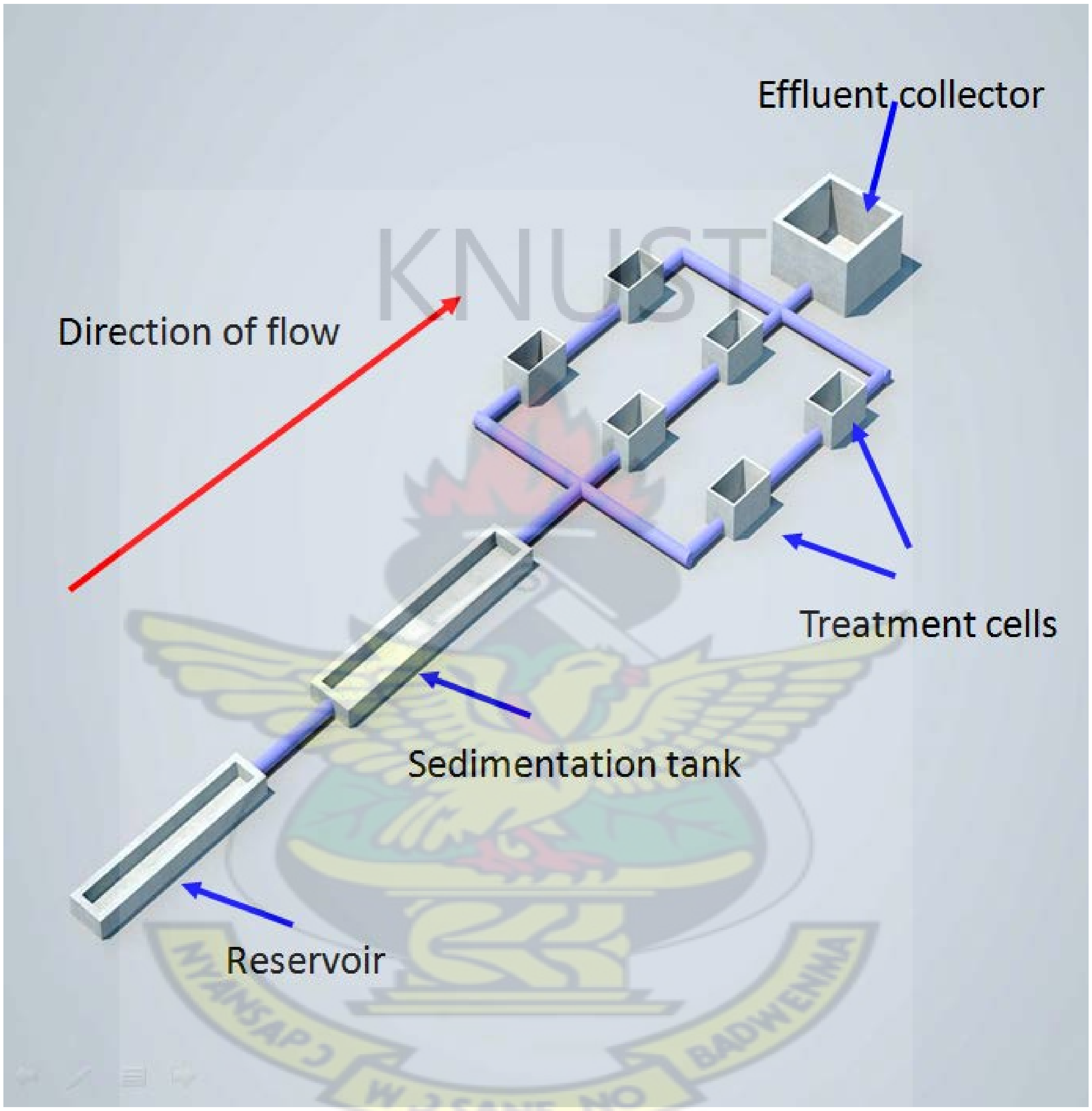
Rule of thumb design criteria for HSF

Criterion	Value range	
	Wood (1995)	Kadlec & Knight (1996)
Hydraulic retention time(days)	2 -7	2-4
Max. BOD loading rate (kg BOD/ ha/ day)	75	n.g
Hydraulic loading rate(cm day-1)	0.2-3.0	8-30
Areal requirement(ha m-3 day)	0.001-0.007	n.g

n.g.: not given

Source: UNESCO-IHE Institution for Water Education, 2010

APPENDIX 7: LAYOUT OF CONSTRUCTED WETLAND



APPENDIX 8: Clearing,Construction and Operation of SSF CW

Plate 2: Digging of SSF CW

Plate 3: Construction of SFF CW

Plate 4: Installation of screens

Plate 5: Transplanting to cells

Plate 6: **Measuring physichochemical Parameters**

Plate 7: Root formation

Plate 8: Shoots and roots after oven drying

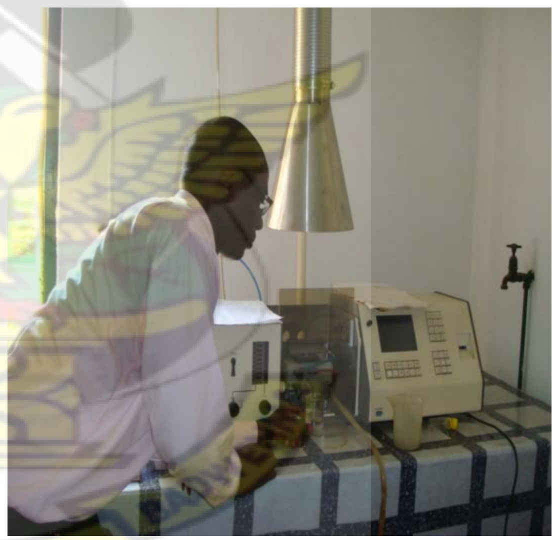


Plate 9: Analysis of samples using AAS

APPENDIX 9: ADDITIONAL RESULTS MEASURED

Mean Monthly Physico-chemical parameter

Typha latifolia	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER
TEMPERATURE	28.9125	29.95	29.25	30.4375	31.53125	31.9625
CONDUCTIVITY(us/cm)	271.175	266.0063	260.0313	257.68125	253.75625	247.1
pH	6.926875	7.090625	7.2375	7.2575	7.355	7.5125
	DECEMBER	JANUARY	FEBRUARY	MARCH		
TEMPERATURE	32.60625	31.39375	29.275	30.3		
CONDUCTIVITY(us/cm)	236.55625	233.875	231.075	227.2063		
pH	7.563125	7.545	7.541875	7.45875		

Limnocharis flava	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER
TEMPERATURE	29.31071	29.95268	29.99821	30.5535714	31.458036	32.1071429
CONDUCTIVITY(us/cm)	202.2299	201.8326	199.5736	198.590972	196.21319	193.332639
pH	61.12069	60.87819	60.40979	60.3147222	59.643472	58.2327083
	DECEMBER	JANUARY	FEBRUARY	MARCH		
TEMPERATURE	32.2839286	31.532143	29.1857143	29.93125		
CONDUCTIVITY(us/cm)	189.399306	181.17847	174.35	165.0972		
pH	56.1605556	53.85125	52.164375	51.12833		

Thalia geniculata	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER
TEMPERATURE	28.68125	29.69375	29.3375	29.61875	29.96875	30.2375
CONDUCTIVITY(us/cm)	263.4063	255.4188	249.8563	246.675	240.8125	236.56875
pH	6.9375	7.001875	7.148125	7.224375	7.31125	7.3975
	DECEMBER	JANUARY	FEBRUARY	MARCH		
TEMPERATURE	30.11875	29.475	28.69375	28.56875		
CONDUCTIVITY(us/cm)	232.8375	228.69375	224.7625	220.3		
pH	7.415625	7.444375	7.434375	7.4075		

Growth Performance Indicators

Typha latifolia	CELL 1						
	WEEK 1			WEEK 3			
	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	
JUNE	59	83	1.2	62	88	1.8	84
JUNE	56	81	1.4	60.5	84	1.8	
JULY	80	117	1.8	99	135	2	127.25
JULY	82.8	119	1.9	100	138	2	
AUGUST	112	156	2.2	128	167	2.3	162.75
AUGUST	115	159	2.2	130	169	2.2	
OCTOBER	140	175	2.3	151	182	2.4	179
OCTOBER	143.4	177	2.4	150.5	182	2.4	
NOVEMBER	162	188	2.4	171	200	2.5	192.25
NOVEMBER	159	186	2.5	169	195	2.8	
DECEMBER	174	207	2.8	184	220	2.7	213.5
DECEMBER	176.8	209	2.8	182.7	218	3	
JANUARY	191	232	2.8	205	248	3	240.5
JANUARY	190	230	3	206	252	3	
FEBRUARY	210	260	3.2	230	308	3.3	287
FEBRUARY	215	275	3.2	229	305	3.5	
MARCH	245	359	3.8	256.5	376	3.7	367.25
MARCH	245	359	3.6	255	375	3.8	

Typha latifolia		CELL 4					
	WEEK 1			WEEK 3			
	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	
JUNE	64	89	1.2	72	93	1.6	89.25
JUNE	60.4	84	1.3	69	91	1.5	
JULY	91	127	1.8	104	140	2	135.5
JULY	89	125	1.8	109	150	2	
AUGUST	129	169	2.2	139	174	2.4	170.5
AUGUST	125	166	2.2	137	173	2.5	
OCTOBER	153.6	184	2.5	155	185	2.8	184
OCTOBER	148	180	2.7	160	187	2.8	
NOVEMBER	180.6	215	3.2	190	230	3.2	224
NOVEMBER	178	211	3.2	195	240	3.3	
DECEMBER	210	258	3.4	216	277	3.5	265
DECEMBER	205	249	3.3	215	276	3.7	
JANUARY	222	290	3.6	230	310	3.6	303
JANUARY	224	296	3.7	234	316	3.8	
FEBRUARY	240	346	3.8	250	454	3.8	400.5
FEBRUARY	242	349	3.8	248	453	3.8	
MARCH	260.2	389	3.8	270.4	408	4	397
MARCH	258	385	3.8	268	406	3.8	

Limnocharis flava	CELL 2						
	WEEK 1			WEEK 3			
	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	
JUNE	15	45	1.3	18	62	1.5	55.75
JUNE	16	50	1.3	19	66	1.6	
JULY	21	72	1.9	24	82	2.3	78.25
JULY	22	74	1.9	25	85	2.4	
AUGUST	27	102	2.6	28	109	2.8	105.25
AUGUST	26	100	2.6	28	110	2.8	
OCTOBER	29	116	3	30.5	118	3	118
OCTOBER	30	118	2.9	31	120	3	
NOVEMBER	30.4	119	3	32	122	3.2	122.25
NOVEMBER	31	120	3	34	128	3.3	
DECEMBER	34	129	3.4	34	129	3.4	128.5
DECEMBER	33	126	3.2	35	130	3	
JANUARY	34	128	3.3	35	131	3	129.75
JANUARY	35	130	3.5	35	130	3	
FEBRUARY	36	133	3.2	36	133	3.3	134.25
FEBRUARY	36	134	3.2	37	137	3.5	
MARCH	39	143	3.8	38	141	3.7	142
MARCH	38	140	3.8	39	144	4	

Limnocharis flava	CELL 2						
	WEEK 1			WEEK 3			
	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	
JUNE	14	35	1.1	18	61	1.5	50.75
JUNE	16	48	1.3	17	59	1.5	
JULY	20	71	1.9	23	79	2.2	77.25
JULY	22	75	2	25	84	2.4	
AUGUST	28	110	2.9	30	119	3	116.25
AUGUST	29	116	2.9	30.6	120	3	
OCTOBER	32	123	3	35	130	3.2	126.5
OCTOBER	33	125	3	34	128	3.2	
NOVEMBER	36.1	134	3.5	36.5	135	3.7	134.5
NOVEMBER	36	134	3.6	36.4	135	3.6	
DECEMBER	37	137	3.9	37	138	3.8	137.5
DECEMBER	36	135	3.3	38	140	3.7	
JANUARY	38	140	3.6	38	141	3.6	141.5
JANUARY	38	141	3.7	39	144	4	
FEBRUARY	39	145	3.8	39.8	150	4.5	149
FEBRUARY	39.5	149	4.2	40	152	4.1	
MARCH	40.5	153	5.2	40.8	154	5	153
MARCH	40	151	4.8	40.5	154	4.8	

Thalia geniculata	CELL 3						
	WEEK 1			WEEK 3			
	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	
JUNE	51	57	1.6	63	69	1.8	63.5
JUNE	50.8	58	1.4	65	70	1.8	
JULY	87	88	2	94	95	2.4	91.25
JULY	85	84	2	96	98	2.5	
AUGUST	110	110	2.5	123	148	2.8	130.5
AUGUST	112	113	2.5	124	151	3	
OCTOBER	132	174	3.3	145	194	3.6	182
OCTOBER	130	170	3.4	143	190	3.6	
NOVEMBER	160.3	211	4.2	170	226	4.3	219
NOVEMBER	161	210	4.2	172	229	4.4	
DECEMBER	175	233	4.5	180	243	4.8	239.75
DECEMBER	177	238	4.5	180.5	245	4.8	
JANUARY	190	269	5	196	278	5.4	274
JANUARY	191.4	270	5.1	195	279	5.4	
FEBRUARY	200	285	5.6	209	296	5.8	290.75
FEBRUARY	201	285	5.7	208	297	5.7	
MARCH	220.5	340	5.8	221	342	5.8	339.5
MARCH	219	337	5.8	220	339	5.9	

Thalia geniculata	CELL 6						
	WEEK 1			WEEK 3			
	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	HEIGHT (cm)	LEAF AREA (cm^2)	STEM DIAMETER (cm)	
JUNE	52	60	1.6	67	70	1.8	65
JUNE	50.5	58	1.5	66	72	1.8	
JULY	88	88	2	96	99	2.5	93.25
JULY	86	86	2	95	100	2.5	
AUGUST	113	116	2.5	125	155	3	137.75
AUGUST	115	120	2.6	127	160	3.2	
OCTOBER	135	178	3.5	146	197	3.7	187.5
OCTOBER	136	180	3.5	145	195	3.6	
NOVEMBER	164	219	4.3	175	232	4.5	225.75
NOVEMBER	163	217	4.2	176	235	4.6	
DECEMBER	180	245	4.8	185	256	4.9	251.25
DECEMBER	181	244	4.7	186	260	4.9	
JANUARY	192	273	5.1	197	280	5.4	277.25
JANUARY	193	276	5.2	196	280	5.4	
FEBRUARY	202.4	289	5.5	210	302	5.8	296.5
FEBRUARY	204	290	5.6	212	305	5.7	
MARCH	223.4	347	5.8	224	350	5.9	350.75
MARCH	224	349	5.9	225	357	6	

APPENDIX 10: ANOVA RESULTS ON TRANSLOCATION OF ELEMENTS IN ROOTS AND SHOOTS

Plant	**Element**	**Root**	**Shoot**
Limnocharis flava (mg/Kg)	Cu	11.19[a]	16.33[b]
	Fe	36.50[b]	12.50[a]
	Hg	8.730[a]	8.645[a]
	Zn	22.20[a]	16.45[a]
	Pb	8.410[a]	8.925[a]
Thalia geniculata (mg/Kg)	Cu	13.8633[a]	13.555[a]
	Fe	36.50 [b]	12.50[a]
	Hg	4.210[a]	6.835[a]
	Pb	2.410[a]	2.550[a]
	Zn	2.1608[a]	2.3892[a]
Typha latifolia (mg/Kg)	Cu	19.5[a]	31.6[b]
	Fe	937[b]	60[a]
	Pb	3.375[a]	3.550[a]
	Hg	36.50[b]	12.50[a]
	Zn	2.35 [a]	5.53[b]

APPENDIX 11: Translocation factors of heavy metals compared for the three candidate phytoremediants

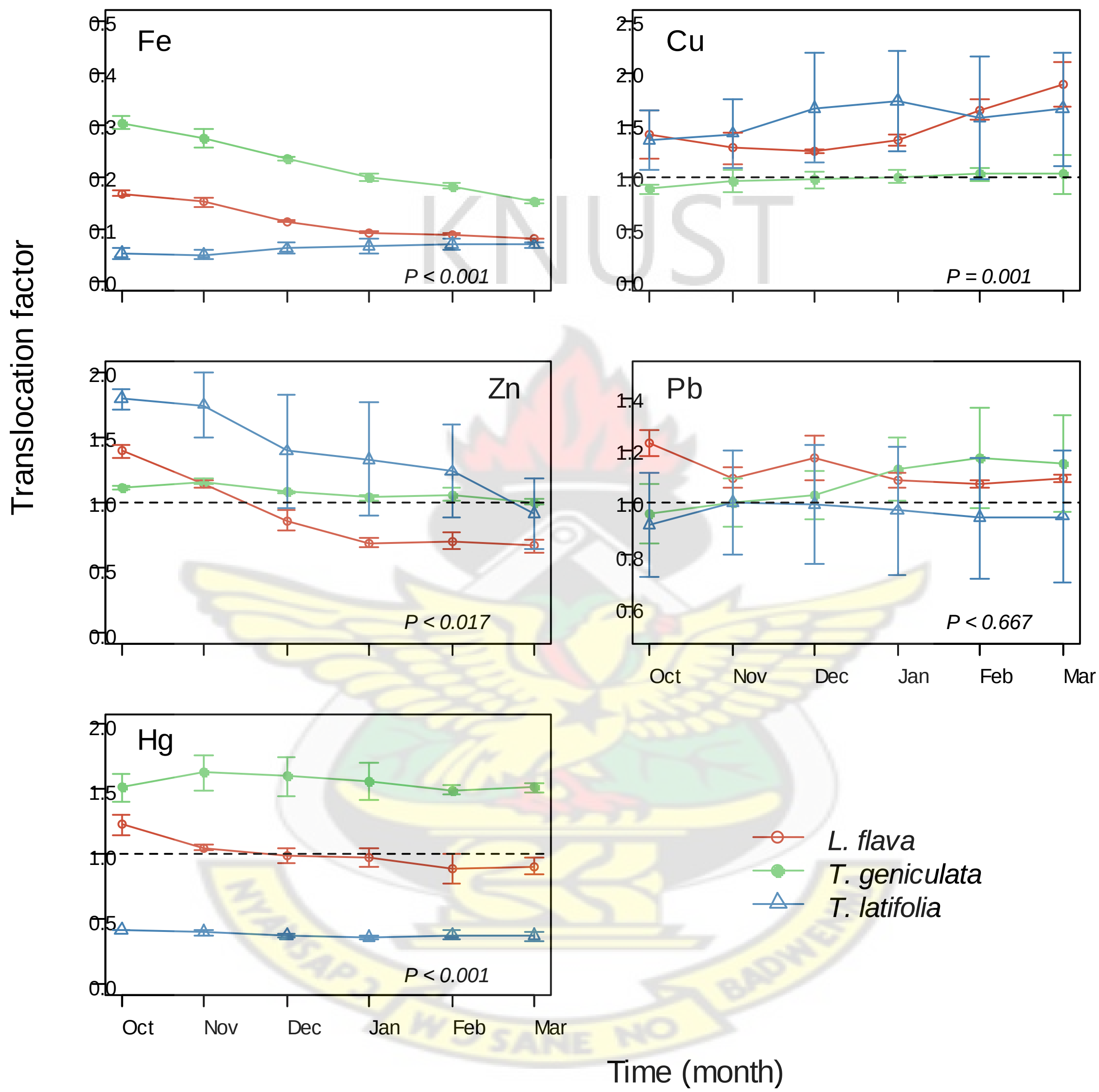


Figure 9: Translocation factors of heavy metals compared for the three candidate phytoremediants (*Limnocharis flava* (Lf), *Thalia geniculata* (Tg)and *Typha latifolia* (Tl)) over time. Error bars represent the standard errors of the means. *P*< 0.05 value indicates statistical difference among the three species. A line above the horizontal dashed line indicates the species effectively translocate heavy metals from its roots to the shoot.