EFFECTS OF COPPER AND LEAD ON THE PHYSIOLOGY OF *SALVINIA MOLESTA, PISTIA STRATIOTES AND LEMNA TRISULCA* AND THEIR PHYTOREMEDIATION POTENTIALS

BY

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Ph.D/Scie/05324/2009-10

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ZARIA

OCTOBER, 2015.

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA,IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN BOTANY (PLANT ECOLOGY).

DEPARTMENT OF BIOLOGICAL SCIENCES,

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NIGERIA.

OCTOBER, 2015

Declaration

I hereby declare that the work in this dissertation titled, "Effects of copper and lead on the physiology of *Salvinia molesta, Pistia stratiotes* and *Lemna trisulca* and their phytoremediation potentials"was performed by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria, under the supervision of Prof. S.P. Bako, Prof. (Mrs) D.N. Iortsuun and Dr. W. S. Japhet. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

__________________ ____________

Adelanwa Esther B. (Mrs.) Date

Certification

This dissertation titled, Effects of copper and lead on the physiology of *Salvinia molesta, Pistia stratiotes* and *Lemna trisulca*and their phytoremediation potentials" meets the regulations governing the award of the degree of Doctor of Philosophy in Botany(Plant Ecology) of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

PROF. S. P. BAKO ________________________ ________________

Chairman, Supervisory Committee Signature Date

PROF. D. N. IORTSUUN ________________________ ________________

Member, Supervisory Committee Signature Date

DR. W. S. JAPHET ________________________ ________________

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PROF. A.K. ADAMU ________________________ ________________

Head of Department Signature Date

PROF. KABIR BALA ________________________ ________________

Dean, School of Postgraduate Studies Signature Date

Dedication

This work is whole heartedly dedicated to my loving husband (Dr. M. A. Adelanwa), children (Tosin, Ope, Seyi, Demilade and Adesewa) for all the sacrifices during the course of this study; also to my parents (Elder and Deaconess Asalu) and siblings.

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Abstract

In the developing countries heavy metal pollution becomes serious due to mining, mineral, smelting, tannery and textile industries. Phytoremediation is a newtechnology in which plants are used to remove pollutants or reduce their effects from water and soil. The use ofmetal-accumulating plants to clean environments contaminated with heavy metals is the most rapidly developing component of this environmentally friendly and cost-effective technology.The effects of copper and lead on the physiology of *Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca* and their phytoremediation potentials were therefore investigated. The experiments were conducted *in vitro* and the parameters evaluated were content of Chlorophyll **a** and **b**, the enzymatic activity of catalase and peroxidase, morphology of the plant (visual signs of chlorosis)and the metal concentration in tissues of *Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca.*There were significant differences ($P \leq 0.05$) in the content of chlorophyll a and b in the three aquatic macrophytes used for this study. In this study, higher contents of chlorophyll **a** and **b** were recorded for the control and treatments with lower concentrations (0.5mg/L of copper and 0.05mg/L of lead). Copper and Lead at higher concentrations (1mg/L and 1.5mg/L) reduced the chlorophyll **a** and **b** contents in the three species. As the concentration of metals increased, the content of chlorophyll a and b reduced drastically. Also, increase in the number of days the plantswere exposed to the metals led to decrease in the content of chlorophyll **a** and **b**.The activities of catalase and peroxidase weresignificantly different ($P \leq 0.05$) in *Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca.*The higher the concentration of copper and lead, the higher the activities of the antioxidant enzymes.

The phytotoxicity effects of the accumulated metals were responsible for a reduction in the pigment content (chlorophyll **a** and **b**); high activity of the catalase and peroxidase in the three

macrophytes species. It was also responsible for the visual toxicity symptoms (chlorosis and necrosis) observed in *P. stratiotes* and *L. trisulca;* although no toxicity symptom/sign was observed in *S. molesta.*All the studied species accumulated copper and lead in their tissues. Also, it was observed that, the accumulation was dependent on the concentrations of the metal(s) in solution and time of exposure to the metals. There was significant difference ($P \leq 0.05$) between the accumulation potentials by the aquatic macrophytes and between the various treatments.*S. molesta* showed highest accumulation of metals (concentrations) in the tissues. *S. molesta* accumulated 1510.71±166.83mg/l of copper, followed by *L. trisulca* (1178.39±146.81mg/l) and the least was in *P. stratiotes* (866.18±101.41mg/l) on day 18after metal application. For lead, the highest accumulation was found in *S. molesta* with 872.22±126.73mg/l followed by *P. stratiotes* (378.44±92.14mg/l) and then L. *trisulca* with 194.77±49.04mg/l on day 18after metal application.*Salvinia molesta* proved to be the best phytoremediator among the aquatic macrophytes used in this study because it accumulated copper and lead more than the other species and there was no change in the morphology of the plants in all the concentrations that the plant was exposed to.

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Abbreviations

mgl^{-1}	milligramme per litre
hrs	hours
kg	kilogramme
mg	milligramme
ml	millilitre
mm	millimetre
mg/kg	milligramme per kilogramme

Acronyms

ANOVA	Analysis of Variance
EPA	Environmental Protection Agency
ppm	parts per million
ROS	Reactive Oxygen Species
rpm	rotation per minute
UNDP	United Nations Development Project
WHO	World Health Organisation

Glossary

CO_2	Carbon IV oxide
ceteris paribus	all things being equal
cum	together/along with
et al	and others

Symbols

+	plus
-	minus
$\pm$	plus or minus
$<$	less than
$>$	greater than
%	percentage
µ	micro
°C	degree Celsius

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Introduction

A pollutant is any substance in the environment, which causes objectionable effects, impairing the welfare of the environment, reducing the quality of life and may eventually cause death. Such a substance has to be present in the environment beyond a set or tolerance limit, which could be either a desirable or acceptable limit. Environment is defined as the totality of circumstances surrounding an organism or group of organisms especially, the combination of external physical conditions that affect and influence the growth, development and survival of organisms (FarlexIncorporated, 2005). It consists of the flora, fauna and the abiotic components, and includes the aquatic, terrestrial and atmospheric habitats. The environment is considered in terms of the most tangible aspects like air, water and food, and the less tangible, though no less important, the communities we live in.

Comprising over 70% of the Earth's surface, water is undoubtedly the most precious natural resource that exists on our planet (Terry, 1996). Population growth, urbanization and industrialization have led to rapid degradation of the environment and publichealth due to improper sewage disposal, especially in developing countries. Conventional solutions are inappropriateand expensive because the infrastructures and skilled labour are lacking.

The development of the intensive agriculture in Nigeria between 1960 and 1990 totally neglected the aspect connected with the negative impact of the chemical compounds toxic on the air, water and soil. As one of the consequences of heavy metal pollution in soil, water and air, plants are contaminated by heavy metals. Contamination of the aquatic environment by the heavy metals has become a serious concern in the developing world(Chandra *et al.,* 1997). Heavy metals unlike organic pollutants are the persistent in nature, therefore, tends to accumulate in the different components of the environment (Chandra *et al.,* 1997). Sources of metals in the environment are widespread and data on typical concentrations in the various media and environmental settings exits worldwide (Mwamburi, 2015).These metals are released from a variety of sources such as mining, urban sewage, smelters, tanneries, textile industry and chemical industry.

Water pollution is the contamination of water bodies (e.g. lakes, rivers, oceans, aquifers and groundwater). Water pollution occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful compounds. Aquatic environments are increasingly affected by human activity because of urban, industrial, mineraland agricultural waste. The use of the ocean as a dumpingground for wastes could lead to high levels of pollution in the aquatic environment (Bramha *et al.*,2014; Bodin *et al.,* 2013). Water pollution affects plants and organisms living in these bodies of water. In almost all cases, the effect is damaging not only to individual species and populations, but also to the natural biological communities.

Water pollution is a major global problem which requires ongoing evaluation and revision of water resource policy at all levels (international down to individual aquifers as well). It has been suggested that it is the leading worldwide cause of deaths and diseases and that it accounts for the deaths of more than 14,000 people daily(Pink, 2006; West, 2006).

The specific contaminants leading to pollution in water include a wide spectrum of chemicals and pathogens. While many of the chemicals and substances that are regulated may be naturally occurring (calcium, sodium, iron, manganese, etc.) the concentration is often the key in determining what is a natural component of water, and what is a contaminant. High concentrations of naturally occurring substances can have negative impacts on aquatic flora and fauna. Oxygen-depleting substances may be natural materials, such as plant matter (e.g. leaves and grass) as well as man-made chemicals. Other natural and anthropogenic substances such as may cause turbidity (cloudiness) which blocks light and disrupts plant growth, and clogs the gills of some fish species (EPA, 2005).

Heavy metal is the term used for a group of elements that have particular weight characteristics. They are on the "heavier" end of the periodic table of elements. Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. The most dangerous heavy metals are Lead, Cadmium, Copper, Chromium, Selenium and Mercury. Some heavy metals – such as Cobalt, Copper, Iron, Manganese, Molybdenum, Vanadium, Strontium, and Zinc – are essential to health in trace amounts. Others are non-essential and can be harmful to health in excessive amounts. These include Cadmium, Antimony, Chromium, Mercury, Lead, and Arsenic – these last three being the most common in cases of heavy metal toxicity.

The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Huton and Symon, 1986). "Heavy metals" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3 , or 5 times or more, greater than water. That is,a specific gravity of greater than 4.0-5.0. The actinides may or may not be included.(Huton and Symon, 1986; Battarbee *et al.*, 1988; Nriagu and Pacyna 1988; Nriagu, 1989; Garbarino *et al.*, 1995).Most recently, the term "heavy metal" has been used as a general term for those metals and semimetals with potential human or environmental toxicity (Chehregani *et al.*, 2005).

All metals, both essential (Cu, Zn, Mg) and toxic (Cd, Pb, Cr, Hg) can cause toxic effects to plants and animals if found in high concentrations in the organisms (when the concentrations exceeds the standard by WHO and EPA).Heavy metals are dangerous because they tend to bio accumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted (http://www.tip2000.com/health/waterpollution.asp). Heavy metal can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down rocks and releasing heavy metals into streams, lakes, rivers and groundwater. Heavy metals present in large water bodies can lead to pollution of the aquatic system, thereby causing several diseases and leading to termination of life of aquatic organisms. It can also in return make fish unsafe for consumption(Xue *et al.*, 2005).

All heavy metals at high concentration have strong toxic effect and are regarded as environmental pollutants (Nedelkoska and Doran,2000; Chehregani *et al.*, 2005). Acute heavy metal intake may damage central nervous function, the cardiovascular and the gastrointestinal (GI) systems, lungs, kidneys, liver, endocrine glands and bones (Jang and Hoffman, 2011; Adal and Wiener, 2013). Chronic heavy metal exposure has been implicated in several degenerative diseases of these same systems and may increase the risk of some cancers (Galanis*et al.*, 2009; Wu*et al*, 2012).

The presence of heavy metals in aquatic ecosystems, causes severe impacts on the biological components of these environments i.e. heavy metals are highly toxic to the aquatic plants and animals as well as they do not vanish easily from the environment. As a result, serious disorders in human health have been observed as a result of biomagnification processes and the toxic effects within the food chain (Xue *et al.*, 2005; Ljung and Vahter 2007).There are two aspects on the interaction of plants with heavy metals: (i) heavy metals show negative effects on plants, and

(ii) plants have their own resistance mechanisms against toxic effects and for detoxifying heavy metal pollution (Cheng, 2003).

There is no doubt that excessive levels of pollution are causing a lot of damage to human and animal health, plants including tropical rainforests, as well as the wider environment. All types of pollution-air, water and soil pollution have an impact on the living environment (Seth *et al.*, 2007).

1.2 Background Information on *Lemna trisulca, Pistia stratiotes* and *Salvinia molesta*

1.2.1 *Lemna trisulca* L.:

Lemna species commonly known as duckweed belong to the genus Lemna and family Lemnaceae. Duckweeds are among the world's smallest flowering plants. Individual lesser duckweed plants are tiny, round, bright green disks, each with a single root. They are found scattered among emergent plants or massed together in floating mats (Appendix XXI). Star duckweed is much less commonly observed. Individual non-flowering plants are longer and narrower than lesser duckweed, commonly floating in masses beneath the water surface. Flowering plants more closely resemble lesser duckweed (Rahman and Hasegawa, 2011).

Lemma species have no true leaves but have a leaf-like body called a thallus, which is flat on the top and slightly rounded on the bottom. Lesser duckweeds are nearly circular to oval, 2-5 mm in diameter; occur as single plants or up-to-five plants may be connected. Star duckweeds are two types: non-flowering plants are elongate or spatula-shaped (6-10 mm long), tapered to a stalked base, connected in branched chains of 8- 30 plants, and submerged beneath the water surface.

Flowering plants are more oval-shaped with a separate margin and a shorter stalk at the base. They float on the water surface (Mkandawire and Dudel, 2005).

Duckweeds have no stem; flowers are tiny and rarely seen and arise from a pouch in the thallus. Fruit is inconspicuous, usually 1 seeded. Root of lesser duckweed is single short rootlet (unbranched root) that hangs from the underside of each plant. Star duckweeds are often rootless (Landolt, 1986; Rahman and Hasegawa, 2011).

Types:

Duckweed comes in many varieties. Only plants belonging to the genus *Lemna* are duckweeds, but a number of similar plants often end up lumped under this name. Watermeal or Wolffia species are a close relative, but lack roots. They are the smallest kind of flowering plant at about ½ mm long. Landoltia and Spirodela species are also similar to duckweed, but have two or more roots (Reid and Stanley, 2003).

Propagation:

Duckweed has a fairly simple life cycle that enables it to spread quickly. New plants bud from pockets on either side of the parent plant and eventually break apart. Overwinters as winterbuds on the lake bottom, but rarely reproduces from seeds (Rahman *et al.*, 2007). Distributed by wind and on the bodies of birds, and aquatic animals. A single lesser duckweed plant can reproduce itself about every 3 days under ideal conditions in nutrient-rich waters. It can be hard to determine the point in the life cycle of a specific duckweed specimen. Botanists primarily use the length of the root to decide the age of a piece of duckweed. Longer roots belong to older plants (Reid and Stanley, 2003; Rahman *et al.*, 2007).

Cloning:

Duckweed is very short lived. *Lemna minor* for instance, survives only five to six weeks. However, the plant almost always appears as a dense mat, since it reproduces primarily asexually. The mature duckweed produces a small outgrowth from a bud on one end, which then breaks off becoming small new duckweed. The new plant produces a root and eventually is indistinguishable from the parent. Immature plants may remain attached to the parent until maturity (Wang *et al.*, 2002).

Importance of plant:

Food for fish and waterfowl and habitat for aquatic invertebrates. Because of its high nutritive value, duckweeds have been used for cattle and pig feed in Africa, India, and Southeast Asia. Sometimes used to remove nutrients from sewage effluent (Wang *et al.*, 2002; Korner *et al.*, 2003).

Distribution: Throughout much of the temperate and subtropical regions of the world.

Habitat: Still and slow moving waters in many freshwater habitats. Often found along the shoreline after water levels have dropped (Wang *et al.*, 2002).

1.2.2 *Pistiastratiotes* L.:

Pistia stratiotes commonly known as water lettuce or shell-flower belongs to the family Araceae.

Description:

Water lettuce is a floating water plant with 15 cm rosettes of ribbed, Ruffles Potato Chip-like leaves (Appendix XXII). The rosettes are connected by stolons that break easily. The leaves are fleshy-thick, pale green and velvety-hairy, which causes water to bead and keeps them from getting wet. The feathery roots are white, purple and black, and quite showy, hanging down a foot or so below the floating rosettes (Ramey, 2001). Water lettuce frequently forms solid mats on the water's surface and can become a serious pest (Skinner *et al.*, 2007).

Location

Water lettuce thrives in still waters in swamps, ponds, lakes, and sluggish rivers in the tropics and subtropics in both the Old and New Worlds. In the United States it is restricted to Peninsular Florida where it probably was introduced (Ramey, 2001).

Culture:

Light: Water lettuce needs full sunlight or slightly filtered sunlight.

Moisture: Water lettuce typically floats on the surface, but can withstand periods of drawdown as long as the mud does not dry out completely.

Propagation: Waterlettuce propagates vegetatively by growing stolons (stem like shoots) which produce new rosettes. Seeds are produced in the tropics and these are said to be easy to germinate (Zimmels *et al.*, 2006).

Usage:

Waterlettuce is difficult to maintain in artificial conditions. It can be grown in tropical or heated aquaria with a glass cover and full sunlight, or in a greenhouse pool. The air must be at least 75ºF (24ºC) degrees and permanently humid above the water (Skinner *et al.,* 2007). Waterlettuce can be grown in a temperate water garden, but must be lifted before frost and overwintered on damp sand or peat at no colder than 50 ºF (10ºC) (Odjegba and Fasidi, 2004; Skinner *et al.,* 2007).

1.2.3 *Salvinia molesta* D. Mitch:

Common name giant *Salvinia*, familySalviniaceae is a non-native, extremely invasiveaquatic fern that has infested the southern UnitedStates. Native to southeastern Brazil, the weedwas introduced for use in aquariums and gardenponds (Jacono, 2002). Giant *Salvinia* may also have been broughtin as packing with fresh, iced fish. It has beensold under many common names, including water velvet, *Salvinia,* African pyle, aquarium watermoss, kariba weed, water fern, and koi kandy. Since its escape, giant *Salvinia*has become a serious problem in rivers, streams, lakes, dams and rice fields (US Army Corps of Engineers, 2002)**.**

Giant *Salvinia*is a free-floating aquatic fern with small, oblong, ½-inch to 1-inch long spongy green leaves along the stem (Appendix XXIII). Young plants are smaller and the leaves lie flat on the water surface. Stems branch in an irregular fashion. The leaves occur in whorls of three: two floating and one submerged. The plant has no flowers, and the submersed leaves are thread-like and look like roots. As the leaves mature, they become thick and curl at the edges in response to crowding. As infestations grow in size, leaves become more vertical, forming upright chains that form mats of floating plants. The leaf surfaces of giant *Salvinia*have rows of hairs that, when magnified, look much like egg beaters in shape. The hairs give the leaves a velvety appearance and repel water. This characteristic is useful in identifying the species (Oliver, 1993).

The root like submerged fronds of giant *Salvinia*often support chains of egg-shaped spore-bearingstructures. Any spores produced, however, appearto be genetically defective, as they do not produceviable plants.Giant *Salvinia* grows best in quiet waters of lakesand ponds, oxbows, ditches, slow-flowing streamsand rivers, marshes, and rice fields. Its growth isfavored in water with a high nutrient content, suchas eutrophic waters or waters polluted by wastes (Land Protection, 2001).

*Salvinia*species reproduces as fragments break offexisting plants as they mature. New plants alsodevelop as dormant buds break away from theoriginal plant. As each node has up to five lateralbuds, the weed has high potential for growth. Asdormant buds, giant *Salvinia* will survive periodsof stress from both low temperature anddewatering.Giant *Salvinia*has small, spongy, vertical leaves.The third leaf forms a root like structure, but theweed is free-floating.Activities that fragment the weed, includingboating, fishing, or intentional harvesting, add toits spread. Giant *Salvinia* is often introduced tonew areas as people empty aquariums or infestedponds into waterways or as infested boats andtrailers are moved to new waters. Spread bywaterfowl may also occur (WAPMS, 2002).

1.2.4 Anti-oxidant Enzymes:

Plants possess several anti-oxidation defence systems and enzymes like Catalase (CAT), Superoxide dismutase (SOD), Glutathione Peroxidase (GPX), Ascorbate Peroxidase (APX), Glutathione Reductase (GR) and Glutathione-S-Transferase (GST) to scavenge toxic reactive oxygen species and to protect themselves from the oxidant stress (Seth *et al.*, 2007). Diverse array of pollutants stimulate a variety of toxicity mechanisms such as oxidative damage to membrane lipids, DNA, Proteins and changes to anti-oxidant enzymes (Valavanidis *et al.*, 2006). Elevated activities of anti-oxidant enzymes may help to alleviate the oxidative damage caused by ROS (Qian *et al.*, 2007). Glutathione –s –Transferase is an enzyme associated with atrazine detoxification in plants by detoxifying ROS through Ascorbate-glutathione cycle (Yadav, 2010). SOD converts superoxide radical into hydrogen peroxide and molecular oxygen, while the Catalase and Peroxidase convert hydrogen peroxide into water. In these two ways, two toxic species, superoxide radical and hydrogen peroxide are converted to the harmless product water (Weydert and Cullen, 2010). Various methods to identify oxidized amino acids in blood proteins as biomarkers of free radical damage, especially for metal-catalyzed oxidations, have been developed recently (Valavanidis *et al.*,2006).

According to Weydert and Cullen (2010), there are three SOD enzymes that are highly compartmentalized, Manganese-containing Superoxide dismutase (MnSOD) is localized in the mitochondria, Copper and Zinc-containing Superoxide dismutase (CuZnSOD) is located in the cytoplasm, nucleus and extracellular SOD (ECSOD). Catalase is found in peroxisomes and cytoplasm. Biological systems have developed during their evolution adequate enzymatic and non-enzymatic anti-oxidant mechanisms to protect their cellular components from oxidative damage (Valavanidis*et al.*, 2006).

The individual toxicity of heavy metals has been assessed, but they can interact and potentially increase their toxicity on plants and microalgae. Correspondingly, the combined effect of metals could be described qualitatively as antagonistic, non-reaction or synergistic. Generally plants are sensitive to pollutants; these pollutants may affect species composition of the plant community (Walsh, 1999). The toxicity of heavy metal (cadmium) in conjunction with atrazine herbicide was inhibited up to 100ppm of metal concentration in bean plant (Azmat *et al.,* 2006). Mathad *et al.* (2006) confirmed that *Helichrysum stuhmannii* and *Scenedesmus quadricauda* exposed to the various concentration of the bi- metallic combinations of chromium- iron, revealed no marked morphological variations at the lower metal concentration but, exhibited distinct abnormalities such as chlorosis, enlargement or reduction of cells, fragmentation and shrinkage of protoplasm or chloroplast, loss of cellular contents and cell lyses at the higher metal concentrations.

1.3 Statement of Research Problem

Environmental pollution by organic compounds and metals became extensive as mining and industrial activities increased in the 19^{th} century and have intensified since then (Torres *et al.*, 2008). These days, environmental problems are multiple and complex, especially those arising from the identification and assessment of the toxicity of chemical substances in the aquatic ecosystem (Ma *et al.,* 2006). Both natural and anthropogenic factors are considered as a major environmental concernfor aquatic ecosystems. They can impact on the aquatic environmentby producing polluting components which may enter into thehuman food chain and result in health problems (Kerambrun*et al.,* 2012). Metallic contamination in aquatic environmentshas received huge concern due to its toxicity (Diop *et al.,* 2014).

Adverse effects of heavy metals such as oxidative stress, inhibition of Hill reaction of photosynthesis, reduction of electron transport through photosystem II leading to photoinhibition, reduce utilization of carbon dioxide, decrease in growth or biomass production, alteration of ultra-structures of the cellular organelles and change in community structure on non-target plants are of particular concern because of the annual, wide spread and increasingly worldwide use of these chemicals (Van der Brink and Ter Braak, 1999). Extensive investments in sewage plants during the last two decades have greatly reduced the organic loading of receiving water bodies in a number of countries. However, an equivalent improvement in water quality has not been achieved since there are many small contributors which still have no cleaning of their wastewater discharge; and since leakage of nitrogen from the agricultural land, as a consequence of the increased use of fertilizers has greatly increased over the last thirty years (Peterson *et al.,* 1987).

There is little information about the joint effect of heavy metals on aquatic organisms. As a food source, microalgae may facilitate the uptake of contaminants into higher organisms, increasing the possibility of toxicity (Okay*et al.,* 2000). Therefore, stress effect of both metals on plants can cascade into the food chain. The stresses are numerous and often plant or location specific. They include increased UV-B radiation, water, high salinity, metal toxicity, herbicides, fungicides, air pollutants, light, temperature, topography and hypoxia that is restricted oxygen supply in waterlogged and compacted soil (Ali and Alqurainy, 2000). They further emphasize that stress depend on tissue and/or species, on membrane properties, on endogenous anti-oxidant content and on the ability to induce the response in the anti-oxidant system.

Low concentrations of trace metal for long periods of time can lead to metabolism modification, elimination of the plant and algae species that are unable to adapt themselves to the new conditions which will eventually affect the biodiversity of the environment (Tripath and Gaur, 2006). Cadmium enters plant cells either by means of active transport or by endocytosis through chelating proteins and affects various physiological and biochemical processes of the plant. The toxicity primarily results from their binding to the sulphydryl group in proteins or disrupting protein structure or displacing essential elements (Arunakumara and Xuecheng, 2007). Heavy metal irons could interrupt routine metabolic processes by competing for the Protein binding sites, active enzymes and various biological reactive groups, causing poor or no growth (Arunakumara and Xuecheng, 2007).

These pollutants stimulate a variety of toxicity mechanisms, such as oxidative damage to membrane lipids, DNA, Proteins, Carbohydrates and changes to anti-oxidant enzymes. Oxygen free radicals are essential in the physiological control of cell function in biological systems and are continuously produced in living cells but, during these metabolic processes, a small proportion (2–3%) of free radicals may escape from the protective shield of anti-oxidant mechanisms, causing oxidative damage to cellular components.

Heavy metal ions can cause plasma membrane depolarization and acidification of the cytoplasm. In fact, membrane injury is one important effect of heavy metal ions that may lead to the disruption of cellular homeostasis (Cardozo *et al.,* 2002).However, study on the biochemical composition changes and anti-oxidant enzymes responses will bring in the effect of metals on plants (Tripathi and Gaur, 2006). Metals may displace or substitute for essential trace metals and interfere with proper functioning of enzymes, associated cofactors and disorganizes chloroplast causing the damage of photosynthetic pigments. Metals trigger changes in the transcript levels of numerous genes encoding proteins and induce the synthesis of several proteins, including metallothionein in plants (Torres *et al.,* 2008).Heavy metals can interfere in the photosynthetic activity by increased photoinhibition from excess of light (Heckathorn *et al.,* 2004).

The disposal of large amounts of sewage and the intensified exploitation of agricultural land involving increased amounts of fertilizers, has resulted in pronounced eutrophication of receiving waters. The effects of eutrophication i.e. lower species diversity and decreasing self–purification capacity have been greatly magnified by destruction of the natural physical heterogeneity of the ecosystem. Streams have been viewed simply as conduits and have been deepened and straightened and their vegetation has been removed to augment the drainage of agricultural land. Natural wetland and marshes have been drained and turned into agricultural land(Peterson *et al.*, 1987).

The causes of water pollution may be due to direct and indirect contaminant sources. The former are effluents outputs from refineries, factories and waste treatment plants. Fluids of differing qualities are emitted to the urban water supplies. In the United States and some other countries, these methods are controlled. However, pollutants can still be found in the water bodies. The latter are the water supply from soils/groundwater systems that have fertilizers, pesticides and industrial wastes. Also those through the atmosphere like bakeries, factories emission and automobile discharge. Contaminants can also be divided into inorganic, organic, acid/base and radioactive.

Municipal, industrial and agricultural sources are the different categories of the causes of water pollution. Municipal causes are related to waste water from homes and commercial establishments. The main aim of handling municipal waste water was to decrease the harmful bacteria, oxygen requiring materials, mixed inorganic compounds and suspended solids. Industrial causes vary as per the biochemical demand, suspended solids, inorganic and organic substances. Agricultural sources include commercial livestock and poultry farming. These lead to organic and inorganic pollutants in surface water and groundwater (http//www.buzzle.com/articles/causes-of-water-pollution.html).

Pollutants have a tremendous impact on the biodiversity and productivity of aquatic communities (Relyea, 2005). Adverse effects of pollutants on non-target organisms of aquatic ecosystems are of special concern. These pollutants cause rapid changes in the communities of macrophytes, phytoplankton and other photosynthetic organisms. A common mechanism of metal toxicity in aquatic plants and algae is inhibition of biological processes such as photosynthesis and mitochondrial electron transport (Babu *et al.,* 2005). This leads to inevitable changes in plant cell physiology, growth and biomass yield (Kuster *et al.,* 2007).

Recently, the value of the biological diversity and complexity which prevail in natural ecosystems have been recognized, and attempts have been made to restore streams, rivers and wetlands in order to regain their heterogeneity, and thereby their self–purification capacity and buffering effects.

1.4 Justification

The problem of environmental pollution is now a worldwide phenomenon that needs to be urgently looked into. In line with the foregoing, the Federal Government of Nigeria recently set up a body-Federal Environmental Protection Agency (FEPA) to look into this problem and proffer solutions with respect to the monitoring and control of pollution sources in the country.

In the early eighties, the local municipalities in Nigeria were met with increasing demands for the removal of nitrogen and phosphorus as well as the organic content of wastewater prior to disposal. The traditional solution for small contributors is to collect the sewage from several small villages in one central – treatment facility. Such a solution is however rather expensive, and therefore the municipalities were, and still are searching for more effective solutions.

The legacy of rapid urbanization, industrialization, fertilizer and pesticide use has resulted in major problems in both terrestrial and aquatic environments. In response, conventional remediation systems based on high physical and chemical engineering approaches have been developed and applied to avert or restore polluted sites (Singh and Laban, 2003; Pilon-Smits 2005). Much as these conventional remediation systems are efficient, they are sparsely adopted because of some economical and technical limitations. Generally, the cost of establishment and running deter their use and meeting the demand particularly in countries with a weak economy.

As one of the consequences of heavy metal pollution in soil, water and air in developing countries (Guo,1994;Liao,1993; Su*et al.,* 1994; Wu *et al.,* 1998), plants were also seen to be polluted by heavy metals (Du*et al.*,1999;Wu *et al.,* 1998; Yin *et al.*,1999;Zhang *et al.*,1996*),* which consequently threatens the health of animals and human beings via the food chain (Wang and Dei, 2001). It is urgently necessary to clean and remediate heavy metals from areas, where crops, vegetables, fruits and pasturages have been grown, in order to protect the health of animals and human beings.

Different methodologies are used for the removal of the different heavy metals viz. electrodialysis, reverse osmosis and adsorption. All of these methodologies are quite costly and energy intensive, none of them could claim to treat all the heavy metals in economically feasible manner (Singh *et al.,* 1996). Economies of developing countries have other investment priority therefore cannot afford the high price involved in the removal of heavy metals from waste water.

Contrary to this phytoremediation which is removal of metals through plants offers an ecofriendly and cost effective methodology for the treatment of heavy metals from waste water.

Plants play an important role in solar energy transport to bio-energy and can clean the environment in an environmentally friendly manner; they would also play an important role in heavy metal remediation (Skinner *et al*., 2007). To understand the effects of heavy metals on plants and resistance mechanisms would be helpful for using plants to clean and remediate heavy metal pollution. Phytoremediation is potentially least harmful method because it uses naturally occurring organisms and preserves the environment in a more natural state (Maine*et al*., 2004; Skinner*et al*., 2007). Therefore, Knowledge of the ability of *Salviniamolesta*, *Pistia stratiotes*and *Lemnatrisulca*to absorb heavy metals would help in pollution control by protecting the environment as well as reducing health hazard.

Having an insight on the factors responsible for differential toxicity and physiological response of plants upon exposure to these metals will improve our ability to predict the impact of aquatic contaminants on freshwater ecosystem. The combined effect of Copper and Lead on the physiological responses of aquatic plants is a subject of intensive investigation, as most studies focus on the individual toxicity of heavy metals on plants. Investigation on the toxicity of heavy metals at environmentally relevant concentrations to fresh water plants will help to provide a scientific basis to assess the ecological risk of the pollutant groups in aquatic ecosystem accurately (He *et al.*, 2012).

Understanding of the regulatory mechanisms of metals tolerance, and the components involved in the mechanism will be helpful in metal removing processes from aquatic ecosystem (Li *et al.*, 2006). The potential of oxygen free radicals and other reactive oxygen species (ROS) to damage tissues and cellular components called oxidative stress, in biological systems has become a topic of significant interest for environmental toxicology studies (Valavanidis*et al.,* 2006). The balance between environmental pollutants and Antioxidant defences (enzymatic and Non-enzymatic) in biological systems can be used to assess toxic effects under stressful environmental conditions, especially oxidative damage induced by different classes of chemical pollutants. Therefore, the role of these Antioxidant systems and their sensitivity can be of great importance in environmental toxicology studies (Valavanidis *et al.,* 2006).

Aquatic ecosystems, especially their biological assemblages, continue to be degraded globally. Anthropogenic enrichment of aquatic ecosystems by heavy metal is an ever increasing phenomenon and, for larger bodies of water, the principal requirement for ecological restoration is the management of degraded water chemistry (Herve *et al.,* 2005). Rivers contribute largely to the biodiversity of macrophytes and macro invertebrates.

Recently, it is evident that durability restoration and long term contamination control in conventional remediation is questionable because in the long run, the pollution problem is only suspended or transferring from one site to another. In view of this, there has been growing interest in the search for alternative remediation technology that is effective, durable and cost-effective. One such technology is phytoremediation, the use of plants and associated microbes for environmental cleanup (Singh and Laban, 2003; Pilon-Smits, 2005).

On an international basis, considerable attention is at present being directed towards the capacity of aquatic macrophytes (Swamp and water plants) to control pollution and to treat municipal and industrial waste water as indicated by the great number of participants at recent international meetings (Athie and Cerri; 1987; Reddy and smith 1987; Hammer, 1989). The interest is partly coupled to the public demand for increasing stringent water quality standards, and partly to the need to develop low–cost decentralized constructions capable of serving small to medium sized-communities. Macrophytes–based wastewater treatment systems have several potential advantages such as low operating costs, low energy requirements, they can often be established at the site where the waste water is produced and they are more flexible and less susceptible to stock loading compared to conventional treatment systems (Brix, 1987).

The utilization of wetland areas as natural filters for the abatement of pollutants transported by water in rivers or lakes is considered to be an effective clean up option to ameliorate the quality of surface waters. Indeed, wetlands have been extensively utilized in the last decades to clean pollutant water almost all over the world (Gopal, 2003).

The vegetation covering the wetland areas plays an important role in sequestering large quantitative of nutrients (Cronk and Fennessy, 2001) and metals (Mays and Edwards, 2001; Baldantoni *et al.,* 2004) from the environment by storing them in the roots and/or shoots. Wetland plants have high remediation potential for macronutrients because of their general fast growth and high biomass production. Some Western Africa estuarine habitats have been seriouslydegraded for the last 30 years by climate changes causing severedroughts with reduction of freshwater flow, combined with theincrease in domestic and industrial effluents (Bouvy *et al.*, 2008).

Bioaccumulation of heavy metals in aquatic ecosystems is gaining tremendous significance globally. Several of the submerged, emergent and free floating aquatic macrophytes are known to accumulate and bioconcentrate heavy metals. Aquatic macrophytes take up metals from the water, producing an internal concentration several fold greater than their surroundings. Many of the aquatic macrophytes are found to be scavengers of heavy metals from water and wetlands (Gopal, 2003).

Shallow, eutrophic, aquatic ecosystems stocked with macrophytes are among the most productive in the world. (Schierup, 1978). Aquatic macrophytes are biological filters that carry out purification of water bodies by accumulating dissolved metals and toxins in their tissues (Begum, 2009). The capacity of such systems to decompose organic matter and assimilate nutrients has long been recognized, and it is well known that streams, lakes, coastal areas, and wetlands contain a considerable self – purification capacity. During the growing season the plants absorb and incorporate the nutrients into their own structures.

The potential of aquatic plants forbio-monitoring of polluted water has increasingly been recognized (Lewis and Wang 1997, Mohan and Hosetti, 1999). Algae have been reported as equally or more sensitive than animals (Lewis, 1992) and have been widely used in toxicity tests for regulatory purposes (ISO 1987; Weber *et al.*., 1987). However, algae may not necessarily be an indication of overall aquatic plant sensitivity to pollution. In fact, some studies have shown a higher sensitivity of macrophytes as compared to algae and animals (Thomas *et al.,* 1986; Roshon *et al.,* 1999).

Algae and aquatic plants play a key role in aquatic ecosystems because they are at the base of food webs. Also, they are a food resource and provide oxygen and shelter for many aquatic organisms. They also contribute to the stabilization of sediments, thus resulting in their accumulation in sediments (Gabas *et al.,* 1991). In aquatic systems, where pollutant inputs are discontinuous and pollutants are quickly diluted, analyses of plants provide a time integrated information about the quality of the system. Phytoremediation has several advantages and the most significant one is study of sub-lethal levels of bio accumulated contaminants within the tissues or components of organisms, which indicate the net amount of pollutants integrated over a period of time. Bio-monitoring of pollutants using some plants as accumulator species, helps to accumulate relatively large amounts of certain pollutants, even from much diluted solutions without obvious noxious effects (Begum, 2009).

Phytoremediation which is the use of plants to remove pollutants from the environment, is a growing field of research in environmental studies because of the advantages of its environmental friendliness, cost effectiveness and the possibility of harvesting the plants for the extraction of the absorbed contaminants such as metals that cannot be easily biodegraded for recycling among others (Maine *et al.*, 2001, 2004; Malik 2007). Phytoremediation work best when the contaminants discharged into the environment are within the reach of the plant roots. Most aquatic plants possess the qualities that favour their potential use in water and wastewater phytoremediation. There are limited data on phytoremediation of contaminated water bodies in Nigeria as against remediation of soil that is common.

Development of aquatic plants-based wastewater treatment systems is now recognized as suitable alternative to cost-effectively and safely treat sewage (Reddy and Smith, 1987; Cooper and Findlater, 1990). The scientific basis and the technical feasibility of this eco-technology are well established (Wolverton, 1987; Tchobanoglous, 1987) and abundant literature exists on the potentials of several aquatic plants to clean water especially in North America and Europe (Kadlek, 1987; Brix and Shierup, 1989; Brix, 1991). The tropical regions offer several advantages for the development of such technology.Aquatic plants are abundant and the suitable climate means that processes are optimum and operational all year round.

The impact of tropical aquatic ecosystems with high concentrations of metals can be reduced through phytoremediation. Some plants have shown great potential for their use in projects involving the sequestering and mitigation of contaminated aquatic effluents (Hasan *et al.* 2007; Alvarado *et al.*, 2008). Butterfly fern, water lettuce and duckweed are among the few popular organisms that can be used as tools to evaluate the presence of heavy metals (Claudia*et al.*, 2008). Enzymes such as catalase and peroxidase participate in protective mechanisms against damage caused by different chemicals (Santandrea *et al.,* 2000). The toxic effect of multiple chemicals plays a vital role in ecotoxicology because chemical mixtures could have a greater negative impact than the individual constituents (Hernando *et al.*, 2003).Several researchers have dealt with toxicity of individual pollutant, but few reports are available on the toxicity of pollutants in combination (Kumar and Han, 2011).

Environmental concerns on heavy metals have been of interest in the world especially in the developing countries including Nigeria. Several studies have described investigations relating to the effects and accumulation of metals in terrestrial and aquatic plants (Fecht-Christoffers *et al.*, 2003; Fernando *et al.,* 2006; Yang *et al.,* 2008; Pollard *et al.*, 2009) outside Nigeria. In Nigeria, some studies have reported the impact of heavy metals in the terrestrial environment using fungi, bryophytes, lichens and plants and very few studies on heavy metal concentrations inaquatic environment. To the best of my knowledge however, no work has been reported in Nigeria on the use of macrophytes for phytoremediation purposes in the aquatic environment and *in vitro*. Therefore, it became necessary to carry out the phytoremediation potential of copper and lead bybutterfly fern, water lettuce and duckweed;thereby making this research work the first in this part of the world.

1.5 Aim

The aim of the study is:

To evaluate the effects of the copper and lead on the physiology and the potential of *Salvinia molesta, Pistia stratiotes and Lemna trisulca* as phytoremediation plants.

1.6 Objectives of the Study

The specific objectives of the study are:-

1. To determine the effects of Cu and Pb on the photosynthetic pigments (chlorophyll a and chlorophyll b) of *Salvinia molesta, Pistia stratiotes* and *Lemna trisulca*.
2. To determine the effects of Cu and Pb on the activity of the antioxidant enzymes (antioxidant responses of catalase and peroxidase) of the three aquatic macrophytes.
3. To determine the effects of Cu and Pb on the morphology(visual signs/symptoms of chlorosis) of the three aquatic macrophytes.
4. To determine the uptake and bioaccumulation (metal sequestration) of the pollutants in the different macrophytes tissues in proportion to exposure concentration over time.
5. To determine which of the species has a potential for phytoremediation in relation to the absorption rate of the heavy metals.

1.7 Research Hypotheses

The hypotheses tested by the research results are:-

1. There is no significant difference in the effect of the metals on the photosynthetic pigment (chlorophyll a and chlorophyll b) of the different aquatic macrophytes

2. There is no significant difference in the effect of the metals on the activity of the antioxidant enzymes of the different aquatic macrophytes.
3. There is no significant difference in the individual effect of the metals and combined effect of the metals on the visual symptoms of the different aquatic macrophytes.
4. There is no significant difference in the uptake and bioaccumulation of the pollutants in the different aquatic macrophytes tissues in relation to exposure concentration over time.
5. There is no significance difference in the absorption rate of the heavy metals by the aquatic macrophytes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Heavy Metal Pollution

Heavy metals in the soil, water and air environment can be involved in a series of complex chemical and biological interactions. Several of the reactions include oxidation-reduction, precipitation-dissolution, volatilization, and surface-solution phase complexation. Heavy metals are conventionally defined as elements withmetallic properties (ductility, conductivity, stability as cations, ligand specificity, etc.) and an atomic number>20 (Banfalvi, 2011). Heavy metal pollution not only affects the production and quality of crops, but alsoinfluences the quality of the atmosphere and water bodies, and threatens the health and life of animals andhuman being. Additional sourcesof heavy metal contamination are rainfall, atmospheric dust, plant protective agents, and fertilizers (Wu *et al*., 1998). The levelof essential elements in plants is conditional, the content being affected by geochemical characteristics ofthe soil and by the ability of plants to selectively accumulate some of these elements (Bastian and Hammer, 1993).

The assimilation of heavy metals in plants isobvious because of widespread heavy metals in the soil due to geo-climatic conditions (Kumar *et al.*, 1995).Heavy metals have a great tendency to accumulate in human organs over prolonged periods of time.The most common heavy metal contaminants are: Arsenic (As), Cadmium(Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Lead (Pb), Nickel (Ni) and Zinc (Zn) (Lasat, 2002). Effects of toxic metals (Cd, Cr, Pb, Ni etc) on human health and their interaction with essentialtrace elements may produce serious consequences.

The environmental impact of heavy metalssuch as Cd, Pb, Hg, Cu and As, as well as their health effects, has been a source of major concern (WHO,1998).

Many of the chemical substances are toxic. Pathogens can produce waterborne diseases in either human or animal hosts (Hogan, 2010). Alteration of water's physical chemistry by chemicals includes acidity (change in pH), electrical conductivity, temperature, and eutrophication. Eutrophication is an increase in the concentration of chemical nutrients in an ecosystem to an extent that increases the primary productivity of the ecosystem. Depending on the degree of eutrophication, subsequent negative environmental effects such as anoxia (oxygen depletion) and severe reductions in water quality may occur, affecting fish and other animal populations.

2.2 Water Pollution

The pressure of increasing population, growth of industries, urbanization, energy intensive life style, loss of forest cover, lack of environmental awareness, lack of implementation ofenvironmental rules and regulations and environment improvement plans, untreated effluent discharge from industries and municipalities, use of non-biodegradable pesticides/fungicides/ herbicides/insecticides, use of chemical fertilizers instead of organic manures, etc are causing water pollution. The pollutants from industrial discharge and sewage besides finding their way to surface water reservoirs and rivers are also percolating into ground to pollute ground water sources (Huang *et al.*,1997).

The polluted water may have undesirable colour, odour, taste, turbidity, organic matter contents, harmful chemical contents, toxic and heavy metals, pesticides, oily matters, industrial waste products, radioactivity, high Total Dissolved Solids (TDS), acids, alkalis, domestic sewage content, virus, bacteria, protozoa, rotifers, worms, etc. The organic content may be biodegradable or non-biodegradable. Pollution of surface waters (rivers, lakes, and ponds), ground waters and sea water are all harmful for human and animal health. Pollution of the drinking water and that of food chain is by far the most worry-some aspect (Huang *et al.*,1997). There are two modes by which pollutants enter a water bodynamely: Point source and non-point source.

2.2.1 Domestic sewage

Domestic sewage is 99.9 percent water, while the other 0.1 percent is pollutants. Although found in low concentrations, these pollutants pose risk on a large scale. In urban areas, domestic sewage is typically treated by centralized sewage treatment plants. In the U.S., most of these plants are operated by local government agencies, frequently referred to as publicly owned treatment works (POTW). Municipal treatment plants are designed to control conventional pollutants: Biochemical oxygen demand (BOD) and suspended solids. Well-designed and operated systems (i.e., secondary treatment) can remove 90 percent or more of these pollutants. Some plants have additional sub-systems to treat nutrients and pathogens. Most municipal plants are not designed to treat toxic pollutants found in industrial wastewater (EPA, 2004).

Cities with sanitary sewer overflows or combined sewer overflows employ one or more engineering approaches to reduce discharges of untreated sewage, including:

- utilizing a green infrastructure approach to improve stormwater management capacity throughout the system, and reduce the hydraulic overloading of the treatment plant (EPA, 2008).
- repair and replacement of leaking and malfunctioning equipment (EPA, 2004).
- Increasing overall hydraulic capacity of the sewage collection system (often a very expensive option).

A household or business not served by a municipal treatment plant may have an individual septic tank, which treats the wastewater on site and discharges into the soil. Alternatively, domestic wastewater may be sent to a nearby privately owned treatment system (e.g. in a rural community).

2.2.2 Industrial wastewater

Some industrial facilities generate ordinary sewage that can be treated by municipal facilities. Industries that generate wastewater with high concentrations of conventional pollutants (e.g. oil and grease), toxic pollutants (e.g. heavy metals, volatile organic compounds) or other nonconventional pollutants such as ammonia, need specialized treatment systems. Some of these facilities can install a pre-treatment system to remove the toxic components, and then send the partially treated wastewater to the municipal system. Industries generating large volumes of wastewater typically operate their own complete on-site treatment systems.

Some industries have been successful at redesigning their manufacturing processes to reduce or eliminate pollutants, through a process called pollution prevention.

Heated water generated by power plants or manufacturing plants may be controlled with:

- cooling ponds, man-made bodies of water designed for cooling by evaporation, convection and radiation
- cooling towers, which transfer waste heat to the atmosphere through evaporation and/or heat transfer
- cogeneration, a process where waste heat is recycled for domestic and/or industrial heating purposes (EPA, 1997).

2.2.3 Agricultural wastes

2.2.3.1 *Non-point source control*:

Sediment (loose soil) washed off fields is the largest source of agricultural pollution in the United States (EPA, 2005). Farmers may utilize erosion controls to reduce runoff flows and retain soil on their fields. Common techniques include contour plowing, crop mulching, crop rotation, planting perennial crops and installing riparian buffers (NRCS, 2009).

Nutrients (nitrogen and phosphorus) are typically applied to farmland as commercial fertilizer; animal manure; or spraying of municipal or industrial wastewater (effluent) or sludge. Nutrients may also enter runoff from crop residues, irrigation water, wildlife and atmospheric deposition. Farmers can develop and implement nutrient management plans to reduce excess application of nutrients (EPA, 2003).

To minimize pesticide impacts, farmers may use Integrated Pest Management (IPM) techniques (which can include biological pest control) to maintain control over pests, reduce reliance on chemical pesticides, and protect water quality (EPA, 2008).

2.2.3.2 *Point source wastewater treatment*

Farms with large livestock and poultry operations, such as factory farms, are called concentrated animal feeding operations or feedlots in the US and are being subject to increasing government regulation (EPA, 2008, IOWA, 2009). Animal slurries are usually treated by containment in anaerobic lagoons before disposal by spray or trickle application to grassland. Constructed wetlands are sometimes used to facilitate treatment of animal wastes. Some animal slurry are treated by mixing with straw and composted at high temperature to produce bacteriologically sterile and friable manure for soil improvement (Gopal, 2003).

Sediment from construction sites is managed by installation of:

- erosion controls, such as mulching and hydroseeding, and
- sediment controls, such as sediment basins and silt fences.

Discharge of toxic chemicals such as motor fuels and concrete washout is prevented by use of:

- spill prevention and control plans, and
- specially designed containers (e.g. for concrete washout) and structures such as overflow controls and diversion berms (EPA, 2006).

2.2.4 Urban runoff (storm water)

Effective control of urban runoff involves reducing the velocity and flow of stormwater, as well as reducing pollutant discharges. Local governments use a variety of stormwater management techniques to reduce the effects of urban runoff. These techniques, called best management practices (BMPs) in the U.S., may focus on water quantity control, while others focus on improving water quality, and some perform both functions (EPA, 1999).

Pollution prevention practices include low-impact development techniques, installation of green roofs and improved chemical handling (e.g. management of motor fuels and oil, fertilizers and pesticides (EPA, 2008). Runoff mitigation systems include infiltration basins, bioretention systems, constructed wetlands, retention basins and similar devices (NJDEP, 2004).

Thermal pollution from runoff can be controlled by stormwater management facilities that absorb the runoff or direct it into groundwater, such as bioretention systems and infiltration basins. Retention basins tend to be less effective at reducing temperature, as the water may be heated by the sun before being discharged to a receiving stream (EPA, 1999).

2.3 Phytoremediation of Toxic Elements by Aquatic Macrophytes

Phytoremediation is derived from (ancient Greek) phyto meaning “plant”, and Latin remedium meaning “restoring balance”. Phytore-mediation describes the treatment of environmental problems (bioremediation) through the use of plants that mitigate the environmental problems without the need to excavate the contaminant materials and dispose of it elsewhere (Burken, *et al.*, 2011).

Phytoremediation consist of mitigating pollutant concentrations in contaminated soils, water or air, with plants able to contain, degrade or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives and various other contaminants from the media that contain them. Phytoremediation may be applied where the soil or static water environment has become polluted or is suffering ongoing chronic pollution. Examples where phytoremediation has been used successfully includes the restoration of pesticides, solvents, explosives (Gopal, 2003). Phytoremediation refers to the natural ability of certain plants called hyper accumulators to bio accumulate, degrade or render harmless contaminants in soils, water or air.

Freshwater as well as seawater resources are being contaminated by various toxic elements through anthropogenic activities and from natural sources. Therefore, remediation of contaminated aquatic environment is important as it is for terrestrial environment. Phytoremediation of the toxic contaminants can be readily achieved by aquatic macrophytes or by other floating plants since the process involves biosorption and bioaccumulation of the soluble and bioavailable contaminants from water (Brooks and Robinson, 1998). In aquatic phytoremediation systems, aquatic plants can be either floating on the water surface or submerged into the water. The floating aquatic hyper accumulating plants absorb or accumulate contaminants by its roots while the submerged plants accumulate metals by their whole body.

Many years ago, Hutchinson (1975) reviewed the ability of aquatic macrophytes to concentrate elements from the aquatic environment and reported that, the levels of potentially toxic elements in the plants were at least an order of magnitude higher than in the supporting aqueous medium. Later on, Outridge and Noller (1991) reviewed the hyper accumulation of toxic trace

elements by aquatic vascular plants and discussed about the pathways and rates of elemental uptake and excretion, environmental factors that control uptake of elements, and the significance of trace elements uptake for the field of wastewater treatment and biomonitoring of pollutants, which is of great interest for bioremediation of aquatic systems.

Several aquatic macrophytes and some other small aquatic floating plants have been investigated for the remediation of natural and wastewater contaminated with Cu(II), Cd(II) and Hg(II) (Sen and Mondal, 1987; Selvapathy and Sreedhar, 1991; Alam *et al.,* 1995). Microspora and *Lemna minor* were studied for Pb and Ni remediation (Axtell *et al.,* 2003). Five common aquatic plant species (*Typha latifolia*, *Myriophyllum exalbescens*, *Potamogeton epihydrus*, *Sparganium angustifolium* and *Sparganium multipedunculatum*) were testedfor Al phytoremediation (Gallon *et al.,* 2004). Parrot feather(*Myriophyllum aquaticum*), creeping primrose (*Ludwigina palustris*),and water mint (*Mentha aquatic*) have been reported to remove Fe,Zn, Cu, and Hg from contaminated water effectively (Kamal *et al.,*2004). The *Lemna minor* was reported to accumulate Cu and Cd fromcontaminated wastewater (Kara, 2004; Hou *et al.*, 2007). The submerged aquatic plant *Myriophyllum spicatum* L. has been reportedas an efficient plant species for the metal-contaminated industrialwastewater treatment (Lesage *et al.,* 2007). The aquatic plants *Rorippa nasturtium-aquaticum* L. and *Mentha spp.* accumulatearsenic from contaminated freshwater (Robinson *et al.,* 2006).The encouraging results of previous studies regarding phytoremediation using aquatic plants gained the attention of researchersand scientists to continue studies in this field. In this review,previous works on phytoremediation of arsenic, one of the majorand widespread contaminants, using aquatic macrophytes has been discussed.

2.3.1 Methods of Phytoremediation

Metals, radionuclides and other inorganic contaminants are among the most prevalent forms of environmental contaminants, and their remediation in soils, water bodies and sediments is rather a difficult task (Cunningham *et al.*, 1997). Heavy metal contamination of the biosphere has increased sharply since 1900 (Nriagu, 1997) and poses major environmental and human health problems worldwide (Ensley, 2000). Unlike many organic contaminants, most metals and radionuclides cannot be eliminated from the environment by chemical or biological transformation (Cunningham and Owe, 1996; NRC, 1997). Although it may be possible to reduce the toxicity of certain metals by influencing their speciation, they do not degrade and are persistent in the environment (NRC, 1999). The various conventional remediation technologies that are used to clean heavy metal polluted environments are soil *in situ*nitrifications, soil incineration, excavation and landfill, soil washing, soil flushing, solidification and stabilization electrokinetic systems. Each of the conventional remediation technology has specific benefits and limitations (EPA, 1997; MADEP, 1993).

All compartments of the biosphere are polluted by a variety of inorganic and organic pollutants as a result of anthropogenic activities and alter the normal biogeochemical cycling. A variety of biological resources have been employed widely both in developed and developing nations for cleanup of the metal polluted sites. These technologies have gained considerable momentum in the last one decade and currently in the process of commercialization (Comis, 1995; Salt*et al.*, 1995; Glass, 1999). The Unites States of America's Environmental Protection Agency´s remediation program included phytoremediation of metals and radionuclides as a thurst area upto 25% during the year 2000. Plants that hyper accumulate metals have tremendous potential for application in remediation of metals in the environment. This approach is emerging as an innovative tool with greater potential for achieving sustainable development and also to decontaminate metal polluted air, soil, water and for other environmental restoration applications through rhizosphere biotechnology (Desouza *et al.*, 1999). Metal hyperaccumulating plants are thus not only useful in phytoremediation, but also play a significant role in biogeochemical prospecting, and have implications on human health through food chain and possibly exhibit elemental allelopathy (metallic compounds leached through plant parts of the hyperaccumulator would supress the growth of other plants growing in the neighbourhood) and resistance against fungal pathogens (Boyd *et al.*, 1994). In order to be realistic about phytoremediation, studiesfocused on factors regulating phytoremediation are necessary.

The term phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) actually refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated environments (Cunninghamet *al.*, 1997; Flathman and Lanza, 1998). The primary motivation behind the development of phytoremediative technologies is the potential for low-cost remediation (Ensley, 2000). Although, the term phytoremediation is a relatively recent invention, it’s an age old practice (Cunningham *et al.*, 1997). Research using semi-aquatic plants for treating radionuclide-contaminated waters existed in Russia at the dawn of the nuclear era (Timofeev-Resovsky *et al.*, 1962; Salt *et al.*, 1995). Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibiting symptoms of toxicity (Reeves and Brooks, 1983; Baker and Brooks, 1989). Chaney, (1983) was the first to suggest using these "hyperaccumulators" for the phytoremediation of metal-polluted sites. However, hyperaccumulators were later believed to have limited potential in this area because of their small size and slow growth, which limit the speed of metal removal (Cunningham *et al.*, 1995; Comis, 1995). By definition, a hyperaccumulator must accumulate at least 100 mg g^{-1} (0.01% dry wt.), Cd, As and some other trace metals, 1000 mg g^{-1} (0.1 dry wt.) Co, Cu, Cr, Ni and Pb and 10,000 mg g^{-1} (1 % dry wt.)Mn and Ni (Wantanabe, 1997).

Phytoremediation consists of four different plant-based technologies each having a different mechanism of action for the remediation of metal-polluted soil, sediment, or water. These include: rhizofiltration, which involves the use of plants to clean various aquatic environments; phytostabilization, where plants are usedto stabilize rather than clean contaminated soil; phytovolatilization, which involves the use of plants to extract certain metals from soil and then release them into the atmosphere through volatilization; and phytoextraction, where plants absorb metals from soil or water and translocate them to the harvestable shoots where they accumulate. Although, plants show some ability to reduce the hazards of organic pollutants (Cunninghamet *al.*, 1995; Gordon *et al.*, 1997), the greatest progress in phytoremediation has been made with metals (Salt *et al.*,1995; Watanabe, 1997). Phytoremediative technologies which are soil-focused are suitable for large areas that have been contaminated with low to moderate levels of contaminants. Sites which are heavily contaminated cannot be cleaned through phytoremediative means because the harsh conditions will not support plant growth (Watanabe, 1997). The depth of soil which can be cleaned or stabilized is restricted to the root zone of the plants being used. Depending on the plant, this depth can range from a few inches to several meters. Phytoremediation should be viewed as a long-term remediation solution because many cropping cycles may be needed over several years to reduce metals to acceptable regulatory levels. This new remediation technology is competitive, and may be superior to existing conventional technologies at sites where phytoremediation is applicable.

2.3.1.1 *Rhizofiltration*

Metal pollutants in industrial-process water and in groundwater are most commonly removed by precipitation or flocculation, followed by sedimentation and disposal of the resulting sludge (Ensley, 1996). A promising alternative to this conventional clean-up method is rhizofiltration, a phytoremediative technique designed for the removal of metals in aquatic environments. The process involves raising plants hydroponically and transplanting them into metal-polluted waters where plants absorb and concentrate the metals in their roots and shoots (Dushenkov *et al.* 1995; Salt *et al.*, 1995). Root exudates and changes in rhizosphere pH also may cause metals to precipitate onto root surfaces. As they become saturated with the metal contaminants, roots or whole plants are harvested for disposal (Flathman and Lanza, 1998). Most researchers believe that plants for phytoremediation should accumulate metals only in the roots (Dushenkov *et al.*, 1995; Salt*et al.*,1995; Flathman and Lanza, 1998). Dushenkov *et al.*, (1995) explains that the translocation of metals to shoots would decrease the efficiency of rhizofiltration by increasing the amount of contaminated plant residue needing disposal. In contrast, Zhu*et al.* (1999) suggest that the efficiency of the process can be increased by using plants which have a heightened ability to absorb and translocate metals within the plant. Despite this difference in opinion, it is apparent that proper plant selection is the key to ensuring the success of rhizofiltration as a water cleanup strategy.

Dushenkov and Kapulnik, (2000) described the characteristics of the ideal plant for rhizofiltration. Plants should be able to accumulate and tolerate significant amounts of the target metals in conjunction with easy handling, low maintenance cost, and a minimum of secondary waste requiring disposal. It is also desirable plants to produce significant amounts of root biomass or root surface area. Several aquatic species have the ability to remove heavy metals from water, including water hyacinth (*Eirchorrnia crassipes* (Mart.) (Zhu*et al.*,1999), and duckweed (*Lemna minor* L.). However, these plants have limited potential for rhizofiltration, because they are not efficient at metal removal, a result of their small, slow-growing roots (Dushenkov *et al.*, 1995). These authors also pointed out that, the high water content of aquatic plants complicates their drying, composting, or incineration. Despite limitations, Zhu*et al.*,(1999) indicated that, water hyacinth is effective in removing trace elements in waste streams. Terrestrial plants are thought to be more suitable for rhizofiltration because they produce longer, more substantial, often fibrous root systems with large surface areas for metal sorption. Sunflower (*Helianthus annuus* L.) and Indian mustard (*Brassica juncea* Czern.) are the most promising terrestrial candidates for metal removal in water. The roots of Indian mustard are effective in the removal of Cd, Cr, Cu, Ni, Pb, and Zn (Dushenkov*et al.*,1995), and sunflower removes Pb (Dushenkov *et al.* 1995), ^{137}Cs, and ^{90}Sr (Dushenkov *et al.*, 1995) from hydroponic solutions.

2.3.1.2 *Phytostabilization*

Sometimes there is no immediate effort to clean metal-polluted sites, either because the responsible companies no longer exist or because the sites are not of high priority on a remediation agenda (Berti and Cunningham, 2000). The traditional means by which metal toxicity is reduced at these sites is by in-place inactivation, a remediation technique that employs the use of soil amendments to immobilize or fix metals in soil. Although, metal migration is minimized, soils are often subject to erosion and still pose an exposure risk to humans and other animals. Phytostabilization, also known as phytorestoration, is a plant-based remediation technique that stabilizes wastes and prevents exposure pathways via wind and water erosion; provides hydraulic control, which suppresses the vertical migration of contaminants into groundwater; and physically and chemically immobilizes contaminants by root sorption and by chemical fixation with various soil amendments (Cunninghamet *et al.*,1995; Berti and Cunningham, 2000). This technique is actually a modified version of the in-place inactivation method in which the function of plants is secondary to the role of soil amendments. Unlike other phytoremediative techniques, the goal of phytostabilization is not to remove metal contaminants from a site, but rather to stabilize them and reduce the risk to human health and the environment.

The most comprehensive and up-to-date explanation of the phytostabilization process is offered by Berti and Cunningham (2000). Before planting, the contaminated soil is plowed to prepare a seed bed and to incorporate lime, fertilizer, or other amendments for inactivating metal contaminants. Soil amendments should fix metals rapidly following incorporation, and the chemical alterations should be long lasting if not permanent. The most promising soil amendments are phosphate fertilizers, organic matter or bio-solids, iron or manganese oxyhydroxides, natural or artificial clay minerals, or mixtures of these amendments. Plants chosen for phytostabilization should be poor translocators of metal contaminants to aboveground plant tissues that could be consumed by humans or animals. The lack of appreciable metals in shoot tissue also eliminates the necessity of treating harvested shoot residue as hazardous waste (Flathman and Lanza, 1998). Selected plants should be easy to establish and care for, grow quickly, have dense canopies and root systems, and be tolerant of metal contaminants and other site conditions which may limit plant growth.

2.3.1.3 *Phytovolatilization*

Some metal contaminants such as As, Hg, and Se may exist as gaseous species in environment. In recent years, researchers have searched for naturally occurring or genetically modified plants that are capable of absorbing elemental forms of these metals from the soil, biologically converting them to gaseous species within the plant, and releasing them into the atmosphere. This process is called phytovolatilization, the most controversial of all phytoremediation technologies. Mercury and Se are toxic (Suszcynsky and Shann, 1995), and there is doubt about whether the volatilization of these elements into the atmosphere is safe (Watanabe, 1997). Selenium phytovolatilization has been given the most attention to date (Lewis *et al.*, 1966), because this element is a serious problem in many parts of the world where there are areas of Se-rich soil. However, there has been a considerable effort in recent years to insert bacterial Hg ion reductase genes into plants for the purpose of Hg phytovolatilization (Heaton *et al.*, 1998). Although, there have been no efforts to genetically engineer plants which volatilize As, it is likely that researchers will pursue this possibility in the future. According to Suszcynsky and Shann(1995), the release of volatile Se compounds from higher plants was first reported by Lewis *et al.* (1966). They reported that members of the Brassicaceae are capable of releasing up to 40 g Se ha-1 day -1 as various gaseous compounds. Some aquatic plants, such as cattail (*Typha latifolia* L.), are also good for Se phytoremediation.Unlike plants that are being used for Se volatilization, those which volatilize Hg are genetically modified organisms. *Arabidopsis thaliana* L. and tobacco (*Nicotiana tabacum* L.) have been genetically modified with bacterial organomecurial lyase (*MerB*) and mercuric reductase (*MerA*) genes (Heaton*et al.*,1998). These plants absorb elemental Hg(II) and methyl mercury (MeHg) from the soil and release volatile Hg(O) from the leaves into the atmosphere (Heaton *et al.*, 1998).

2.3.1.4 *Phytoextraction*

Phytoextraction is the most commonly recognized of all phytoremediation technologies. The terms phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept while phytoextraction is a specific cleanup technology. The phytoextraction process involves the use of plants to facilitate the removal of metal contaminants from a soil matrix or water (Kumar*et al.* 1995). In practice, metal-accumulating plants are seeded or transplanted into metal-polluted soil and are cultivated using established agricultural practices. The roots of established plants absorb metal elements from the soil and translocate them to the above-ground shoots where they accumulate. If metal availability in the soil is not adequate for sufficient plant uptake, chelates or acidifying agents may be used to liberate them into the soil solution (Huang*et al.*, 1997). After sufficient plant growth and metal accumulation, the above-ground portions of the plant are harvested and removed, resulting in the permanent removal of metals from the site. As with soil excavation, the disposal of contaminated material is a concern. Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring disposal (Kumar *et al.* 1995). In some cases valuable metals can be extracted from the metal-rich ash and serve as a source of revenue, thereby offsetting the expense of remediation (Comis, 1995; Cunningham and Owe, 1996). Phytoextraction should be viewed as a long-term remediation effort, requiring many cropping cycles to reduce metal concentrations (Kumar *et al.*, 1995) to acceptable levels. The time required for remediation is dependent on the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years (Kumar *et al.*, 1995; Blaylock and Huang, 2000). This technology is suitable for the remediation of large areas of land that are contaminated at shallow depths with low to moderate levels of metal-contaminants (Kumar *et al.*, 1995; Blaylock and Huang, 2000). Many factors determine the effectiveness of phytoextraction in remediating metal-polluted sites (Blaylock and Huang, 2000). The selection of a site that is conducive to this remediation technology is of primary importance. Phytoextraction is applicable only to sites that contain low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils. Soil metals should also be bio-available, or subject to absorption by plant roots. The land should be relatively free of obstacles, such as fallen trees or boulders, and have an acceptable topography to allow for normal cultivation practices, which employ the use of agricultural equipment. As a plant-based technology, the success of phytoextraction is inherently dependent upon several plant characteristics. The two most important characters include the ability to accumulate large quantities of biomass rapidly and the ability to accumulate large quantities of environmentally important metals in the shoot tissue (Kumar *et al.*, 1995; Cunningham and Owe, 1996; Blaylock and Huang, 2000). It is the combination of high metal accumulation and high biomass production that results in the most metal removal. Ebbs *et al.* (1997) reported that *Brassica juncea*, while having one-third the concentration of Zn in its tissue, is more effective at Zn removal from soil than *T. caerulescens*, a known hyperaccumulator of Zn. This advantage is due primarily to the fact that *B. juncea* produces ten-times more biomass than *Thlaspi caerulescens*. Plants being considered for phytoextraction must be tolerant of the targeted metal, or metals, and be efficient at translocating them from roots to the harvestable above-ground portions of the plant (Blaylock and Huang, 2000). Other desirable plant characteristics include the ability to tolerate difficult soil conditions (*i.e.*, soil pH, salinity, soil structure, water content), the production of a dense root system, ease of care and establishment, and few disease and insect problems. Although, some plants show promise for phytoextraction, there is no plant which possesses all of these desirable traits.

The other three processes of phytoremediation employed by plants and algae are:

2.3.1.5 Phytotransformation

Chemical modification of environmental substances as a direct result of plant metabolism often resulting in their inactivation, degradation (phytodegradation) or immobilization (phyto stabilization)(Susarla *et al.,* 2002).

2.3.1.6 *Phytostimulation*

Enhancement of soil microbial activity for the degradation of contaminants, typically by organism that associate with roots. This process is known as rhizophere degradation. Phyto stimulation can also involve aquatic plants supporting active populations of microbial Degraders, as in the stimulation of Atrazine degradation by hornworts (Rupassara*et al.,* 2002).

2.3.1.7 *Phytosequestration*

"Phytochemical complexation in the root zone, reduce the fraction of the contaminant that is bioavailable. Transport protein inhibition on the root membrane-preventing contaminants from entering the plant. Vacuolar storage in root cells-contaminants can be sequestered into the vacuoles of root cells.

Finding the perfect plant continues to be the focus of many plant-breeding and genetic-engineering research efforts.

2.3.2 Advantages and limitations of phytoremediation

2.3.2.1 *Advantages:*

i. The cost of the phytoremediation is lower than that of traditional processes both in-site and ex-site.
ii. The plants can be easily monitored.
iii. The possibility of the recovery and re-use of valuable metals (by companies specializing in "phytomining").
iv. It is potentially the least harmful method because it uses natural occurring organisms and preserves the environment in a more natural state.

2.3.2.2 *Limitations*

i. Phytoremediation is limited to the surface area and depth occupied by the roots
ii. Slow growth and low biomass require a long-term commitment.
iii. With plant-based systems of remediation, it is not possible to completely prevent the leaching of contaminants into the ground water (without the complete removal of contaminated ground, which in itself doesn't resolve the problem of contamination).

iv. The survival of the plants is affected by the toxicity of the contaminated land and water; and the general condition of the environment.

v. Bio-accumulation of contaminants chelates into the plant tissues which then pass into the food chain, from primary level consumers upwards or requires the safe disposal of the affected plant material (Rupassara *et al.,* 2002).

2.4 Oxidative Stress

The damage caused by reactive oxygen species (ROS) is known as oxidative stress. Cells contain a large number of antioxidants to prevent or repair the damage caused by ROS, as well as to regulate redox sensitive signalling pathways (Wu*et al.,* 2008).Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species and a biological system. The inability to readily detoxify the reactive intermediates or to repair the resulting damage, will cause disturbance in the normal redox state of cells through the production of peroxides and free radicals that damage all components of the cell, including Proteins, Lipids and DNA.

Reactive oxygen species are produced in many aerobic cellular metabolic processes; they include but are not limited to species such as superoxide and hydrogen peroxide which react with various intracellular targets including Lipids, Proteins and DNA. The biological effects of ROS on these intracellular targets are dependent on their concentration and increase level of these species present during oxidative stress (Weydert and Cullen, 2010). Formation of ROS is a normal process in photosynthesis, but can become a problem under stress conditions when production is increased (Asada, 1999).

Increased levels of ROS are cytotoxic while lower levels are necessary for the regulation of several key physiological mechanisms. Increased levels of ROS can result in ROS –induced damage such as cell death, mutations, chromosomal aberrations and carcinogenesis (Weydert and Cullen, 2010).All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. To cope with metal stress, plants possess mechanisms to protect themselves from metal injury and poisoning, these mechanisms include extra cellular and intracellular sequestration of metal ions (Tripathi and Gaur, 2006). Freshwater algae form the base of aquatic food webs and changes in algal community structure and composition may have cascading effects on other component of the community due to changes in food availability and important community level interactions (Lockert*et al.*, 2006).

2.5 Toxicity of Copper and Lead

A toxic heavy metal is any relatively dense metal or metalloid that is noted for its potential toxicity, especially in environmental contexts (Baldwin and Marshall, 1999). The term has particular application to cadmium, mercury, lead and arsenic, (Banfalvi, 2011) all of which appear in the World Health Organisation's list of 10 chemicals of major public concern. Other examples include manganese, chromium, cobalt, nickel, copper, zinc, selenium, silver, antimony and thallium.

Toxic heavy metals are found naturally in the earth, and become concentrated as a result of human activities. They enter plant, animal and human tissues via inhalation, diet and manual handling, and can bind to, and interfere with the functioning of vital cellular components. The toxic effects of arsenic, mercury and lead were known to the ancients but methodical studies of the toxicity of some heavy metals appear to date from only 1868. In humans, heavy metal poisoning is generally treated by the administration of chelating agents. Some elements regarded as toxic heavy metals are essential, in small quantities, for human health.

2.5.1 Effects of Copper and Lead

2.5.1.1 *Copper (Cu)*

Excess of Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (Lewis *et al.*, 2001). Exposure of plants to excess Cu generates oxidative stress and ROS (Stadtman and Oliver, 1991). Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus *et al*., 2001). Qian *et al.* (2007) stated that, exposure to atrazine increased the level of malondialdehyde in *Chlorella vulgaris*, suggesting potential oxidative damage to the alga.

Afkar *et al.* (2010) reported that inhibitory and stimulatory effect of heavy metals depends on concentration, different organisms have different sensitivities to some metals and some organisms may be less or more damaged by different metals. According to Qian *et al.* (2009) copper and cadmium independently inhibits photosystem II activities and CO_2 assimilation but synergistically increased ROS contents to disrupt chlorophyll synthesis and inhibit cell growth in *Chlorella vulgaris*. Various sources of input such as mining, milling and surface finishing industries have contributed to metal pollution in the environment (Wang and Robert, 2000). The co-existence of a multiplicity of metal ions is often considered to give rise to distinct interactive effects (Mathad *et al.,* 2006).

Copper toxicity, also called copperiedus, refers to the consequences of an excess of copper in the body. Copperiedus can occur from eating acid foods cooked in uncoated copper cookware or from exposure to excess copper in drinking water or other environmental sources. It can also result from the genetic condition known as ‘Wilson's disease’.

2.5.1.2 *Lead (Pb)*

Lead is a commutative poison and a possible human carcinogen (Bakare - Odunola, 2005). It may also cause the development of autoimmunity which can lead to joint diseases and ailment of kidneys and circulatory system.At higher concentrations, lead can cause irreversible brain damage (EPA, 2015).

The general population is exposed to lead from air and food in roughly equal proportions. Earlier, lead in foodstuff originated from pots used for cooking and storage, and lead acetate was previously used to sweeten port wine (EPA, 1987). During the last century, lead emissions to ambient air have further polluted our environment, over 50% of lead emissions originating from petrol.

Occupational exposure to inorganic lead occurs in mines and smelters as well as welding of lead painted metal, and in battery plants. Low or moderate exposure may take place in the glass industry. High levels of air emissions may pollute areas near lead mines and smelters. Airborne lead can be deposited on soil and water, thus reaching humans *via* the food chain (Kunkel, 1986).

Up to 50% of the inhaled inorganic lead may be absorbed in the lungs. Adults take up 10–15% of lead in food, whereas children may absorb up to 50% *via* the gastrointestinal tract. Lead in blood is bound to erythrocytes, and elimination is slow and principally *via* urine. Lead is accumulated in the skeleton, and is only slowly released from this body compartment. Half-life of lead in blood is about 1 month and in the skeleton 20–30 years (WHO, 1995).

In adults, inorganic lead does not penetrate the blood–brain barrier, whereas this barrier is less developed in children. The high gastrointestinal uptake and the permeable blood–brain barrier make children especially susceptible to lead exposure and subsequent brain damage. Organic lead compounds penetrate body and cell membranes. Tetramethyl lead and tetraethyl lead penetrate the skin easily (EPA, 2015). These compounds may also cross the blood–brain barrier in adults, and thus adults may suffer from lead encephalopathy related to acute poisoning by organic lead compounds.

2.5.2.1 *Health effects*

Signs and Symptoms of lead include combinations of gastrointestinal complaints, hypertension, fatigue, haemolytic anaemia, abdominal pain, nausea, constipation, weight loss, peripheral neuropathy, cognitive dysfunction, arthralgias, headache, weakness, convulsions, irritability, impotence, loss of libido, depression of thyroid and adrenal function, chronic renal failure, gout. A patient with lead poisoning may have a combination of symptoms – or no symptoms at all until the condition has progressed. Mental symptoms include restlessness, insomnia, irritability, confusion, excitement, anxiety, delusions, and disturbing dreams (US DHHS, 2007).

The symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system. Lead encephalopathy is characterized by sleeplessness and restlessness (EPA, 2015). Children may be affected by behavioural disturbances, learning and concentration difficulties. In severe cases of lead encephalopathy, the affected person may suffer from acute psychosis, confusion and reduced consciousness. People who have been exposed to lead for a long time may suffer from memory deterioration, prolonged reaction time and reduced ability to understand. Individuals with average blood lead levels under3 μmol/l may show signs of peripheral nerve symptoms with reduced nerve conduction velocity and reduced dermal sensibility. If the neuropathy is severe the lesion may be permanent. The classical picture includes a dark blue lead sulphide line at the gingival margin. In less serious cases, the most obvious sign of lead poisoning is disturbance of haemoglobin synthesis, and long-term lead exposure may lead to anaemia.

Recent research has shown that long-term low-level lead exposure in children may also lead to diminished intellectual capacity (WHO, 1995). Acute exposure to lead is known to cause proximal renal tubular damage (WHO, 1995). Long-term lead exposure may also give rise to kidney damage and, in a recent study of Egyptian policemen, urinary excretion of NAG was positively correlated with duration of exposure to lead from automobile exhaust, blood lead and nail lead (Mortada *et al.*, 2001).

Despite intensive efforts to define the relationship between body burden of lead and blood pressure or other effects on the cardiovascular system, no causal relationship has been demonstrated in humans (WHO, 1995).

Using routinely collected data on mortality (1981–96), hospital episode statistics data 1992–1995 and statutory returns to the Health and Safety Executive (RIDDOR), one death and 83 hospital cases were identified (Elliott *et al.,* 1999). The authors found that, mortality and hospital admission ascribed to lead poisoning in England were rare, but that cases continue to occur and that some seem to be associated with considerable morbidity.

Lead poisoning is a medical condition in humans and other vertebrates caused by increased levels of the heavy metallead in the body. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders. Symptoms include abdominal pain, confusion, headache, anaemia, irritability, and in severe cases seizures, coma, and death (US DHHS, 2007).

Blood lead levels in children below 10 µmg/dl have so far been considered acceptable, but recent data indicate that there may be toxicological effects of lead at lower levels of exposure than previously anticipated. There is also evidence that certain genetic and environmental factors can increase the detrimental effects of lead on neural development, thereby rendering certain children more vulnerable to lead neurotoxicity (Lidsky and Schneider, 2003).

IARC classified lead as a ‘possible human carcinogen’ based on sufficient animal data and insufficient human data in 1987. Since then a few studies have been published, the overall evidence for lead as a carcinogen being only weak, the most likely candidates are lung cancer, stomach cancer and gliomas (Steenland and Boffetta, 2000).

2.6 Photosynthetic Pigment

Fifty percent of pollutants act via inhibition of photosynthesis at the photosystem-I (PSI) and PSII levels by replacing PSI's ultimate electron acceptor or by blocking PSII-catalyzed photosynthetic electron transport(He *et al.,* 2012). Pigments have often been used as biomarkers of exposure to different class of herbicides in autotrophic plants including algae (He *et al.,* 2012). Carotenoid protects chlorophyll and photosynthetic membrane from photo oxidative damage. Therefore decline in carotenoid content could have serious consequences on chlorophyll, as well as thylakoid membrane which may lead to the reduction in photosynthetic efficiency of *Plectonema boryanum* (Prasad and Zeeshan, 2005).Total chlorophyll contents of *Cladophora fracta* exposed to lead and cadmium at higher concentrations decreased significantly from those of controls after two days of exposure (Lamai *et al.,* 2005).

2.7 Effect on Antioxidant Enzymes

Oxidative stress has become an important subject for terrestrial and aquatic toxicology (Ames*et al.,* 1993). Regulated production of free radicals in organisms and maintenance of "redox homeostasis" are essential for the physiological health of organisms. Superoxide dismutase (SOD) is generally accepted as a first defence line protector against reactive oxygen species (ROS) and it is responsible for catalyzing the dismutation of oxygen to form oxygen and hydrogen peroxide which are decomposed by Catalase and Peroxidase into oxygen and water (Torres*et al.,* 2008). According to Anderson *et al.* (1995) Glutathione-s-Transferase exhibited clear logical response to copper, the response being greater in higher exposure group. In addition to anti-oxidant enzymes, photosynthetic organisms reduce concentrations of ROS with low molecular weight compounds such as ascorbate, glutathione, flavonoids, tocopherols and carotenoids (Collen*et al.,* 2003).

The activities of the anti-oxidant enzymes, Superoxides dismutase, Peroxidase and Catalase also increased markedly in the presence of glufosinate. Protein, carbohydrates, photosynthetic pigments levels, uptake and assimilation of nitrates, photosynthetic oxygen evolution and respiration, and growth of *Scenedesmus species* were inhibited by copper and zinc (Tripathi and Guar, 2006).

Valavanidis *et al.* (2006) suggested that, the balance between environmental pollution and anti-oxidant defence (enzymatic and non-enzymatic) in biological systems can be used to assess toxic effects under stressful environmental conditions by different classes of chemical pollutants. Cellular antioxidant defence systems in biological systems when exposed to environmental pollutants are depleted but levels of this anti-oxidant in living organisms may also redress the imbalance caused by the oxidative damage (Collen*et al.,* 2003).

2.8 Potency of *Lemna* as a Bioremediation Agent

The efficiency of carbon assimilation (primary production) in decontamination and detoxification from the water pathway has many aspects which includes: (1) generation of bio-mass for accumulation and immobilization of contaminants;(2) production of organic carbon in the form of litter for durable fixation depending on biomass quality and stability against decay; (3)quick removal of excess nutrients from the medium to fuel fast growth and primary production; and(4)production of a *Lemna*mat that has bio-redox advantages by reducing gas exchange, and harbouring microflora (Salt *et al*., 1995; Ensley *et al.,* 1996). No doubt growth and primary production is an important deciding factor for selecting phytoremediation species.

Generally, *Lemna* species are considered as very fast growing, thereby a high turnover and yield (Landolt, 1980,1986). Most *Lemna* species have a mean specific growth rate range of 0.2-0.3 d^{-1} with a doubling time in the range between 0.7 and 2 days (Landolt, 1980, 1986). However,*L. minor* and *L. gibba* can reach specific growth rate of about 0.6d^{-1}under ideal conditions, rich in nutrients (Reid and Stanley, 2003). Maximum relative growth rates (RGR) of 0.7 to0.79 have been observd in *L. aequinoctialis* and*Wolffia* microscopic which corresponds to doubling times between 20 and 24h (Korner *et al.*, 2003). Landolt and Kandeler (1987) estimated annual mean yield of *Lemna spp*. of 73 tonnes $ha^{-1}yr^{-1}$ dry biomass. Some yields of above 180 tonnes $h\text{-}^{1}yr^{-1}$ drymass have been recorded.

2.8.1 Hyperaccumulationin *Lemna* species

Plant species that accumulate appreciable quantities of metal in their tissues regardless of the concentration of metal in the media are classified as hyperaccumulators. As a rule of thumb, plant species should accumulate above 1000mg kg^{-1} of dry biomass (Wang *et al.*,2002). About a thousand plant species are known as bioaccumulators. The dominating species are from the Asteraceae, Brasssicaceae, Flacourtiaceae, Lamiaceae, Euphobiaceae, Fabiaceae, Poaceae and Lemnaceae. *Lemna* species can formally be categorised in the hyperaccumulator category.Normally, hyperaccumulator plants have co-evolved in a metal rich environment like bed rock (Baker and Walker, 1990). Hyperaccumulation in*Lemna* species is related to direct contact between the plant and contaminants, which are controlled by the water chemical conditions (anaerobic and micro-anaerobic) below the *Lemna* canopy. These conditions are triggered by *Lemna*'s organic matter production and they can be especiallyinduced and regulated by changing the physicochemical conditions, nutrient regime and contaminant loading in the milieu (Mkandawire and Dudel, 2005).

2.9 Potency of *Salvinia* for phytoremediation studies

Salvinia is a free-floating aquatic macrophyte of the family Salviniaceae. Its wide distribution, faster growth rate and close relation with other water ferns, including *Azolla* and *Lemna*, make it capable for phytoremediation. A number of species of this aquatic plant have been studied for phytoremediation of toxic elements (Banerjee and Sarker, 1997; Espinoza-Quinones *et al.*, 2005; Sanchez-Galvan *et al.*, 2008; Dhir, 2009; Wolff *et al.*, 2009). Only two species, *S. natans* and *S. minima*, have been investigated for arsenic remediation (Hoffmann *et al.*, 2004; Rahman *et al.*, 2008). Arsenic uptake kinetics of *S. minima*reveals that, arsenic uptake was increased with increasing exposure time and arsenic concentration in the growth solution (Hoffmann *et al.*, 2004). The increasing phosphate concentration decreased arsenic uptake in *S. minima* (Hoffmann *et al.*, 2004), which is similar to that of other aquatic floating macrophytes. Influence of phosphate and iron ions in selective uptake of arsenic species by *S. natans* L. was studied by Rahman *et al.* (2008). The results showed that, increasing phosphate concentration in culture solution decreased the arsenic uptake into the plant significantly, when the source of arsenic was As (V). On the other hand, arsenic uptake was not influenced by phosphate when the source was dimethylamylamine (DMAA). Significant correlation was reported between arsenic and iron content in tissues of *S. natans* L. when the sourcewas As(V), while the correlation was not significant when thesource was DMAA (Rahman *et al.,* 2008). The results indicate thatDMAA uptake mechanisms into *S. natans* L. are diferent from thoseof As (V), which is also same for *S. polyrhiza* (Rahman *et al.*, 2007).

Ferns of the *Salvinia* genus have also been shown to have a useful application in removing metals from the environment. These plants are considered to be promising plants because of their high ability to accumulate great quantities of metals in their tissues, including Cd (Olguín *et al.*, 2002), As and Pb (Hoffmann *et al.*, 2004), Cr (Nichols *et al.*, 2000), and atrazine (Guimarães *et al.*, 2011).

2.10 Potency of Water lettuce (*P. stratiotes*) for Phytoremediation Studies

Pistia is a genus of aquatic plant in the family Araceae, comprisinga single species, *P. stratiotes*, often called water cabbage orwater lettuce. It floats on the surface of the water, and its rootshanging submersed beneath floating leaves. It is a common aquaticweed in the United States, particularly in Florida where it may clogwaterways.Although,*P. stratiotes* mats degrade water quality by blockingthe air–water interface, reducing oxygen levels in the water, andthus threatening aquatic life, it has been tested for metal remediation(Odjegba and Fasidi, 2004; Skinner *et al.*, 2007), metal detoxification(Tewari *et al.*, 2008), and treatment of urban sewage

(Zimmels *et al.*, 2006). Arsenic uptake by *P. stratiotes* has not beenstudied extensively. A field study carried out by Lee *et al.* (1991)reported that, the average arsenic enrichment (bioaccumulation)factor of *P. stratiotes* was 8632 in roots and 2342 in leaf. It appearsthat, arsenic translocation in *P. stratiotes* was slow and most of thearsenic was strongly adsorbed onto root surfaces from solution.This agrees with the earlier findings that arsenic compounds areless readily translocated through the root system of aquatic plants.

CHAPTER THREE

3.0 MATERIALSANDMETHODS

3.1 Description of Study Area:

The practical set up was carried out inthe Physiology Laboratory,Department of Biological Sciences, Ahmadu Bello University, Zaria. The University is located at latitude 122^0 12^{11} N and longitude 07^0 37^{11} E, altitude of 550 -700m above sea level. Zaria is located within the Northern Guinea savanna of Nigeria characterized by distinct rainy season (May – October) and dry season (Nov – April).

3.2 Experimental Plants

Three aquatic macrophytes namely *Pistia stratiotes*,*Lemna trisulca*and *Salvinia molesta* were collected from their natural habitat and introduced into plastic containers to assess their heavy metal removal capacities for two metals (lead and copper) in a laboratory experiment.*Lemna trisulca* were collected from the pond in thegarden of Biological sciences department, *Pistia stratiotes* were collected from their natural habitat (pond) in Hanwa extension, near Limi Hospital, Zaria and *Salvinia molesta* were collected from river Galma.

The aquatic macrophytes that are used in any phytoremediation process must meet two main criteria: First, they must present a high growth rate, and second, high absorption ability for the analyzed pollutant (Vissottiviseth *et al.*, 2002). Thus, the present research assessed the ability of three macrophytes to sequester lead and copper. The leaf colour, content of pigments (chlorophyll a and chlorophyll b), anti-oxidant response and content of Cu and Pb accumulated in the vegetal tissues will be used as parameters for the analyses.

3.3 Experimental Design

This consists of a factorial combination of the two metals in different concentrations. Treatments were replicated three times and laid in a complete randomized design (CRD).

Experimental Combinations for Lead and Copper

	Cu0	Cu1	Cu2	Cu3
Pb0	Pb0Cu0	Pb0Cu1	Pb0Cu2	Pb0Cu3
Pb1	Pb1Cu0	Pb1Cu1	Pb1Cu2	Pb1Cu3
Pb2	Pb2Cu0	Pb2Cu1	Pb2Cu2	Pb2Cu3
Pb3	Pb3Cu0	Pb3Cu1	Pb3Cu2	Pb3Cu3

Key:

Pb0 = Lead at 0mg/L

Pb1 = Lead at 0.05mg/L

Pb2 = Lead at 1.0mg/L

Pb3 = Lead at 1.5mg/L

Cu0 = Copper at 0mg/L

Cu1 = Copper at 0.5mg/L

Cu2 = Copper at 1.0mg/L

Cu3 = Copper at 1.5mg/L

3.4 The Acquisition and Acclimation of the Plants

The species *Lemna trisulca, Pistia stratiotes* and *Salvinia molesta* were obtained from their natural habitats. To meet the experimental conditions and obtain enough biomass, the plants were pre-cultivated in a Knop's nutrient medium/solution, consisting of $Ca(NO_3)_2$ (0.492g), KH_2PO_4 (0.136g), KCl (0.075g), MgSO4 (0.06g) and FeCl3 (0.025g) per liter of water at a P^H of 6.5±0.5. One Normal base (NaOH) and acid (H_2SO_4) were prepared by dissolving 20g and 13.9ml of the base and acid respectively in distilled water. These were used to maintain the P^H of the medium. The plants were placed in plastic containers containing 7L of the prepared Knop nutrientsolution in a growth room at temperatures of 25±1°C, illuminated with fluorescent bulbs for 21 days. The nutrient solution was renewed every 4 days and the pH maintained at 6.5±0.5.

3.5 Experimental Set–up and Introduction of Metals

The experiment was performed under the same microclimatic conditions established during the acclimation period. This experiment was conducted for all the species of macrophytes. At the beginning of the experiment, 7L of Knop's solution were measured into each 10L plastic container (bowl). The plants/macrophytes were then introduced to fill the surface of the containers of Knop's solution. The plants were allowed to acclimatize/stabilize in the growth medium for seven days before the metals were introduced. The metals were then introduced in the form of copper nitrate and lead nitrate at concentrations specified above. The control groups were cultivated only in the Knop's solution, and the pH was maintained at 6.5±0.5. The experiment was conducted with three repetitions, using a completely randomized design, under the same conditions.

The applied concentrations were 0.5, 1.0 and 1.5 mg/L of Copper nitrate and 0.05, 1.0 and 1.5 mg/L of Lead nitrate. For lead, 0.35, 7 and 10.5ml per 7L of water were introduced while 3.5ml, 7ml and 10.5ml of the copper nitrate stock were introduced into containers containing 7L of water.Controls without heavy metals were run simultaneously. The control groups were cultivated in the Knop's solution without the addition of Cu and Pb. The pH of each solution were measured daily and, whenever necessary, corrected with HCl (1 M) or NaOH (1 M) to 6.5±0.5. The experiment had duration of 21 days.

The evaluation included chlorophyll **a** and **b**contents, Catalase and Peroxidase activity, leaf morphology/color and analysis of the metal contents over the period of time that the experiment was set up.

3.6 Standard preparation:

All chemicals and reagents were of the analytical grade and were obtained from Chemicalstore in Zaria.Stock solutions of copper and lead (1000mg/kg) were prepared bydissolving in a 1 litre volumetric flask 3.80g and 1.60g of copper nitrateand Lead nitrate respectively with 1000ml of distilled water.

3.7 The Determination of Pigments:Chlorophyll a and Chlorophyll b

The chlorophylla and chlorophyll b contents of each species were determined with 0.5g of the fresh vegetal matter. The samples were placed in a plastic centrifuge tube, mashed/crushed with a glass rod and and 3mls of 80% (v/v) acetone were added, centrifuged at 2500 rpm for 10 minutes. The absorbance reading of the supernatant was conducted at wavelengths of 645 nm and 663 nm, in a UV visible Spectrophotometer (UVmini-1240, Shi-madzu, Japan). The total contents of chlorophyll a and b were calculated using equationsproposed by Arnon(1949). These equations were proven to be correct and reliable by Hiscox and Israelstam (1979) and Mackinney (1941).

Chl**a** (mg g^{-1}) = 0.0127 A663 – 0.00269 A645

Chl**b** (mg g^{-1}) = 0.0029 A645 – 0.00468 A663

3.8 Antioxidant enzyme extraction and assays

3.8.1 Assay of Catalase

Catalase activity in the selected macrophytes samples were determined by the method of Luck (1974).

3.8.1.1 *Principle*

The UV light absorption of hydrogen peroxide can be easily measured between 230– 250 nm. On decomposition of hydrogen peroxide by Catalase, the absorption decreases with time. The enzyme activity can be estimated by this decrease in absorption.

3.8.1.2 *Reagents*

1. Phosphate buffer 0.067 M (pH 7.0)
2. Hydrogen peroxide in phosphate buffer (2mM)

3.8.1.3 *Procedure*

A 20% homogenate (0.2g) of the plant samples were prepared in phosphate buffer (0.067M, PH 7.0) and the homogenate was employed for the assay. The samples were read against a control without homogenate, but containing the H_2O_2-phosphate buffer in a UV VIS Spectrophotometer (Spectrum lab 752S).

To the experimental cuvette, 2.9mL of H_2O_2 phosphate buffer was added, followed by the rapid addition of 0.1mlcatalase enzyme extract and mixed thoroughly. The samples were read against a control containing 3mL H_2O_2phosphate buffer without enzyme extract. The time interval required for a decrease in absorbance by 0.05 units was recorded at 240 nm. The enzyme solution containing H_2O_2 free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

The Catalase activity was calculated as follows:

Catalase activity (units/mL enzyme) = (3.45) / (min) (0.1)

Where,

3.45 correspond to the decomposition of 3.45 micromoles of hydrogen peroxide in a 2.0 ml of reaction mixture.

0.1= Volume of enzyme used (in milliliters)

Min = time interval required for a decrease in absorbance by 0.05 units

3.8.2 Assay of Peroxidase

The activity of Peroxidase was measured by themethod of Reddy *et al.* (1995).

3.8.2.1 *Principle*

Peroxidase catalyses the conversion ofH_2O_2 to H_2O and O_2in the presence of the hydrogen donor pyrogallol. The oxidation of pyrogallol to a coloured product called purpurogalli can be measured spectrophotometrically at 430 nm with the specified time interval. The intensity of the product is proportional to the activity of the enzyme

3.8.2.2 *Reagents*

1. Pyrogallol (0.05 M in 0.1 M phosphate buffer, pH 6.5)

2. H_2O_2 (1% in 0.1M phosphate buffer, pH 6.5)

3.8.2.3 *Procedure*

The plant samples were prepared as 20% homogenate (0.2g) in 0.1M phosphate buffer (PH 6.5)and used for the assay in a UV VIS Spectrophotometer (Spectrum lab 752S). Pyrogallol solution (3.0mL) and enzyme extract (0.1 mL) was pipette into a cuvette. The spectrophotometer was adjusted to read zero at 430 nm followed by the addition of 0.5 ml of 1% H_2O_2 and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes. One unit of Peroxidase activity was defined as the change in absorbance per minute at 430 nm.

$$\text{Peroxidase activity (units/mg)} = \frac{\Delta A_{430}/\text{mins}}{6.58 \times \text{ml enzyme/mL reaction mixture}}$$

Where,

A = Absorbance

3.9 Determination of Morphological Variation

The leaf colours of the macrophytes (controls and treatments) were observed before and 5 days after the addition of the metals. Observations were recorded according tothe pattern of Lizieri *et al.*(2012).

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3.10 Collection of Plant Samples for Determination of Metal Accumulation

Plant samples were collected five times (initial, days 9, 12, 15 and 18) during the experimental set-up to determine the uptake of metals by the aquatic macrophytes. The collected samples from the plastic bowls were cleaned properly and kept in paper envelopes. The enveloped samples wereoven dried to a constant weight at 70^{0}C for 72 hours.Dried samples were ground to fine powdermanually using the pestle and mortars and weighed.

3.11 Digestion and Analysis of Samples

The determination of the contents of lead and copper accumulated in the plants wasdone through the humid mineralization of dried samples. Using a sample volume of 100mL, 5 volume of concentrated HCl and 1 volume of concentrated HNO_3, the samples were acid digested in an autoclave at a temperature of 120°C until the complete digestion of the organic matter (Sivaci *et al.* 2004; ASTM D1971 -91, 1991). The acid digested samples were re-filtered and the filtrateswere topped up to 100 mL with deionized water. The determination of the total accumulated metals in the plants was done using Atomic Absorption Spectrophotometry (AAS) (AA 240 FS, Varian Australia) at Centre for Energy Research and Training (CERT) A.B.U and Multi User Research Laboratory, Department of Chemistry, A.B.U., Zaria, Kaduna State.

3.12 Data Analyses

The significant differences among the different treatments weredetermined by one-way analysis of variance (ANOVA). Where significant differences exist, Duncan Multiple Range Test was used to separate the means. A significant relationship/correlation between the treatments and response variables were determined using a correlation based principal components analysis (PCA).

CHAPTER FOUR

4.0 RESULTS

4.1 Chlorophyll Content Determination at Different Copper and Lead Concentrations

4.1.1 Chlorophyll **a**

In *Salvinia molesta*, chlorophyll **a** content is higher in the control.With increase in the concentration of the metals (lead and copper), there was reduction in the photosynthetic pigment (chlorophyll a) (Figure 4.1). Chlorophyll a production was negatively associated with copper and lead concentrations. The longer the plants stayed in the medium containing the metals, the lower the chlorophyll a content of the plant. The lower value was observed in the treatment with the highest concentration of the two metals (6.30±0.01) as seen in Fig. 4.1.There were significant differences in the various treatments with the control having the highest value (8.40±0.00) and the treatment with the highest concentrations of the two metals (CU3PB3) having the least value of chlorophyll a (6.30±0.01), followed by CU2PB3 (6.58±0.03).

In *Pistia stratiotes*, chlorophyll **a** content was highest in the control (5.84±0.01) followed by the treatment with only lead at concentration 1 without copper (5.48±0.02), then copper at concentration 1 without lead (Figure 4.2). As concentration of the metals increased, the pigment content reduced in the treatments. The longer the plants stayed in the medium containing the pollutants, the lower the chlorophyll a. There were significant differences in the chlorophyll a value with the control having the highest value and it continued to decrease as the concentration of metals increased (Fig. 4.2).

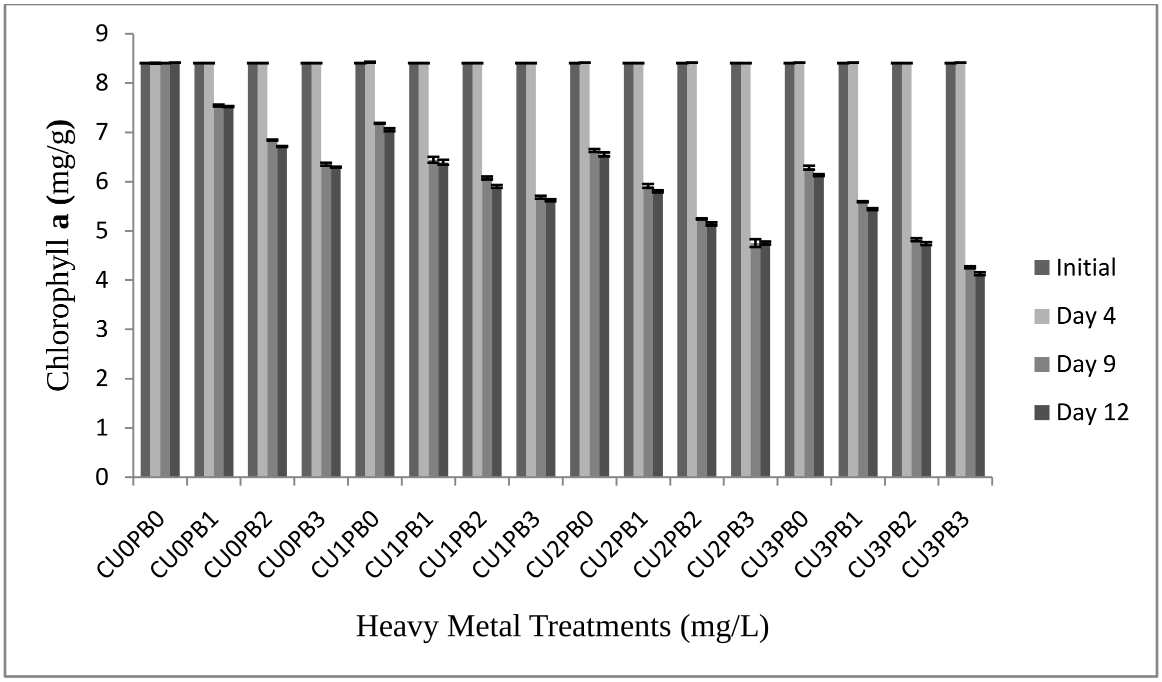

Fig. 4.1: Chlorophyll **a** content in *Salvinia molesta* at different Copper and Lead concentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

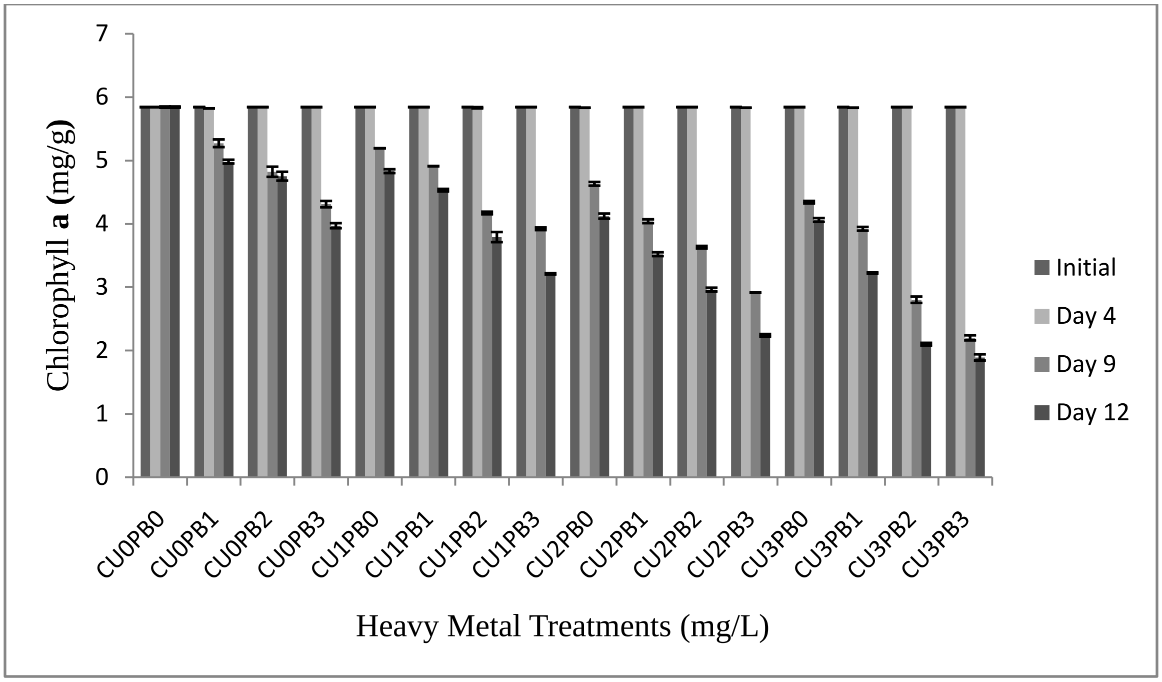

Fig. 4.2: Chlorophyll **a** contents in *Pistia stratiotes* at different Copper and Leadconcentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

For *Lemna trisulca*, the value of the chlorophyll **a** decreased with increase in the concentration of the metals (Fig. 4.3). The control had the highest value of 12.64±0.08 followed by CU0PB1 11.38±0.03, then CU1PB0 (11.18±0.01). The least value was recorded In CU3PB3 (8.03±0.02) i.e the treatment with the highest concentration of copper and lead. There were significant differences (P≤0.05) in the value of chlorophyll a in *Lemna trisulca* (Fig. 4.3).

In overall chlorophyll **a**, there was no significant difference in the various treatment before the introduction of metals (day 4) but after the introduction of the metals, the value of chlorophyll a continued to decrease with increase in concentration, and also increase in the days the plants stayed in the containers (Table 4.1). The longer the plants stayed in the medium, the higher the reduction in the value chlorophyll a. There were significant differences in the comparison between chlorophyll a levels among the different plants. *Lemna trisulca*, has higher mean value of chlorophyll a **(**10.15±0.16**)** followed by *Salvinia molesta*(7.24±0.08) and then *Pistia stratiotes***(**4.9±0.07**)**had the least value chlorophyll a (Table 4.2).

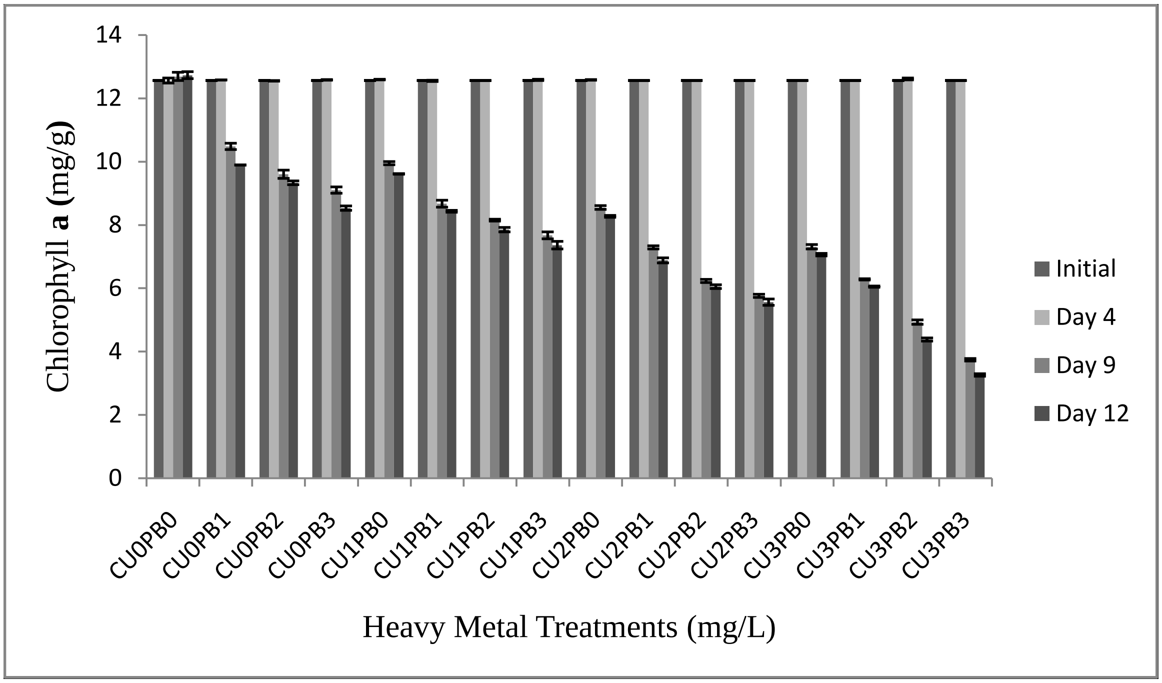

Fig. 4.3: Chlorophyll **a** contents in *Lemna trisulca*at different Copper and Leadconcentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.1: Overall Chlorophyll **a** content of the 3 macrophytesat different Copper and Leadconcentrations

Treatments	**Chlorophyll a (mg/g)**				
	Initial	**Day 4**	**Day 9**	**Day 12**	**Mean**
CU0PB0	8.93±0.98	8.93±0.98	$8.98{\pm}1.00^{a}$	$8.99{\pm}1.00^{a}$	$8.96{\pm}0.99^{a}$
CU0PB1	8.93±0.98	8.93±0.98	$7.76{\pm}0.76^{ab}$	$7.46{\pm}0.71^{ab}$	$8.27{\pm}0.86^{ab}$
CU0PB2	8.93±0.98	8.93±0.98	$7.09{\pm}0.69^{bcd}$	$6.93{\pm}0.66^{bc}$	$7.97{\pm}0.83^{ab}$
CU0PB3	8.93±0.98	8.94±0.98	$6.59{\pm}0.69^{bcde}$	$6.26{\pm}0.66^{bcd}$	$7.68{\pm}0.83^{ab}$
CU1PB0	8.93±0.98	8.95±0.98	$7.44{\pm}0.69^{abc}$	$7.16{\pm}0.69^{bc}$	$8.12{\pm}0.84^{ab}$
CU1PB1	8.93±0.98	8.93±0.98	$6.67{\pm}0.55^{bcde}$	$6.45{\pm}0.56^{bcd}$	$7.75{\pm}0.77^{ab}$
CU1PB2	8.93±0.98	8.93±0.98	$6.13{\pm}0.57^{bcdef}$	$5.85{\pm}0.59^{bcde}$	$7.46{\pm}0.78^{ab}$
CU1PB3	8.93±0.98	8.94±0.98	$5.75{\pm}0.54^{cdefg}$	$5.40{\pm}0.60^{cdef}$	$7.26{\pm}0.77^{ab}$
CU2PB0	8.93±0.98	8.94±0.98	$6.60{\pm}0.57^{bcde}$	$6.32{\pm}0.60^{bcd}$	$7.70{\pm}0.78^{ab}$
CU2PB1	8.93±0.98	8.93±0.98	$5.75{\pm}0.47^{cdefg}$	$5.40{\pm}0.50^{cdef}$	$7.25{\pm}0.72^{ab}$
CU2PB2	8.93±0.98	8.94±0.98	$5.03{\pm}0.38^{efgh}$	$4.72{\pm}0.46^{defg}$	$6.91{\pm}0.69^{ab}$
CU2PB3	8.93±0.98	8.93±0.98	$4.47{\pm}0.42^{fgh}$	$4.18{\pm}0.50^{efg}$	$6.63{\pm}0.71^{ab}$
CU3PB0	8.93±0.98	8.94±0.98	$5.98{\pm}0.44^{bcdefg}$	$5.75{\pm}0.44^{bcde}$	$7.40{\pm}0.70^{ab}$
CU3PB1	8.93±0.98	8.93±0.98	$5.26{\pm}0.35^{defg}$	$4.90{\pm}0.43^{defg}$	$7.01{\pm}0.67^{ab}$
CU3PB2	8.93±0.98	8.95±0.99	$4.18{\pm}0.35^{gh}$	$3.74{\pm}0.41^{fg}$	$6.45{\pm}0.65^{ab}$
CU3PB3	8.93±0.98	8.93±0.98	$3.40{\pm}0.31^{h}$	$3.09{\pm}0.33^{g}$	$6.09{\pm}0.59^{b}$
Mean	**8.93±0.98**	**8.94±0.23**	**6.07±0.18**	**5.79±0.19**	**7.43±0.19**
P value	**1.000ns**	**1.000ns**	**0.000****	**0.000****	**0.562ns**

Means with the same superscript along column are not significant at P>0.05. ns – not significant at P>0.05, ** - highly significant at P ≤ 0.01. CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.2: Comparison of Chlorophyll **a** content among the 3 macrophytes

Plants	**Chlorophyll a (mg/g)**				
	Initial	**Day 4**	**Day 9**	**Day 12**	**Mean**
Salvinia	8.40±0.00[b]	8.41±0.00[b]	6.12±0.15[b]	6.03±0.16[b]	7.24±0.08[b]
Pistia	5.84±0.00[c]	5.83±0.00[c]	4.18±0.14[c]	3.75±0.16[c]	4.90±0.07[c]
Lemna	12.56±0.00[a]	12.57±0.01[a]	7.90±0.32[a]	7.58±0.33[a]	10.15±0.16[a]
Mean	**8.93±0.23**	**8.94±0.23**	**6.07±0.18**	**5.79±0.19**	**7.43±0.19**
P value	**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

4.1.2 Chlorophyll **b**

The chlorophyll **b** value (level) in *Salvinia molesta* did not show great variation before the introduction of metals i.e day 4 in Table 4.6. After the metals were introduced, the value reduced drastically in the various treatments except in the control. With increase in the concentration of the metals, there was decrease in the level of chlorophyll **b.** Treatment with only one of the metals did not show reduction in chlorophyll **b**value like those with the two metals especially the ones with the highest concentration of the metals. At day 9 and 12, there were significant difference in the level of chlorophyll **b** between the various treatments. The control had the highest value (17.16±0.02 and 17.21±0.05) and CU3PB3 having the least value (10.86±0.11 and 10.58±0.11) at day 9 and 12 respectively (Fig.4.4).

For *Pistia stratiotes*, there was no significant difference in chlorophyll **b** value before the introduction of metals, but after the introduction of metals there were significant differences in the various treatments (Fig.4.5). The higher the concentration of the metals, the lower the value of chlorophyll **b** pigment.

In Figure 6, *Lemna trisulca* showed significant difference in the value of chlorophyll **b** after the introduction of the metals. The value of chlorophyll **b** continued to decrease with increase in concentration of the metals (Table 4.3, 4.4). The value were higher in the control at day 9 and 12 (28.16±0.04 and 28.36±0.16), followed by CU0PB1 (26.88±0.08 and 26.32±0.45) followed by CU1PB0 (25.62±0.09 and 25.07±0.02) and the least value was recorded in CU3PB3 (12.71±0.07 and 12.36±0.15) (Table 4.3).

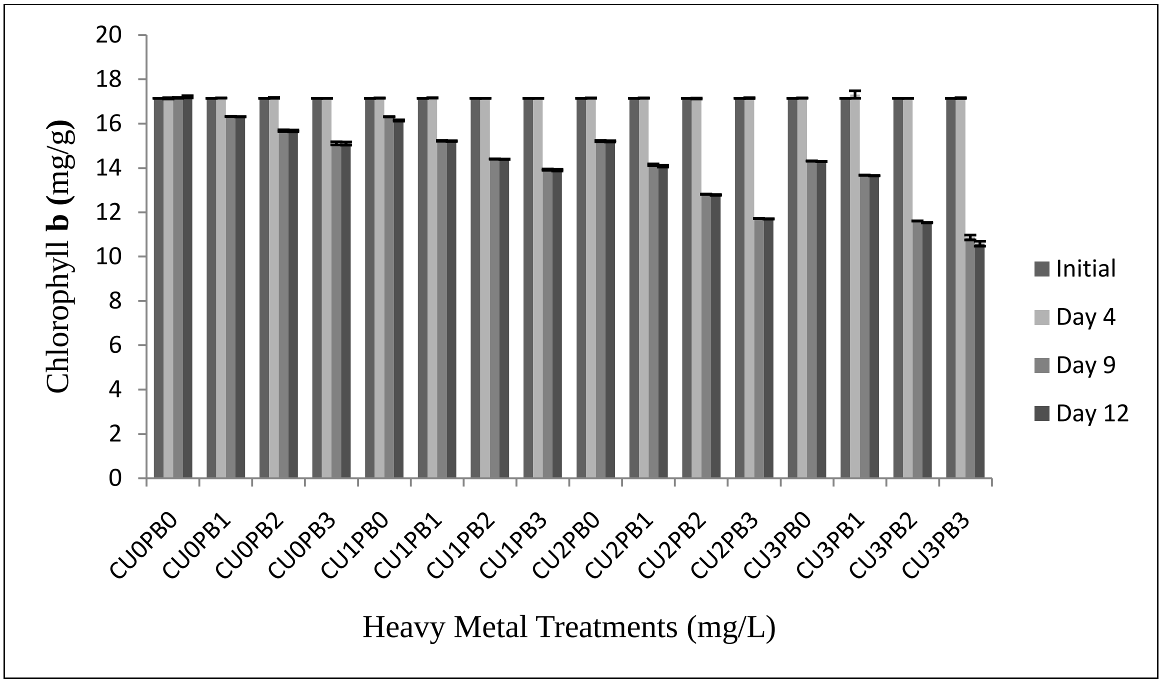

Fig. 4.4: Chlorophyll **b** contents in *Salvinia molesta* at different Copper and Leadconcentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

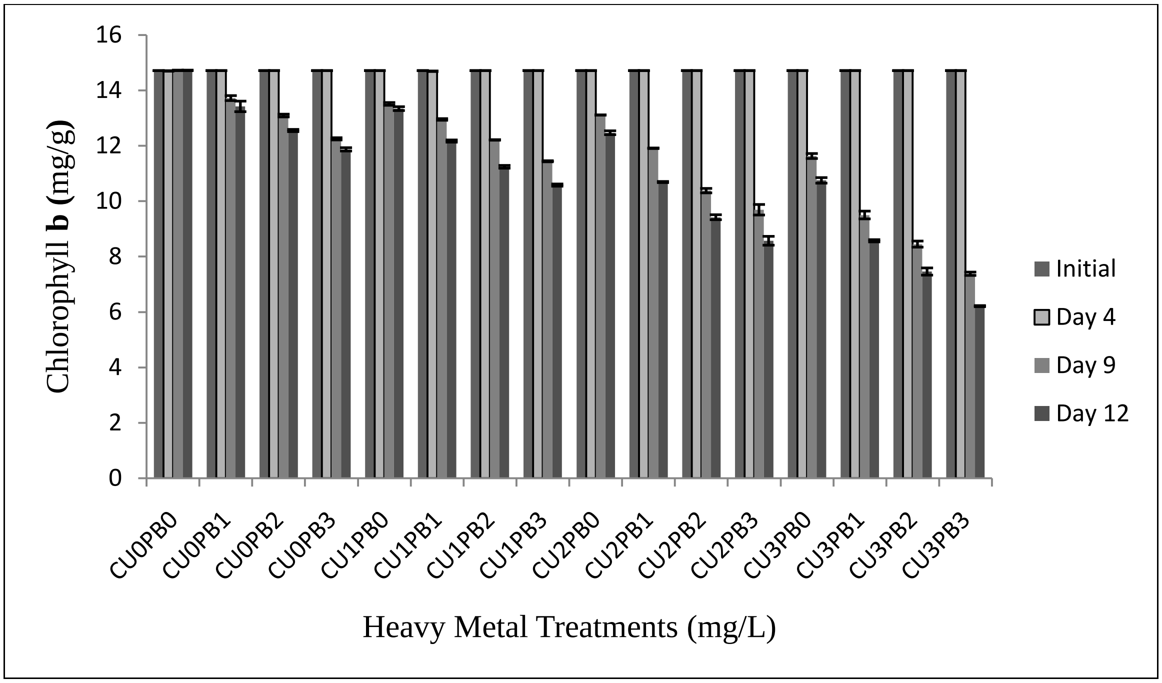

Fig. 4.5: Chlorophyll **b** contents in *Pistia stratiotes* at different Copper and Leadconcentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L,

PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

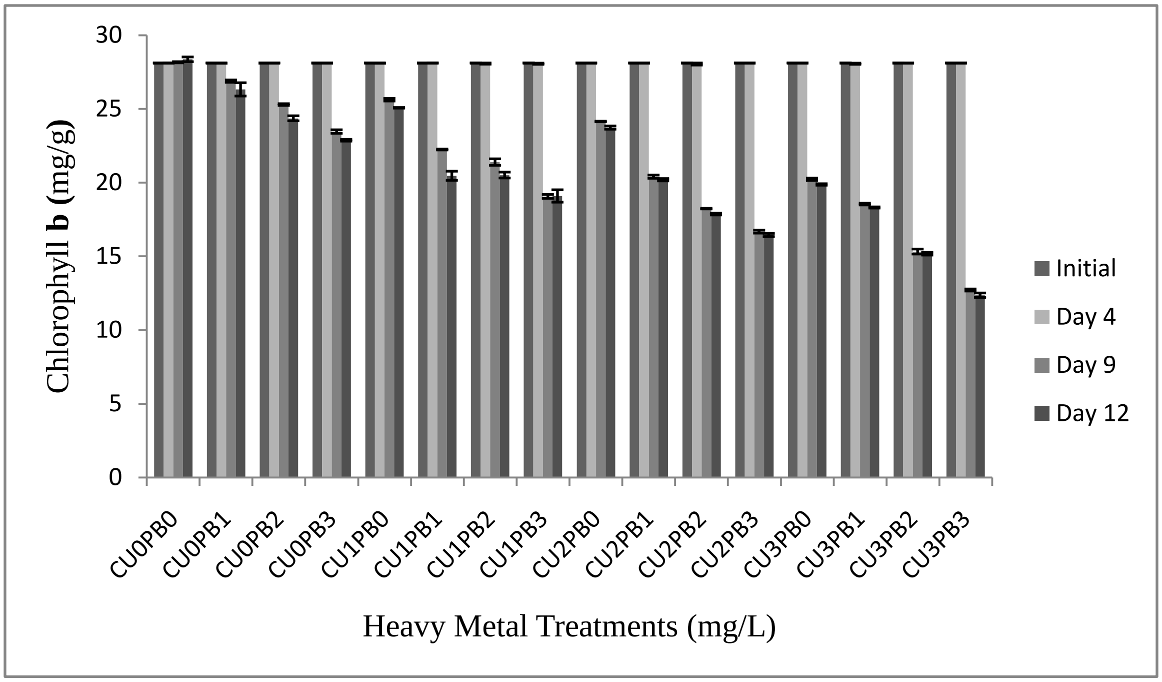

Fig. 4.6: Chlorophyll **b** contents in *Lemna trisulca* at different Copper and Leadconcentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

With increase in the concentration of the metals (copper and lead), there was a decrease in the value of chlorophyll **b**; with increase in the number of days, there was reduction in the chlorophyll b content. This implies that chlorophyll **b** content was negatively associated with exposure time (Table 4.3).

Table 4.4 showed the comparison between chlorophyll **b** levels among the three plants species. *Lemna* species had the highest chlorophyll **b** mean value (24.47±0.31) followed by *Salvinia molesta*(15.70±0.13) and the least were recorded in *Pistia stratiotes* (12.75±0.18).

Table 4.3: Overall Chlorophyll **b** contentof the 3 macrophytes at different Copper and Leadconcentrations

Treatments	**Chlorophyll b(mg/g)**				
	Initial	**Day 4**	**Day 9**	**Day 12**	**Mean**
CU0PB0	19.98±2.06	19.99±2.06	20.01±2.07^{a}	20.10±2.10^{a}	20.02±2.07^{a}
CU0PB1	19.98±2.06	19.99±2.06	18.97±2.01^{ab}	18.69±1.96^{ab}	19.41±2.02^{a}
CU0PB2	19.98±2.06	16.72±3.64	18.02±1.85^{abc}	17.53±1.77^{abc}	18.06±2.25^{a}
CU0PB3	19.98±2.06	19.98±2.06	16.94±1.68^{abcd}	16.61±1.63^{abcd}	18.38±1.86^{a}
CU1PB0	19.98±2.06	18.35±3.01	18.48±1.83^{abc}	18.19±1.77^{abc}	18.75±2.10^{a}
CU1PB1	19.98±2.06	19.99±2.06	16.80±1.40^{abcd}	15.95±1.21^{abcde}	18.18±1.68^{a}
CU1PB2	19.98±2.06	19.97±2.06	16.00±1.38^{abcde}	15.38±1.36^{abcde}	17.83±1.71^{a}
CU1PB3	19.98±2.06	19.97±2.05	14.81±1.12^{bcdef}	14.52±1.24^{bcde}	17.32±1.61^{a}
CU2PB0	19.98±2.06	19.99±2.06	17.49±1.69^{abcd}	17.13±1.70^{abcd}	18.65±1.88^{a}
CU2PB1	19.98±2.06	19.99±2.06	15.48±1.27^{abcde}	14.99±1.39^{bcde}	17.61±1.69^{a}
CU2PB2	19.98±2.06	19.96±2.05	13.81±1.16^{cdef}	13.35±1.23^{cdef}	16.78±1.62^{a}
CU2PB3	19.98±2.06	19.99±2.06	12.69±1.04^{def}	12.24±1.15^{def}	16.23±1.57^{a}
CU3PB0	19.98±2.06	19.10±1.80	15.39±1.27^{abcde}	14.97±1.33^{bcde}	17.36±1.58^{a}
CU3PB1	19.98±2.06	20.03±2.04	13.90±1.31^{cdef}	13.51±1.41^{cdef}	16.86±1.68^{a}
CU3PB2	19.98±2.06	19.98±2.06	11.80±0.99^{ef}	11.39±1.11^{ef}	15.79±1.54^{a}
CU3PB3	19.98±2.06	19.99±2.06	10.31±0.78^{f}	9.72±0.92^{f}	15.00±1.41^{a}
Mean	**19.98±0.49**	**19.62±0.54**	**15.68±0.41**	**15.27±0.42**	**17.64±0.43**
P value	**1.000ns**	**1.000ns**	**0.000****	**0.000****	**0.909ns**

Means with the same superscript along column are not significant at P>0.05. ns – not significant at P>0.05, ** - highly significant at P ≤ 0.01. CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.4: Comparison of Chlorophyll **b**content among the 3 macrophytes

	Chlorophyll b(mg/g)				
Plants	**Initial**	**Day 4**	**Day 9**	**Day 12**	**Mean**
Salvinia	17.14 ± 0.00^{b}	17.16 ± 0.01^{b}	14.28 ± 0.26^{b}	14.23 ± 0.26^{b}	15.70 ± 0.13^{b}
Pistia	14.71 ± 0.00^{c}	13.79 ± 0.52^{c}	11.62 ± 0.29^{c}	10.88 ± 0.33^{c}	12.75 ± 0.18^{c}
Lemna	28.11 ± 0.00^{a}	27.93 ± 0.17^{a}	21.14 ± 0.61^{a}	20.69 ± 0.60^{a}	24.47 ± 0.31^{a}
Mean	**19.98±0.49**	**19.62±0.54**	**15.68±0.41**	**15.27±0.42**	**17.64±0.43**
P value	**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at $P>0.05$. ** - highly significant at $P \leq 0.01$.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 - 0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

4.2 Antioxidant Enzyme Activities

4.2.1 Catalase (CAT)

Fig. 4.7 showed the activity of the enzyme catalase in *Salvinia molesta*. There were significant differences (at $P \leq 0.05$) in the activities of the enzymes after the introduction of the metals (copper and lead) into a containers/medium. The activities of the enzyme catalase increase greatly with increase in the concentration of the metals.

The higher the concentration of the metals, the higher the activities of the enzyme, and the longer the plants stayed in the medium, the higher the activities of the enzyme catalase. i.e*Salvinia* plants collected on day 14 (1.841±0.00) had higher activities than that of day 11 (1.840±0.00) which in turn had the enzyme activities higher than that of day 8 (1.839±0.00) Fig. 4.7. The activities of catalase in *Salvinia molesta* were highly significant at $P \leq 0.05$ in day 8, day 11 and day 14.

For *Pistia stratiotes*, the higher the concentration of the metals (copper and lead) the higher the activity of catalase. These were significant differences ($P \leq 0.05$) in the activities of enzyme at the different days (Fig. 4.8). The activities of catalase was higher in the treatment with the highest concentrations of metals; CU3PB3 (lead at 1.5 mg/L and copper at 1.5 mg/L) in day 11 and 14 1.847 ± 0.00 and it is least in CU0PB1 (1.840 ± 0.00) although this is also higher than the control (1.836 ± 0.00). In day 8, 11 and Day 14, the activities of catalase was higher in CU3PB3 with 1.846 ± 0.00, 1.847 ± 0.00 and 1.847 ± 0.00 reading respectively and they were highly significant at ($P \leq 0.05$) in the three days.

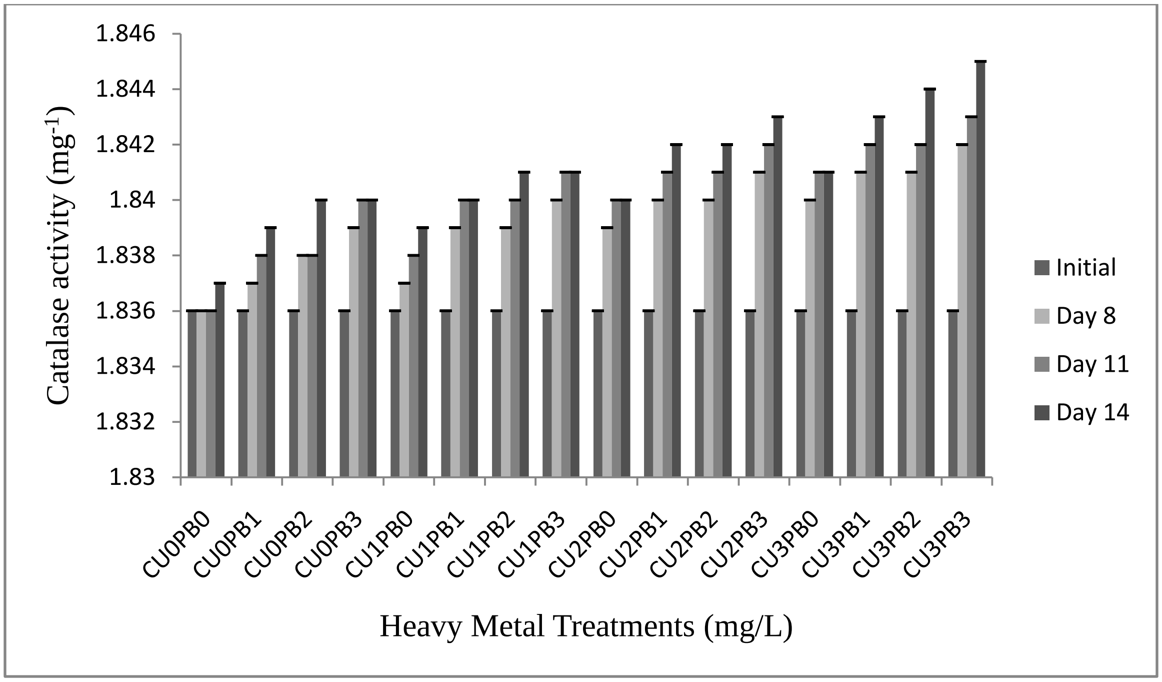

Fig. 4.7: Catalase activity of *Salvinia molesta* as a function of different Copper and Lead concentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

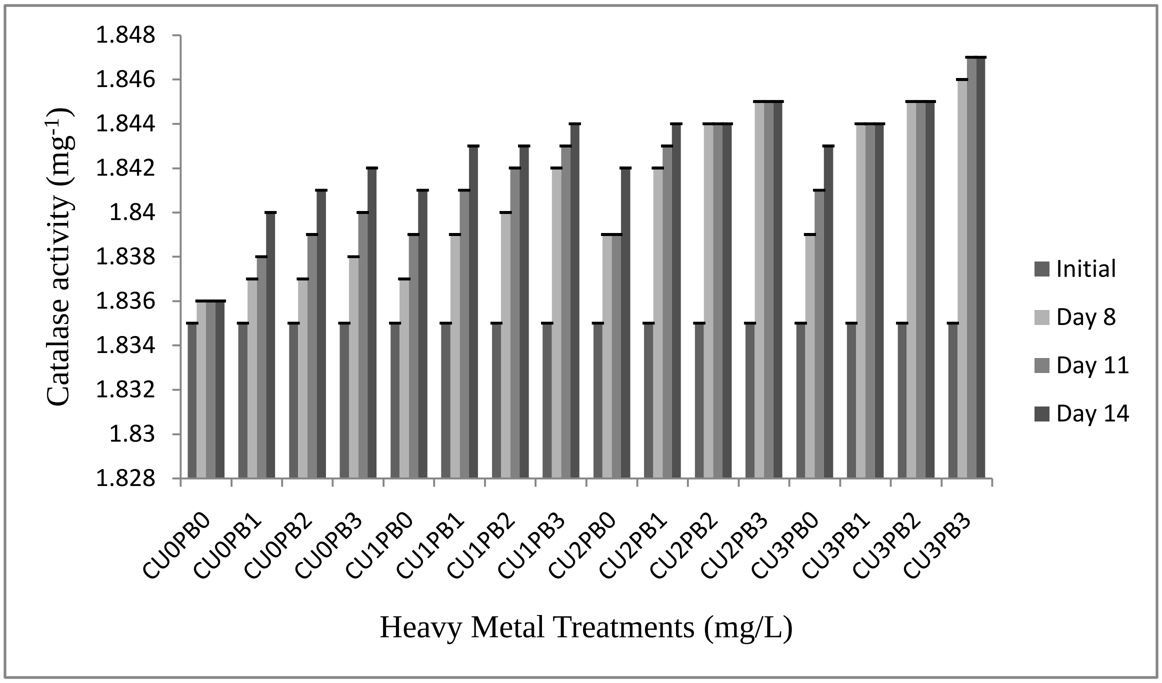

Fig. 4.8: Catalase activity of *Pistia stratiotes* as a function of different Copper and Lead concentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Figure 4.9 shows the activities of catalase in *Lemnatrisulca*. There were significant differences ($P \leq 0.05$) between the various treatments/concentration of the metals. The highest increase in the activities was recorded in CU3PB3 and also in day 14. This implies that the longer the macrophytes were left in the medium containing the pollutants, the activities of catalase increased especially at the highest concentrations.

The activity of the overall catalase was shown in table 4.5. This table showed which of the treatment perform better or averagely on the different days i.e day 8, day 11 and day 14, not minding the plant whether it is *Salvinia molesta, Lemna mimor* or *Pistia stratiotes.*

It can be seen from Table 4.5 that the activities of catalase was higher in the treatment with the highest concentration (CU3PB3). As the concentration of the metals reduced, there was also reduction in the activities of catalase. There were significant differences at $P \leq 0.05$ in day 8, 11 and 14 (Table 4.5).

Table 4.6 is a table showing the comparison of catalase activities the three plants on the different days. There was no significant difference ($P \leq 0.05$) in catalase activities between *Salvinia molesta* and *Lemna trisulca* i.e both have value or readings that were higher than that of lemna species in Day 8, 11 and 14. The value in *Lemnatrisulca* was far lower than that of *Salviniamolesta* and *Pistiastratiotes*.

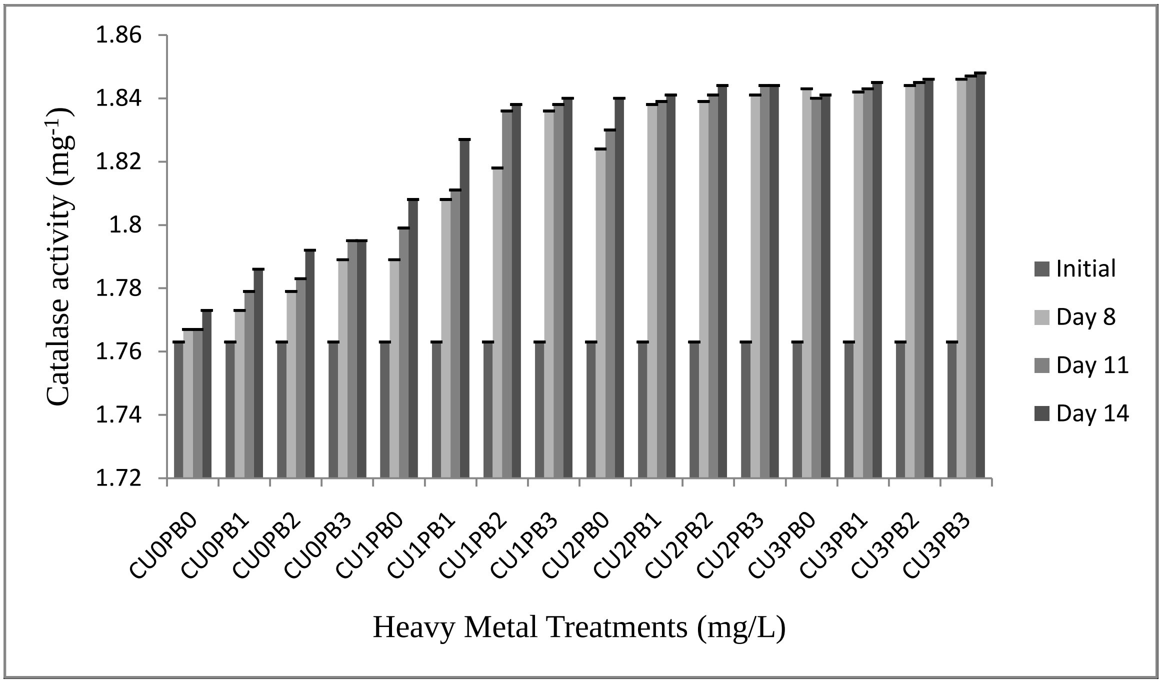

Fig. 4.9: Catalase activity of *Lemna trisulca* as a function of different Copper and Lead concentrations.

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.5: Overall Catalase activities for the various treatments in relation to days

	Catalase activities (mg^{-1})				
Treatments	**Initial**	**Day 8**	**Day 11**	**Day 14**	**Mean**
CU0PB0	1.811±0.01	1.813 ± 0.01^{f}	1.813 ± 0.01^{e}	1.815 ± 0.01^{d}	1.813 ± 0.01^{b}
CU0PB1	1.811±0.01	1.815 ± 0.01^{ef}	1.819 ± 0.01^{de}	1.821 ± 0.01^{d}	1.817 ± 0.01^{ab}
CU0PB2	1.811±0.01	1.818 ± 0.01^{def}	1.820 ± 0.01^{cde}	1.824 ± 0.01^{cd}	1.818 ± 0.01^{ab}
CU0PB3	1.811±0.01	1.822 ± 0.01^{bcdef}	1.825 ± 0.01^{bcde}	1.826 ± 0.01^{cd}	1.821 ± 0.01^{ab}
CU1PB0	1.811±0.01	1.821 ± 0.01^{cdef}	1.825 ± 0.01^{bcde}	1.829 ± 0.01^{bcd}	1.822 ± 0.01^{ab}
CU1PB1	1.811±0.01	1.829 ± 0.01^{abcdef}	1.831 ± 0.01^{abcd}	1.837 ± 0.01^{abc}	1.827 ± 0.01^{ab}
CU1PB2	1.811±0.01	1.832 ± 0.01^{abcde}	1.839 ± 0.01^{ab}	1.841 ± 0.01^{ab}	1.831 ± 0.00^{ab}
CU1PB3	1.811±0.01	1.839 ± 0.01^{abc}	1.841 ± 0.01^{ab}	1.842 ± 0.01^{ab}	1.833 ± 0.00^{ab}
CU2PB0	1.811±0.01	1.834 ± 0.01^{abcd}	1.836 ± 0.01^{abc}	1.841 ± 0.01^{ab}	1.831 ± 0.00^{ab}
CU2PB1	1.811±0.01	1.840 ± 0.01^{ab}	1.841 ± 0.01^{ab}	1.842 ± 0.01^{ab}	1.834 ± 0.00^{ab}
CU2PB2	1.811±0.01	1.841 ± 0.01^{a}	1.842 ± 0.01^{ab}	1.843 ± 0.01^{ab}	1.834 ± 0.00^{a}
CU2PB3	1.811±0.01	1.842 ± 0.01^{a}	1.844 ± 0.01^{a}	1.844 ± 0.01^{ab}	1.835 ± 0.00^{a}
CU3PB0	1.811±0.01	1.841 ± 0.01^{ab}	1.841 ± 0.01^{ab}	1.842 ± 0.01^{ab}	1.833 ± 0.00^{ab}
CU3PB1	1.811±0.01	1.842 ± 0.01^{a}	1.843 ± 0.01^{a}	1.844 ± 0.01^{ab}	1.835 ± 0.00^{a}
CU3PB2	1.811±0.01	1.843 ± 0.01^{a}	1.844 ± 0.01^{a}	1.845 ± 0.01^{ab}	1.836 ± 0.00^{a}
CU3PB3	1.811±0.01	1.845 ± 0.01^{a}	1.846 ± 0.01^{a}	1.847 ± 0.01^{a}	1.837 ± 0.00^{a}
Mean	**1.811±0.01**	**1.832±0.01**	**1.834±0.01**	**1.836±0.01**	**1.829±0.00**
P value	**1.000ns**	**0.000****	**0.000****	**0.000****	**0.089ns**

Means with the same superscript along column are not significant at P>0.05. ns – not significant at P>0.05, ** - highly significant at P ≤ 0.01. CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.6: Comparison in catalase activitiesbetween the 3 macrophyte species

Plants	**Catalase activities (mg^{-1})**				
	Initial	**Day 8**	**Day 11**	**Day 14**	**Mean**
Salvinia	1.836 ± 0.00^{a}	1.839 ± 0.00^{a}	1.840 ± 0.00^{a}	1.841 ± 0.00^{a}	1.839 ± 0.00^{a}
Pistia	1.835 ± 0.00^{b}	1.841 ± 0.00^{a}	1.842 ± 0.00^{a}	1.843 ± 0.00^{a}	1.840 ± 0.00^{a}
Lemna	1.763 ± 0.00^{c}	1.817 ± 0.00^{b}	1.821 ± 0.00^{b}	1.826 ± 0.00^{b}	1.807 ± 0.00^{b}
Mean	**1.811±0.00**	**1.832±0.00**	**1.834±0.00**	**1.836±0.00**	**1.829±0.00**
P value	**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 - 0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

4.2.2 Peroxidase (POX)

The activity of peroxidase in *Salvinia molesta* was presented in figure 4.10. Peroxidase (POX) activity significantly increased ($P \leq 0.05$) with increasing copper and lead concentrations in *Salvinia molesta* (Figure 10) the higher POD was in CU3PB3 and the least was observed in the control ($P<0.05$). The activity of POX was highest in $P>0.05$ in CU3PB3 on day 10, day 13 and 15 in *Salvinia molesta* and *Lemna trisulca* for *Pistia stratiotes*, it was highest $P \leq 0.05$ in CU3PB3 on day 10 and 13 in CU0PB3 on day 15. The POX level in the three plants used did not increase with increase in concentration. Though there was increase in the POX level, it did not follow a particular pattern, it was haphazard. In all the plants used, the POX readings in the control were the least in all the plants.

In Figure 4.11, the peroxidase (POX) activity of *Pistia stratiotes*increased with the number of days. For day 10, CU3PB3 and CU1PB3 has the highest POD level 1.00±0.00 followed by CU1PB2 (0.98±0.00) and the least value was recorded in the control (0.88±0.00). CU0PB3 had the highest POD level (1.10±0.00) followed by CU3PB3 (1.08±0.00), then CU0PB2 (1.07±0.01).the least value ($P<0.05$) was observed in the control (0.88±0.00).

The highest POD activity in *Lemna trisulca* was observed in the treatment with the highest concentration of metals (copper and lead) at 1.5mg/L each for the days. The lowest POX activity was recorded in the control (Fig. 412). CU3PB3 had the highest POX level (1.07±0.01), followed by CU3PB2 (1.00±0.00), then CU2PB3 (0.96±0.00) and the least was observed in the control (0.21±0.00). As the copper and lead stress increased, the activity of the enzyme increased.

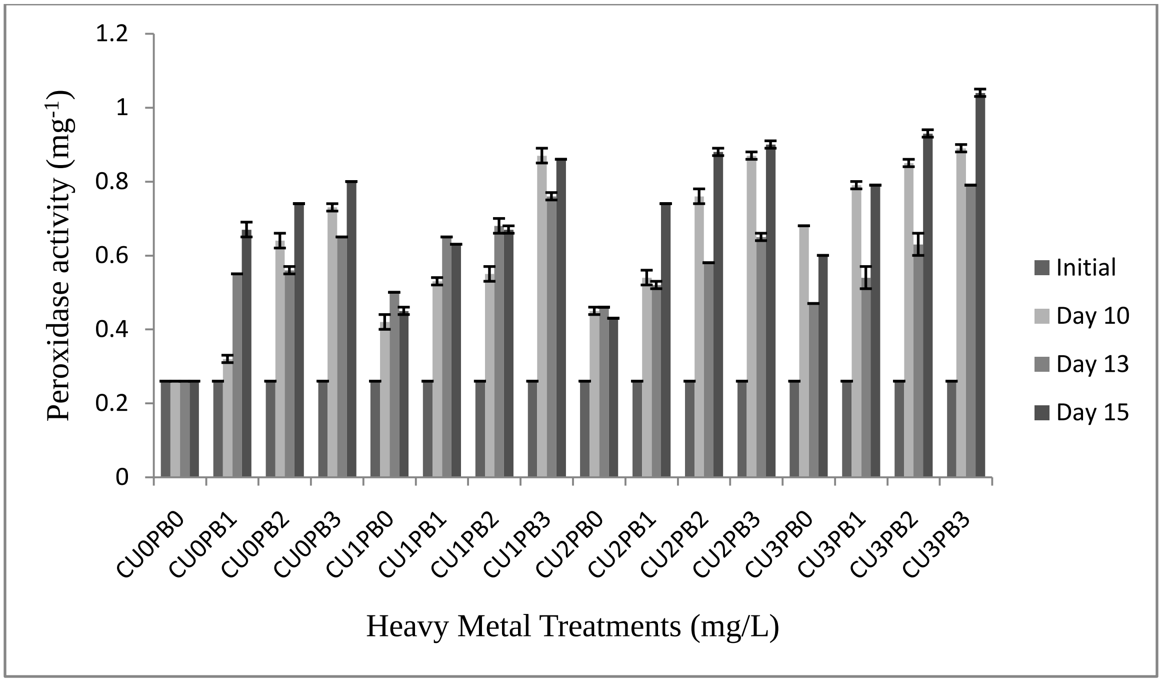

Fig. 4.10: Peroxidase activity of *Salvinia molesta* at different Copper and Leadconcentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

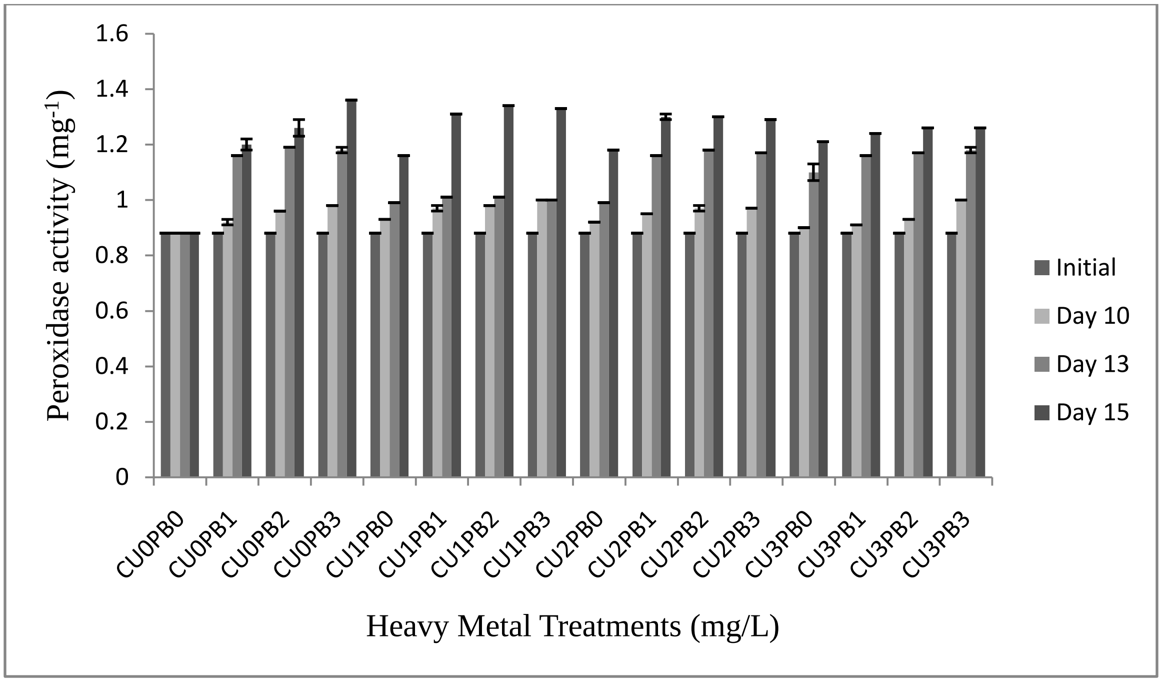

Fig. 4.11: Peroxidase activity of *Pistia stratiotes* at different Copper and Leadconcentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

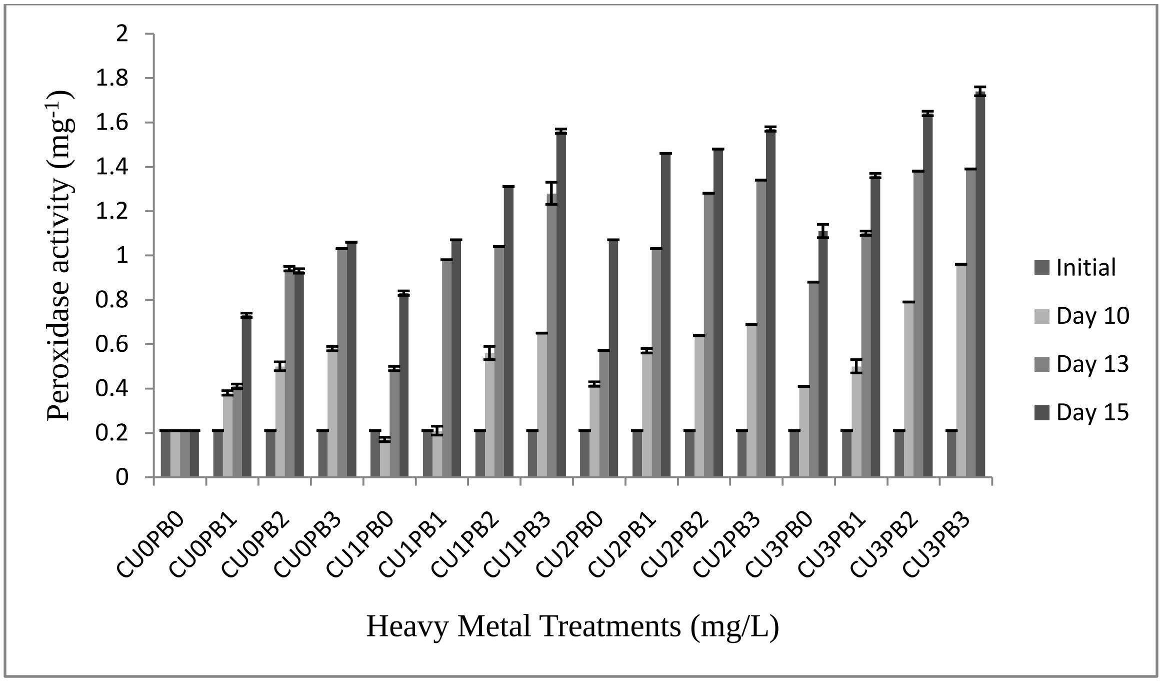

Fig. 4.12: Peroxidase activity of *Lemna trisulca* at different Copper and Leadconcentrations

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.7 showed the overall POD activity of the treatment in the different days after exposure to different concentration of copper and lead. CU3PB3 (treatment with the highest concentration of copper and lead 1.5mg/L each) had the highest activity (0.97±0.06) followed by CU3PB2 (0.91±0.06). The least was recorded in the control (0.45±00.11) followed by CU0PB1 (0.64±0.10).

The increase in POD activity was highest in *Pistia stratiotes* (1.04±0.01), followed by *Lemna trisulca* (0.72±0.03) and it was least in *Salvinia molesta* (0.55±0.02). There were differences in the activity of POD in the three plants species used ($P<0.05$) and ($P\geq0.01$) (Table 4.8).

Table 4.7: Overall Peroxidase activity for the different treatments in relation to days

Treatments	**Peroxidase activity (mg^{-1})**				
	Initial	**Day 10**	**Day 13**	**Day 15**	**Mean**
CU0PB0	0.45±0.11	0.45±0.11^{f}	0.45±0.11^{d}	0.45±0.11^{e}	0.45±0.11^{e}
CU0PB1	0.45±0.11	0.54±0.10^{def}	0.71±0.11^{bcd}	0.87±0.08^{cd}	0.64±0.10^{cde}
CU0PB2	0.45±0.11	0.70±0.07^{bcde}	0.90±0.09^{abc}	0.97±0.08^{bcd}	0.76±0.08^{abcd}
CU0PB3	0.45±0.11	0.77±0.06^{abcd}	0.96±0.08^{abc}	1.08±0.08^{abcd}	0.81±0.07^{abcd}
CU1PB0	0.45±0.11	0.51±0.11^{ef}	0.66±0.08^{cd}	0.82±0.10^{d}	0.61±0.10^{de}
CU1PB1	0.45±0.11	0.57±0.11^{cdef}	0.88±0.06^{abc}	1.00±0.10^{bcd}	0.73±0.08^{abcd}
CU1PB2	0.45±0.11	0.70±0.07^{bcde}	0.91±0.06^{abc}	1.11±0.11^{abcd}	0.79±0.07^{abcd}
CU1PB3	0.45±0.11	0.84±0.05^{ab}	01.01±0.08^{ab}	1.25±0.10^{ab}	0.89±0.05^{abc}
CU2PB0	0.45±0.11	0.60±0.08^{cdef}	0.67±0.08^{cd}	0.89±0.12^{cd}	0.65±0.09^{bcde}
CU2PB1	0.45±0.11	0.69±0.07^{bcdef}	0.91±0.10^{abc}	1.17±0.11^{abc}	0.80±0.08^{abcd}
CU2PB2	0.45±0.11	0.79±0.05^{abc}	1.01±0.11^{ab}	1.22±0.09^{ab}	0.87±0.07^{abcd}
CU2PB3	0.45±0.11	0.85±0.04^{ab}	1.05±0.10^{a}	1.26±0.10^{ab}	0.90±0.06^{abc}
CU3PB0	0.45±0.11	0.66±0.07^{bcdef}	0.81±0.09^{abc}	0.98±0.09^{bcd}	0.73±0.08^{abcd}
CU3PB1	0.45±0.11	0.74±0.06^{abcde}	0.94±0.10^{abc}	1.13±0.09^{abcd}	0.81±0.07^{abcd}
CU3PB2	0.45±0.11	0.86±0.02^{ab}	1.06±0.11^{a}	1.27±0.10^{ab}	0.91±0.06^{ab}
CU3PB3	0.45±0.11	0.95±0.02^{a}	1.12±0.09^{a}	1.35±0.10^{a}	0.97±0.06^{a}
Mean	**0.45±0.03**	**0.70±0.02**	**0.88±0.03**	**1.05±0.03**	**0.77±0.02**
P value	**1.000ns**	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ns – not significant at P>0.05, ** - highly significant at P ≤ 0.01. CU0 -0mg/L, CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 – 0mg/L,PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.8: Comparison of peroxidase activities among macrophyte species

	Peroxidase activity (mg^{-1})				
Plants	**Initial**	**Day 10**	**Day 13**	**Day 15**	**Mean**
Salvinia	0.26 ± 0.00^{b}	0.63 ± 0.03^{b}	0.58 ± 0.02^{c}	0.71 ± 0.03^{b}	0.55 ± 0.02^{c}
Pistia	0.88 ± 0.00^{a}	0.95 ± 0.01^{a}	1.10 ± 0.01^{a}	1.24 ± 0.02^{a}	1.04 ± 0.01^{a}
Lemna	0.21 ± 0.00^{c}	0.51 ± 0.03^{c}	0.96 ± 0.05^{b}	1.20 ± 0.06^{a}	0.72 ± 0.03^{b}
Mean	**0.45±0.03**	**0.70±0.02**	**0.88±0.03**	**1.05±0.03**	**0.77±0.02**
P value	**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at $P>0.05$. ** - highly significant at $P \leq 0.01$. CU0 – 0mg/L, CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 - 0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

4.3 Morphological Observations/Visual Symptoms by *Salvinia Molesta, Pistia Stratiotes* and *Lemna Trisulca*

The ferns of *Salvinia molesta* did not show any change in their leaves and roots. The plants remained the same throughout the experimental period and no change was recorded in the morphology of the plants in all the treatments (Table 4.9).

For *Pistia stratiotes* and *Lemna trisulca*, the plants showed various degrees of chlorosis with increase in the concentration of copper and lead. From CU2PB2 up to the highest concentrations, many of the plant samples showed symptoms of chlorosis. Necrosis was later observed in CU2PB2, CU2PB3, CU3PB2 and CU3PB3 as from day 15 upward (Table 4.10 and 4.11).

Table 4.9. Symptoms of Chlorosis/Necrosis in *Salvinia molesta*after copper and lead treatment

Treatment	**Symptoms in *Salvinia molesta***
CU0PB0	Fresh, Green, no symptom of chlorosis
CU0PB1	Fresh, Green, no symptom of chlorosis
CU0PB2	Fresh, Green, no symptom of chlorosis
CU0PB3	Fresh, Green, no symptom of chlorosis
CU1PB0	Fresh, Green, no symptom of chlorosis
CU1PB1	Fresh, Green, no symptom of chlorosis
CU1PB2	Fresh, Green, no symptom of chlorosis
CU1PB3	Fresh, Green, no symptom of chlorosis
CU2PB0	Fresh, Green, no symptom of chlorosis
CU2PB1	Fresh, Green, no symptom of chlorosis
CU2PB2	Fresh, Green, no symptom of chlorosis
CU2PB3	Fresh, Green, no symptom of chlorosis
CU3PB0	Fresh, Green, no symptom of chlorosis
CU3PB1	Fresh, Green, no symptom of chlorosis
CU3PB2	Fresh, Green, no symptom of chlorosis
CU3PB3	Fresh, Green, no symptom of chlorosis

Table 4.10. Symptoms of Chlorosis/Necrosis in *Pistia stratiotes*after copper and lead treatment

Treatment	**Symptoms in *Pistia stratiotes***
CU0PB0	Fresh, Green, no symptom of chlorosis
CU0PB1	Fresh, Green, no symptom of chlorosis
CU0PB2	Fresh, Green, no symptom of chlorosis
CU0PB3	Green but chlorosis seen
CU1PB0	Green but chlorosis seen
CU1PB1	Green but chlorosis seen
CU1PB2	Green but chlorosis seen
CU1PB3	Green but chlorosis seen
CU2PB0	Chlorosis observed in few leaves
CU2PB1	Chlorosis observed
CU2PB2	Chlorosis observed
CU2PB3	Chlorosis and necrosis observed
CU3PB0	Chlorosis observed
CU3PB1	Chlorosis observed
CU3PB2	Chlorosis and necrosis observed
CU3PB3	Chlorosis and necrosis observed

Table 4.11. Symptoms of Chlorosis/Necrosis in *Lemna trisulca*after copper and lead treatment

Treatment	Symptoms in *Lemna trisulca*
CU0PB0	Fresh, Green, no symptom of chlorosis
CU0PB1	Fresh, Green, no symptom of chlorosis
CU0PB2	Fresh, Green, no symptom of chlorosis observed
CU0PB3	Green but chlorosis observed in some leaves
CU1PB0	Fresh, Green, no symptom of chlorosis observed
CU1PB1	Green but chlorosis observed in some leaves
CU1PB2	Green but chlorosis observed in some leaves
CU1PB3	Green but chlorosis observed in some leaves
CU2PB0	Green but chlorosis observed in some leaves
CU2PB1	Green but chlorosis observed in some leaves
CU2PB2	Chlorosis and necrosis observed
CU2PB3	Chlorosis and necrosis observed
CU3PB0	Chlorosis was observed
CU3PB1	Chlorosis was observed
CU3PB2	Chlorosis and necrosis observed
CU3PB3	Chlorosis and necrosis observed

4.4 Metal Accumulation/Concentration in the Aquatic Macrophytes Species.

In *Salvinia molesta*, copper accumulation increased with increase in the concentration of the copper. Figure 4.13 reveals that there was a significant difference in the concentration of copper in the different treatments. On day 9, CU3PB3 had the highest accumulation of copper (2465.16 ±169.25) followed by CU3PB1 (2265.73±3.37) and the least was recorded in CU0PB1 (60.51±11.47). On day 15 it was highest in CU3PB2 (3015.43±207.72) and on day 18, it was highest in CU3PB3 (3286.83±45.23).

There was a significantdifference ($P \leq 0.5$) in the value of copper concentration and it showed that the higher the concentration of lead, the higher the accumulation. Also the longer the plants stayed in the medium, the higher the content of lead accumulated. Figure 4.14 shows the accumulation of lead by *Salvinia molesta*. On day 9, 12, 15 and 18, lead absorption was highest in CU3PB3 with 1212.10±88.16, 1458.48±129.62, 2068.08±46.34 and 2526.95±24.77 value respectively. There was a significant difference ($P \leq 0.5$) in lead accumulation in *Salvinia molesta* (Figure 4.14). The mean value showed that lead accumulation was highest in CU3PB3 (14.61.14±49.32) followed by CU2PB3 (1355.19±16.29) and it was least in the control CU0PB0 (45.74±3.07).

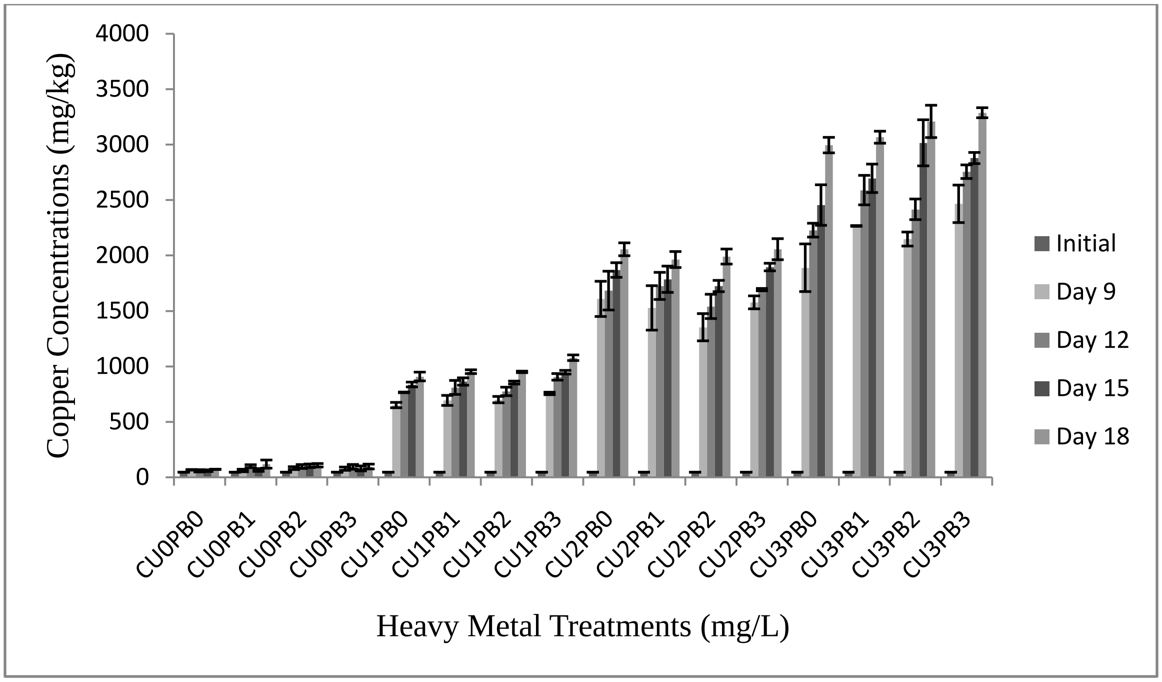

Fig. 4.13: Copper concentrations in *Salvinia molesta* in 18 days period *invitro*.

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

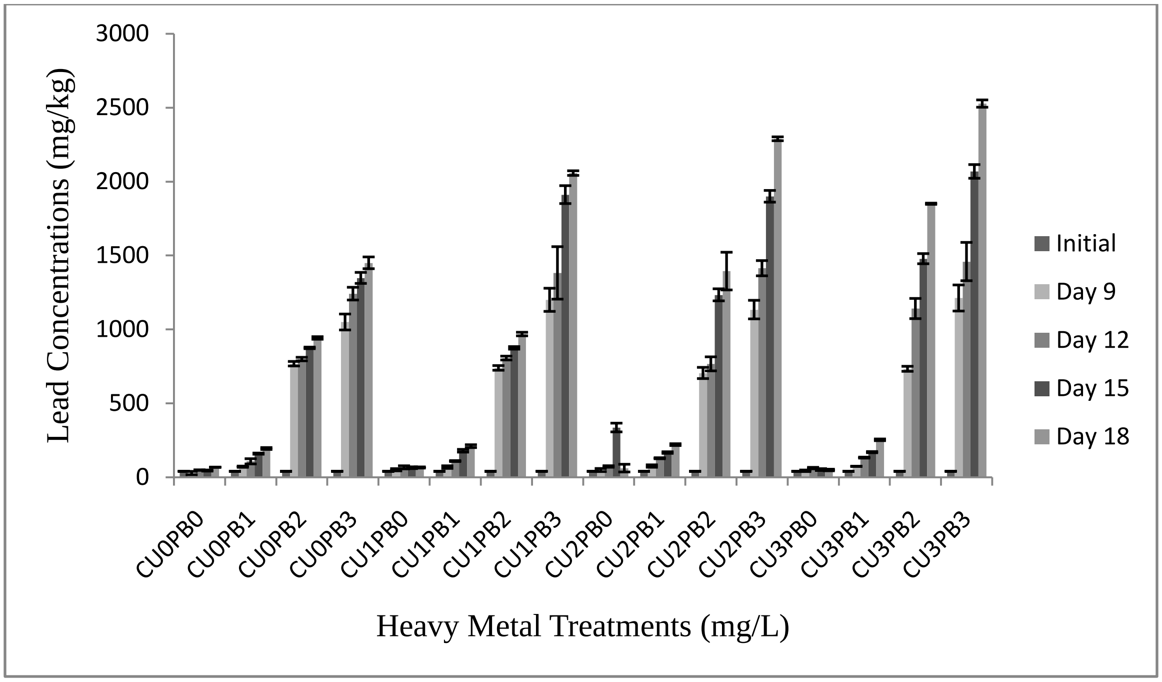

Fig. 4.14: Lead concentrationsin *Salvinia molesta* in 18 days period *invitro*

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

For *Pistia stratiotes*, Figure 4.15 revealed that the accumulation of copper was highest in the treatments with the highest concentration of copper CU3PB0, CU3PB1, CU3PB2 and CU3PB3 in all the days. There was no significant difference between the four treatment with the highest concentration of metals but they were different significantly ($P \leq 0.5$) from other treatments with reduced concentration of copper.

Figure 4.16 shows the accumulation of lead by *Pistia stratiotes*. There were significant differences between the various treatments ($P \leq 0.5$). On day 9, CU3PB3 had the highest accumulation of lead 718.82±20.62 followed by CU3PB2 and CU1PB3 (592.03±43.39 and 591.77±64.40) respectively. On day 12 CU2PB3 (1182.75±296.08) had thehighest followed byCU1PB3(1144.07±399.00) followed by CU3PB3 and CU3PB2. For day15 and 18 CU3PB2 had the highest lead accumulation followed by CU3PB3 and CU2PB3. The least in all the days is CU0PB0 (control).

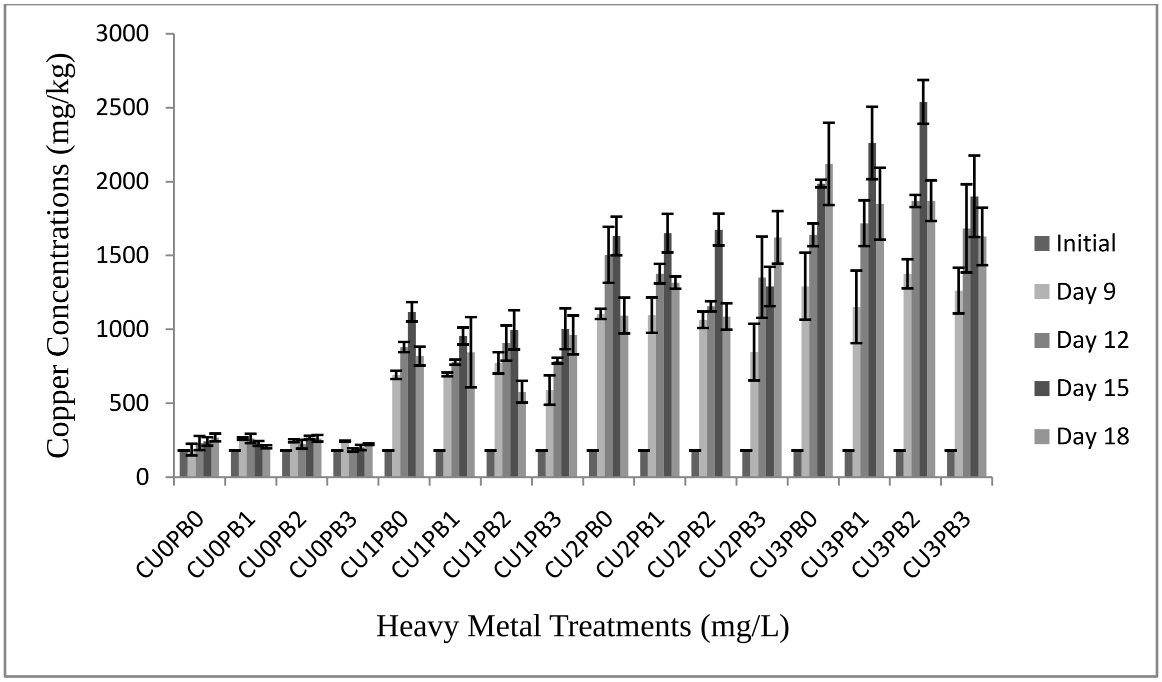

Fig. 4.15: Copper concentrations in *Pistia stratiotes* in 18 days period *in vitro*

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

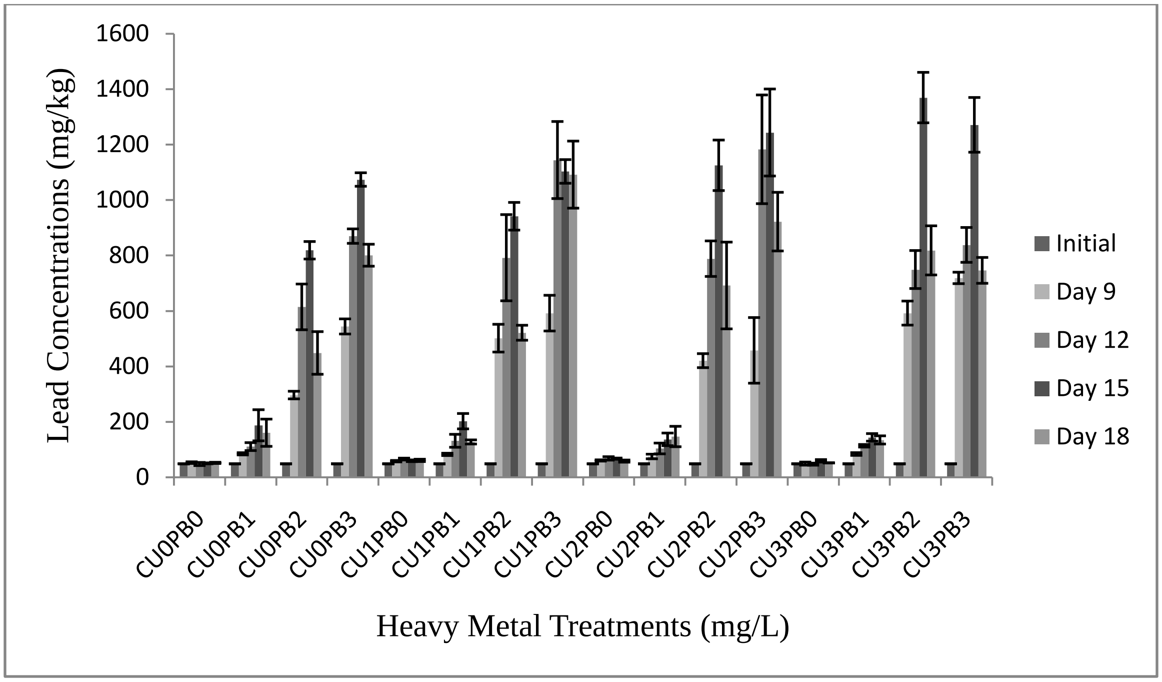

Fig. 4.16: Lead concentrationsin *Pistia stratiotes*in 18 days period *invitro*

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

For *Lemna trisulca*, there was a significant difference in the different treatment with regards to the content of copper accumulated (Figure 4.17). CU3PB3 and CU3PB2 had the highest accumulated lead value in all the days (day 9, 12, 15, and 18) and the least value were recorded CU0PB0, CU0PB1, CU0PB2 and CU0PB3).

For lead accumulation in *Lemna trisulca*, significant differences ($P \leq 0.05$) were also recorded for the different treatments (Figure 4.18). The highest lead accumulation was recorded in CU3PB3 in day 9, 12, 15, and 12 with value of 1191.69±116.25, 1324.39±138.59, 1606.78±128.95 and 552.03±32.36 respectively. This was followed by CU2PB3 and CU3PB2. The lowest lead accumulation level was recorded in CU0PB0. i.e the control.

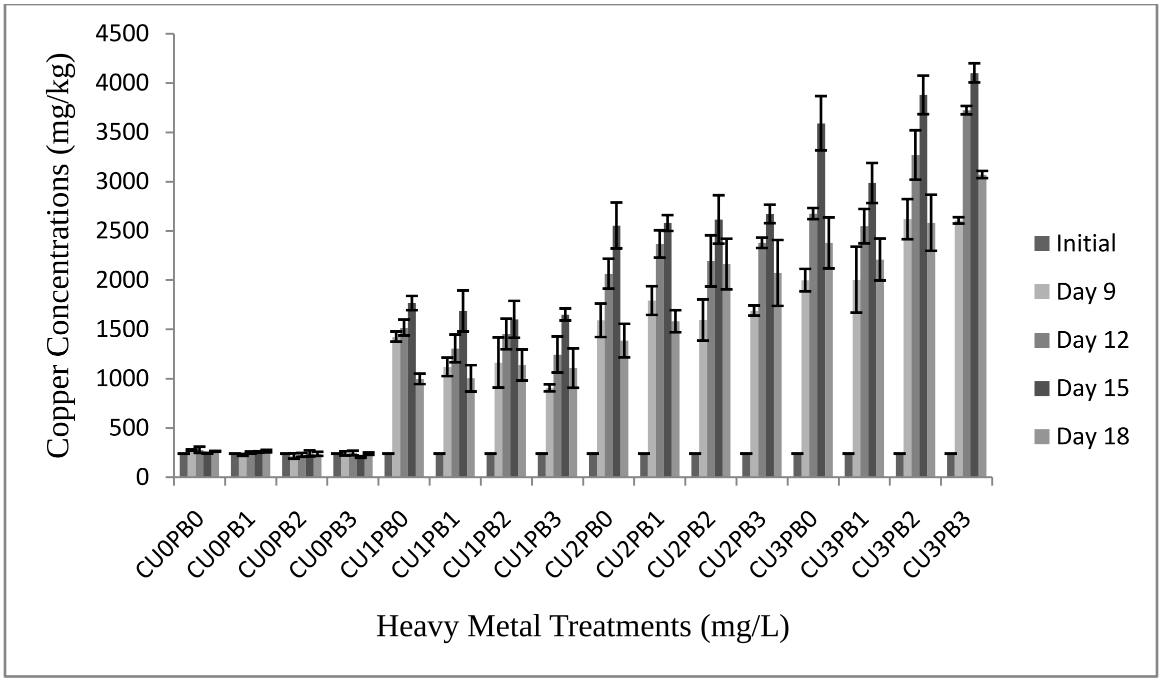

Fig. 4.17: Copper concentrationsin *Lemna trisulca* in 18 days period *invitro*

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

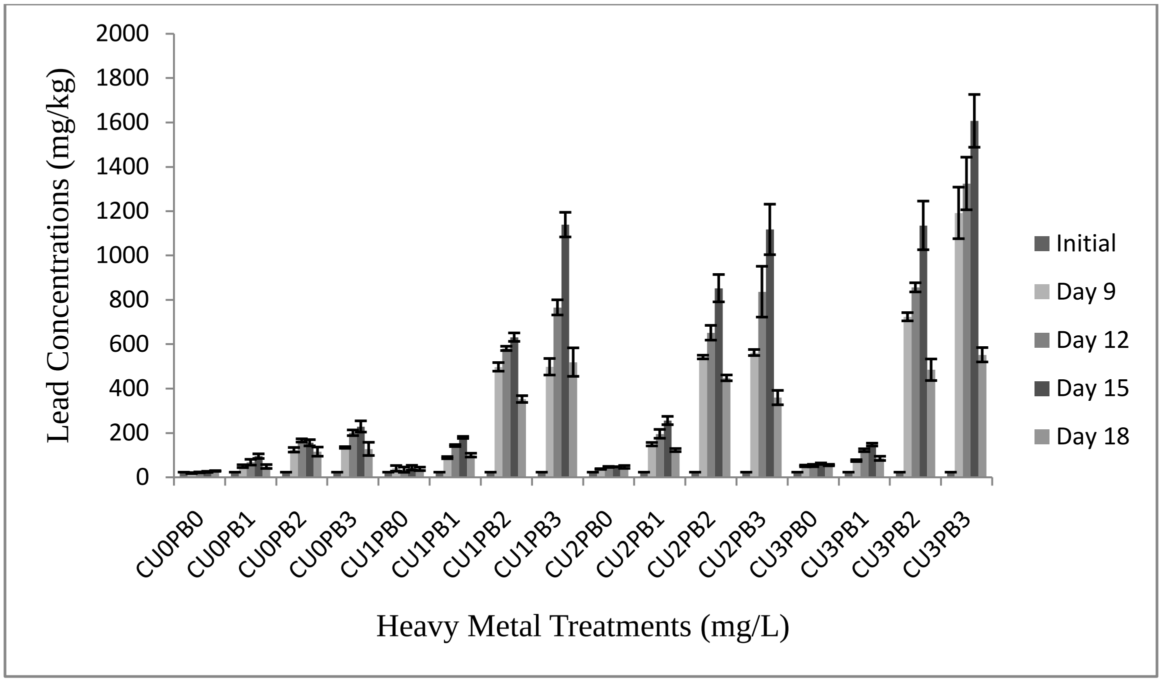

Fig. 4.18: Lead concentrationsin *Lemna triculca*in 18 days period *invitro*

CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

4.4.1 Comparison of metal uptake by plants in the different days

Table 4.12 shows the comparison of copper uptake by *Salvinia molesta* between treatment and days. There were significant difference ($P \leq 0.5$) between the treatment and days. In the treatments, day18 had the highest copper uptake followed by 15, then 12 and day 9, the initial has the least in all.

For lead uptake by *Salvinia molesta*, there were significant difference ($P \leq 0.5$) between treatments and days (Table 4.13). Day18 had the highest uptake in all the treatments, followed by day 15, 12 and 9. The accumulated copper content was also least in the initial i. e reading before the introduction of the metals.

Pistia stratiotes showed highest accumulation of copper and lead on day 15, followed by day 18 and 12. This implies that after day 15, the accumulated copper and lead reduced in Pistia stratiotes. The concentration of copper and lead increased from the onset (day 9, 12) up to day 15. But after day 15, the concentration or absorption reduced (Table 4.14 and 4.15).

Table 4.16 showed copper uptake in *Lemna trisulca*, the concentration increased from initial up to day 9, 12 and 15. On day 15 copper uptakes decreased from CU1PB0 to CU3PB3 and it was significantly different ($P \leq 0.5$). Lead uptake in *Lemna trisulca* also increased from the beginning up to day 15; but a decrease was also recorded on day 18 as seen in table 4.17. There were significant differences ($P \leq 0.5$) between the treatment and days. Day 15 is significantly higher than day 18.

Table 4.12. Comparison of Copper accumulationin *Salvinia molesta* in different days

Treatment	Copper accumulation (mg/kg)						
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**	**P value**
CU0PB0	45.51±0.00b	66.57±2.68a	57.36±10.42ab	58.72±7.65ab	70.53±1.31a	**59.74±3.87**	**0.097ns**
CU0PB1	45.51±0.00b	60.51±11.47ab	99.13±13.37ab	65.10±12.44ab	118.11±36.79a	**77.67±9.15**	**0.113ns**
CU0PB2	45.51±0.00b	81.72±12.83ab	96.59±17.56a	102.92±16.29a	107.71±15.61a	**86.89±5.36**	**0.061ns**
CU0PB3	45.51±0.00a	76.75±14.85a	93.32±21.53a	79.35±22.54a	96.11±21.80a	**78.21±14.33**	**0.364ns**
CU1PB0	45.51±0.00d	650.54±24.30c	765.04±3.21b	835.91±21.81b	908.25±39.40a	**641.05±8.60**	**0.000****
CU1PB1	45.51±0.00f	692.55±45.17c	809.72±63.15bc	862.84±33.34ab	951.56±17.16a	**672.43±28.83**	**0.000****
CU1PB2	45.51±0.00e	699.75±28.61d	773.48±38.28c	853.47±13.44b	949.82±6.86a	**664.41±16.63**	**0.000****
CU1PB3	45.51±0.00d	755.44±11.37c	904.98±29.82b	946.66±16.64b	1077.38±25.38a	**746.00±12.80**	**0.000****
CU2PB0	45.51±0.00c	1608.03±158.85b	1682.70±174.91b	1868.38±65.95ab	2055.29±58.19a	**1451.98±91.20**	**0.000****
CU2PB1	45.51±0.00c	1526.77±200.39b	1725.19±122.17ab	1785.17±118.63ab	1962.70±72.11a	**1409.07±98.72**	**0.000****
CU2PB2	45.51±0.00d	1352.54±122.74c	1540.20±109.45bc	1723.97±50.95b	1989.92±67.97a	**1330.43±58.82**	**0.000****
CU2PB3	45.51±0.00c	1576.76±58.48b	1691.36±10.64b	1895.13±34.19a	2056.20±94.93a	**1452.99±194.20**	**0.000****
CU3PB0	45.51±0.00d	1888.80±214.88c	2227.81±62.44bc	2454.19±182.77b	2994.76±69.96a	**1922.21±80.02**	**0.000****
CU3PB1	45.51±0.00d	2265.73±3.37c	2589.03±132.99b	2695.35±128.24b	3065.89±54.04a	**2132.30±62.65**	**0.000****
CU3PB2	45.51±0.00c	2147.86±63.33b	2415.73±93.00b	3015.43±207.72a	3208.49±145.67a	**2166.60±78.70**	**0.000****
CU3PB3	45.51±0.00d	2465.16±169.25c	2754.93±61.46b	2878.20±50.36b	3286.83±45.23a	**2286.13±61.29**	**0.000****

Means with the same superscript along rows are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.13. Comparison of Lead accumulationin *Salvinia molesta* in different days

Treatment	**Lead accumulation (mg/kg)**						
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**	**P value**
CU0PB0	40.08±0.00bc	27.94±11.38c	48.10±1.93b	45.05±3.72bc	67.54±0.95a	**45.74±3.07**	**0.006****
CU0PB1	40.08±0.00e	71.20±4.15d	107.42±17.68c	159.48±3.92b	194.11±6.33a	**114.46±2.22**	**0.000****
CU0PB2	40.08±0.00e	767.65±15.38d	799.05±11.90c	874.59±5.41b	941.74±8.69a	**684.62±5.21**	**0.000****
CU0PB3	40.08±0.00d	1049.68±53.94c	1241.49±42.97b	1347.97±37.23ab	1449.97±39.80a	**1025.84±29.20**	**0.000****
CU1PB0	40.08±0.00b	50.43±7.42ab	66.90±10.43a	64.18±6.03a	65.70±3.99a	**57.46±0.88**	**0.062ns**
CU1PB1	40.08±0.00e	68.35±8.86d	109.18±3.30c	178.91±9.27b	209.89±9.77a	**121.28±5.48**	**0.000****
CU1PB2	40.08±0.00e	739.17±15.78d	805.87±13.07c	874.56±8.67b	968.23±11.99a	**685.58±3.70**	**0.000****
CU1PB3	40.08±0.00c	1199.84±78.22b	1381.78±177.45b	1911.30±60.53a	2056.84±15.72a	**1317.97±36.09**	**0.000****
CU2PB0	40.08±0.00a	48.49±10.45a	72.58±5.09a	336.06±259.63a	61.45±26.04a	**111.73±52.13**	**0.383ns**
CU2PB1	40.08±0.00e	75.55±8.24d	128.23±3.55c	166.73±5.40b	220.41±6.12a	**126.20±3.77**	**0.000****
CU2PB2	40.08±0.00c	705.05±37.90b	766.64±47.38b	1233.38±40.81a	1393.98±127.50a	**827.83±19.12**	**0.000****
CU2PB3	40.08±0.00e	1133.57±62.84d	1413.49±51.44c	1899.93±39.65b	2288.89±13.06a	**1355.19±16.29**	**0.000****
CU3PB0	40.08±0.00b	43.36±5.31b	61.70±4.94a	51.56±6.17ab	48.81±5.41ab	**49.10±1.30**	**0.078ns**
CU3PB1	40.08±0.00e	73.65±0.82d	132.42±3.44c	169.56±3.97b	252.81±5.76a	**133.70±2.35**	**0.000****
CU3PB2	40.08±0.00e	733.07±16.98d	1140.79±68.26c	1477.99±34.25b	1849.50±4.10a	**1048.29±2.78**	**0.000****
CU3PB3	40.08±0.00e	1212.10±88.16d	1458.48±129.62c	2068.08±46.34b	2526.95±24.77a	**1461.14±49.32**	**0.000****

Means with the same superscript along rows are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.14. Comparison of Copper accumulationin *Pistia stratiotes* in different days

Treatment	Copper accumulation (mg/kg)						
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**	**P value**
CU0PB0	180.87±0.00a	186.73±39.09a	231.08±47.63a	241.70±29.22a	269.27±26.37a	**221.93±15.98**	**0.328ns**
CU0PB1	180.87±0.00b	261.48±8.85a	261.82±31.40a	227.97±16.80ab	206.24±10.54ab	**227.68±7.17**	**0.029***
CU0PB2	180.87±0.00b	246.86±10.52a	222.67±29.78ab	268.41±11.35a	263.02±22.36a	**236.36±13.07**	**0.036***
CU0PB3	180.87±0.00c	243.81±3.00a	184.28±11.80c	200.60±17.52bc	223.07±5.84ab	**206.53±4.94**	**0.005****
CU1PB0	180.87±0.00d	691.74±27.77c	880.32±34.26b	1118.66±65.86a	818.80±63.36bc	**738.08±27.95**	**0.000****
CU1PB1	180.87±0.00b	695.21±12.28a	777.60±17.24a	955.24±57.64a	845.74±237.44a	**690.93±54.58**	**0.005****
CU1PB2	180.87±0.00c	773.37±72.31ab	907.14±119.73a	996.63±133.07a	578.00±73.43b	**687.20±78.56**	**0.001****
CU1PB3	180.87±0.00c	589.42±99.90b	788.16±19.37ab	1004.42±137.97a	962.76±131.59a	**705.13±76.36**	**0.001****
CU2PB0	180.87±0.00c	1104.41±34.41b	1503.70±189.46a	1631.66±130.25a	1093.68±120.59b	**1102.86±50.76**	**0.000****
CU2PB1	180.87±0.00c	1096.03±120.23b	1376.67±66.03b	1650.63±130.50a	1316.12±41.80b	**1124.06±31.24**	**0.000****
CU2PB2	180.87±0.00a	1064.77±55.74b	1156.18±34.39b	1674.66±107.67a	1087.10±89.67b	**1032.72±10.92**	**0.000****
CU2PB3	180.87±0.00b	846.06±191.26ab	1352.14±274.87a	1290.04±132.63a	1621.90±378.06a	**1058.20±86.78**	**0.011***
CU3PB0	180.87±0.00b	1291.63±226.84a	1639.45±76.44a	1986.47±25.46a	2118.97±578.00a	**1443.48±135.02**	**0.004****
CU3PB1	180.87±0.00c	1152.01±245.13b	1718.18±154.58ab	2260.44±244.81a	1849.50±243.16a	**1432.20±105.40**	**0.000****
CU3PB2	180.87±0.00c	1376.21±98.33b	1868.32±400.84ab	2538.37±548.15a	1870.19±337.37ab	**1566.79±220.49**	**0.007****
CU3PB3	180.87±0.00b	1262.37±154.17a	1682.98±298.31a	1899.92±295.34a	1628.42±194.09a	**1330.91±150.31**	**0.002****

Means with the same superscript along rows are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.15. Comparison of Lead accumulation (mg/kg) in *Pistia stratiotes* in different days

Treatment	Lead accumulation (mg/kg)						
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**	**P value**
CU0PB0	48.41±0.00a	52.57±2.96a	47.61±5.74a	49.97±1.79a	51.69±2.25a	**50.05±0.82**	**0.776ns**
CU0PB1	48.41±0.00b	84.41±3.99ab	110.38±14.29ab	187.27±56.21a	160.38±49.14ab	**118.17±22.01**	**0.091ns**
CU0PB2	48.41±0.00d	296.27±13.79c	614.32±82.52b	818.55±31.53a	448.20±76.88bc	**445.15±27.29**	**0.000****
CU0PB3	48.41±0.00d	543.75±27.22c	869.68±26.06b	1073.78±24.34a	800.71±39.61b	**667.27±23.40**	**0.000****
CU1PB0	48.41±0.00b	57.80±2.68ab	65.39±3.40a	59.34±3.08a	60.67±4.24a	**58.32±2.48**	**0.030***
CU1PB1	48.41±0.00c	82.28±4.21bc	131.35±23.37b	202.06±27.71a	127.14±7.40b	**118.25±10.22**	**0.001****
CU1PB2	48.41±0.00c	501.51±50.02b	791.72±155.46a	941.26±49.96a	521.10±27.04b	**560.80±86.64**	**0.000****
CU1PB3	48.41±0.00b	591.77±64.40ab	1144.07±399.00a	1102.96±42.64a	1091.45±250.98a	**795.73±139.36**	**0.018***
CU2PB0	48.41±0.00b	60.36±2.35a	67.74±6.18a	66.96±2.18a	58.09±3.94ab	**60.31±1.16**	**0.020***
CU2PB1	48.41±0.00b	74.74±8.29ab	103.71±19.44ab	136.51±22.82a	146.74±36.85a	**102.02±11.85**	**0.044***
CU2PB2	48.41±0.00d	420.43±25.25c	788.26±64.06b	1125.05±91.09a	691.57±156.52bc	**614.75±32.37**	**0.000****
CU2PB3	48.41±0.00c	457.48±118.36bc	1182.75±296.08a	1243.40±186.88a	921.87±105.82ab	**770.78±49.75**	**0.003****
CU3PB0	48.41±0.00a	49.08±5.61a	47.08±3.76a	58.99±4.52a	51.42±0.14a	**51.00±2.67**	**0.226ns**
CU3PB1	48.41±0.00c	83.68±4.90b	113.35±4.71ab	143.71±13.55a	134.42±14.80a	**104.71±0.89**	**0.000****
CU3PB2	48.41±0.00d	592.03±43.39c	749.10±68.52bc	1369.35±91.00a	818.15±88.64b	**715.41±33.74**	**0.000****
CU3PB3	48.41±0.00c	718.82±20.62b	837.94±62.87b	1270.95±98.74a	746.06±46.66b	**724.44±33.32**	**0.000****

Means with the same superscript along rows are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.16. Comparison of Copper accumulation in *Lemna trisulca* in different days

Treatment	Copper accumulation (mg/kg)						
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**	**P value**
CU0PB0	239.67±0.00a	276.17±6.65a	277.26±32.00a	243.31±3.07a	262.76±3.95a	**259.83±7.21**	**0.291ns**
CU0PB1	239.67±0.00ab	225.61±10.51b	250.33±10.49ab	256.89±7.68ab	264.80±12.40a	**247.46±6.56**	**0.092ns**
CU0PB2	239.67±0.00a	215.40±27.50a	226.31±19.73a	241.76±30.78a	236.43±20.86a	**231.91±18.53**	**0.910ns**
CU0PB3	239.67±0.00a	242.51±21.77a	245.57±22.66a	204.61±9.51a	238.44±12.89a	**234.16±6.22**	**0.397ns**
CU1PB0	239.67±0.00d	1426.77±52.12b	1518.53±79.80b	1766.90±71.77a	997.44±52.60c	**1189.86±21.05**	**0.000****
CU1PB1	239.67±0.00c	1119.23±93.47b	1306.40±140.02ab	1685.84±208.70a	1002.92±134.51b	**1070.81±105.72**	**0.000****
CU1PB2	239.67±0.00b	1163.78±255.12a	1453.15±154.62a	1601.20±187.17a	1138.21±157.18a	**1119.20±149.85**	**0.002****
CU1PB3	239.67±0.00c	906.97±35.61b	1245.73±183.03b	1651.56±60.86a	1107.11±200.32b	**1030.21±69.10**	**0.000****
CU2PB0	239.67±0.00c	1591.64±169.52b	2064.46±151.70ab	2553.34±233.03a	1385.94±369.68b	**1567.01±133.74**	**0.000****
CU2PB1	239.67±0.00c	1792.21±146.04b	2366.29±139.00a	2578.95±80.56a	1582.90±111.77b	**1712.00±14.41**	**0.000****
CU2PB2	239.67±0.00b	1594.59±209.56a	2193.88±360.16a	2614.91±245.99a	2162.62±556.25a	**1761.13±64.70**	**0.004****
CU2PB3	239.67±0.00b	1690.50±51.44a	2377.96±52.31a	2670.94±93.42a	2072.16±834.65a	**1810.25±157.96**	**0.008****
CU3PB0	239.67±0.00c	1999.60±113.46b	2674.91±56.49b	3591.00±375.43a	2377.02±458.25b	**2176.44±125.04**	**0.000****
CU3PB1	239.67±0.00b	2003.64±334.67a	2547.14±174.16a	2984.98±403.11a	2208.35±512.27a	**1996.76±213.16**	**0.002****
CU3PB2	239.67±0.00c	2618.30±203.44b	3268.97±250.66ab	3878.33±195.49a	2580.94±284.69b	**2517.24±57.41**	**0.000****
CU3PB3	239.67±0.00e	2605.48±32.41d	3723.79±42.68b	4101.77±97.14a	3071.01±36.45c	**2748.35±20.14**	**0.000****

Means with the same superscript along rows are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Table 4.17. Comparison of Lead accumulation in *Lemna trisulca* in different days

Treatment	Lead accumulation (mg/kg)						
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**	**P value**
CU0PB0	22.67±0.00ab	18.67±2.11b	21.71±1.99b	24.18±2.43ab	27.98±1.67a	**23.04±0.51**	**0.052ns**
CU0PB1	22.67±0.00c	49.58±6.45bc	67.67±13.00ab	94.89±10.41a	48.22±8.76bc	**56.61±5.71**	**0.002****
CU0PB2	22.67±0.00c	123.60±10.39b	165.62±7.37a	155.26±14.04ab	115.27±20.57b	**116.48±6.04**	**0.000****
CU0PB3	22.67±0.00c	134.06±3.76b	200.30±12.79a	228.68±25.31a	127.62±29.73b	**142.66±14.05**	**0.000****
CU1PB0	22.67±0.00a	39.87±12.75a	33.93±11.86a	42.69±10.85a	37.65±7.87a	**35.36±8.42**	**0.654ns**
CU1PB1	22.67±0.00d	88.21±4.81c	142.67±4.53b	179.42±4.54a	99.45±8.64c	**106.48±2.61**	**0.000****
CU1PB2	22.67±0.00e	497.44±19.30c	580.73±9.66b	631.52±18.96a	352.41±15.21d	**416.95±9.12**	**0.000****
CU1PB3	22.67±0.00d	498.00±37.27c	765.61±34.18b	1138.80±55.54a	519.05±64.18c	**588.82±27.84**	**0.000****
CU2PB0	22.67±0.00b	36.86±1.64a	46.44±2.35a	46.75±0.75a	46.17±6.49a	**39.78±1.14**	**0.001****
CU2PB1	22.67±0.00d	148.84±7.85c	195.59±19.91b	255.58±18.76a	122.25±6.92c	**148.98±9.63**	**0.000****
CU2PB2	22.67±0.00d	541.73±8.37c	651.38±33.28b	852.19±61.62a	447.85±13.10c	**503.16±12.12**	**0.000****
CU2PB3	22.67±0.00d	562.20±13.60bc	836.46±114.53b	1117.12±153.91a	358.91±32.49c	**579.47±62.08**	**0.000****
CU3PB0	22.67±0.00b	50.81±3.94a	52.64±5.33a	60.93±3.30a	54.78±3.38a	**48.37±2.35**	**0.000****
CU3PB1	22.67±0.00d	74.76±4.13c	121.60±6.43b	147.04±6.51a	84.91±9.51c	**90.20±1.45**	**0.000****
CU3PB2	22.67±0.00d	723.43±18.57bc	856.05±20.61ab	1135.33±209.68a	484.49±48.60c	**644.39±50.97**	**0.000****
CU3PB3	22.67±0.00d	1191.69±116.25b	1324.39±138.59ab	1606.78±128.95a	552.03±32.36c	**939.51±80.86**	**0.000****

Means with the same superscript along rows are not significant at P>0.05. * – significant at P

≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

4.5: Comparison of Copper and Lead Bioaccumulation by *Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca.*

Salvinia molesta and *Lemna trisulca* had the highest accumulation potential for copper ($P \leq 0.05$). The value of copper accumulated by *Salvinia molesta* on 18 days *in vitro* experiment is 1510.71 ±294.49 and that of *Lemna trisulca* is 1178.39 ±229.27. The least accumulator of copper is *Pistia stratiotes* with 866.18 ±159.95 (Fig. 4.19).

For lead, highest accumulation was recorded in Salvinia *molesta*. This plant had an absorbed value of 872.22±223.96on Day 18, which was statically different ($P \leq 0.05$) from that of *Pistia stratiotes* (378.44±92.14) and *Lemna trisulca* with 194.77±49.04 (Fig. 4.20). On Day 15, *Salvinia molesta*absorbed 763.63±188.11, *Pistia stratiotes* (567.22±133.74) and *Lemna trisulca* (459.65±129.60).

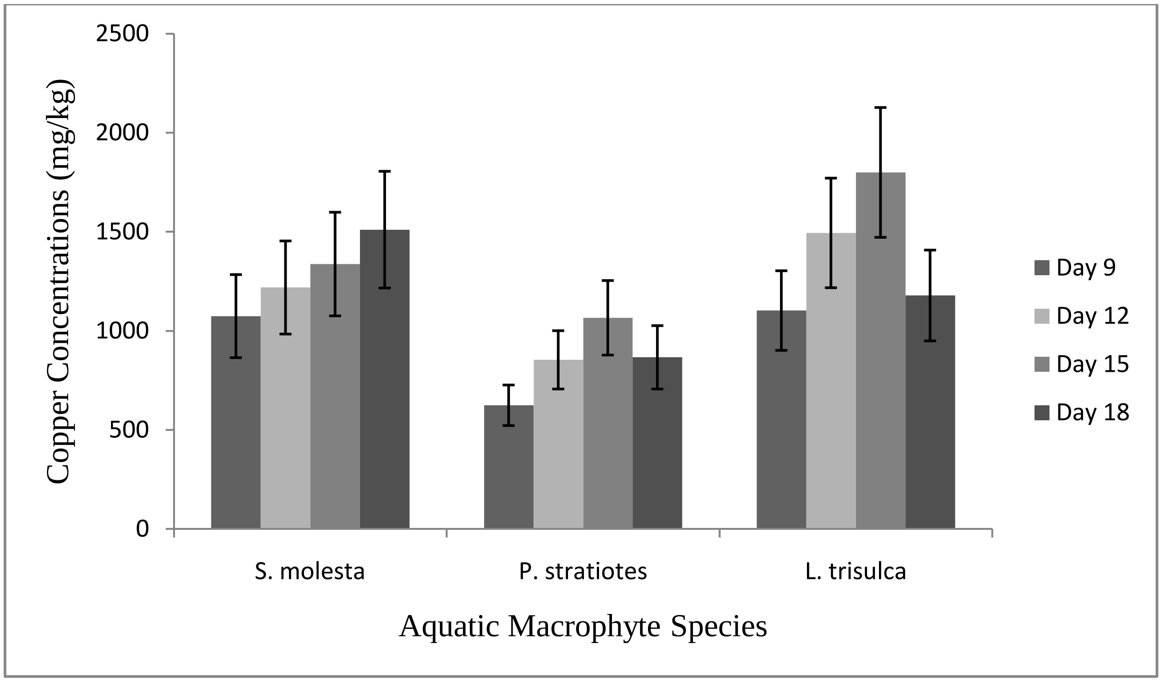

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Fig. 4.19: Comparison of Copper bioaccumulation by *Salvinia molesta*, *Pistia stratiotes* and*Lemna trisulca*

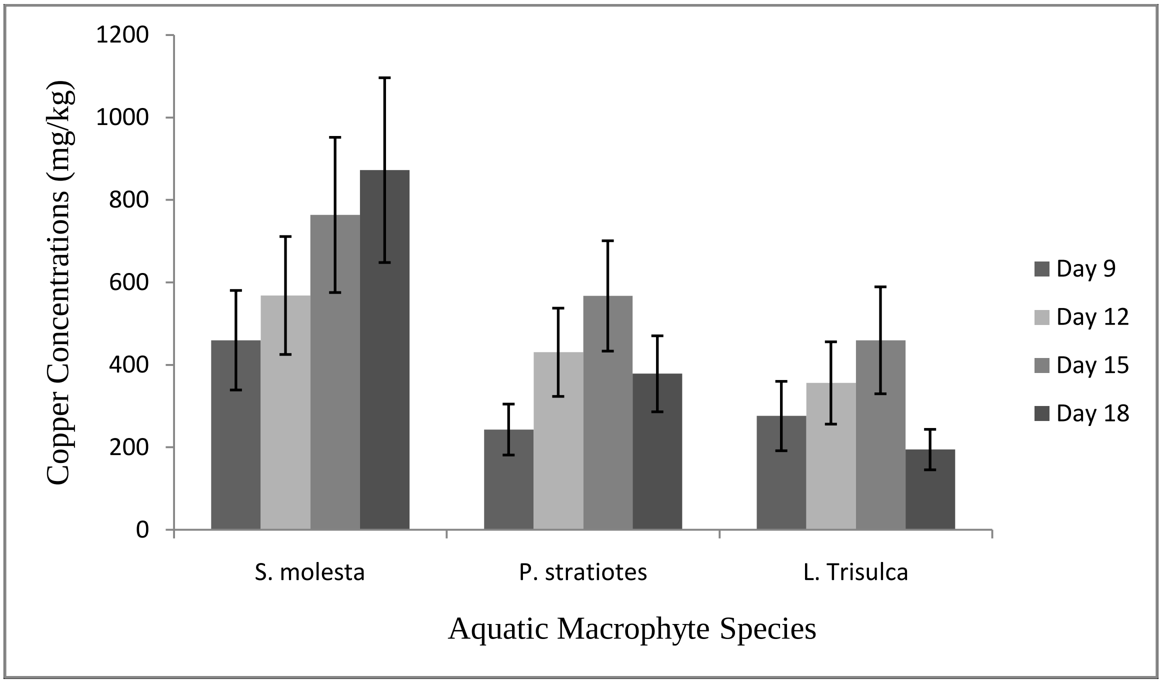

Fig. 4.20:Comparison of lead bioaccumulation by *Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca*

Table 4. 18: Comparison of the accumulation of copper and lead in the 3macrophytes

Plants	Copper	Lead
S. molesta	1510.71±294.49[a]	872.22±223.96 [a]
P. stratiotes	866.18±159.95[a]	378.44±92.14 [b]
L. trisulca	1178.39±229.27[a]	194.77±49.04 [b]
Mean ± SE	1185.09±137.89	481.81±90.75
P value	**0.163ns**	**0.005***

Means with the same superscript along column are not significant at P>0.05.* – significant at P ≤ 0.05.

4.6 Principal Component Analysis (PCA)

Principal Component Analysis is a multiple correlation that takes the proximity of all the treatments/factors and the parameters into consideration.

The whole parameters are grouped into two components and explanation is based on/relative to component 1 and 2 (Fig. 4.21). Parameters in the first quadrant (S Pb, P Pb and L Pb) are positively correlated with factors in the 1st quadrant (CU2PB3, CU2PB2, CU3PB3 and CU3PB2). The parameters and factors in the 1st quadrantare also positively correlated with parameters and factors in the second quadrant (L Cu, S Cu, P Cu, CU2PB1, CU3PB1, CU2PB0, CU3PB0, Catalase, Peroxidase, Chlorophyll *a* and chlorophyll *b*).

The factors in the 3rd and 4th quadrant are negatively correlated with parameters and factors in the 1st and 2nd quadrant (Fig.4.21).

Overlapping of catalase, peroxidase, chlorophyll *a* and *b* is because of the close relationship of the values and the data.

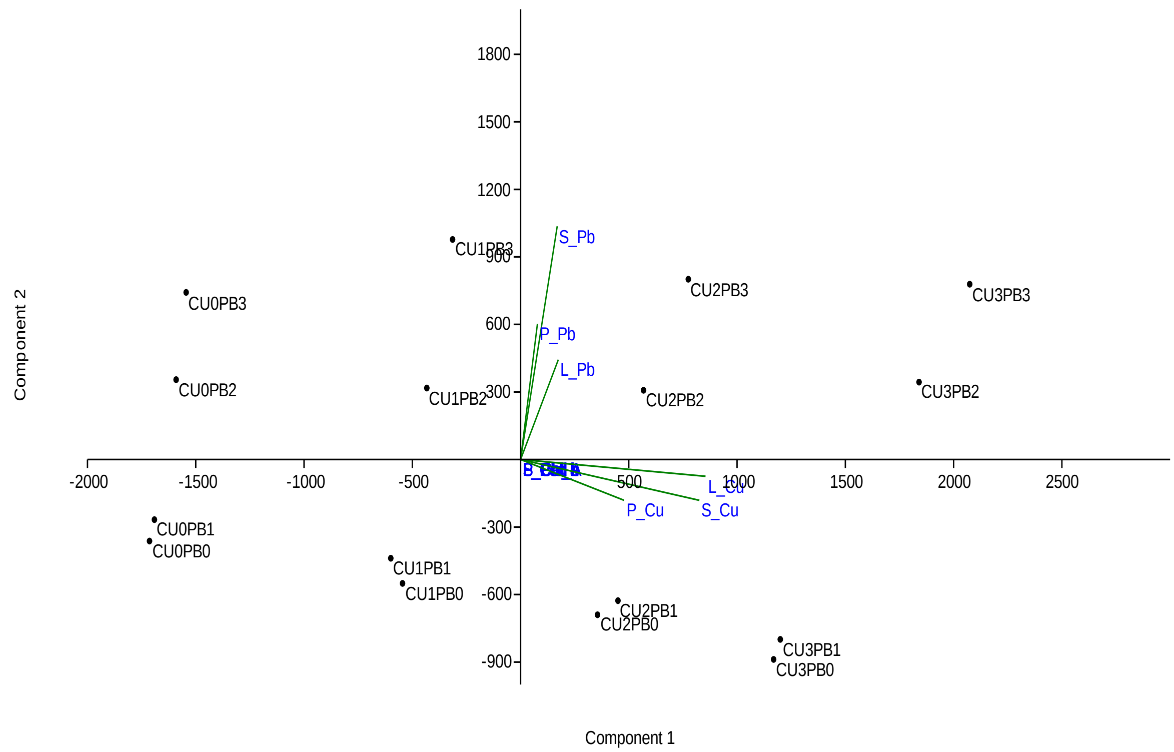

Fig. 4.21: Principal Components Analysis (PCA) showing significant correlation between physiological responses and metal uptake/absorption by the macrophytes

KEY: SCu – *Salvinia* Cu, SPb – *Salvinia* Pb, PCu – *Pistia* Cu, PPb – *Pistia* Pb, LCu – *Lemna* Cu, LPb – *Lemna* Pb,
Cat – Catalase, ChlA – Chlorophyll A, ChlB – Chlorophyll B, P – Pod.

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Chlorophyll Content (Photosynthetic Pigment)

5.1.1 Chlorophyll **a** content

A decrease in chlorophyll **a** content was recorded in this study. The decrease in chlorophyll **a**concentrations recorded in this study in the presence of different copper and lead combinations may be because these metals are capable of destroying and decreasing plant chlorophyll content. This is mainly because of the reduction and oxidation of various components involved in the biosynthesis pathway of pigments (Battah 2010). According to Wang and Dei(2001) the reduction in cell density observed in the presence of increasing metal contents may be due to the blocking of the intake of other nutrients into the cell. This is because of the binding of metal ions to the cell nutrient transport systems.

For all the macrophytes studied, the chlorophyll content decreased with an increase in metal(s) concentration. It was only the control that had stability in the chlorophyll a content. With increase in the concentration of copper and lead (individually and combination of the two), the content of chlorophyll a drastically reduce *in Salvinia molesta, Pistia stratiotes* and *Lemna trisulca* (Figure 4.1, 4.2 and 4.3). Significant differences ($p>0.05$) were recorded in the content of chlorophyll a. The higher the concentrations of copper and lead applied, the lower the content of chlorophyll a. The reduction of chlorophyll a content in the macrophytes may be attributed to inhibition of chlorophyll synthesis which results in the loss of photosynthetic activity due to the disruption of chloroplast.

The lowest content of chlorophyll a was observed in day 12. This implies that the number of days in which the plants are exposed to the heavy metals will determine the extent to which the pigment will be affected. The longer the plants were left in the aquaria containing the metals, the higher the reduction in the pigment content (Fig. 4.4).A decline in chlorophyll **a** content suggests that the metals affected the chlorophyll **a** synthesizing system and chlorophyllase activity. This is in agreement with the work of Singh *et al.*, (2012) where they reported that Pb and Ni reduced the chlorophyll a in black gram plant (*Vignamungo* L). At higher concentration of the metal, the reduction in chlorophyll a content increased drastically.

The negative effect of metals on the production of chlorophyll *a*obtained in the present study agrees with the results of He *et al.*,(2012), who showed a decrease in chlorophyll *a* concentration in cultures exposed to atrazine. Also Ma *et al.* (2006) showed that decreased chlorophyll **a**concentrations can lead to a decline in photosynthesis, thereby affecting growth rate and biomass concentrations in the cultures. Similar findings were recorded by Maine*et al.*, (2004) where they found that chlorophyll a content reduced in *Salvinia herzogii* and *P. stratiotes* due to chromium accumulation. Mishra and Tripathi (2009) also recorded decrease in Chlorophyll **a** content in *Eirchorrniacrassipes* with increase in the concentration of chromium and zinc.

Lemnatrisulca had the highest chlorophyll a content among all the macrophytes used. As a result of that, it was the plant with the highest means when the plants were compared (Fig. 4.4). Megateli *etal*. (2009) also reported in their findings that there was a reduction in chlorophyll **a** content in *Lemnagibba* when they were exposed to different concentration of cadmium, copper and zinc.Babu *etal*. (2005) found that chlorophyll a content was affected in the same proportion and that it was accompanied by leaf necrosis, a possible indicator of cell death.

5.1.2 Chlorophyll **b** content determination

In allthe three macrophytes studied, the content of chlorophyll **b** reduced with increase in the concentration of the copper and lead. The content of chlorophyll **b** in the control in all the days did not show any significant difference, but as the concentration increased with the exposure time, the content of chlorophyll b reduced drastically and it showed a significant difference ($P<0.05$ and $P\geq0.01$). The combination of the two metals had more effect on the content of chlorophyll b, than the individual effect of each of the metals.

In all the macrophytes, there was a reduction in the chlorophyll **b** content on all the plants exposed to concentration of 0.05, 1.0, and 1.5 mg/L of lead and 0.5, 1.0 and 1.5mg/L of copper.Decreased chlorophyll **b** content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. This is in agreement with the work of Singh *et al.*, (2012) where it reported that Pb and Ni reduced the chlorophyll b in black gram plant (*Vignamungo* L). At higher concentration of the metal, the reduction in chlorophyll b increased drastically.

The negative effect of metals on the production of chlorophyll **b**obtained in the present study agrees with the results of He *et al.*(2012), who showed a decrease in chlorophyll **b**concentration in cultures exposed to atrazine. Also Ma *et al.* (2006) showed that decreased chlorophyll**b** concentrations can lead to a decline in photosynthesis, thereby affecting growth rate and biomass concentrations in the cultures. A reduction of the total chlorophyll content in *A. caroliniana, S. minima* and *S. polyrhiza* was verified by Lizieri *et al.*, 2012. Several studies have demonstrated that excess metals reduce the chlorophyll content in plants (Sinha *et al.*, 1994, Gonzales *et al.*, 1998; Hua *et al.*,2011). It is believed that this process occurs as a result of the involvement of more than one mechanism and may be interpreted as a process of both direct and indirect actions of metals on chlorophyll molecules.

The longer the plants were exposed to the pollutants (heavy metals), the more the reduction in the chlorophyll **b**content. The chlorophyll b content of the macrophytes was greatly affected on day 9 and 12. There were significant differences in the content of chlorophyll b between the treatments when compared with the time of exposure (Table 4.3).In all the studied plants, the excess of copper and lead in solution decreased the chlorophyll content. These declines occurred differently among the species; treatment and time of exposure (Table 4.3 and 4.4).Similar findings were reported by Lizieri *et al*. (2012) where they subjected some macrophytes to different concentration of manganese; Hassan *et al.*, 2007; Mishra *et al.*, 2008; Cheng *et al.*, 2007 and Mishra and Tripathi, 2009. Mishra and Tripathi (2009) discovered that there was a decline in chlorophyll contents with increase in the concentration of Cr and Zn and also with increasing duration of exposure.

Lemnatrisulca had a content that was far higher than the other macrophytes. When compared statistically, it still showed that the content was higher than other macrophytes even after subjecting them to different concentration of copper and lead. There was a significant difference in the content of chlorophyll **b** among the different plants (Table 4.4).*Lemna trisulca* had the highest content followed by *Salviniamolesta* and it was least in *Pistiastratiotes*. Sudhaka Babu *etal*. (2003) found that growth decreased significantly and that chlorophyll content was affected in the same proportion in the plants they worked on.

The mechanisms of toxicity of metalcompounds (including Cu and Pb) on photosynthetic processes are still subject to discussions they are capable of decreasing the activity of photosystem II (PSII). Baron *et al.,* (1995) showed that Cu has a direct impact on photosynthesisby inhibiting photosynthetic electron transport, especiallyin PSII.

The effect of metal ions on plants includes thedisruption of many physiological functions such as water uptake,respiration, mineral nutrient uptake and photosynthesis (Burzynskiand Zurek, 2007). Lead altered theactivity of the oxygen-evolving complex in pea and broad bean,ultimately causing the disassembly of their PSII (Geiken *et al.,* 1998; Herbette *et al.,* 2006).

5.2 Antioxidant enzyme activity

Oxidative stress has become an important subject for terrestrial and aquatic toxicology (Ames*et al.,* 1993).This present study demonstrated an up regulation of the important antioxidant enzymes CAT and PODafter exposure to copper and lead stress. This increased activity of these enzymes is indicative of the toxic effect of copper and lead. The up-regulation of antioxidant enzyme activities means there was an increasedreactive oxygen species(ROS) content, which are able to disrupt chlorophyll synthesis and inhibit cell growth (Qian *et al.* 2009). Many studies have shown that ROS are produced when plant was exposed to different pollutants (Qian *et al*. 2009). Reactive oxygen species (ROS)are formed and degraded by all aerobic organisms, leadingto either physiological concentrations required for normalcell function or excessive quantities, a state called oxidativestress (Pinho and Ladeiro 2012).

5.2.1 Catalase (CAT) Activity

The enzymatic activity of the CAT showed statistical differences ($P \leq 0.05$) among the different treatments. With increase in the concentration of the metals (copper and lead) there were significant increases in the activity of catalase. Also, time exposure was another important factor to consider with reference to the activity of the enzyme catalase.Figure 4.7, 4.8 and 4.9 showed that in all the plants studied, the activity of catalase increased drastically with increase in the concentration of the metals and also with the increase in incubation period.Thus the increased activities of this enzyme suggest increased production of H_2O_2. To cope with metal stress, plants possess mechanisms to protect themselves from metal injury and poisoning, these mechanisms include extra cellular and intracellular sequestration of metal ions (Tripathi and Gaur, 2006).

Analysis of variance showed significant difference ($P \leq 0.05$) between the catalase activity and the incubation period, for all the experimental sets. Analysis of catalase activity with increasing time has suggested that activities increased from day 1 to 8, 11 and finally to day 14 in different experimental sets (Table 4.5). Lizieri *et al*. (2012) reported a contrary view in the leaves of *S. polyrhiza* that were subjected to 0.4mM of Mn; there was a significant reduction in the catalase enzymatic activity when compared with the control.

When the catalase enzymatic activities were compared among the plant, *Pistia stratiotes* and *Salvinia molesta*had the highest values (1.840 ± 00 and 1.839 ± 00) respectively and it was least in *Lemna trisulca* with a value of 1.829±00 (Table 4.15). In all the studied plants, catalase activity was least in the control. All the other treatments had values that were higher than that of the control, the value increase with increase in the concentration of metals.In agreement with our findings, Li *et al.*, (2006) showed that copper at the greatest dose caused a similar increase in antioxidantenzymes (SOD, CAT and GPX) compared to the control,which was accounted for a circumstantial evidencefor enhanced production of free radicals under metal stress. A number of studies further supported our findings that when faced with stresses, these enzymes are synthesized in high quantity to reduce the effect of ROS and further reduced oxidative damage (Collen *et al.,* 2003; Qian *et al*. 2007; Branco *et al*. 2010).

5.2.2 Peroxidase (POD) Activities

Enzymes such as POD participate in protective mechanisms against damage caused by free radicals. The antioxidant activity of the enzymes usually increase under stress, thereby conferring protection to plants (Santandrea *etal*. 2000, Fecht-christoffers *etal*. 2003) in the present study the result revealed that excess copper and lead altered the POD activity.

There were significant differences (P>0.05) in the peroxidase activity in *Salvinamolesta*, *Pistiastratiotes* and *Lemnatrisulca*. The activities were highest in CU3PB3 and least in CU0PB0 (Fig. 4.10, 4.11and 4.12). This disagrees with the work of Lizieri *et al.* (2012) where the enzyme activity of POD did not show statistical difference among plants exposed to the Mn concentration of 0.4mM and that of the control plant.Studies conducted with woody and herbaceous plant (Panda *et al.,* 1986, Shi *et al.,* 2006) demonstrated that excess Mn led to lipid peroxidation, indicating an increase production of reactive oxygen species and, consequently, an increase in peroxidase activity.

The interaction between the metals (copper and lead) generally had an additive effect on the activity of the two enzymes investigated in this study. Consequences of ROS formation include the gradual peroxidation of lipidstructures (Baryla *et al.,* 2000), oxidative DNA damage (Kasprzak, 2002) and photosynthetic apparatus damage (Dewez *et al.,* 2005).Devos *et al*. (1991)showed that Cudisturbed the integrity of thylakoid membranes. Yruela (2005)showed that ROS interfered with the biosynthesisof photosynthetic machinery and decreased the photosynthetic rate.

The enzymatic activity of the POD showed statistical difference $P>0.05$ among plants exposed to the different concentration of copper and lead compared with the control (Table 4.7, 4.8). The mechanism of defense of *Salvinia molesta, Pistia stratiotes and Lemna trisulca* to copper and lead toxicity is associated with the activities of these antioxidant enzymes as they showed a significant association with increasing copper and lead concentrations and the time of exposure. CAT and POD activities increased throughout the experimental period, indicating that these enzymes played a more important role in the regulation of oxidative stress in *Salvinia molesta, Pistia stratiotes and Lemna trisulca,* therefore it may be considered as stress biomarkers.

5.3 Effect of Copper and Lead on the Visual Symptoms

In the present study, *Salvinia molesta* did not have their growth affected throughout the period of the study (incubation period). The morphology of the ferns of *Salviniamolesta* did not change in the leaves or roots. With increase in concentration of the metals (copper and lead), there is no change in the appearance or morphology of the leaves and root(Table 4.9). This suggests that *Salviniamolesta* has an exclusive mechanism in response to stress imposed by excess metals (copper and lead). Thus, this strategy may grant greater tolerance to the plant. This is in line with the findings of Mishra and Tripathi, 2009 where it was recorded that *E. crassipes* did not show any morphological symptoms to Zn at any of the applied concentrations.

Pistiastratiotes and *Lemnatrisulca* showed visual symptoms from the day eleven of exposure to copper and lead. Chlorotic spots were observed on the leaves of these two species especially on those plants subjected to a concentration of 1.5mg/L. Chlorosis was less intense on those subjected to a concentration of 0.5 and 0.05mg/L of copper and lead respectively. For *Pistiastratiotes*, chlorosis was observed in all the treatment except in the control (Table 4.10).

For *Lemnatrisulca*, chlorosis was not observed in the control, in CU0PB1 and CU0PB2. All the other treatment showed symptom of chlorosis (Table 4.11).

Symptom of chlorosis became visible when the plants were exposed to concentration of 1.0mg/L and it became more pronounced at concentration of 1.5mg/L of both copper and lead. From the fourth day of exposure to copper and lead, the leaves presented chlorosis in the based region gradually extending to the apical region during the experiment. Similarly observations were reported by Lizieri *et al*.(2012) where they were subjected *Azollacaroliniana*Wild, *Salvniaminima* Baker and *SpirodelaPolyrhiza* (L) to different concentration of manganese. With increase in the concentration of manganese, there was increase in the severity of chlorotic symptoms

Chlorosis in response to copper is a commonly observed symptom in various aquatic species (Lewis, 1995; Vavilin *et al.,* 1995). In this study, there was degradation in the structure /morphology of the plants or photosynthetic pigments with increase in the concentration of copper and lead. This confirms a possible directed link between pigment degradation due to oxidative stress and toxicity of the metals (Maksymiec, 1997; Teisseire and Vernet, 2000).

Mishra and Tripathi (2009) found that *E. crassipes* subjected to different concentrations of chromium showed morphological symptoms like yellowing, chlorosis of leaves and root shedding. Uysal and Taner (2007) reported various visual toxicity symptoms (Pale and transparent plants) in *Lemnaminor* after 48h in the presence of cadmium. The same visual symptoms were observed in this research for *Pistiastratiotes* and *Lemnatrisulca*, along with a root loss in plants exposed to 1.5mg/L of copper and lead.

Salvinia molesta did not show any visual toxicity symptoms in all the concentrations the macrophytes were exposed to. This is in agreement with the findings of Mishra and Tripathi (2009) where they recorded that no symptom was observed in the aquatic plant *Eirchorrnia crassipes* at 20mg/L of Zn.

5.4 Metals Accumulation in the Aquatic Macrophytes

It is common knowledge that metal concentrations in aquatic plants vary considerably according to the plant part as well as to the type of element (Larsen and Schierup1981, Stoltz and Greger, 2002). Results revealed increasing trend of removal with the increasing incubation period. Analysis of variance showed significant ($P>0.05$) difference between the absorption of metals and incubation period for all species in day 9, 12, and 15. High uptake and accumulation of copper and lead by *Salviniamolesta*, *Pistiastratiotes* and *Lemnatrisulca* were observed at 0.5, 1.0, 1.5mg/L and 0.05, 1.0 and 1.5mg/L for copper and lead respectively (Fig. 4.13- 4.18). It was established by Kamal *et al*. (2004) that the removal rate of metals was dependent on the contamination/concentration. With increase in metal concentrations, the three plants were able to remove and accumulate high amount of heavy metals. This clearly shows that the metal sequestration also increases with increasing concentration of copper and lead in the medium.This result finding is in line with the findings of Miretzy *et al.*, (2004);Denga *etal*. (2004) and Mishra *et al.*, (2008).

The metal absorption/accumulation efficiency of copper and lead increase with increasing day and concentration for *Pistiastratiotes* and *Lemnatrisulca*. It increased in day 9, 12 and 15 but there was a decline in the bioaccumulation of metals in day 18 (Figure 4.13 to 4.18). The reduction in the accumulation of the metals on day 18may be due to hyper accumulation of the metals in the *P. stratiotes* and *Lemna trisulca*. It is also as a result of the chlorotic and necrotic signs shown by the plants. Once the plants start dying, they will not be able to absorb the metals into their tissues again.

For *Salviniamolesta*, the accumulation of copper and lead continued to increase and there was no decline in the absorption capacity of the metals (Fig. 4.13 and 4.14). There was greater absorption capacity in the plants that were exposed to concentration of 1.0mg/L and 1.5mg/L in all the species of aquatic, macrophytes. Higher remover of metals may be attributed to the several special characters possess by these species like fibrous and dense root system broadleaves by *Salviniamolesta* and *Pistiastratiotes* and fast growth in the three species (Mant *et al.*, 2007).

In a study comparing the effect of several metals on Lemna, Drost *etal*. (2007) found that after 7 days that Cd and Cu showed the highest toxicity (EC50 of 1.9and 9.7Um respectively) while Zn had a lower toxicity (EC 50 of 46.1Um). Other plants such as rapeseed were able to survive in the presence of 2mM (130mg/L) of Zn (Ben Glinaya *et al.*, 2007).

The macrophytes resulted highly effective in the uptake of heavy metals (Table 4.18).*Salvinia molesta*had the highest accumulation potential. These ferns were able to remove copper and lead from the water and accumulated it into the tissues (Fig. 4.19 and 4.20). The high difference between the final metals concentration in the plants species and the initial metal concentration ratifies that the main process of metals removal from water is macrophytes uptake. The fact that they have been able to remove all metals simultaneously constitutes a strong indication of their potential for advanced treatment of polluted waters. This is in line with the findings of Dipu *et al.*, 2010 where they reported that *Eirchorrnia* and *Salvinia* species were more effective for treatment of industrial effluent particularly heavy metal uptake than the other plants studied. However, the economic potential of the studied macrophytes may not be fully realized until they can be economically reduced to a dried, compact commodity.

It was established by Kamal *et al.,* (2004) that the removal rate of metals was dependent on the contaminant or the concentration of the metal. Removal of heavy metals by *Salviniamolesta*, *Pistiastratiotes* and *Lemnatrisulca* in the present study was high and this agrees with the work of Denga *etal*. (2004); Miretzky *etal*. (2004); Mishra and Tripathi (2009)and Mishra *et al.,* (2008). The higher the concentration of copper and lead, the greater the amount of metal removed by the plants with *Salviniamolesta* exhibiting the largest uptake and accumulation capability overall followed by *Lemnatrisulca*, then *Pistiastratiotes* for copper; while for lead, *Pistia stratiotes* had greater accumulation potential than *Lemna trisulca.*

A decrease in metals (copper and lead) removal was observed on day 18 by *Pistiastratiotes* and *Lemnatrisulca* (Table 4.24, 4.25, 4.26 and 4.27). The decrease in metals accumulation efficiency on day 18 may be due to the saturation of copper and lead in the macrophytes species, and also the tolerance of the plants towards the metals when the days were further increased. The accumulations on day 15 were higher than that of day 18 (Table 4.23, 4.24, 4.26 and 4.27). A similar result was reported by Mishra and Tripathi (2009) when they exposed *Eirchorrniacrassipes* to zinc at concentration of 20mgL^{-1}.Similar findings were also reported by Hassan *et al*. (2007) and Hua *et al*.(2011).

All the macrophytes used for this study were efficiently able to absorb or accumulate copper and lead from the medium into their tissues. This can be seen in Figure 13 to 18, the higher the concentration of the metal, the more the amount or content of metals absorb into the tissues of the plants. It was observed that even when the plants have accumulated metals, that is, when they may have been suffering toxic effects due to bioaccumulation of copper and lead, they still continued to remove the metals from the water. After they started showing visual symptoms like chlorosis from day eleven, the content of metals still continue to increase in the macrophytes. This implied that bioaccumulation still continued even when they were affected by high copper and lead concentration. This is in line with the findings of Maine *et al.*, 2004.

Copper is an essential micronutrient for normal plant metabolism, playingan important role in large number of metalloenzymes, photosynthesis related plastocyanin and membrane structure. It is also known to be a toxic heavy metal (Li and Xiong, 2004). Indeed, excessive Cu accumulation in plant tissues can affect several physiological and biochemical processes such as changes in nitrogen metabolism with reduction of total nitrogen, increase of free amino acids (Llorens *et al.*,2000; Mazen, 2004) reduction of photosynthetic activity (Frankart *et al.*, 2002; Olette *et al.*, 2008) and growth (Weckx and Clijsters, 1996; Sudhaker Babu *et al.*, 2003). In this experiment, copper proved to be toxic for *Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca*.

In plants, lead can be adsorbed by carboxyl groups (-COOH) from carbohydrates present in the cellular walls of the roots. It can also be bound to different functional groups from internal structures or tissues such as thiol (-SH) from cysteine and glutathione, amino acids of phytochelatins, hydroxyl (-OH) and carboxyl (-COOH) groups from malate, citrate and Oxalate, which are accumulated mainly in vacuoles and carboxyl (-COOH) and amino ($-NH_2$) group from amino acids (Lasat, 2000; Clemens, 2001; Ahalya*et al.*, 2003; Mari and Lebrun, 2005; Sharma and Dubey, 2005).

It has been estimated that in some terrestrial plant the majority of absorbed metals are immobilized in the cellular walls and accumulated in vacuoles, both in root and leaves (Mari and Lebrun, 2005; Sharma and Dubey, 2005) although the proportion appearing in different tissues and cellular structures depend on the species of plant, the type of metal and plant phenology.

5.5 Potential of Macrophytes for Phytoremediation Studies

Research by Kamal *et al*. (2004) has shown that some species of plants can absorb metals at a higher efficiency than the others. *Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca* can all be used for phytoremediation of copper and lead. On the 18th day, *S. molesta* was able to absorb the highest amount of copper (1510.71±294.49) followed by *L. trisulca* (1178.39±229.27) and the least was *P. stratiotes* (866.18±159.95). For lead, *S. molesta*also had the highest (872.22±223.96), followed by *P. stratiotes* (378.44±92.14) and *L. trisulca* (194.77±49.04) on day 18. The fact that *S.molesta* did not show any sign of chlorosis and necrosis in all the treatments also proves that the species is the best phytoremediator of copper and lead. This is in line with the findings of Dipu *et al.*, 2010 where it was reported that *Eirchorrnia* species and *Salvinia* species were more effective for treatment of industrial effluent particularly heavy metal uptake than the other plants studied.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendations

6.1 Summary

Investigations on the effects of copper and lead on the aquatic macrophytes (*Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca*) showed that photosynthetic pigments (Chlorophyll A and

Chlorophyll B) reduced/decreased with increasing copper and lead concentrations. Decrease in the photosynthetic pigment was observed in the three plants as the concentration of copper increased from 0.5mg/L to 1.0mg/L and then to 1.5mg/L and as the concentration of lead increased from0.05mg/L to 1.0mg/L and finally to 1.5mg/L. With increase time of exposure to copper and lead, chlorophyll content also reduced from day 4 to 9 and 12.

Catalase and peroxidase activities generally increased with increase in copper and lead concentration and with time of exposure (initial, day 8, 11, and 14 for catalase; initial, day 10, 13 and 15 for peroxidase). The enzyme activities showed significant positive correlation with copper and lead stress.

Some morphological symptoms of metal toxicity such as yellowing, chlorosis of leaves and root shedding were apparent *in Pistia stratiotes* and *Lemna trisulca* especially those expose to 1.0 and 1.5mg/L of copper and lead (combined). Salvinia molesta did not show any morphological symptoms in all the treatments expose to different concentration.

There was uptake and accumulation of copper and lead by *Salvinia molesta, Pistia stratiotes* and *Lemna trisulca.* Accumulations of the metals were concentration dependent. At a greater concentration of copper and lead, greater uptake and bioaccumulation were recorded in the three species. The time of exposure to the metals also determine/played a role in the accumulation. *Pistia stratiotes* and *Lemna trisulca* had a decrease in accumulation of metals on day 18.

Salvinia molesta, Pistia stratiotes and *Lemna trisulca* all proved to good phytoremediator of copper and lead considering the accumulated copper and lead in their tissues. All the three plants can accumulate the metals into their tissues especially copper.

The combined effect of these metals was greater than their individual effect. Therefore, individual and combined toxicity of copper and lead depend on the concentration of metals and duration of exposure to the metals.

6.2 Conclusions

- Chlorophyll a and b decreased with increasing concentrations of copper and lead and their various combinations and also with the exposure time in *S. molesta, P. stratiotes* and *L. trisulca.*
- The activities of the antioxidant enzyme (catalase and peroxidase) were dependent on the concentration of metals. The activities increased with increase in the concentration of the metals and also with increase in the exposure time. The higher the concentration of copper and lead, the higher the activities of catalase and peroxidase in *S. molesta, P. stratiotes* and *L. trisulca*.
- Copper and lead accumulation at concentrations of 1.0 and 1.5mg/L are associated with production of some morphological symptoms of toxicity such as yellowing of leaves and chlorosis in *P. stratiotes* and *L. trisulca.*
- Copper and Lead accumulation were concentration dependent in *S. molesta, P. stratiotes* and *L. trisulca*. At a greater concentration of copper and lead (1.0 and 1.5 mg/L), greater uptake and bioaccumulation rates were recorded in the three species of aquatic macrophytes. The more the plants stayed in the medium, the more the absorption of the metals.
- The three species can be used for phytoremediation purpose. *S. molesta* was found to be best for phytoremediation studies in relation to the absorption rate of the heavy metals followed by *L. trisulca* and *P. stratiotes* had the least absorption.

6.3 Recommendations

- *S. molesta, P. stratiotes* and *L. trisulca* can be recommended for the removal of copper and lead from polluted aquatic environment. Metal uptake feature in plants can be used for phytoremediation technology in the aquatic environment.
- The results highlighted the importance of studying interactions of heavy metals toxicity in parallel with their individual effects. Studies in multi- species exposure to metals should be investigated to get further information on the effects of metals on water plants.
- Further works using other species of aquatic plants should be encouraged in order to know if these plants can also be used for phytoremediation studies.
- To achieve better remediate effects, more effort is required to accelerate the pace of phytoremediation techniques from laboratory experiment to practical use.
- Macrophytes replacement should be done at the right time in order to develop a more efficient, natural and economic integrated macrophytes based system most advantageous for heavy metal removal.

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APPENDIX I

Chlorophyll acontentin *Salvinia molesta* at different Cu and Pb concentrations

Treatments	Chlorophyll a content (mg g^{-1})				
	Initial	**Day 4**	**Day 9**	**Day 12**	**Mean**
CU0PB0	8.40±0.00	8.40±0.01^{b}	8.40±0.00^{a}	8.41±0.00^{a}	8.40±0.00^{a}
CU0PB1	8.40±0.00	8.40±0.00^{b}	7.54±0.02^{b}	7.52±0.01^{b}	7.96±0.00^{b}
CU0PB2	8.40±0.00	8.40±0.00^{b}	6.84±0.01^{d}	6.71±0.01^{d}	7.59±0.00^{d}
CU0PB3	8.40±0.00	8.40±0.00^{b}	6.35±0.03^{fg}	6.29±0.01^{g}	7.36±0.00^{g}
CU1PB0	8.40±0.00	8.42±0.01^{a}	7.18±0.01^{c}	7.05±0.03^{c}	7.77±0.01^{c}
CU1PB1	8.40±0.00	8.40±0.00^{b}	6.44±0.06^{f}	6.39±0.05^{f}	7.41±0.02^{f}
CU1PB2	8.40±0.00	8.40±0.00^{b}	6.07±0.03^{h}	5.90±0.03^{i}	7.20±0.01^{i}
CU1PB3	8.40±0.00	8.40±0.00^{b}	5.68±0.03^{j}	5.62±0.02^{k}	7.03±0.00^{k}
CU2PB0	8.40±0.00	8.41±0.00^{b}	6.63±0.03^{e}	6.55±0.04^{e}	7.50±0.01^{e}
CU2PB1	8.40±0.00	8.40±0.00^{b}	5.91±0.04^{i}	5.80±0.02^{j}	7.13±0.01^{j}
CU2PB2	8.40±0.00	8.41±0.00^{b}	5.24±0.01^{k}	5.14±0.03^{m}	6.80±0.01^{m}
CU2PB3	8.40±0.00	8.40±0.00^{b}	4.75±0.08^{l}	4.75±0.03^{n}	6.58±0.03^{n}
CU3PB0	8.40±0.00	8.41±0.00^{b}	6.28±0.04^{g}	6.13±0.02^{h}	7.31±0.01^{h}
CU3PB1	8.40±0.00	8.41±0.00^{b}	5.59±0.01^{j}	5.44±0.02^{l}	6.96±0.00^{l}
CU3PB2	8.40±0.00	8.40±0.00^{b}	4.82±0.03^{l}	4.74±0.03^{n}	6.59±0.02^{n}
CU3PB3	8.40±0.00	8.41±0.00^{b}	4.26±0.02^{m}	4.13±0.03^{o}	6.30±0.01^{o}
Mean	**8.40±0.00**	**8.41±0.00**	**6.12±0.15**	**6.03±0.16**	**7.24±0.08**
P value		**0.044***	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX II

Chlorophyll acontentin *Pistia stratiotes* at different Cu and Pb concentrations

Treatments	Chlorophyll a content (mg g^{-1})				
	Initial	**Day 4**	**Day 9**	**Day 12**	**Mean**
CU0PB0	5.84±0.00	5.84±0.00^{ab}	5.84±0.01^{a}	5.84±0.01^{a}	5.84±0.01^{a}
CU0PB1	5.84±0.00	5.82±0.00^{c}	5.27±0.06^{b}	4.98±0.03^{b}	5.48±0.02^{b}
CU0PB2	5.84±0.00	5.84±0.00^{ab}	4.82±0.08^{c}	4.75±0.07^{c}	5.31±0.04^{d}
CU0PB3	5.84±0.00	5.84±0.00^{a}	4.31±0.05^{e}	3.97±0.04^{f}	4.99±0.02^{f}
CU1PB0	5.84±0.00	5.84±0.00^{a}	5.19±0.00^{b}	4.83±0.03^{c}	5.42±0.01^{c}
CU1PB1	5.84±0.00	5.84±0.00^{ab}	4.91±0.00^{c}	4.53±0.02^{d}	5.28±0.01^{d}
CU1PB2	5.84±0.00	5.83±0.01^{bc}	4.17±0.02^{f}	3.79±0.08^{g}	4.91±0.03^{g}
CU1PB3	5.84±0.00	5.84±0.00^{a}	3.92±0.02^{h}	3.21±0.01^{i}	4.70±0.01^{i}
CU2PB0	5.84±0.00	5.83±0.00^{ab}	4.63±0.03^{d}	4.12±0.04^{e}	5.11±0.02^{e}
CU2PB1	5.84±0.00	5.84±0.00^{a}	4.04±0.03^{g}	3.52±0.03^{h}	4.81±0.01^{h}
CU2PB2	5.84±0.00	5.84±0.00^{a}	3.63±0.02^{i}	2.96±0.03^{j}	4.57±0.01^{j}
CU2PB3	5.84±0.00	5.83±0.00^{ab}	2.91±0.00^{j}	2.24±0.02^{k}	4.21±0.00^{k}
CU3PB0	5.84±0.00	5.84±0.00^{a}	4.34±0.02^{e}	4.06±0.03^{ef}	5.02±0.01^{f}
CU3PB1	5.84±0.00	5.83±0.00^{ab}	3.92±0.03^{h}	3.22±0.01^{i}	4.70±0.01^{i}
CU3PB2	5.84±0.00	5.84±0.00^{ab}	2.80±0.05^{k}	2.10±0.02^{l}	4.15±0.02^{l}
CU3PB3	5.84±0.00	5.84±0.00^{ab}	2.20±0.04^{l}	1.89±0.05^{m}	3.94±0.02^{m}
Mean	**5.84±0.00**	**5.83±0.00**	**4.18±0.14**	**3.75±0.16**	**4.90±0.07**
P value		**0.005****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 - 0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX III

Chlorophyll acontentin *Lemna trisulca*at different Cu and Pb concentrations

	Chlorophyll a content (mg g^{-1})				
Treatments	**Initial**	**Day 4**	**Day 9**	**Day 12**	**Mean**
CU0PB0	12.56±0.00	$12.56±0.08^{a}$	$12.69±0.13^{a}$	$12.73±0.11^{a}$	$12.64±0.08^{a}$
CU0PB1	12.56±0.00	$12.58±0.00^{a}$	$10.48±0.10^{b}$	$9.89±0.00^{b}$	$11.38±0.03^{b}$
CU0PB2	12.56±0.00	$12.55±0.01^{a}$	$9.60±0.13^{d}$	$9.33±0.06^{d}$	$11.01±0.04^{d}$
CU0PB3	12.56±0.00	$12.58±0.01^{a}$	$9.10±0.10^{e}$	$8.53±0.07^{e}$	$10.69±0.04^{e}$
CU1PB0	12.56±0.00	$12.59±0.01^{a}$	$9.95±0.05^{c}$	$9.61±0.01^{c}$	$11.18±0.01^{c}$
CU1PB1	12.56±0.00	$12.55±0.02^{a}$	$8.67±0.11^{f}$	$8.43±0.03^{ef}$	$10.55±0.04^{f}$
CU1PB2	12.56±0.00	$12.56±0.00^{a}$	$8.15±0.03^{g}$	$7.85±0.07^{g}$	$10.28±0.02^{g}$
CU1PB3	12.56±0.00	$12.58±0.02^{a}$	$7.67±0.11^{h}$	$7.36±0.12^{h}$	$10.04±0.05^{h}$
CU2PB0	12.56±0.00	$12.58±0.01^{a}$	$8.55±0.06^{f}$	$8.27±0.03^{f}$	$10.49±0.02^{f}$
CU2PB1	12.56±0.00	$12.56±0.00^{a}$	$7.29±0.05^{i}$	$6.88±0.08^{j}$	$9.82±0.03^{i}$
CU2PB2	12.56±0.00	$12.56±0.00^{a}$	$6.23±0.05^{j}$	$6.05±0.06^{k}$	$9.35±0.03^{j}$
CU2PB3	12.56±0.00	$12.56±0.00^{a}$	$5.76±0.05^{k}$	$5.56±0.10^{l}$	$9.11±0.04^{k}$
CU3PB0	12.56±0.00	$12.56±0.00^{a}$	$7.31±0.07^{i}$	$7.06±0.04^{i}$	$9.87±0.03^{i}$
CU3PB1	12.56±0.00	$12.56±0.00^{a}$	$6.28±0.02^{j}$	$6.05±0.02^{k}$	$9.36±0.01^{j}$
CU3PB2	12.56±0.00	$12.61±0.03^{a}$	$4.93±0.07^{l}$	$4.38±0.05^{m}$	$8.62±0.03^{l}$
CU3PB3	12.56±0.00	$12.56±0.00^{a}$	$3.74±0.04^{m}$	$3.26±0.04^{n}$	$8.03±0.02^{m}$
Mean	**12.56±0.00**	**12.57±0.01**	**7.90±0.32**	**7.58±0.33**	**10.15±0.16**
P value		**0.953ns**	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ns – Not significant at P>0.05, ** - highly significant at P ≤ 0.01. CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX IV

Chlorophyll b content in *Salvinia molesta* at different Cu and Pb concentrations

Treatments	Chlorophyll b content (mg g^{-1})				
	Initial	Day 4	Day 9	Day 12	Mean
CU0PB0	17.14±0.00	$17.14±0.03^{b}$	$17.16±0.02^{a}$	$17.21±0.05^{a}$	$17.16±0.02^{a}$
CU0PB1	17.14±0.00	$17.15±0.00^{b}$	$16.32±0.01^{b}$	$16.31±0.01^{b}$	$16.73±0.01^{b}$
CU0PB2	17.14±0.00	$17.16±0.02^{b}$	$15.68±0.04^{c}$	$15.67±0.04^{d}$	$16.41±0.02^{c}$
CU0PB3	17.14±0.00	$17.14±0.00^{b}$	$15.11±0.07^{d}$	$15.10±0.07^{e}$	$16.12±0.04^{d}$
CU1PB0	17.14±0.00	$17.15±0.01^{b}$	$16.31±0.01^{b}$	$16.14±0.03^{c}$	$16.69±0.01^{c}$
CU1PB1	17.14±0.00	$17.16±0.01^{b}$	$15.22±0.02^{d}$	$15.21±0.02^{e}$	$16.18±0.01^{d}$
CU1PB2	17.14±0.00	$17.14±0.00^{b}$	$14.40±0.01^{e}$	$14.39±0.01^{f}$	$15.77±0.00^{e}$
CU1PB3	17.14±0.00	$17.14±0.00^{b}$	$13.92±0.03^{g}$	$13.90±0.04^{h}$	$15.52±0.02^{g}$
CU2PB0	17.14±0.00	$17.15±0.01^{b}$	$15.21±0.03^{d}$	$15.20±0.03^{e}$	$16.18±0.01^{d}$
CU2PB1	17.14±0.00	$17.15±0.01^{b}$	$14.14±0.04^{f}$	$14.08±0.04^{g}$	$15.63±0.02^{f}$
CU2PB2	17.14±0.00	$17.13±0.02^{b}$	$12.81±0.01^{i}$	$12.78±0.02^{j}$	$14.96±0.01^{i}$
CU2PB3	17.14±0.00	$17.15±0.02^{b}$	$11.72±0.00^{j}$	$11.70±0.01^{k}$	$14.43±0.00^{j}$
CU3PB0	17.14±0.00	$17.15±0.01^{b}$	$14.31±0.01^{e}$	$14.29±0.00^{f}$	$15.72±0.00^{e}$
CU3PB1	17.14±0.00	$17.31±0.17^{a}$	$13.67±0.01^{h}$	$13.65±0.01^{i}$	$15.44±0.05^{h}$
CU3PB2	17.14±0.00	$17.14±0.00^{b}$	$11.61±0.00^{j}$	$11.53±0.01^{l}$	$14.35±0.00^{k}$
CU3PB3	17.14±0.00	$17.15±0.02^{b}$	$10.86±0.11^{k}$	$10.58±0.11^{m}$	$13.93±0.04^{l}$
Mean	**17.14±0.00**	**17.16±0.01**	**14.28±0.26**	**14.23±0.26**	**15.70±0.13**
P value		**0.592ns**	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ns – not significant at P>0.05, ** - highly significant at P ≤ 0.01. CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX V

Chlorophyll bcontentin *Pistia stratiotes* at different Cu and Pb concentrations

Treatments	Chlorophyll b content (mg g^{-1})				
	Initial	**Day 4**	**Day 9**	**Day 12**	**Mean**
CU0PB0	14.71±0.00	14.70±0.01^{a}	14.72±0.01^{a}	14.72±0.01^{a}	14.71±0.01^{a}
CU0PB1	14.71±0.00	14.71±0.00^{a}	13.72±0.09^{b}	13.42±0.19^{b}	14.14±0.07^{ab}
CU0PB2	14.71±0.00	14.71±4.00^{a}	13.09±0.05^{c}	12.55±0.04^{c}	13.76±0.29^{abc}
CU0PB3	14.71±0.00	14.71±0.00^{a}	12.25±0.04^{d}	11.87±0.06^{e}	13.38±0.03^{abc}
CU1PB0	14.71±0.00	14.71±4.00^{a}	13.51±0.05^{b}	13.34±0.07^{b}	14.07±0.19^{ab}
CU1PB1	14.71±0.00	14.69±0.01^{a}	12.95±0.03^{c}	12.17±0.04^{d}	13.63±0.01^{abc}
CU1PB2	14.71±0.00	14.71±0.00^{a}	12.21±0.01^{d}	11.24±0.05^{f}	13.22±0.01^{abcd}
CU1PB3	14.71±0.00	14.71±0.00^{a}	11.44±0.02^{f}	10.58±0.04^{g}	12.86±0.02^{abcd}
CU2PB0	14.71±0.00	14.71±0.00^{a}	13.11±0.00^{c}	12.47±0.07^{c}	13.75±0.02^{abc}
CU2PB1	14.71±0.00	14.71±0.00^{a}	11.91±0.01^{e}	10.69±0.02^{g}	13.00±0.01^{abcd}
CU2PB2	14.71±0.00	14.71±0.00^{a}	10.38±0.08^{g}	9.42±0.09^{h}	12.30±0.04^{bcde}
CU2PB3	14.71±0.00	14.71±0.00^{a}	9.69±0.19^{h}	8.57±0.16^{i}	11.92±0.07^{cde}
CU3PB0	14.71±0.00	14.71±0.00^{a}	11.63±0.09^{f}	10.75±0.10^{g}	12.95±0.05^{bcd}
CU3PB1	14.71±0.00	14.71±0.00^{a}	9.50±0.14^{h}	8.57±0.04^{i}	11.87±0.04^{abcd}
CU3PB2	14.71±0.00	14.71±0.00^{a}	8.45±0.11^{i}	7.46±0.13^{j}	11.33±0.06^{de}
CU3PB3	14.71±0.00	14.71±0.00^{a}	7.38±0.06^{j}	6.21±0.02^{k}	10.75±0.02^{e}
Mean	**14.71±0.00**	**14.71±0.00**	**11.62±0.29**	**10.88±0.33**	**12.98±0.17**
P value		**0.630***	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at $P>0.05$. * – significant at $P \leq 0.05$, ** - highly significant at $P \leq 0.01$. CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX VI

Chlorophyll b contentin *Lemna trisulca* at different Cu and Pb concentrations

Treatments	Chlorophyll b content (mg g^{-1})				
	Initial	**Day 4**	**Day 9**	**Day 12**	**Mean**
CU0PB0	28.11±0.00	$28.11±0.01^{a}$	$28.16±0.04^{a}$	$28.36±0.16^{a}$	$28.19±0.05^{a}$
CU0PB1	28.11±0.00	$28.10±0.01^{a}$	$26.88±0.08^{b}$	$26.32±0.45^{b}$	$27.35±0.13^{b}$
CU0PB2	28.11±0.00	$28.11±0.00^{a}$	$25.29±0.06^{d}$	$24.36±0.17^{d}$	$26.46±0.06^{cd}$
CU0PB3	28.11±0.00	$28.11±0.00^{a}$	$23.46±0.12^{f}$	$22.87±0.06^{f}$	$25.64±0.04^{e}$
CU1PB0	28.11±0.00	$28.10±0.01^{a}$	$25.62±0.09^{c}$	$25.07±0.02^{c}$	$26.73±0.03^{c}$
CU1PB1	28.11±0.00	$28.11±0.01^{a}$	$22.24±0.03^{g}$	$20.46±0.31^{gh}$	$24.73±0.08^{f}$
CU1PB2	28.11±0.00	$28.07±0.04^{a}$	$21.39±0.22^{h}$	$20.51±0.20^{g}$	$24.52±0.11^{f}$
CU1PB3	28.11±0.00	$28.06±0.04^{a}$	$19.06±0.13^{j}$	$19.09±0.42^{i}$	$23.58±0.14^{g}$
CU2PB0	28.11±0.00	$28.11±0.00^{a}$	$24.14±0.02^{e}$	$23.73±0.11^{e}$	$26.02±0.03^{de}$
CU2PB1	28.11±0.00	$28.11±0.00^{a}$	$20.40±0.11^{i}$	$20.19±0.08^{gh}$	$24.20±0.05^{f}$
CU2PB2	28.11±0.00	$28.04±0.07^{a}$	$18.23±0.02^{l}$	$17.86±0.06^{j}$	$23.06±0.02^{g}$
CU2PB3	28.11±0.00	$28.11±0.00^{a}$	$16.67±0.10^{m}$	$16.44±0.11^{k}$	$22.33±0.05^{h}$
CU3PB0	28.11±0.00	$28.11±0.00^{a}$	$20.22±0.08^{i}$	$19.87±0.05^{h}$	$23.41±0.67^{g}$
CU3PB1	28.11±0.00	$28.06±0.04^{a}$	$18.54±0.06^{k}$	$18.31±0.04^{j}$	$23.25±0.02^{g}$
CU3PB2	28.11±0.00	$28.10±0.00^{a}$	$15.32±0.17^{n}$	$15.17±0.09^{l}$	$21.68±0.06^{i}$
CU3PB3	28.11±0.00	$28.11±0.00^{a}$	$12.71±0.07^{o}$	$12.36±0.15^{m}$	$20.32±0.05^{j}$
Mean	**28.11±0.00**	**28.10±0.06**	**21.14±0.61**	**20.69±0.60**	**24.47±0.31**
P value		**0.635ns**	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ns – not significant at P>0.05, ** - highly significant at P ≤ 0.01. CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX VII

Catalase activity of *Salvinia molesta* as a function of different Cu and Pb concentrations

Treatments	Catalase activity (mg^{-1})				
	Initial	**Day 8**	**Day 11**	**Day 14**	**Mean**
CU0PB0	1.836±0.00	$1.836±0.00^{g}$	$1.836±0.00^{h}$	$1.837±0.00^{h}$	$1.836±0.00^{h}$
CU0PB1	1.836±0.00	$1.837±0.00^{f}$	$1.838±0.00^{g}$	$1.839±0.00^{g}$	$1.837±0.00^{g}$
CU0PB2	1.836±0.00	$1.838±0.00^{e}$	$1.838±0.00^{g}$	$1.840±0.00^{f}$	$1.838±0.00^{f}$
CU0PB3	1.836±0.00	$1.839±0.00^{d}$	$1.840±0.00^{ef}$	$1.840±0.00^{ef}$	$1.839±0.00^{e}$
CU1PB0	1.836±0.00	$1.837±0.00^{e}$	$1.838±0.00^{g}$	$1.839±0.00^{g}$	$1.838±0.00^{g}$
CU1PB1	1.836±0.00	$1.839±0.00^{d}$	$1.840±0.00^{f}$	$1.840±0.00^{ef}$	$1.839±0.00^{e}$
CU1PB2	1.836±0.00	$1.839±0.00^{d}$	$1.840±0.00^{ef}$	$1.841±0.00^{de}$	$1.839±0.00^{e}$
CU1PB3	1.836±0.00	$1.840±0.00^{c}$	$1.841±0.00^{de}$	$1.841±0.00^{cd}$	$1.839±0.00^{d}$
CU2PB0	1.836±0.00	$1.839±0.00^{d}$	$1.840±0.00^{f}$	$1.840±0.00^{ef}$	$1.839±0.00^{e}$
CU2PB1	1.836±0.00	$1.840±0.00^{c}$	$1.841±0.00^{de}$	$1.842±0.00^{c}$	$1.840±0.00^{cd}$
CU2PB2	1.836±0.00	$1.840±0.00^{c}$	$1.841±0.00^{cd}$	$1.842±0.00^{c}$	$1.840±0.00^{c}$
CU2PB3	1.836±0.00	$1.841±0.00^{b}$	$1.842±0.00^{b}$	$1.843±0.00^{b}$	$1.841±0.00^{b}$
CU3PB0	1.836±0.00	$1.840±0.00^{c}$	$1.841±0.00^{de}$	$1.841±0.00^{cd}$	$1.839±0.00^{d}$
CU3PB1	1.836±0.00	$1.841±0.00^{b}$	$1.842±0.00^{bc}$	$1.843±0.00^{b}$	$1.840±0.00^{b}$
CU3PB2	1.836±0.00	$1.841±0.00^{b}$	$1.842±0.00^{b}$	$1.844±0.00^{b}$	$1.841±0.00^{b}$
CU3PB3	1.836±0.00	$1.842±0.00^{a}$	$1.843±0.00^{a}$	$1.845±0.00^{a}$	$1.842±0.00^{a}$
Mean	**1.836±0.00**	**1.839±0.00**	**1.840±0.00**	**1.841±0.00**	**1.839±0.00**
P value		**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 - 0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX VIII

Catalase activity of *Pistia stratiotes*as a function of different Cu and Pb concentrations

Treatments	Catalase activity (mg^{-1})				
	Initial	**Day 8**	**Day 11**	**Day 14**	**Mean**
CU0PB0	1.835±0.00	$1.836±0.00^{i}$	$1.836±0.00^{k}$	$1.836±0.00^{j}$	$1.835±0.00^{k}$
CU0PB1	1.835±0.00	$1.837±0.00^{h}$	$1.838±0.00^{j}$	$1.840±0.00^{i}$	$1.837±0.00^{j}$
CU0PB2	1.835±0.00	$1.837±0.00^{h}$	$1.839±0.00^{j}$	$1.841±0.00^{h}$	$1.838±0.00^{i}$
CU0PB3	1.835±0.00	$1.838±0.00^{g}$	$1.840±0.00^{i}$	$1.842±0.00^{g}$	$1.839±0.00^{h}$
CU1PB0	1.835±0.00	$1.837±0.00^{h}$	$1.839±0.00^{j}$	$1.841±0.00^{h}$	$1.838±0.00^{i}$
CU1PB1	1.835±0.00	$1.839±0.00^{ef}$	$1.841±0.00^{hi}$	$1.843±0.00^{f}$	$1.839±0.00^{g}$
CU1PB2	1.835±0.00	$1.840±0.00^{e}$	$1.842±0.00^{g}$	$1.843±0.00^{f}$	$1.840±0.00^{f}$
CU1PB3	1.835±0.00	$1.842±0.00^{d}$	$1.843±0.00^{ef}$	$1.844±0.00^{cd}$	$1.841±0.00^{e}$
CU2PB0	1.835±0.00	$1.839±0.00^{fg}$	$1.839±0.00^{j}$	$1.842±0.00^{g}$	$1.839±0.00^{h}$
CU2PB1	1.835±0.00	$1.842±0.00^{d}$	$1.843±0.00^{fg}$	$1.844±0.00^{ef}$	$1.841±0.00^{e}$
CU2PB2	1.835±0.00	$1.844±0.00^{c}$	$1.844±0.00^{de}$	$1.844±0.00^{de}$	$1.841±0.00^{d}$
CU2PB3	1.835±0.00	$1.845±0.00^{b}$	$1.845±0.00^{bc}$	$1.845±0.00^{b}$	$1.842±0.00^{bc}$
CU3PB0	1.835±0.00	$1.839±0.00^{ef}$	$1.841±0.00^{h}$	$1.843±0.00^{f}$	$1.839±0.00^{fg}$
CU3PB1	1.835±0.00	$1.844±0.00^{bc}$	$1.844±0.00^{cd}$	$1.844±0.00^{bc}$	$1.842±0.00^{c}$
CU3PB2	1.835±0.00	$1.845±0.00^{b}$	$1.845±0.00^{b}$	$1.845±0.00^{b}$	$1.842±0.00^{b}$
CU3PB3	1.835±0.00	$1.846±0.00^{a}$	$1.847±0.00^{a}$	$1.847±0.00^{a}$	$1.844±0.00^{a}$
Mean	**1.835±0.00**	**1.841±0.00**	**1.842±0.00**	**1.843±0.00**	**1.840±0.00**
P value		**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ** - highly significant at P ≤ 0.01. CU0 – 0mg/L, CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 - 0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX IX

Catalase activity of*Lemna trisulca*as a function of different Cu and Pb concentrations.

Treatments	Catalase activity (mg^{-1})				
	Initial	**Day 8**	**Day 11**	**Day 14**	**Mean**
CU0PB0	1.763±0.00	1.767±0.00^{h}	1.767±0.00^{i}	1.773±0.00^{j}	1.767±0.00^{k}
CU0PB1	1.763±0.00	1.773±0.00^{gh}	1.779±0.00^{h}	1.786±0.00^{i}	1.775±0.00^{j}
CU0PB2	1.763±0.00	1.779±0.00^{g}	1.783±0.00^{h}	1.792±0.00^{h}	1.779±0.00^{i}
CU0PB3	1.763±0.00	1.789±0.00^{f}	1.795±0.00^{g}	1.795±0.00^{h}	1.786±0.00^{h}
CU1PB0	1.763±0.00	1.789±0.00^{f}	1.799±0.00^{g}	1.808±0.00^{g}	1.790±0.0^{g}
CU1PB1	1.763±0.00	1.808±0.00^{e}	1.811±0.00^{f}	1.827±0.00^{f}	1.802±0.00^{f}
CU1PB2	1.763±0.00	1.818±0.00^{d}	1.836±0.00^{d}	1.838±0.00^{e}	1.814±0.00^{e}
CU1PB3	1.763±0.00	1.836±0.00^{c}	1.838±0.00^{cd}	1.840±0.00^{cde}	1.819±0.00^{d}
CU2PB0	1.763±0.00	1.824±0.00^{d}	1.830±0.00^{e}	1.840±0.00^{de}	1.814±0.00^{e}
CU2PB1	1.763±0.00	1.838±0.00^{bc}	1.839±0.00^{cd}	1.841±0.00^{bcde}	1.820±0.00^{d}
CU2PB2	1.763±0.00	1.839±0.00^{abc}	1.841±0.00^{cd}	1.844±0.00^{abcde}	1.822±0.00^{cd}
CU2PB3	1.763±0.00	1.841±0.00^{abc}	1.844±0.00^{abc}	1.844±0.00^{abcd}	1.823±0.00^{bc}
CU3PB0	1.763±0.00	1.843±0.00^{abc}	1.840±0.00^{cd}	1.841±0.00^{cde}	1.822±0.00^{cd}
CU3PB1	1.763±0.00	1.842±0.00^{abc}	1.843±0.00^{abc}	1.845±0.00^{abc}	1.823±0.00^{bc}
CU3PB2	1.763±0.00	1.844±0.00^{ab}	1.845±0.00^{ab}	1.846±0.00^{ab}	1.825±0.00^{ab}
CU3PB3	1.763±0.00	1.846±0.00^{a}	1.847±0.00^{a}	1.848±0.00^{a}	1.826±0.00^{a}
Mean	**1.763±0.00**	**1.817±0.00**	**1.821±0.00**	**1.826±0.00**	**1.807±0.00**
P value		**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 - 0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX X

Peroxidase activity of *Salvinia molesta* at different Cu and Pb concentrations

Treatments	Peroxidase activity (mg^{-1})				
	Initial	**Day 10**	**Day 13**	**Day 15**	**Mean**
CU0PB0	0.26±0.00	$0.26±0.00^{h}$	$0.26±0.00^{i}$	$0.26±0.00^{l}$	$0.26±0.00^{j}$
CU0PB1	0.26±0.00	$0.32±0.01^{g}$	$0.55±0.00^{de}$	$0.67±0.02^{g}$	$0.45±0.00^{h}$
CU0PB2	0.26±0.00	$0.64±0.02^{d}$	$0.56±0.01^{de}$	$0.74±0.00^{f}$	$0.55±0.00^{f}$
CU0PB3	0.26±0.00	$0.73±0.01^{c}$	$0.65±0.00^{bc}$	$0.80±0.00^{e}$	$0.61±0.00^{de}$
CU1PB0	0.26±0.00	$0.42±0.02^{f}$	$0.50±0.00^{fg}$	$0.45±0.01^{j}$	$0.41±0.01^{i}$
CU1PB1	0.26±0.00	$0.53±0.01^{e}$	$0.65±0.00^{bc}$	$0.63±0.00^{h}$	$0.52±0.00^{g}$
CU1PB2	0.26±0.00	$0.55±0.02^{e}$	$0.68±0.02^{b}$	$0.67±0.01^{g}$	$0.54±0.00^{f}$
CU1PB3	0.26±0.00	$0.87±0.02^{a}$	$0.76±0.01^{a}$	$0.86±0.00^{d}$	$0.69±0.00^{b}$
CU2PB0	0.26±0.00	$0.45±0.01^{f}$	$0.46±0.00^{h}$	$0.43±0.00^{k}$	$0.40±0.00^{i}$
CU2PB1	0.26±0.00	$0.54±0.02^{e}$	$0.52±0.01^{ef}$	$0.74±0.00^{f}$	$0.52±0.00^{g}$
CU2PB2	0.26±0.00	$0.76±0.02^{bc}$	$0.58±0.00^{d}$	$0.88±0.01^{d}$	$0.62±0.01^{d}$
CU2PB3	0.26±0.00	$0.87±0.01^{a}$	$0.65±0.01^{bc}$	$0.90±0.01^{c}$	$0.67±0.01^{c}$
CU3PB0	0.26±0.00	$0.68±0.00^{d}$	$0.47±0.00^{gh}$	$0.60±0.00^{i}$	$0.50±0.00^{g}$
CU3PB1	0.26±0.00	$0.79±0.01^{b}$	$0.54±0.03^{e}$	$0.79±0.00^{e}$	$0.60±0.01^{e}$
CU3PB2	0.26±0.00	$0.85±0.01^{a}$	$0.63±0.03^{c}$	$0.93±0.01^{b}$	$0.67±0.01^{c}$
CU3PB3	0.26±0.00	$0.89±0.01^{a}$	$0.79±0.00^{a}$	$1.04±0.01^{a}$	$0.75±0.00^{a}$
Mean	**0.26±0.00**	**0.63±0.03**	**0.58±0.02**	**0.71±0.03**	**0.55±0.02**
P value		**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 – 0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX XI

Peroxidase activity of *Pistia stratiotes* at different Cu and Pb concentrations

Treatments	Peroxidase activity (mg^{-1})				
	Initial	Day 10	Day 13	Day 15	Mean
CU0PB0	0.88±0.00	0.88±0.00^{j}	0.88±0.00^{e}	0.88±0.00^{i}	0.88±0.00^{g}
CU0PB1	0.88±0.00	0.92±0.01^{h}	1.16±0.00^{b}	1.20±0.02^{fg}	1.04±0.01^{d}
CU0PB2	0.88±0.00	0.96±0.00^{de}	1.19±0.00^{a}	1.26±0.03^{e}	1.07±0.01^{b}
CU0PB3	0.88±0.00	0.98±0.00^{bc}	1.18±0.01^{ab}	1.36±0.00^{a}	1.10±0.00^{a}
CU1PB0	0.88±0.00	0.93±0.00^{gh}	0.99±0.00^{d}	1.16±0.00^{h}	0.99±0.00^{f}
CU1PB1	0.88±0.00	0.97±0.01^{d}	1.01±0.00^{d}	1.31±0.00^{cd}	1.04±0.00^{d}
CU1PB2	0.88±0.00	0.98±0.00^{b}	1.01±0.00^{d}	1.34±0.00^{ab}	1.05±0.00^{c}
CU1PB3	0.88±0.00	1.00±0.00^{a}	1.00±0.00^{d}	1.33±0.00^{bc}	1.05±0.00^{c}
CU2PB0	0.88±0.00	0.92±0.00^{h}	0.99±0.00^{d}	1.18±0.00^{gh}	0.99±0.00^{f}
CU2PB1	0.88±0.00	0.95±0.00^{f}	1.16±0.00^{b}	1.30±0.01^{d}	1.07±0.00^{b}
CU2PB2	0.88±0.00	0.97±0.01^{d}	1.18±0.00^{ab}	1.30±0.00^{d}	1.08±0.00^{b}
CU2PB3	0.88±0.00	0.97±0.00^{cd}	1.17±0.00^{ab}	1.29±0.00^{d}	1.08±0.00^{b}
CU3PB0	0.88±0.00	0.90±0.00^{i}	1.10±0.03^{c}	1.21±0.00^{f}	1.02±0.01^{e}
CU3PB1	0.88±0.00	0.91±0.00^{h}	1.16±0.00^{ab}	1.24±0.00^{e}	1.05±0.00^{c}
CU3PB2	0.88±0.00	0.93±0.00^{g}	1.17±0.00^{ab}	1.26±0.00^{e}	1.06±0.00^{c}
CU3PB3	0.88±0.00	1.00±0.00^{a}	1.18±0.01^{ab}	1.26±0.00^{e}	1.08±0.00^{b}
Mean	**0.88±0.00**	**0.95±0.01**	**1.10±0.01**	**1.24±0.02**	**1.04±0.01**
P value		**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ** - highly significant at P ≤ 0.01. CU0 -0mg/L,. CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 - 0mg/L,PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX XII

Peroxidase activity of*Lemna trisulca* at different Cu and Pb concentrations

	Peroxidase activity (mg^{-1})				
Treatments	**Initial**	**Day 10**	**Day 13**	**Day 15**	**Mean**
CU0PB0	0.21±0.00	0.21±0.00^{h}	0.21±0.00^{k}	0.21±0.00^{l}	0.21±0.00^{m}
CU0PB1	0.21±0.00	0.38±0.01^{g}	0.41±0.01^{j}	0.73±0.01^{k}	0.43±0.00^{l}
CU0PB2	0.21±0.00	0.50±0.02^{f}	0.94±0.01^{f}	0.93±0.01^{i}	0.65±0.01^{i}
CU0PB3	0.21±0.00	0.58±0.01^{de}	1.03±0.00^{e}	1.06±0.00^{h}	0.72±0.00^{h}
CU1PB0	0.21±0.00	0.17±0.01^{h}	0.49±0.01^{i}	0.83±0.01^{j}	0.43±0.00^{l}
CU1PB1	0.21±0.00	0.21±0.02^{h}	0.98±0.00^{f}	1.07±0.00^{h}	0.62±0.01^{j}
CU1PB2	0.21±0.00	0.56±0.03^{ef}	1.04±0.00^{e}	1.31±0.00^{f}	0.78±0.02^{g}
CU1PB3	0.21±0.00	0.65±0.00^{c}	1.28±0.05^{c}	1.56±0.01^{c}	0.92±0.01^{d}
CU2PB0	0.21±0.00	0.42±0.01^{g}	0.57±0.00^{h}	1.07±0.00^{h}	0.57±0.00^{k}
CU2PB1	0.21±0.00	0.57±0.01^{ef}	1.03±0.00^{e}	1.46±0.00^{d}	0.82±0.00^{f}
CU2PB2	0.21±0.00	0.64±0.00^{cd}	1.28±0.00^{c}	1.48±0.00^{d}	0.90±0.00^{e}
CU2PB3	0.21±0.00	0.69±0.00^{c}	1.34±0.00^{b}	1.57±0.01^{c}	0.96±0.00^{c}
CU3PB0	0.21±0.00	0.41±0.00^{g}	0.88±0.00^{g}	1.11±0.03^{g}	0.65±0.01^{i}
CU3PB1	0.21±0.00	0.50±0.03^{f}	1.10±0.01^{d}	1.36±0.01^{e}	0.80±0.01^{g}
CU3PB2	0.21±0.00	0.79±0.00^{b}	1.38±0.00^{ab}	1.64±0.01^{b}	1.00±0.00^{b}
CU3PB3	0.21±0.00	0.96±0.00^{a}	1.39±0.00^{a}	1.74±0.02^{a}	1.07±0.01^{a}
Mean	**0.21±0.00**	**0.51±0.03**	**0.96±0.05**	**1.20±0.06**	**0.72±0.03**
P value		**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. ** - highly significant at P ≤ 0.01. CU0 -0mg/L, CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 - 0mg/L,PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX XIII

Copper concentrations in *Salvinia molesta* in 18 days period *in vitro*

Treatment	Copper concentrations (mg/kg)					
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**
CU0PB0	45.51±0.00	66.57±2.68g	57.36±10.42f	58.72±7.65f	70.53±1.31f	59.74±3.87e
CU0PB1	45.51±0.00	60.51±11.47g	99.13±13.37f	65.10±12.44f	118.11±36.79f	77.67±9.15e
CU0PB2	45.51±0.00	81.72±12.83g	96.59±17.56f	102.92±16.29f	107.71±15.61f	86.89±5.36e
CU0PB3	45.51±0.00	76.75±14.85g	93.32±21.53f	79.35±22.54f	96.11±21.80f	78.21±14.33e
CU1PB0	45.51±0.00	650.54±24.30f	765.04±3.21e	835.91±21.81e	908.25±39.40e	641.05±8.60d
CU1PB1	45.51±0.00	692.55±45.17f	809.72±63.15e	862.84±33.34e	951.56±17.16e	672.43±28.83d
CU1PB2	45.51±0.00	699.75±28.61f	773.48±38.28e	853.47±13.44e	949.82±6.86e	664.41±16.63d
CU1PB3	45.51±0.00	755.44±11.37f	904.98±29.82e	946.66±16.64e	1077.38±25.38e	746.00±12.80d
CU2PB0	45.51±0.00	1608.03±158.85de	1682.70±174.91d	1868.38±65.95d	2055.29±58.19d	1451.98±91.20bc
CU2PB1	45.51±0.00	1526.77±200.39e	1725.19±122.17d	1785.17±118.63d	1962.70±72.11d	1409.07±98.72bc
CU2PB2	45.51±0.00	1352.54±122.74e	1540.20±109.45d	1723.97±50.95d	1989.92±67.97d	1330.43±58.82c
CU2PB3	45.51±0.00	1576.76±58.48e	1691.36±10.64d	1895.13±34.19d	2056.20±94.93d	1452.99±194.20bc
CU3PB0	45.51±0.00	1888.80±214.88cd	2227.81±62.44c	2454.19±182.77c	2994.76±69.96c	1922.21±80.02ab
CU3PB1	45.51±0.00	2265.73±3.37ab	2589.03±132.99ab	2695.35±128.24bc	3065.89±54.04bc	2132.30±62.65a
CU3PB2	45.51±0.00	2147.86±63.33bc	2415.73±93.00c	3015.43±207.72a	3208.49±145.67ab	2166.60±78.70a
CU3PB3	45.51±0.00	2465.16±169.25a	2754.93±61.46a	2878.20±50.36ab	3286.83±45.23a	2286.13±61.29a
Mean	**45.51±0.00**	**1119.72±120.29**	**1264.16±1333.74**	**1382.55±148.83**	**1556.22±166.83**	**1136.38±132.65**
P value		**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX XIV

Lead concentrations in *Salvinia molesta* in 18 days period *in vitro*

Treatment	**Lead concentrations (mg/kg)**					
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**
CU0PB0	40.08±0.00	27.94±11.38d	48.10±1.93e	45.05±3.72f	67.54±0.95h	45.74±3.07h
CU0PB1	40.08±0.00	71.20±4.15d	107.42±17.68e	159.48±3.92ef	194.11±6.33g	114.46±2.22fgh
CU0PB2	40.08±0.00	767.65±15.38c	799.05±11.90d	874.59±5.41d	941.74±8.69f	684.62±5.21e
CU0PB3	40.08±0.00	1049.68±53.94b	1241.49±42.97bc	1347.97±37.23bc	1449.97±39.80e	1025.84±29.20c
CU1PB0	40.08±0.00	50.43±7.42d	66.90±10.43e	64.18±6.03f	65.70±3.99h	57.46±0.88gh
CU1PB1	40.08±0.00	68.35±8.86d	109.18±3.30e	178.91±9.27ef	209.89±9.77g	121.28±5.48fg
CU1PB2	40.08±0.00	739.17±15.78c	805.87±13.07d	874.56±8.67d	968.23±11.99f	685.58±3.70e
CU1PB3	40.08±0.00	1199.84±78.22a	1381.78±177.45ab	1911.30±60.53a	2056.84±15.72c	1317.97±36.09b
CU2PB0	40.08±0.00	48.49±10.45d	72.58±5.09e	336.06±259.63e	61.45±26.04h	111.73±52.13fgh
CU2PB1	40.08±0.00	75.55±8.24d	128.23±3.55e	166.73±5.40ef	220.41±6.12g	126.20±3.77fg
CU2PB2	40.08±0.00	705.05±37.90c	766.64±47.38d	1233.38±40.81c	1393.98±127.50e	827.83±19.12d
CU2PB3	40.08±0.00	1133.57±62.84ab	1413.49±51.44ab	1899.93±39.65a	2288.89±13.06b	1355.19±16.29b
CU3PB0	40.08±0.00	43.36±5.31d	61.70±4.94e	51.56±6.17f	48.81±5.41h	49.10±1.30h
CU3PB1	40.08±0.00	73.65±0.82d	132.42±3.44e	169.56±3.97ef	252.81±5.76g	133.70±2.35f
CU3PB2	40.08±0.00	733.07±16.98c	1140.79±68.26c	1477.99±34.25b	1849.50±4.10d	1048.29±2.78c
CU3PB3	40.08±0.00	1212.10±88.16a	1458.48±129.62a	2068.08±46.34a	2526.95±24.77a	1461.14±49.32a
Mean	**40.08±0.00**	**499.94±68.63**	**608.38±81.74**	**803.71±107.26**	**912.30±126.73**	**572.88±75.89**
P value		**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX XV

Copper concentrations in *Pistia stratiotes* in 18 days period *in vitro*

Treatment	**Copper concentrations (mg/kg)**					
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**
CU0PB0	180.87±0.00	186.73±39.09e	231.08±47.63e	241.70±29.22f	269.27±26.37ef	221.93±15.98e
CU0PB1	180.87±0.00	261.48±8.85de	261.82±31.40e	227.97±16.80f	206.24±10.54f	227.68±7.17e
CU0PB2	180.87±0.00	246.86±10.52de	222.67±29.78e	268.41±11.35f	263.02±22.36ef	236.36±13.07e
CU0PB3	180.87±0.00	243.81±3.00de	184.28±11.80e	200.60±17.52f	223.07±5.84f	206.53±4.94e
CU1PB0	180.87±0.00	691.74±27.77c	880.32±34.26cd	1118.66±65.86de	818.80±63.36cdef	738.08±27.95d
CU1PB1	180.87±0.00	695.21±12.28c	777.60±17.24d	955.24±57.64e	845.74±237.44	690.93±54.58d
CU1PB2	180.87±0.00	773.37±72.31bc	907.14±119.73	996.63±133.07e	578.00±73.43def	687.20±78.56d
CU1PB3	180.87±0.00	589.42±99.90cd	788.16±19.37a	1004.42±137.97e	962.76±131.59cdef	705.13±76.36d
CU2PB0	180.87±0.00	1104.41±34.41ab	1503.70±189.46ab	1631.66±130.25cd	1093.68±120.59cde	1102.86±50.76bc
CU2PB1	180.87±0.00	1096.03±120.23ab	1376.67±66.03abc	1650.63±130.50cd	1316.12±41.80bcd	1124.06±31.24bc
CU2PB2	180.87±0.00	1064.77±55.74ab	1156.18±34.39cd	1674.66±107.67cd	1087.10±89.67cde	1032.72±10.92c
CU2PB3	180.87±0.00	846.06±191.26bc	1352.14±274.87abc	1290.04±132.63de	1621.90±378.06abc	1058.20±86.78bc
CU3PB0	180.87±0.00	1291.63±226.84a	1639.45±76.44ab	1986.47±25.46abc	2118.97±578.00a	1443.48±135.02a
CU3PB1	180.87±0.00	1152.01±245.13ab	1718.18±154.58a	2260.44±244.81ab	1849.50±243.16ab	1432.20±105.40a
CU3PB2	180.87±0.00	1376.21±98.33a	1868.32±400.84a	2538.37±548.15a	1870.19±337.37ab	1566.79±220.49a
CU3PB3	180.87±0.00	1262.37±154.17a	1682.98±298.31a	1899.92±295.34bc	1628.42±194.09abc	1330.91±150.31ab
Mean	**180.87±0.00**	**805.13±62.62**	**1034.42±89.52**	**1246.61±113.00**	**1047.05±101.41**	**862.82±68.83**
P value		**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX XVI

Lead concentrations in *Pistia stratiotes* in 18 days period *in vitro*

Treatment	**Lead concentrations (mg/kg)**					
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**
CU0PB0	48.41±0.00	52.57±2.96f	47.61±5.74c	49.97±1.79e	51.69±2.25e	50.05±1.30c
CU0PB1	48.41±0.00	84.41±3.99f	110.38±14.29c	187.27±56.21e	160.38±49.14e	118.17±18.60c
CU0PB2	48.41±0.00	296.27±13.79e	614.32±82.52b	818.55±31.53d	448.20±76.88d	445.15±73.23b
CU0PB3	48.41±0.00	543.75±27.22bc	869.68±26.06ab	1073.78±24.34bc	800.71±39.61b	667.27±94.83ab
CU1PB0	48.41±0.00	57.80±2.68f	65.39±3.40c	59.34±3.08e	60.67±4.24e	58.32±1.88c
CU1PB1	48.41±0.00	82.28±4.21f	131.35±23.37c	202.06±27.71e	127.14±7.40e	118.25±15.22c
CU1PB2	48.41±0.00	501.51±50.02bcd	791.72±155.46ab	941.26±49.96cd	521.10±27.04cd	560.80±86.64ab
CU1PB3	48.41±0.00	591.77±64.40b	1144.07±399.00a	1102.96±42.64bc	1091.45±250.98a	795.73±139.36a
CU2PB0	48.41±0.00	60.36±2.35f	67.74±6.18c	66.96±2.18e	58.09±3.94e	60.31±2.31c
CU2PB1	48.41±0.00	74.74±8.29f	103.71±19.44c	136.51±22.82e	146.74±36.85e	102.02±12.80c
CU2PB2	48.41±0.00	420.43±25.25d	788.26±64.06ab	1125.05±91.09bc	691.57±156.52bcd	614.75±102.12ab
CU2PB3	48.41±0.00	457.48±118.36cd	1182.75±296.08a	1243.40±186.88ab	921.87±105.82ab	770.78±137.92a
CU3PB0	48.41±0.00	49.08±5.61f	47.08±3.76c	58.99±4.52e	51.42±0.14e	51.00±1.78c
CU3PB1	48.41±0.00	83.68±4.90f	113.35±4.71c	143.71±13.55e	134.42±14.80e	104.71±9.99c
CU3PB2	48.41±0.00	592.03±43.39b	749.10±68.52ab	1369.35±91.00a	818.15±88.64b	715.41±116.18a
CU3PB3	48.41±0.00	718.82±20.62a	837.94±62.87ab	1270.95±98.74ab	746.06±46.66bc	724.44±107.02a
Mean	**48.41±0.00**	**291.69±35.83**	**479.03±66.57**	**615.63±76.81**	**426.85±55.06**	**372.32±27.03**
P value		**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX XVII

Copper concentrations in *Lemna trisulca* in 18 days period *in vitro*

Treatment	Copper concentrations (mg/kg)					
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**
CU0PB0	239.67±0.00	276.17±6.65g	277.26±32.00g	243.31±3.07d	262.76±3.95g	259.83±7.21f
CU0PB1	239.67±0.00	225.61±10.51g	250.33±10.49g	256.89±7.68d	264.80±12.40g	247.46±6.56f
CU0PB2	239.67±0.00	215.40±27.50g	226.31±19.73g	241.76±30.78d	236.43±20.86g	231.91±18.53f
CU0PB3	239.67±0.00	242.51±21.77g	245.57±22.66g	204.61±9.51d	238.44±12.89g	234.16±6.22f
CU1PB0	239.67±0.00	1426.77±52.12cde	1518.53±79.80f	1766.90±71.77c	997.44±52.60fg	1189.86±21.05e
CU1PB1	239.67±0.00	1119.23±93.47ef	1306.40±140.02f	1685.84±208.70c	1002.92±134.51fg	1070.81±105.72e
CU1PB2	239.67±0.00	1163.78±255.12def	1453.15±154.62f	1601.20±187.17c	1138.21±157.18defg	1119.20±149.85e
CU1PB3	239.67±0.00	906.97±35.61f	1245.73±183.03f	1651.56±60.86c	1107.11±200.32efg	1030.21±69.10e
CU2PB0	239.67±0.00	1591.64±169.52bcd	2064.46±151.70f	2553.34±233.03b	1385.94±369.68cdef	1567.01±133.74d
CU2PB1	239.67±0.00	1792.21±146.04bc	2366.29±139.00cde	2578.95±80.56b	1582.90±111.77bcdef	1712.00±14.41cd
CU2PB2	239.67±0.00	1594.59±209.56bcd	2193.88±360.16de	2614.91±245.99b	2162.62±556.25abcde	1761.13±64.70cd
CU2PB3	239.67±0.00	1690.50±51.44bc	2377.96±52.31cde	2670.94±93.42b	2072.16±834.65abcdef	1810.25±157.96cd
CU3PB0	239.67±0.00	1999.60±113.46b	2674.91±56.49c	3591.00±375.43a	2377.02±458.25abc	2176.44±125.04b
CU3PB1	239.67±0.00	2003.64±334.67b	2547.14±174.16cd	2984.98±403.11b	2208.35±512.27abcd	1996.76±213.16bc
CU3PB2	239.67±0.00	2618.30±203.44a	3268.97±250.66b	3878.33±195.49a	2580.94±284.69ab	2517.24±57.41a
CU3PB3	239.67±0.00	2605.48±32.41a	3723.79±42.68a	4101.77±97.14a	3071.01±36.45a	2748.35±20.14a
Mean	**239.67±0.00**	**1342.03±117.43**	**1733.79±159.25**	**2039.14±189.15**	**1418.07±146.81**	**1354.54±117.82**
P value		**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX XVIII

Lead concentrations in *Lemna triculca* in 18 days period *in vitro*

Treatment	Lead concentrations (mg/kg)					
	Initial	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Mean**
CU0PB0	22.67±0.00	18.67±2.11f	21.71±1.99f	24.18±2.43e	27.98±1.67f	23.04±0.51g
CU0PB1	22.67±0.00	49.58±6.45def	67.67±13.00ef	94.89±10.41e	48.22±8.76def	56.61±5.71efg
CU0PB2	22.67±0.00	123.60±10.39def	165.62±7.37ef	155.26±14.04e	115.27±20.57de	116.48±6.04efg
CU0PB3	22.67±0.00	134.06±3.76de	200.30±12.79e	228.68±25.31e	127.62±29.73d	142.66±14.05ef
CU1PB0	22.67±0.00	39.87±12.75ef	33.93±11.86f	42.69±10.85e	37.65±7.87ef	35.36±8.42g
CU1PB1	22.67±0.00	88.21±4.81def	142.67±4.53ef	179.42±4.54e	99.45±8.64def	106.48±2.61efg
CU1PB2	22.67±0.00	497.44±19.30c	580.73±9.66d	631.52±18.96d	352.41±15.21c	416.95±9.12d
CU1PB3	22.67±0.00	498.00±37.27c	765.61±34.18bc	1138.80±55.54b	519.05±64.18ab	588.82±27.84bc
CU2PB0	22.67±0.00	36.86±1.64ef	46.44±2.35ef	46.75±0.75e	46.17±6.49def	39.78±1.14g
CU2PB1	22.67±0.00	148.84±7.85d	195.59±19.91e	255.58±18.76e	122.25±6.92de	148.98±9.63e
CU2PB2	22.67±0.00	541.73±8.37c	651.38±33.28cd	852.19±61.62c	447.85±13.10b	503.16±12.12cd
CU2PB3	22.67±0.00	562.20±13.60c	836.46±114.53b	1117.12±153.91b	358.91±32.49c	579.47±62.08bc
CU3PB0	22.67±0.00	50.81±3.94def	52.64±5.33ef	60.93±3.30e	54.78±3.38def	48.37±2.35fg
CU3PB1	22.67±0.00	74.76±4.13def	121.60±6.43ef	147.04±6.51e	84.91±9.51def	90.20±1.45efg
CU3PB2	22.67±0.00	723.43±18.57b	856.05±20.61b	1135.33±209.68b	484.49±48.60ab	644.39±50.97b
CU3PB3	22.67±0.00	1191.69±116.25a	1324.39±138.59a	1606.78±128.95a	552.03±32.36a	939.51±80.86a
Mean	**22.67±0.00**	**298.73±47.97**	**378.92±57.21**	**482.32±74.89**	**217.44±28.22**	**280.02±41.04**
P value		**0.000****	**0.000****	**0.000****	**0.000****	**0.000****

Means with the same superscript along column are not significant at P>0.05. * – significant at P ≤ 0.05, ** - highly significant at P ≤ 0.01.CU0 -0mg/L,CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

APPENDIX XIX

Comparison of Copper Bioaccumulation by *Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca.*

Plants	**Copper Bioaccumulation (mg/kg)**				
	Day 9	**Day 12**	**Day 15**	**Day 18**	**Mean**
S. molesta	1074.20 ± 209.64^{a}	1218.65 ± 234.98^{a}	1337.04 ± 261.53^{a}	1510.71 ± 294.49^{a}	1285.15 ± 124.67^{a}
P. stratiotes	624.26 ± 102.32^{a}	853.55 ± 147.10^{a}	1065.74 ± 188.06^{a}	866.18 ± 159.95^{a}	852.43 ± 76.99^{b}
L. Trisulca	1102.36 ± 200.87^{a}	1494.12 ± 276.55^{a}	1799.47 ± 327.65^{a}	1178.39 ± 229.27^{a}	1393.59 ± 133.60^{a}
Mean ± SE	933.61±105.36	1188.77±133.33	1400.75±156.24	1185.09±137.89	1177.06±67.76
P value	**0.114ns**	**0.144ns**	**0.153ns**	**0.163ns**	**0.002****

Means with the same superscript along column are not significant at P>0.05. * – significant at P≤0.05

APPENDIX XX

Comparison of lead Bioaccumulation by *Salvinia molesta*, *Pistia stratiotes* and *Lemna trisulca*

Plants	Lead Bioaccumulation (mg/kg)				
	Day 9	**Day 12**	**Day 15**	**Day 18**	**Mean**
S. molesta	459.86±120.68[a]	568.30±142.95[a]	763.63±188.11[a]	872.22±223.96[a]	666.00±87.11[a]
P. stratiotes	243.28±61.77[a]	430.62±107.00[a]	567.22±133.74[a]	378.44±92.14[b]	404.89±51.89[b]
L. Trisulca	276.06±84.11[a]	356.25±99.77[a]	459.65±129.60[a]	194.77±49.04[b]	321.69±48.05[b]
Mean ± SE	326.40±53.86	451.733±67.93	596.83±88.27	481.81±90.75	464.19±38.69
P value	**0.212ns**	**0.443ns**	**0.370ns**	**0.005***	**0.001****

Means with the same superscript along column are not significant at P>0.05.* – significant at P ≤ 0.05.

APPENDIX XXI

Picture of *Lemnatrisulca* L.

*Lemna trisulca*L

APPENDIX XXII

Picture of *Pistia stratiotes* L.

*Pistia stratiotes*L

APPENDIX XXIII

Picture of *Salvinia molesta* D. Mitch

*Salvinia molesta*D. Mitch