SUSTAINABLE BIOREMEDIATION OF SOIL, CONTAMINATED WITH PETROLEUM SLUDGE: A CASE STUDY OF SULTAN HAMUD - KENYA

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Sustainable Bioremediation of Soil Contaminated with Petroleum Sludge: A Case Study of Sultan Hamud. Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this thesis to God Almighty my Creator, my Strong Tower, my Redeemer, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program.

I also dedicate this work to my family, especially my mother and dear sisters, Lillo and Cheru who have encouraged me to remain focused.

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ABBREVIATIONS/ACRONYMS

AAS	Atomic Absorption Spectrometry
As	Arsenic
ANOVA	Analysis of Variance
BCF	Bioconcentration Factors
BTF	Biotranslocation Factor
BOD	Biochemical Oxygen Demand
Btu	British Thermal Unit
Ca	Calcium
Cd	Cadmium
CFC	Chloroflorocarbon
Co	Cobalt
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
Cr	Chromium
Cu	Copper
EM	Effective Microorganism
EMCA	Environmental Management and Coordination Act
EMA	Environmental Protection Agency of the USA
FAO	Food & Agricultural Organization
Fe	Iron
FTG	Tensor Gravity Gradiometry

GHG	Green House Gases
HDPE	Hight Density Polythene
K	Potassium
Km^2	Square Kilometers
Km^3	Kilometers cubed
KPC	Kenya Pipeline Company Limited
Mg	Magnesium
mg	milligrams
ml	millimeters
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
N_2O	Nitrous Oxide
NEMA	National Environmental Management Authority
$(NH_4)_2SO_4$	Ammonium Sulphate
Ni	Nickel
NPK	Nitrogen, Potassium and Calcium
P	Phosphorous
Pb	Lead
PCRA	Progress on the Convention of Rio Summit
Ppm	Parts per million
S	Sulphur

SNK	Student-Newman-Keuls test
SOC	Soil Organic Carbon
SPSS	Statistical Package for Social Sciences
TPH	Total Petroleum Hydrocarbon
UNESCO	United Nations Education Scientific & Cultural Organization
WHO	World Health Organization
WARMA	Water Resource Management Authority
WSRP	Water Services Regulatory Board (Kenya)
WWAP	World Water Assessment Program
WWTP	Wastewater Treatment Plant
Zn	Zinc

DEFINITIONS OF TERMS

Biodegradation	The process by which organic substances are broken down by living organisms (Nkeng, Nkwelang, & Mattew 2012)
Bioremediation	Is the use of living organisms for the treatment of polluted soils or water (Nkeng, Nkwelang, & Mattew 2012)
Biotransformation	The process, which uses green plants for the relief, transfer, stabilization or degradation of pollutants from soil, sediments, surface waters, and groundwater (Nkeng, Nkwelang, & Mattew 2012).
Carcinogenic	Capable of causing the development or increase in the incidence of occurrence of cancer (Battikhi & Mohammed, 2014)
Heavy metals	Refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations (Vdovenko, Boichenko, & Kochubei, 2015)
Hydrocarbons	These are organic molecules that consist exclusively, or primarily, of carbon and hydrogen atoms (Vdovenko, Boichenko, & Kochubei, 2015)
Leaching	Natural process by which water soluble substances (such as calcium, fertilizers, pesticides) are washed out from soil or wastes. These leached out chemicals (called leachites) cause pollution of surface and sub-surface water (Schoumans, 2015)
Mutagenic	An agent such as a chemical, ultraviolet light, or radioactive element that can induce or increase the frequency of mutation in an organism (Nkeng, Nkwelang, & Mattew, 2012)
Percolation	Percolation is part of the water cycle that occurs after precipitation and before storage during which water filters down through aerated soil due to gravity. After percolation, water is stored in groundwater reservoirs until it reaches a point where

	sunlight warms it and the water evaporates (Schoumans, 2015)
Petroleum Sludge	A complex petroleum industries mixture, containing different quantities of waste oil, wastewater, sand, and mineral matter (Vdovenko, Boichenko, & Kochubei, 2015)
Phytoaccumulation	The process of uptake of contaminants by plant roots and the translocation/accumulation (phytoextraction) of contaminants into plant shoots and leaves (Khan *et al*., 2015).
Phytoremediation	The process, which uses green plants for the relief, transfer, stabilization or degradation of pollutants from soil, sediments, surface waters, and groundwater (Chen *et al*., 2015).
Phytostabilization	Reduction of the mobility of heavy metals in soil (Bian *et al*., 2018)
Rhizofiltration	Refers to the use of plant roots to absorb, concentrate, and precipitate toxic metals from contaminated groundwater (Bian *et al*., 2018).
Soil Saturation	A condition in which all easily drained voids (pores) between soil particles are temporarily or permanently filled with water, significant saturation during the growing season is usually one week or more (Verbruggen *et al*., 2009)
Toxic	Capable of causing injury or death especially by chemical means (Battikhi & Mohammed, 2014)

ABSTRACT

Petroleum sludge is a residue extracted mostly from petroleum storage. Contaminants in petroleum sludge (heavy metals and hydrocarbons) may exist in the soil for long periods and may be transmitted to plants and tissues of living organisms within the soil ecosystem through ground water or food chain. Disposal of raw petroleum sludge to the ground exposes the soil and underground water to pollution since contaminants in the sludge can leach and get to underground water. Alternatively, the contaminants can be taken up by plants growing in the contaminated soil depending on the concentration of the heavy metals in the soil. The goal of this Thesis was to investigate sustainable technology for enhancing bioremediation of soil contaminated with petroleum sludge. The research involved planting of bamboo and papyrus plants in soil polluted with petroleum sludge. The plants were managed in line with good agronomic practices as well as plant science during the entire period of the research. Parts of the plants (roots, stem and leaves) were periodically harvested and prepared for analysis for heavy metals (Lead, Copper and Chromium) by Atomic Absorption Spectrophotometry (AAS). The concentrations of the heavy metals from the analysis were compared to the initial concentration of the same metals in the polluted soil as well as in the plants that were used in the research. The polluted soil used in the study was sampled from Sultan Hamud dumping site. The soil was analyzed for presence and concentration of lead, copper and chromium. The soil had 107 mg/g of Lead, 151mg/g of Chromium and 204 mg/g of Copper. At the end of the experiment, Bamboo was able to accumulate a maximum of 0.055mg/g (54.45%) of lead, 0.093mg/g (53.45%) of copper, and 0.02mg/g (14.60%) of chromium. Papyrus on the other hand accumulated a maximum of 0.018mg/g (17.82%) of lead, 0.028mg/g (16.09%) of copper and 0.015mg/g (10.95%) of chromium. Notably leaching caused the washing away pf significant amounts of the metals. The success of this research so much depended on several variables and, more importantly, the time factor. This is because bio-accumulation and the rate at which plants accumulate heavy metals in their biomass, are directly dependent on the duration of growth period of the plants in the medium that is contaminated with heavy metals. Other variables such as soil characteristics and leaching are equally important. The growth rate of the plants is another aspect that is influenced by agronomical practices for the plants, besides the species of the plants used. The same factors, consequently affected the rate of accumulation of heavy metals from the soil by the plants. It is thus evident that in the long run, the output of the plants, particularly regarding bio-accumulation, is directly influenced by: the green house agronomic practices employed to the plant species, leaching of the heavy metals (Cu, Pb, and Cr) from the soil where the plants were grown to the pores at the bottom of the containers, plant growth rate, absorption rate and the duration of growth during research.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Petroleum industries produce a great deal of sludge that contains various pollutants, such as heavy metals. In addition, petroleum sludge contains phenols, suspended and dissolved solids, sulphides, BOD-bearing materials, grease and oil. The heavy metals and hydrocarbons from the petroleum sludge contaminate waters and soils in many parts of the world either through sludge disposal or petroleum product spillage (Vdovenko, Boichenko, & Kochubei, 2015). It is worth noting that both petroleum sludge being disposed and product spillage on soil cause the similar form of pollution of the environment as petroleum sludge. Some of the common heavy metals from petroleum sludge that are predominantly found in contamination studies include cadmium, copper, mercury, chromium and lead.

In various countries across the world, the sludge is directly disposed into the surface waters or landfills. In other areas such as Eastern Africa, it is disposed in a designated remote site, but still manages to contaminate the soil and ground water through leaching and surface runoff (Mwanguni & Munga, 2015). No doubt, the heavy metals have detrimental effects on microorganisms, plant, animal as well as human health. Therefore, it is important for governments to take urgent measures to come up with technologies that can manage pollution from petroleum sludge and eliminate or detoxify these heavy metals from the environment. Standard environmental regulation guidelines that are adopted and used by most countries state that the metal ions concentration should be about 1ppm each and not exceeding 5ppm in soil (Anifowose, Lawler, Van & Chapman, 2016).

Notably, petroleum sludge continues to be a threat to the environment particularly regarding the potentials of oil exploration in East Africa and Kenya, in that matter, is not secluded. A report by The Deloitte Guide to Oil and Gas in east Africa established that since the year 2006, a lot of oil reserves have been discovered in Uganda, Tanzania, Mozambique, and Kenya. A further emphasis on oil exploration in Uganda and Kenya noted that indeed the two countries have the potential of expanding their economies beyond agriculture. The report by East Africa Oil and Gas Summit & Exhibition, emphasized that discoveries have found billion-barrel potential at Uganda's Lake Albert and Kenya's Lokichar basin (*Africa News Service, 2013*).

Kenya hosts several both offshore and inland oil basins some of which are already under piloting by companies such as Tullow. Tullow has successfully used full Tensor Gravity Gradiometry (FTG) seismic survey to discover oil basins in Kenya. The main offshore basin is along Indian Ocean including the disputed maritime border between Kenya and Somalia. Most recent independent resource study highlighted growth at the Ekales, Twiga, Ngamia -1, Agete, Amosing, Etuko, Ewoi, Etom and Lamu (Mugendi, 2020; Johannes, Zulu, & Kalipeni, 2015). So far, with the rising resources at South Lokichar, planned drilling, a pipeline arrangement and upstream researches are up-scaling into ultimate investment decision expected to have materialize in early 2018. In the year 2012, exploration of Ngamia-1 well was successfully launched and over 200 million of net oil pay was faced. This marked the very first and beginning of significant programme of drilling activities in Kenya (Christoforou, 2014).

One important point to note is that the main means of transport in Kenya remains road. It is also obvious that the exploration will produce a lot of petroleum wastes and thus a domestic precaution should be thought about in relation to how petroleum waste will be managed to minimize possible pollution of the environment as much as possible. Onsite pollution may result from extraction processes while offsite may occur during storage and transit and most probably via spillages. In either case precautionary measures are inevitable.

The origin of the heavy metals in the petroleum sludge is mainly the feedstock. Others are because of corrosion of pipes and equipment, and other chemical additives used during the processing (Vdovenko, *et al*., 2015). Some of the additives used include catalysts, such as, Chromium, Selenium, Vanadium, Cadmium, Lead, Nickel Copper, Zinc and Mercury. Petroleum sludge also contains both organic and inorganic matter, including bacteria and viruses, oil and grease, nutrients such as nitrogen and phosphorus, and organo-chlorine compounds (Stout, & Wang, 2016).

Contamination of soils by petroleum products such as spills or by-products has been a major environmental concern for a long period of time. Although no studies have documented effects of the petroleum sludge dumping, observation evidence from Sultan Hamud damping site showed limited growth of shrubs unlike the surrounding area. This is basically so because petroleum by-products and spills persist in the soil as hazardous contaminants and hydrocarbon respectively for very long periods of time. Worse still is the fact that the compounds are either insoluble or slightly soluble in soil water, and they also exhibit low volatility.

1.2 Bioremediation

Bioremediation is considered environment friendly and cost effective. Bioremediation process can be implemented at the site of the pollution (*in-situ*) or away from the site of pollution (*ex-situ*). In either case, bioremediation can be categorized as microbial remediation, mycoremediation or phytoremediation. Microbial remediation uses microorganisms such as yeast, bacteria, archaebacteria, and algae while phytoremediation uses plants. Mycoremediation on the other hand relies on the use of fungi (Battikhi & Mohammed 2014). The challenge of using microorganisms is the production of more toxic and mobile intermediates than the parent compound. For example, reductive dehalogenation of TCE can produce vinyl chloride, which is a toxic and carcinogenic product (Megharaj, Venkateswarlu, Naidu, 2014). Consequently microbial remediation is not utterly safe. This studied employed phytoremediation

1.2.1 Phytoremediation

Due to the concern of unfulfilled factors of outcomes such as biomass concentration, population diversity, metabolic pathways, production of toxic by-products and bacterial growth (Chachina, Voronkova, & Baklanova, 2016), the use of plants has been advanced as a safer remediation strategy. Phytoremediation is the use of plants to remediate pollution in the soil and water. Phytoremediation works by binding, extracting, and removing of the contaminants, such as hydrocarbons, pesticides, and heavy metals from the soil or water. This study chose on *Phyllostachys pubescens* (bamboo) *and Papyrus cyperus* (papyrus) for five main reasons. First, literature confirms their high tolerance to heavy metals (Bian et al., 2021; Emamverdian, Ding, & Xie, 2018; Dewedar *et al*., 2018; Chen *et al*., 2015; Liu *et al*., 2015). Secondly, the plants are locally available and adapted to local ecological patterns. Thirdly, the plants are not known to form part of human food chain in Kenya. Fourthly, the combination of the two plants is deemed adequately suitable to remediate both pollution on dry land and wetlands because whenever pollution occur on dry lands, the pollutants find their way into aquatic systems. Lastly, the choice of papyrus was informed by the fact that it is a fast-growing plant and consequently would proportionately accumulate the metals (Dewedar *et al*., 2018)

It is reported that about 8 million tons of petroleum is spilled into the environment every year (Anifowose, *et al*., 2016). Contamination of soil with oil derivatives has often been witnessed in cities particularly around industrial facilities and in areas where crude oil and earth gas drilling occur. Good examples of petroleum and petroleum products pollution are: Ecuador in which oils wastes (17 million gallons) of crude oil were placed in ground holes resulting to contamination of forest land and rivers (Stout, & Wang 2016). In Nigeria, oil exploration has led to destruction of wildlife and biodiversity, loss of fertile soils, pollution of air and drinking water, degradation of farmland and damage to aquatic ecosystem. These problems have majorly been caused by gas flaring, above ground pipeline leakage; oil waste dumping and oil spills (Audu, 2016). Colombia on the other side experienced pollution of no less magnitude. It is reported that the guerrilla squads dynamited Colombia’s main oil 346 times, spilling slightly more than 1.2 million barrels of crude oil (Tejeda-Benitez, Flegal, Odigie, & Olivero-Verbel, 2016).). There are many other countries which have suffered the wrath of oil petroleum product pollution across the globe. These include, but not limited to Kazakhstan, Azerbaijan, Falkland Island, Kuwait, and the South Sudan.

In Kenya, the case is no big difference. Incidences of ground water pollution by petroleum products occur through accidents during transportation by road such as, Sachangwany and Busia tragedies (Daily Nation, June 19, 2009, and February 03, 2009 respectively) and via oil pipeline in Konza and Coastal regions. Kenya Pipeline Company Limited (KPC) sludge dumping site between Sultan Hamud and Emali, Makueni County is another case of pollution since the wastes is not treated before dumping (Cooper, 2016). These are just but a few examples of instances of petroleum product pollution. One can imagine the global case, it is not a little matter about which governments, or activists or NGOs should afford to remain silent about.

In Kenya, the problem of petroleum waste management has not been properly addressed despite the existence of several laws and regulations that protect the soil and water. Much of this could be blamed on the environmental enforcement agencies, while most of the damage is because of low or no goodwill on environmental conservation. The Environmental Management and Coordination Act (EMCA) of 1999 provide a good legal and institutional framework for the protection of the environment. Article 93(1) of the act prohibits the discharge of any hazardous substance, chemical, oil or mixture containing oil into any waters or any other segments of the environment (EMCA, 1999). There exist water quality regulations, 2006, waste management regulations 2006, controlled substance regulations 2007 and other sectorial laws as land act that also regulates on land use practices essential for proper management of the environment. Generation of petroleum sludge in Kenya overtime has not been matched to its mode of disposal. Sludge of this nature is disposed of in open grounds often in remote arid lands. This could be argued to present quick and cheap way of disposing these hazardous compounds, while at the same time provide an alternative use of these fallow, arid and semi-arid vast lands of Kenya. This argument however defeats logic when it is known for a fact that there exists great danger to the nearby ecosystems that could be harmed immediately either through direct exposure to the toxins of the sludge, or through intake of contaminated water of from the soils.

Healthy soil supports the growth of plants, while contaminated ones are detrimental to plant growth, human and animal health. In most cases, the characteristics of contaminated soil is determined after carrying out various laboratory tests on samples collected from polluted sites, such as those with municipal sewerage waste, as well as petroleum sludge. In areas affected by industrial activity, a study by (Clifton, 2014) , showed that soil pH varied from 4.1 to 4.5, and that the sulphur content increased considerably. This is attributed to the availability of binding surfaces available on sulphur for subsequent oxidation and bacterial absorption.

The use of plants in decontaminating soils is referred to as phytoremediation or botanical bioremediation. This is an emerging technique that can be used for both organic and inorganic contaminants present in soil (Nkeng Nkwelang, & Mattew, 2012). The ability of plants to accumulate heavy metals varies with various plant species, moisture content of soil, and soil composition.

Soil remediation techniques can be grouped into: *insitu* and *ex-situ* techniques. Soil remediation is described as ex-situ when the contaminated soil is excavated for treatment either on site or off-site. In-situ methods comprise strategies whereby the contaminated soil is treated without any excavation (Battikhi & Mohammed, 2014). In most cases, the in-situ techniques are favoured over the ex-situ due to their minimal cost and lesser impacts on the environment. Remediation of contaminated soils usually involves excavating soil and taking it to a designated landfill site or managing it on-site. Mainly, off-site management entails taking out the soil contaminated with heavy metals from the site and burying it in a landfill. However, one disadvantage associated with this strategy is that it involves shifting the problem elsewhere. Furthermore, transporting contaminated soil is associated with numerous hazards, as well as the adverse effects on the adjacent environment (Malik, & Biswas, 2012).

On-site management involves using clean soil dilatants to minimize the contaminants to safe levels. Other remediation strategies involve stripping top spills and stockpiling them and then redistributing them over the landfill. To dilute the heavy metal contents, deep ploughing is carried out because it helps in mixing the less contaminated soils in the lower layers with the heavily contaminated on upper layers. Biological remediation involves using microorganisms to detoxify the heavy metals from the soil through volatilization, extracellular chemical precipitation and valance transformation. Another way is using specific plants to decontaminate the soil through translocation into aerial parts or inactivating them into the rhizosphere (Malik, & Biswas, 2012).

Ground water is an important source of water supply for municipalities, agricultural and industrial sectors. Primary users are agriculture, municipalities, industry, and rural areas where alternate surface supplies are inadequate. Preservation of good quality groundwater is therefore essential in ensuring high public health standards. Scientific and technological input must therefore be applied in trying to prevent its contamination, while at the same time preserving the integrity of the ecosystem from which most living organisms derive their living (Nkeng et al, 2012).

1.3 Statement of the Problem

Contamination of soil with petroleum sludge is a real phenomenon as indeed confirmed by NEMA (2016), since sludge recovered from tanks used for storage of petroleum products was previously   disposed of through weathering site (NEMA, 2016) or oily waste discharged into storm drains. Further contamination of the environment comes from used oil from ships at the port of Mombasa due to poor handling (NEMA, 2016), A more practical evidence is the case study of a plot in Port Reitz, Mombasa in which 60-70% of the soil was found contaminated with oil and consequently could not support growth and development of plants (Mwanguni & Munga, 2015). It is therefore evident that petroleum sludge has detrimental effect particularly in dump site ecosystem where disposal of petroleum sludge is done. The contamination of soil must consequently be looked at in a broader scope. Currently in Kenya and surrounding regions, the disposal method of this waste is poorly carried out and it can be harmful to the environment.

If plants in contaminated soil die, then animals in the food chain including human are affected in no lesser magnitude. Moreover, the contaminants can find their way into the ground water table and aquifer through percolations, and users of the water end up becoming vulnerable to contamination and as such much bigger and direct danger is posed (Stout, & Wang, 2016).

Previously, biodegradation, physical and chemical methods have been used to remediate the pollution. Biodegradation is often friendly to the environment, and cheap. The only disadvantage of dependence on Bacteria for example is the unknown effect of their mutational nature and the release of harmful hydrocarbons into the atmosphere. Moreover, there is a setback of unfulfilled factors of outcomes such as biomass concentration, population diversity, metabolic pathways and bacterial growth (Battikhi & Mohammed, 2014). Physical methods are also options because they at least reduce and curtail spread of pollution, but they are expensive and equally pollution risk factors. Chemical methods on the other hand are expensive and require a lot of technical knowledge to use efficiently and effectively (Khalid *et al*, 2016). Urgent and sustainable mitigation strategy must therefore be put in place to remedy the situation. Phytoremediation technology using non-edible plants thus proves to be the safest, most environmentally friendly and most sustainable techniques which can be adopted. However, in Kenya, very minimal exploration of such plants has been done particularly with regards to pollution caused by petroleum sludge and thus this was the main focus of study.

Making the soil favorable for the growth of plants may encourage growth of native plants but it will not lower the risk of contamination effects of the pollutants in the soil. Additionally, it may not be practical to always make the soils favorable in the same way the study did. That notwithstanding, it would require a research study to establish the facts right.

1.4 Objectives

1.4.1 General objective

To investigate the effectiveness of phytoremediation as a technology for sustainable bioremediation of soil contaminated with petroleum sludge.

1.4.2 Specific Objectives

1. To examine the presence and concentration of lead, copper and chromium metals in soil that has been contaminated with petroleum sludge at Sultan Hamud dumping site.
2. To investigate the trend of uptake of Lead, Chromium and Copper by Bamboo and Papyrus plant species from the contaminated soil.
3. To examine the effect of leaching on the concentration/bioavailability of the heavy metals in the soil that has been contaminated with petroleum sludge.
4. To formulate/devise an appropriate Bioremediation strategy for soil contaminated with petroleum sludge containing the heavy metals.

1.5 Research Questions

1. How much of Copper, Lead and Chromium is present in the soil contaminated with petroleum sludge?
2. What is the rate at which Bamboo and Papyrus plant species take up the heavy metals from soil contaminated with petroleum sludge?
3. What is the effect of leaching on the concentration of heavy metals within 300mm and 600 mm depth of soil contaminated with petroleum sludge?
4. How effective is the use of bamboo and papyrus as bioremediation strategy in soils contaminated with petroleum sludge?

1.6 Null Hypothesis (H_0)

1. H_0 There is no significant impact of leaching on the uptake of heavy metals by Bamboo and Papyrus from soil contaminated with petroleum sludge.
2. H_0 There is no significant variation in the rate at which Bamboo and Papyrus take up heavy metals from Soil contaminated with petroleum sludge
3. H_0 Bamboo and Papyrus are not effective in sustainable bioremediation of soil contaminated with petroleum sludge.

1.6.1 Alternative Hypothesis (H_A)

1. H_{A1}There is significant impact of leaching on the uptake of heavy metals by Bamboo and Papyrus from soil contaminated with petroleum sludge
2. H_{A2} There is significant variation in the rate at which Bamboo and Papyrus take up heavy metals from Soil contaminated with petroleum sludge
3. H_{A3} Bamboo and Papyrus are not effective in sustainable bioremediation of soil contaminated with petroleum sludge.

1.7 Justification

Heavy metals that contaminate the soil are a major threat to human health and the environment. Any soil contaminants can potentially pollute ground water (Clifton, 2014). This study examines the level of Copper, Lead and Chromium in soil that has been contaminated with petroleum sludge. This gives an indication of the threat posed by wrong and careless disposal of petroleum sludge. The study also investigates the possibility of using plants under varying levels of moisture content to remediate contaminated soil (Nkonge, 2012). This will hopefully provide a sustainable solution to the pollution problem associated with petroleum sludge. The two plant species chosen were *Phyllostachys pubescens* and *Papyrus cyperus* because they are highly tolerant to heavy metals, they are readily available, do not form part of human food chain in Kenya, have massive root system and lastly, they are non-invasive (Bian *et al*., 2021; Emamverdian, Ding, & Xie, 2018). A wide category of parties would therefore draw benefits from this research. These include: Oil deports (Kenya pipeline and Oil Multinational Companies), petrol stations, oil exploitation institutions such as Tullow, Lapset Project, Seaports for instance KPA, and the agricultural industry that need to minimize soil contamination on arable land. Furthermore, the agricultural industry that needs to minimize soil contamination on arable land and companies that handle petroleum sludge will consequently be better equipped to handle the hazardous substance and prevent or minimize soil and groundwater contamination through leaching.

1.8 Scope of the Study

This research explores concepts such as: Bioremediation, Phytoremediation, Basic Plant and Soil Science (agronomy), Atomic Absorption Spectrophotometry and Good Laboratory Practices (GLPs), with the view of exploring the potentials of sustainable green remediation of soil contaminated with petroleum sludge using bamboo and papyrus plant species. Contaminated soil samples from Sultan Hamud were used in this experiment. Two plant species namely: Bamboo (*Phyllostachys pubescens*) also known as Moso Bamboo, and papyrus (*Papyrus cyperus*) were adopted for the study since they are some of the most tolerant to high levels of heavy metals (Liu et al., 2015). Additionally, they are readily available in Kenya and particularly within reach around JKUAT, and Isinya, not part of human food chain in Kenya, they are massively rooted and lastly, they are non-invasive. Having the two plants would take care of remediation of dry land and wet land, particularly where the pollutants fins their way into wetlands through surface runoffs. The experiment was set up partly in a greenhouse in Kitil Farm - Isinya and another greenhouse at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja, Kiambu County- - Kenya. A total of thirty-two (32) containers were used as seed beds for the plants named above. In the experiment, four variables were considered namely: leaching, heavy metal concentration in contaminated soil, plant species and time. The Study focused on only three heavy metals, namely Copper, Lead and Chromium. The choice of the metals was informed by cost and time contains with regard to Atomic Absorption Spectroscopy (AAS) for heavy metals, the need to narrow down the scope of the study for efficiency and lastly, the availability of the standards at the time of the experiment. Although hydrocarbons forms part of the pollution concern, the scope of this study could not allow focus on remediating pollution which could be caused by them. The study has however recommended further studies with regards to the other metals and hydrocarbons.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This study draws from several theoretical concepts and past research regarding remediation of soil contaminated with petroleum sludge. This chapter is therefore divided into two main sections: Theoretical review and empirical review. The theoretical reviews captured some of the theoretical information based on chemical composition of petroleum sludge, disposal and pollution effect of petroleum sludge, and bioremediation technology of soil polluted with petroleum sludge. Empirical review on the other hand captures such concepts as composition of petroleum sludge, detrimental effect of contaminants in petroleum sludge, characteristics of soil polluted with petroleum sludge, and remediation techniques available for exploration. It further reviewed heavy metal uptake mechanisms, optimization of phytoremediation and phytoremediatory potentials of bamboo and papyrus. Finally, empirical review also investigates disposal of phytoremediatory plants upon harvesting and summary of research gaps.

2.2 Theoretical Review

Phytoremediation is a phenomenon which has been used for many years in environmental conservation sciences across the globe. This is considering the high concentration of some of the contaminants in major soil and water pollutants far beyond the permissible levels. It employs the use of some plants such as papyrus and bamboo, to degrade, extract, contain, or immobilize contaminants from soil and water (United States Environmental Protection Agency (USEPA), 2000). Various plants differ in their ability to extract, degrade, contain and immobilize contaminants, depending on factors such as, weather, topography, type and concertation of the contaminants, plants species, and agronomy (Malik, & Biswas, 2012). Leaching plays a major role in determining concentration and bioavailability of heavy metals within the reach of plant roots.

Leaching on the other hand is dependent on how much water is held in the soil, and the chemical characteristics of the soil. This explains the wide scope of phytoremediation, ranging from; rhizodegradation, phytodegradation, phytoextraction, bioaccumulation, to phytovolatilization in treatment the of soil and water contaminants. Nevertheless, the technology has not been fully explored and studies are ongoing on best practices of adopting the technology (United States Environmental Protection Agency (USEPA), 2000). The study seeks to experimentally quantify the efficacy of a few selected plant species in phytoremediation.

2.2.1 Mechanism and Action

Uptake of metals by moso bamboo and papyrus takes place through the root and the rate of uptake commonly depends on the ionic potential of the element in question. Sometimes metals can be absorbed actively or passively or undergo accumulation together with a macronutrient cation. There occurs a competition among the metals for chelation, movement to the root and uptake at the root-tip. Root cell walls of moso bamboo and papyrus initially bind metal ions from the soil. The metal ions are then taken up across plasma membrane through high affinity binding sites with the help of plasma membrane localized transport systems. Secondary transporters such as channel proteins and/or H^+-coupled carrier proteins aid the process of the uptake. For example, uptake of cations through secondary transporters is facilitated by the membrane potential of the plasma membrane, which is negatively charged on the inner side of the membrane (Shruti, & Dubey, 2010). According to Williams *et al*., (2000), several different types of metal transporters are involved in plants and often more than one transport system exists for one metal.

The absorption of lead by roots of bamboo and papyrus takes place through the apoplastic pathway or via Ca^{2+-}permeable channels. The uptake is controlled by speciation of the metal and other factors such as soil pH, cation-exchange capacity, and soil particle size, surface area of the root, exudation by the roots, and extent of mycorrhizal transpiration. After uptake, a lot lead primarily accumulates in root cells but small quantities can be translocated to the shoot but the process is inhibited by Casparian strips found in the endodermis (Pourrut *et al*., 2011). The uptake of Chromium on the other hand is aided by specific carries which target essential ions. The uptake through the plasma membrane involves carriers which facilitate uptake of sulfates and phosphates. This implies that the uptake and translocation is both passive and active. This happens due to structural similarity of Cr (VI), with sulfates and phosphates. Consequently the uptake of Cr involves phosphate and sulfate transporters. For the same reason, the translocation of Cr to the shoot is aided by sulfate and phosphate transporters (de Oliveira *et al*., 2014). Notably Cr toxicity inhibits plant growth by triggering ultrastructural changes in the cell membrane and chloroplast, inducing leaf chlorosis, root cell damage, reducing pigment content, impedes transpiration and nitrogen assimilation (Sharma *et al*., 2020).

Copper is taken up in the roots of bamboo and papyrus in the Cu+ form by highly selective Cu-transporters - COPT proteins. Additionally, Cu uptake system is aided by non-selective ZIP proteins whereas Cu^{2+-}efflux is mediated by H^{+}/Cu^{2+} antiporters. Cu-acquisition from soil depends essentially on the protein COPT1. COPT1 is a plasma membrane-localized high-affinity Cu(I) transport found in many organs located at the root tips (Rodrigo-Moreno et al., 2013). The direct interaction of OH · with the cytosolic OH ·-binding site of non-selective cationic channels (NSCC), being plasma membrane ion channels, may then activate the influx of Ca^{2+} to allow root growth and thus increase the root prospection area for mineral acquisition. However, when excessive Cu is available, the massive entry of Cu(I) generates a burst of OH · radicals, leading to the activation of Ca^{2+}-influx channels on one side and to the opening of K^{+} efflux channels on the other side, thereby activating a caspase-like driven programmed cell death (PCD) and inhibiting root elongation (Rodrigo-Moreno et al., 2013). In addition, excess Cu may disturb root elongation by preventing auxin redistribution through interaction with Pinformed1 (PIN1), an auxin efflux carrier responsible for acropetal auxin flow in the root stele.

2.2.2 Bamboo and Papyrus Mechanisms of Remediation

Both Bamboo and Papyrus exhibit phytoremediation mechanisms. These mechanisms include Phytoextraction in which the two plants absorb the heavy metals from the soil through their roots translocating them to the shoot of the plant (Rafati *et al*., 2011). Phytoextraction relies on the metals uptake mechanism which has been discussed above. In addition to phytoextraction, papyrus also pytovolatilize the metals translocated to the shoot. Phytovolatilization involves the conversion of the pollutants into a form that can volatilize together with transpiring materials from the leaves of the plant. Also notable in the phytodegradation and phytostabilization characteristics of the roots of papyrus and bamboo respectively (Mburu *et al*., 2015; Emamverdian, Ding, & Xie, 2018). According to Mburu et *al*., (2015) and Emamverdian, Ding, & Xie (2018), this explains the high tolerance of the two plants to high amounts of the toxic metals. Notably, no single phytoremediation mechanism works independently, in most cases several mechanism complement each other. Most commonly, phytodegradation, phytoextraction, and phytovolatilization work together.

2.2.3 Remediation techniques

Contaminated soil is usually cleaned up using various remediation techniques. Biological, chemical or physical techniques are used to attain complete/ partial elimination of the heavy metals from the soil or reduce their bioavailability, thereby minimizing toxicity (Tivoli, *et al*., 2016). Chemical remediation involves addition of chemicals to the soil to transform the contaminants into less toxic and harmful states. Physical methods on the other hand involve excavating the soil, mixing or capping it to clean it up. The selection of the most effective remediation technique that can be used to remediate contaminated soil depends largely on the nature of the contaminants, the contaminated site characteristics, the type of soil, operational cost, availability of the required materials and the applicable regulations. For the sake of this piece of work biological method is the target technique.

Bioremediation is a biological technique, in which living organisms are used to reduce or eliminate environmental hazards resulting from accumulations of toxic and harmful chemicals or other hazardous wastes. Battikhi and Mohammed, (2014), have observed that bioremediation is an attractive approach of cleaning up petroleum hydrocarbons because it is simple to maintain, applicable over large areas, cost-effective and leads to the complete elimination of the contaminants.

Bacteria are generally used for bioremediation, but fungi, algae and plants could also be used. If plants are used, then the science is called phytoremediation. Bioremediation is not a new technology but there are different perspectives on the use of bio-remedial technologies to treat contaminants (Khalid *et al*., 2016). There are three classifications of bioremediation. The first is bio-transformation which is the alteration of contaminant molecules into less or non-hazardous molecules. The second is biodegradation which is the breakdown of substances into smaller organic or inorganic molecules while the third is bioremediation which is mineralization which is the complete biodegradation of organic materials into inorganic constituents such as carbon dioxide (CO_2) or water (H_2O) (Battikhi & Mohammed, 2014). These three classifications of bioremediation can occur either in situ (at the site of contamination) or ex situ (contaminant taken out of the site of contamination and treated elsewhere. There are various bioremediation techniques which have been adopted but the most dominant are microbial degradation and phytoremediation. This study is interested in phytoremediation.

2.1.3.1 Phytoremediation.

The idea of using plants in cleaning up contaminated environment can be traced back to around 300 years ago when they were proposed to be used in treating wastewater. By the close of the 19th century, *Viola calaminaria* and *Thlaspicaerulescens* became the first plant species to be documented for accumulating high metal levels in their leaves. Other studies that followed, such as one carried by Byers in 1935, identified that the plant genus of *Astragalushad* had the capability of accumulating 0.6 percent Selenium in their shoots (Chen et al., 2015). Later in 1984, Vergnano and Minguzzi identified other plants that were capable of accumulating 1 % Ni within their shoots (Nkeng *et al*., 2012). A recent study by Rascio has showed that *Thlaspicaerulescens* species have a high tolerance to high levels of zinc accumulation.

2.1.3.2 Optimum Growth Conditions for Bamboo.

Most bamboo species grow well in moderately acidic loamy soil or sandy loam soil. Bamboo requires 3-5 times watering per week which should be equivalent of 800-1000mm of annual rainfall. Water logging must be avoided. The plants should be spaced at 1-1.5 meters apart. NPK (21:5:6) fertilizer application should be done at a rate of Half pound/100sq feet. Shade may be necessary for young plants during summer (Chen *et al*., 2015)

2.1.3.3 Optimum Growth Condition for Papyrus.

Papyrus requires moist planting sandy soil with pH of between 6.0 and 8.5. The plants should be fully exposed to sunlight, but the temperature should be maintained between 20°C-30°C during the growth period. Individual plants should be spaced at 0.5-0.9m for small species and 0.6-1.2m for large species. Liquid fertilizer spays can be applied to encourage faster growth (Home & Muthigo, 2013)

2.2.4 Benefits of Phytoremediation

Malik, & Biswas, (2012), state that it is quite challenging to remediate soil that is contaminated by heavy metals. The current technologies used involve either chemical or physical separation of the contaminants from the soil. More so, the cost of any of these remediation techniques depends on the conditions of the site, the soil properties and nature of contaminants. Using conventional engineering and chemical technologies to remediate the soil is prohibitively expensive. Nevertheless, there is still need to come up with cheaper and sustainable technologies to clean the soil. Phytoremediation has emerged as one of the most cost-effective technique to achieve this goal. Moreover, it also prevents a dramatic disruption of the contaminated site and preserves the surrounding ecosystem because it is an *in-situ* strategy (Khalid *et al*., 2016). Phytoremediation can occur either by rhyzofiltration, phytostabilization, phytoextraction, or phytovolatilization.

2.3 Empirical Review

A lot of research studies and experiments have been carried out to justify technologies advanced towards solving the problem of soil pollution with petroleum sludge. Among the technologies are incineration, thermal desorption, chemical oxidation, immobilization and solvent extraction. Though these technologies are effective, they are not efficient because they are expensive, energy intensive and thus not sustainable (Battikhi & Mohammed, 2014). The costs of chemical and physical methods are very high, the methods are energy intensive and they are also feared to cause recontamination (Khalid *et al*., 2016). Biodegradation on the other hand is environmentally friendly but the unknown results of evolutionary nature of microorganism poses a challenge. It has also been reported that biodegradation also leads to the emissions of pollutants hydrocarbon into the atmosphere (Tivoli, *et al*., 2016). A main challenge is to search for a more economical, environmentally friendly and sustainable substitute to these traditional methods. This explains the absoluteness of phytoremediation as the best tool.

2.3.1 Chemical Pollutants in Petroleum Sludge

Previous studies have established that petroleum sludge has numerous chemicals and physical substances some of which occur in significant proportions of concerns although some occur in negligible quantities. Oily sludge basically comprises of about 55.13% of water, 9.246% of sediments, 1.9173% of asphaltenes, 10.514% of wax and 23.19% of light hydrocarbons and a high concentration of heavy metals for instance, Lead is 809.79 ppm, Copper is 108.99ppm and Chromium is 88.97 ppm (Duan, Gao, Sipra, Tong, et al., 2022). However these differ from Xiao,Yao, & Zhang, (2019) who found 333 ppm of Lead, 825 ppm of Copper, and 282 ppm of Chromium in oily sludge. The quantities of the heavy metals are variables that depend on differing industry practices. According to studies by Nkeng *et al*., (2012), petroleum sludge is a complex mixture of hydrocarbons, water, metals, and suspended fine solids that have high pollution potentials and so need treatment before disposal (Nkeng *et al.*, 2012). The nature of industrial sludge depends on the source of the wastewater. It could contain not only organic and inorganic matter, but also bacteria and viruses, oil and grease, nutrients such as nitrogen and phosphorus, heavy metals and organochlorine compounds (Jafarinejad, 2016). Sludge disposal is a worldwide problem and a variety of disposal routes have been adopted as dictated by local conditions. However, they have often failed due to huge amount of sludge being generated against the cost of treatment (Khalid *et al*., 2016). There is therefore, need to effect proper treatment of sludge before disposal and reuse.

A study to determine the characteristics of sludge from a petroleum industry in Nigeria by Hu *et al.*, (2016), found out that the pH was at 5.48, indicating that fresh petroleum sludge is acidic. Other studies have also shown that low pH is toxic to fish and other aquatic lives (Baker & Schofield, 1982). The sludge also had a high turbidity, an indication that fresh sludge has high solid concentration. BOD and COD were high beyond the acceptable standards, while the dissolved oxygen was very low (Hu *et al*., 2016).

2.3.1.1 Heavy Metals in Petroleum.

The process of refining crude oil generates a large amount of sludge as the waste product. The problem with this product is that it is poorly biodegradable and poses serious challenges to human health, the environment and has substantial implications on the economy. A study carried out by Nkeng, *et al*. (2012), on the contents of petroleum sludge showed that it is mainly made up of a very high solid content and a smaller TPH content. According to the analysis by the above study, petroleum sludge contained four other predominant elements of heavy metals as shown in Table 2.1. The metals include

Chromium, Lead, Barium, Copper, Zinc, Arsenic, and Nickel. Other studies have also revealed the presence of other heavy metals including zinc, and copper. Testing for heavy metals was carried out in the laboratory in most of these studies (Nkeng *et al*., 2012). The presence and concentration of metals in petroleum sludge is summarized in Table 2.2. In another study by Akpoveta, & Osakwe, (2014), on heavy metal content in refined petroleum products, the residual levels of the metals were generally high as can be observed from Table 2.2. Table 2.2 is an indicator that the source of the heavy metals in the sludge as indicated in Table 2.1, through individual refined petroleum products which are held in the tanks from which the sludge is retrieved.

Both WHO and FAO have always published standards limits and maximum allowable limits of certain heavy metals with regards to the implication of animal's health and most specifically to human. The maximum allowable limit for instance means that any level above it in the food chain of human may pose gross health concerns as was mentioned in the statement of problems. The standards thus help in prediction of possible implication of the metals if they should find their way into the food chain of human. Column 2 of Table 2.3 shows the normal levels of some heavy metals in a non-polluted soil. Any concentration above these levels would imply pollution and thus would raise the concern of contamination if food crops are grown in such soil or if rainwater run-offs from such soil may find their way down stream.

2.3.2 Disposal and Pollution Effect of Petroleum Sludge

Petroleum sludge is often disposed in weathering sites or landfills located in areas judged to be less productive and inhabited by man. In Kenya for instance, unusable petroleum sludge is disposed at the only known landfills between Sultan Hamud and Emali in Makueni County (NEMA, 2014). This disposal poses challenges in the sense that contaminants in petroleum sludge can percolate and seep into water aquifers which may end up being used for domestic and industrials purposes. This implies that the contaminants can find their way into the ecosystem on and in the ground, hence threatening living things in such an environment.

Depending on the components of petroleum sludge, it can be classified as soil, air or water contaminant. The contaminants are toxic, mutagenic and carcinogenic (Battikhi & Mohammed, 2014). The petroleum products in the sludge may contaminate water, soil and air thus endangering the lives of living organisms living in those ecosystems. It reduces oxygen concentration in the soil and water and thus poses danger of suffocation of organisms in these habitats. Hydrocarbons in the sludge are volatile and upon irradiation by ultra-violet radiations from the sun, they are degraded into emissions which are carcinogenic and can cause cancer when breathed in from the air. The heavy metals in the sludge could percolate with leaching water to the water table and thus contaminate the water. The heavy metals can also be taken up by plants from soil contaminated with petroleum sludge depending on the concentration of the contaminants in the soil and consequently get into the food chains of animals and humans and become harmful (Verbruggen, Hermans &, Schat, 2009). These facts magnified the need to handle the contaminants to mitigate the risk of harm to the environment or ecosystem.

2.3.3 Detrimental Effects of Petroleum Sludge

The heavy metals present in the petroleum sludge contaminate the soil when it is disposed in a designated site or landfill. Depending on the amount of the contaminants, the pollution may or may not allow any growth of plants and the threat to human food chain is dependent on the types and amount of the heavy metals in the pollutant. Notably, the contaminants threat is not limited to plant-based food chain. They pose threat when they get their way into domestic water through borehole water, or through aquatic based food chain. Contamination of soil is further caused by runoff water that may carry the heavy metals and other pollutants downstream. Additionally, contents of petroleum sludge from any source released into the soil leach down into the soil by gravity. The characteristics of the subsoil will determine how far the contaminants from the sludge leach downwards. At times, they can reach the water table or the impermeable layer, which can lead to the pollution of groundwater (Vdovenko, *et al*., 2015). The pollution of the environment by heavy metals is a worldwide concern due to their toxic nature. High levels of heavy metals lead to the formation of free radicals, which can lead to oxidative stress. When heavy metals accumulate in the soil, they replace the essential metals that are considered important in maintaining fertility. The mobility and level of toxicity of heavy metals are influenced by environmental factors and the nature of the soil. In small quantities, some metals are an essential component of the soil and are commonly known as micronutrients or trace elements. However, the non-essential metals are very toxic and pose a serious threat to soil and all the living organisms relying on it (Mwanguni & Munga, 2015).

According to Tejeda-Benitez *et al*., (2016), the high COD values and low DOD values indicate the high potential of the aqueous effluents to cause gross inorganic and organic pollution to the receiving surface water bodies. This means that discharge of such sludge into a water body could cause a reduction in the population of fish, other aquatic organism, and algae "boom" in surface water bodies, a condition known as eutrophication of water bodies. Vdovenko, *et al*., (2015) established that conductivity values were higher than the acceptable limits, which show that the sludge had high concentration of ions. Oil and grease values were also high, while salinity was low. The sludge also contained a large amount of heavy metals including, iron, calcium, magnesium, manganese, copper, cadmium, chromium, lead and zinc.

Clifton, (2014) also indicates that land as a component of the environment deserves the same attention and protection as water and air. This realization could be because of increased incidents of land pollution, scarcity, awareness and concern about long-term effects of its pollution on terrestrial and aquatic ecosystems (Clifton, 2014). The adverse effects of petroleum sludge on soil ecology and fertility have been crucial in the development of efficient technologies for the degradation of these contaminants into the environment.

2.3.4 Bioremediation of Soil Contaminated with Petroleum Sludge

Bioremediation is a broad field of mitigation that has been employed in solving or minimizing the problem of soil and water contamination particularly with petroleum sludge or associated products. It makes use of living organism to degrade and transform some or most of the contaminants into harmless or less harmful forms (Dindar, Topaç Şağban, & Başkaya, 2015). In most common cases, bioremediation involves microbial remediation using microorganisms such as yeast, bacteria, archaebacteria, and algae while phytoremediation uses plants. Mycoremediation on the other hand is a bioremediation technique which relies on the use of fungi (Battikhi & Mohammed 2014). Bioremediation of soil contaminated with hydrocarbon is carried out by various microorganisms (microbial remediation). However, the success microbial remediation depends on the use of biostimulators which can significantly improves the efficiency of hydrocarbons remediation (Villalba *et al*., 2021). Examples of bacteria which can effectively remediate petroleum hydrocarbon include bacteria such as *Achromobacter, Alteromonas, Acinetobacter, Arthrobacter, Alkanindiges, Burkholderia, Enterobacter, Dietzia, Kocuria, Mycobacterium, Marinobacter, Pandoraea, Staphylococcus, Pseudomonas, Streptobacillus, , Rhodococcus, and Streptococcus* (Sarkar et al., 2017; Varjani, 2017; Xu et al., 2017). In phytoremediation, some plant species have been studied and found to be very effective in remediation. For example, Indian mustard (*Brassica juncea L*.) (Rathore *et al*., 2019), Willow (*Salix species*) (White Willow) (Robichaud *et al*., 2019), *Populus deltoids* (Nikolić *et al*., 2017), Indian grass (Sorghastrum nutans) (Bharti *et al*., 2017), Bamboo (*Phyllostachys pubescens*) (Bian *et al*., 2021; Emamverdian, Ding, & Xie, 2018) and *Papyrus cyperus* (Dewedar *et al*., 2018; Mburu et *al*., (2015) among others.

2.3.5 Characteristics of Phytoremediation Plants

Plants suitable for phytoremediation should possess a series of characteristics which enable them to effectively and efficiently remediate contaminated soil or water. These characteristics are the factors considered in mearing the suitability of the plants to phytoremediation. According to Anyasi, and Atagana, (2018), phytoremediation plants should: (i) have ability to accumulate metals preferably in the in their biomass; (ii) be tolerant to high concentration of heavy metals; (iii) be faster in growth and high biomass; (iv) have widespread, deep and highly branched root system (v) adapted to the weather condition of the polluted area and, (vi) be easy to harvest, and not edible by humans and animals. To measure the phytomediatiory potential of a plant, the tolerance to the heavy metals and accumulation of the heavy metals in the biomass are the major parameter of consideration. These conditions were considered in the choice of moso bamboo and papyrus. Bamboo is well adapted to grow in dry land like areas around Sultan Hamud as evidenced by bamboo farms in Isinya which is in same weather zone as Sultan Hamud. Papyrus on the other hand is adapted to growing in marshy areas and thus suitable for remediation downstream and thus was chosen to target pollutants which could be carried by surface runoffs downstream. Both the plants have ability to accumulate high amount of heavy metals in their biomass without them suffering significant physiological stress. They equally have widespread, deep and highly branched and massive root system, easy to harvest, and not edible by humans in Kenya.

2.3.6 Characteristics of Soil Contaminated with Petroleum Sludge

Contamination of the soil by petroleum sludge results in biological and chemical disturbances. The changes in the soil will depend on the characteristic of the contaminants found in the petroleum sludge. The characteristics of contaminated soil are determined by carrying out various lab tests that measure the temperature, pH, moisture, organic matter content. Petroleum product contaminated soil has a pH of 7.2, with total organic carbon at 0.9%, NO_3 at 6.3 mg/kg, P_2O_5at 156 mg/kg, and water content of about 1.8%. The particle-size distribution as <0.25 mm, 53.6%; 0.25–0.5 mm, 19%; 0.5–1.0 mm, 11.5%; 1.0–5.0, mm 14.2%; and >5 mm, 1.8% (Muratova *et al*., 2008).

2.3.6.1 Soil Enzymes.

Dindar *et al.,* (2015) in their study carried out to ascertain the microbial characteristics of contaminated sandy soil revealed that enzyme activities in the soil are an indication of soil ecological stress (Dindar *et al.,* (2015). Enzyme activity is measured using dehydrogenase, which reflects the microorganisms' respiratory activity. Contaminated soils show a lower level of dehydrogenase activities compared to their healthy counterparts. The decreased activities are attributed to the fact that toxics from contaminants lead to the death of microbial functions that are extremely sensitive, and because of enzyme degradation. Most studies have showed that dehydrogenase activity is very sensitive to contaminants. The microorganisms' response to contaminants can be measured by estimating phosphatase activities as well as the extracellular enzymes (Dindar *et al*., 2015).

2.3.6.2 Rates of Ammonification and Nitrification.

According to Dindar *et al.*, (2015), the concentration of nitrates in the soil decreased in contaminated soil. However, there were higher rates of ammonification, which is attributed to the killing the microbial biomass part in the mineralized soil. This leads to the immobilization of nitrogen in the new microbial biomass that is not affected by the contaminants. This unaffected population uses the nutrients released to support their development and growth (Plaza et al., 2010).

2.3.6.3 Microbial Population.

The study by Jakubauskaite *et al.* (2016), showed that contaminants changed the quantity of bacteria that help in the soil nitrogen transformation. Indeed, contaminated soil exhibited lower levels of nitrifying bacteria compared to the healthy sample. Results from this study also showed that higher levels of contaminants led to the lower numbers of the nitrifying bacteria. This is attributed to the fact that contaminants inhibited the nitrogenase activity and growth of the vital nitrifying bacteria predominant in the soil. However, contaminants do not have any effects on the denitrifying bacteria because they remained unaffected (Jakubauskaite *et al*., 2016).

2.3.6.4 High Levels of Heavy Metals.

Vdovenko *et al*. (2015), in their study of characterization of soils contaminated by hydrocarbons discovered that the samples collected contained high water extractable and total heavy metal contents. The soil had high concentrations of Zinc, Lead, Cadmium, Iron, Barium, and Strontium. The soil samples with high levels of petroleum hydrocarbons also had a lower pH, an indication that they were acidic (Vdovenko *et al*., 2015). These physicochemical and ecotoxicological soil properties were a clear indication that petroleum sludge negatively altered the conventional characteristics of the soil.

2.3.7 Disposal of Phytoremediating Plants

In phytoremediation, the plant absorbs the heavy metals from the soil through the roots and translocates them into the shoots above the ground where they will accumulate. After the plant has attained certain size, the shoots are harvested and safely disposed. By so doing, the heavy metals are permanently eliminated from the site. Most researches have showed that incineration of the harvested plant tissue is the best way of reducing the quantity of material that will require disposal. Moreover, valuable metals can be extracted from the ash and used to offset the cost of the expenses associated with the process of mining. According to Cooper, (2016) a researcher must be careful in selection of the phytoremediating plant by choosing a species that is easy to incinerate or utilize for other economic activities such as construction, or furniture.

2.3.7.1 Optimizing Phytoremediation.

Several factors influence the success of phytoremediation. These factors include but not limited to:

Plant Selection. Battiki and Nizar (2014), emphasize that the most important factor that will affect the level of metal removal from the soil is the selection of the phytoremediation species. The primary consideration for the selection of any plant species is its potential to extract metals from the soil. However, it is equally important to always consider native species as opposed to exotic protection of the ecosystem. With this in mind, the general rule is always to consider the native species as opposed to exotic plants, which might be invasive, thereby endangering the harmony within the ecosystem. Crops are usually preferred instead of propagating the weedy species.

Another important consideration in the selection of the most ideal species is the physical characteristics of the contaminated soil. Shallow rooted plant species are more ideal for subsurface contaminated soils, whereas their deeper-rooted counter parts are a good choice for contamination which has gone deeper into the soil.

Soil conditioning and fertilization. No doubt phytoremediation is an agronomic approach and its success is pegged on applying certain practices on the site. A study carried out by Montiel-Rozas, Madejón, & Madejón, (2016), that investigated the effects of acidifying the soil on phytoextraction of Cd and Zn proposed the application of $(NH_4)_2SO_4$ as a soil additive. This additive would help in providing the necessary nutrients for higher yields and in making the soil more acidic to allow for a higher metal bioavailability (Montiel-Rozas *et al*., 2016). However, this must be handled with a lot of caution because the increased solubility could increase the leaching of the heavy metals into ground water, which is a serious environmental hazard.

Soil Moisture Content. All contaminants in the soil are either dissolved or suspended materials within water. Soil water is thus the medium that defines the presence and availability of contaminants. Soil moisture is a vital aspect that allow for the movement of the soil solution to the root surface. Maintaining of soil moisture is of utmost importance and irrigation is necessary depending on the local climate (Tivoli *et al*, 2016). More so, the method of irrigation must also be carefully selected to minimize evapotranspiration and avoid inhibition of leaf transpiration. In most cases, drip irrigation is the most ideal technique because it has minimal effect on the humidity of air.

2.4 Conceptual Framework

This research specifically considers phytoremediation as a key sustainable tool in redeeming land that has suffered pollution by heavy metal introduced from petroleum sludge. The study acknowledges that there are a lot of metals in petroleum sludge and consequently soil contaminated by the former, however, the study focuses on only three of the metals which are classified as heavy metal. The heavy metals are considered more harmful pollutants than other metals and thus, this research finds it more relevant to minimise the scope of interest about targeted metals. Figure 2.1 shows the representation of the concept of the study.

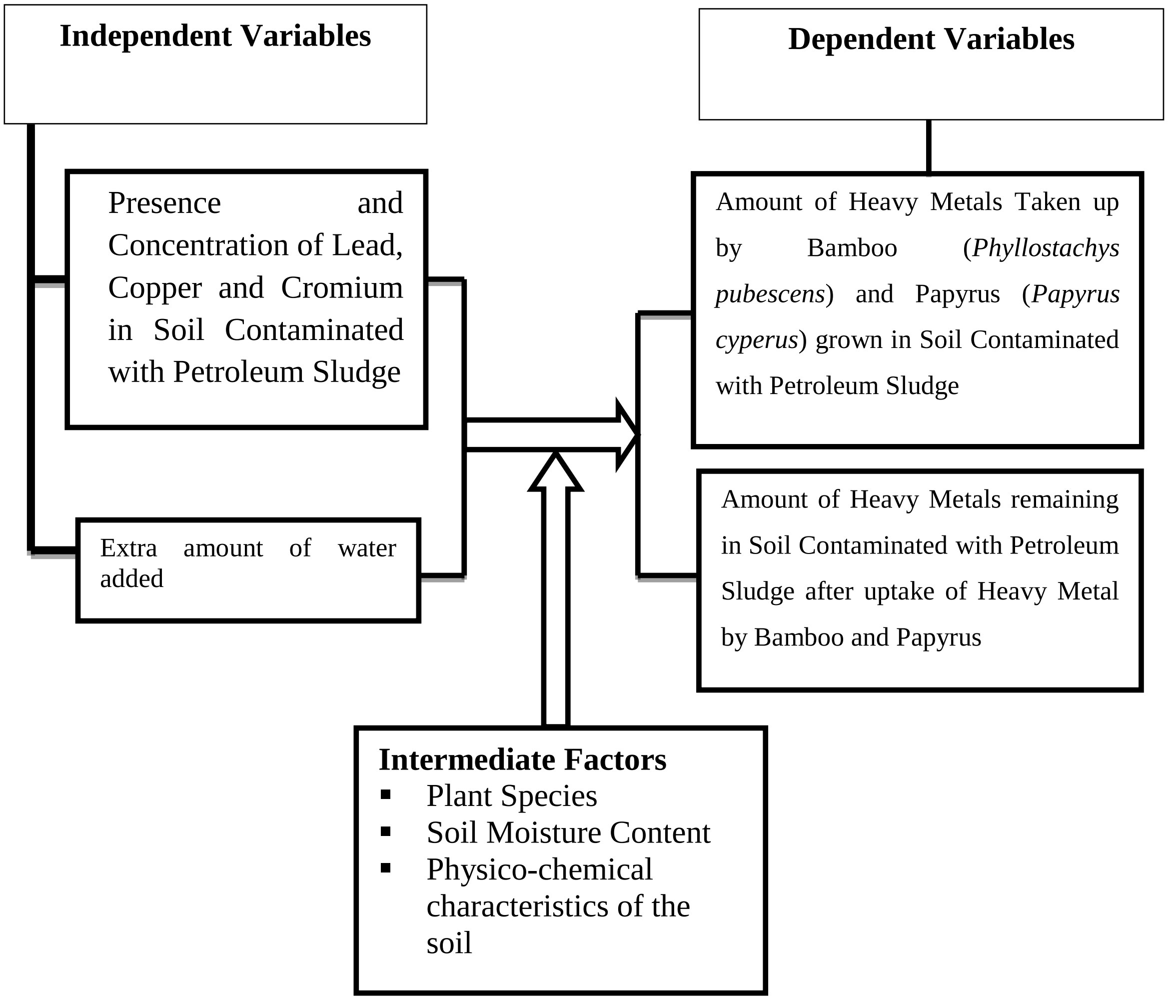


Figure 2 1: Conceptual Framework

2.5 Research Gaps

Physical and chemical methods are possible options, but not the best to adopt because of the high cost of energy associated with them. Furthermore, they do not address the question of re-contamination. Microbial remediation on the other hand, though effective and efficient, is faced with the challenge of fear of unknown outcomes of evolutionary nature of microorganisms. Limited research has established what could be the results of the evolutionary nature of microorganism when used to remediate soils contaminated with petroleum sludge hence creating a gap in. Likewise, in most of the researches, the remediation of soil contaminated with petroleum sludge has not been fully studied. Finally, phytoremediation is reliable, but concerns on use of plants forming food chains are inevitable. Wide varieties of plant species have been effective and efficient in phytoremediation, but for health, safety and environmental reasons, one is bound to ask about the risk of adopting edible plants, and the risk of recontamination. This explains why this study adopted plants (Moso Bamboo and Papyrus) with history of effectiveness in phytoremediation but no known history of being used as food by humans in Kenya. Depending on the level of contamination of the medium in question, the plants can be used for phytomining to extract metals.

2.6 Research Summary

At the centre of this study, is not only phytoremediation in general, but the use of Bamboo and Papyrus plant species to redeem soil that is contaminated with petroleum sludge. Petroleum sludge is a major by-product of crude oil processing plants, transporting and storage facilities. Other sources of petroleum sludge include the processed petroleum products at oil deports, gas filling stations and generally, the motor industries which produce tonnes of oil wastes., Whichever way we look at it, suitable disposal strategies are inadequate or lacking leading to disposal of the wastes in weathering sites, eventually leading to soil and water pollution. Notably, Kenya lacks Sanitary landfill in which the wastes can be safely disposed and therefore licenced enterprises find it relatively very expensive or impossible to disposes the wastes safely.

Crude oil spillages also contribute to pollution. That justifies the need to find a solution to the pollution caused by disposing the sludge in weathering sites. One of the best approaches towards solving the problem is phytoremediation using plants which do not form part of human food chain. Bamboo and papyrus were chosen because they are readily available, they are easy to grow and take care of, and they have been used in phytoremediation sciences for a long time. Many studies have shown the efficacy and effectiveness of the two species in treatment of sewage wastes, and polluted rivers. The two species have high tolerance to toxic wastes including heavy metals.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter seeks to clarify subjects such as research design that was adopted for the study, Soil and plants sample collection strategy that were used, set up of the experiment, sample preparation and analysis, and finally, data collection and presentation. At the same time, the chapter sets straight precautionary measures that were observed to minimize errors as much as humanly possible

3.2 Research Design

This study took a three-level experimental design: (i) Soil sampling from petroleum sludge damping site. The sampling was done ones and the samples analysed for lead, copper and chromium; (ii) Controlled gardening and study of bamboo and papyrus in line with the plants' agricultural Agronomical requirements and (iii) Analysis of the accumulation of the heavy metals by the plants and the gradual variation in level of concentration of lead, copper and chromium in the contaminated soil.

3.3 Material Collection

Since the study was an applied science research which required quantitative data, primary data collection was used. The primary data collection involved various experimental sequences as described in the subsequent sections.

3.3.1 Sample Collection

Soil sample contaminated with petroleum sludge were sampled from Kenya Pipeline Company Limited (KPC) sludge dumping site between Sultan-Hamud and Emali, in Makueni County. Soil auger was used to scoop contaminated soil from about 30-60 cm of depth from the site. The samples were then put in cooler boxes for transport and temporary storage at 4°C.

Red clay soil was sampled from JKUAT soil stock used for horticultural studies using the same method used for the contaminated soil. Same precaution measures were taken for the same reasons.

Both the soil samples were safely stored in tightly sealed glass containers to avoid any contamination. Half of the contaminated soil and the virgin soil were safely transported to Kitil farm, and carefully stored there.

Bamboo seedlings; *Phyllostachys pubescens* (moso bamboo) were sampled from Kitil Farm in Isinya- Kajiado County, and the seedlings were carefully isolated into a greenhouse within the farm. Bamboo was chosen for its ready availability, affordability, adaptability to the experimental conditions, effectiveness in treatment of raw sewage and river polluted by toxic wastes and its ability to tolerate high level of heavy metals. Papyrus rhizomes (*Papyrus cyperus*) on the other hand were obtained from parts of the paddy farms within JKUAT. These rhizomes were used to grow and develop seedlings which were then used for the study. Papyrus was also chosen for its ready availability, affordability, adaptability to the experimental conditions, effectiveness in treatment of raw sewage and river polluted by toxic wastes and its ability to tolerate high level of heavy metals.

3.3.2 Experimental Set-up

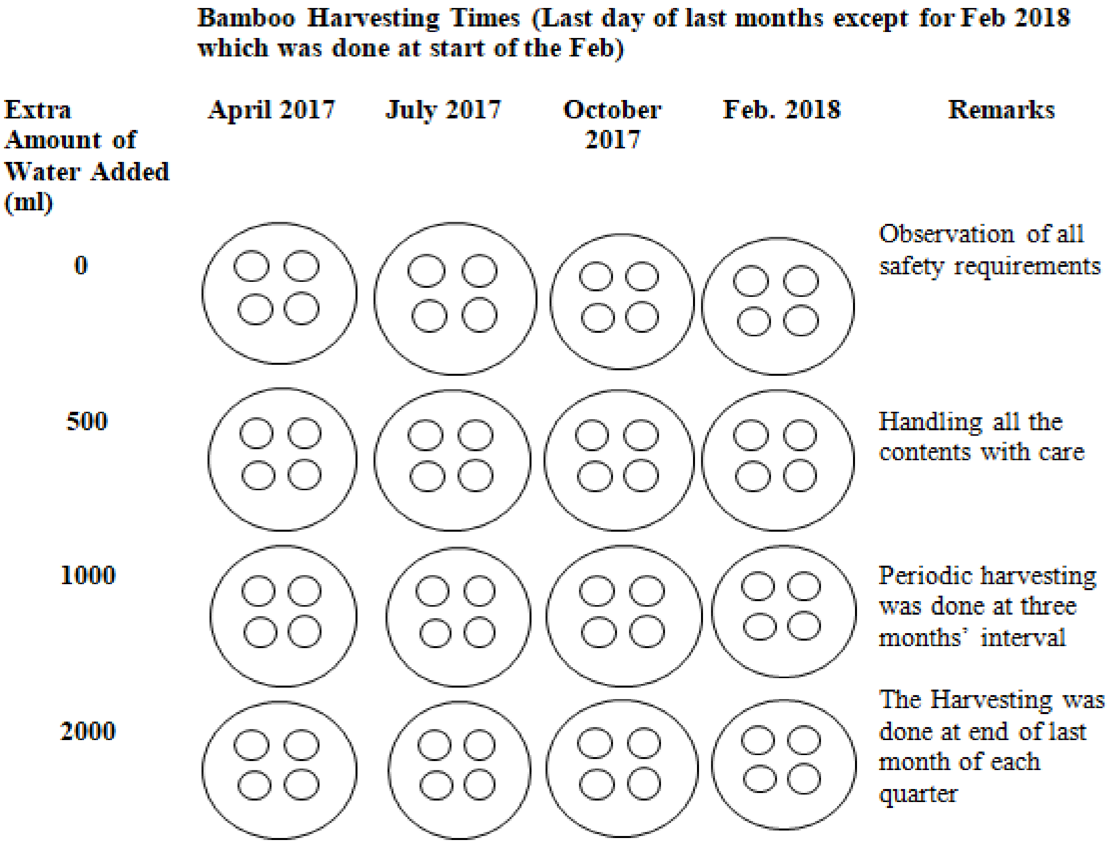
On a thick and firm plastic sheet of paper, the red clay soil from virgin land was thoroughly mixed with the contaminated soil contaminated, compost manure and NPK fertilizer (elements needed for the optimum growth of the crops) using a shovel ensuring that the mixture was fully homogeneous (Emamverdian, Ding, & Xie, 2018; Liu *et al*., 2015). This was done at the ratio of 3:3:1:0.1 (by volume). The decision on the blending ratios was experimental as no previous studies made any guidance on the same. The modification of the soils was to serve two purposes. Firstly, it was meant to improve the condition of the soils so that it could sustain the growth of the two plant species. Secondly the blending and the addition of the fertilizer and compost manure was to accelerate the growth rate of the plant so as to accelerate the rate of bioaccumulation in the speedily increasing biomass. Equal amounts of the blend were put in 32 plastic containers and water added just to make the blend homogeneously moist.

Four bamboo plant seedlings, about 30cm tall, were planted in each of the first 16 containers with the soil blend, and papyrus seedlings of about the same size were also planted in each of the remaining 16 containers. This ensured that for every specific treatment to be done for the plants, there were four sets (quadruplet). Planting one seedling would possibly lead to more absorption but that would mean too little samples for triplicate analysis hence four seedlings were planted and harvested from each container. These containers were placed on benches within one of the green houses at Kitil farm for bamboo, and another green house in JKUAT for papyrus.

In addition to the normal agronomical water requirement (>2,000 mm per year (Kleinhenz, Midmore, 2011), varying amount of extra water were periodically added through drip method to the containers with the plants in three days per week interval for bamboo seedlings, and daily for papyrus. For every four pairs of containers with both bamboo and papyrus, 0ml, 500ml, 1000ml and 2000ml of water was added respectively for the containers through the growth period. Plate 3.1 shows an example of the set-up.

Plate 3.1: Experimental Setup in the Greenhouse

Both bamboo and papyrus grow well indoors where there is natural day light; implying that they could do well in the greenhouses (Cooper, 2016). Ten, 1mm diameter holes were bored at the bottom of the containers to facilitate drainage. The experimental set up is summarised in Figure 3.1



Bamboo Harvesting Times (Last day of last months except for Feb 2018 which was done at start of the Feb)

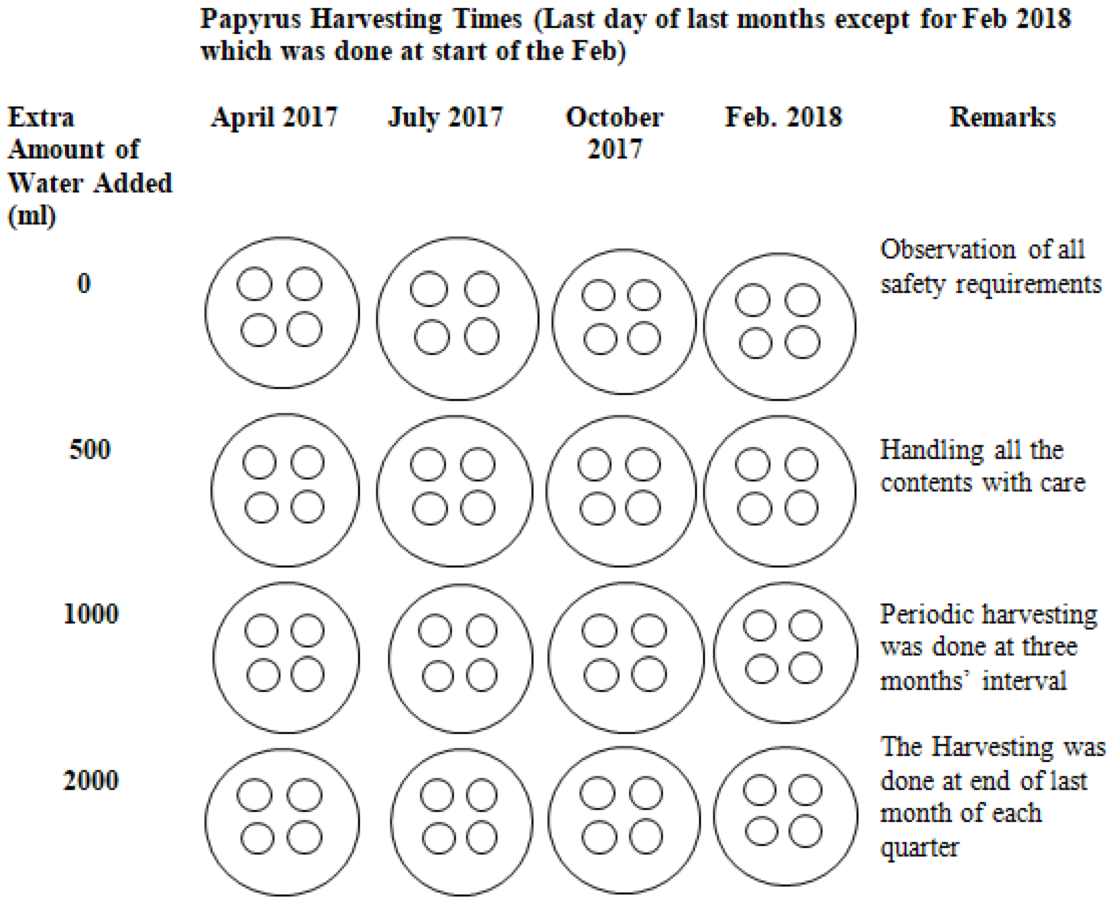
Observation of all safety requirements

Handling all the contents with care

Periodic harvesting was done at three months' interval

The Harvesting was done at end of last month of each quarter

Figure 3.1: Outlook of Setup of Pots of the Bamboo Plants



Papyrus Harvesting Times (Last day of last months except for Feb 2018 which was done at start of the Feb)

Observation of all safety requirements

Handling all the contents with care

Periodic harvesting was done at three months' interval

The Harvesting was done at end of last month of each quarter

Figure 3.2: Outlook of Setup for Pots of Papyrus Plant

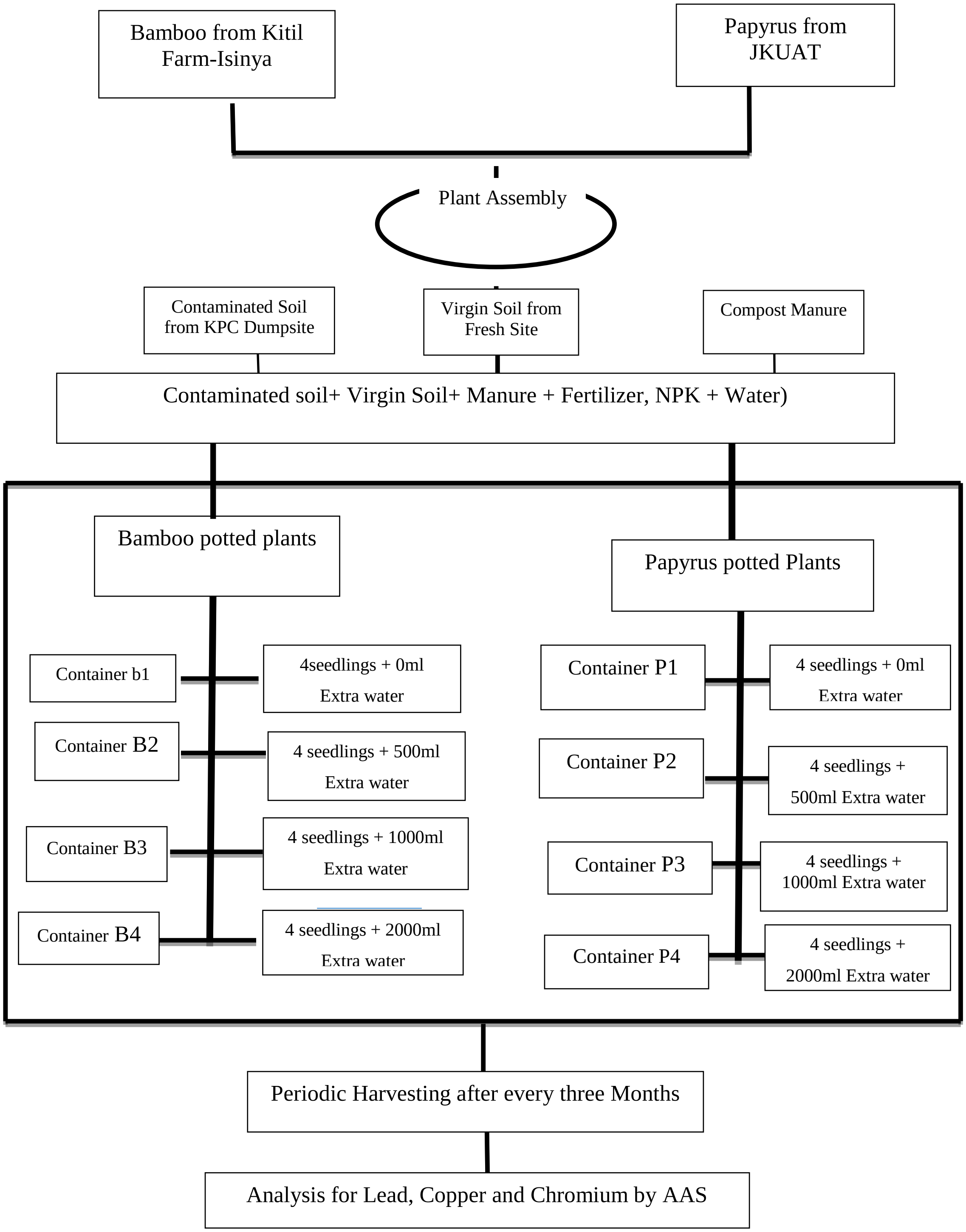


Figure 3.3: Experimental Design

3.5 Sample Preparation for Analysis

Harvesting was done quarterly (at intervals of 3 months) as per the extra amount of water that was being added to the plants. Thus, 4 pieces of bamboo seedlings were harvested from different containers to which varying extra volumes of water were added; all the four plants were harvested from the containers with 0ml extra water added, the one with extra 500ml, the 3rd one with additional 1000ml, and the 4th one with 2000ml. The harvesting was done wholesomely, that is, the whole plant including the roots. They were each inserted in clean, well labeled A5 size self-sealing paper bags. A similar procedure carried out simultaneously for the papyrus plants. The process was repeated after every three months. After harvesting a total of the 32 plants, 16 of which were bamboo and the other 16 were papyrus. These plants were each stored in different containers which were labeled differently as per the date of harvesting and type. In every container, the plants were cut in to 3 parts: the roots, stem and leaves; these were stored in three different clean, well labeled 4 inch by 4 inch self-sealing paper bags small transparent bags marked as R, S and L respectively and allowed to dry further. Thus, the three parts of the plants were in each of the 32 containers; hence a total of 96 samples parts (32 roots, 32 stems and 32 leaves). Implying that there were 16 small bags of bamboo roots, 16 small bags of bamboo stems, 16 small bags of bamboo leaves, 16 small bags of papyrus roots, 16 small bags of papyrus stems and 16 small bags of papyrus roots all labeled as per the initial 32 main containers.

Laboratory crucibles were thoroughly cleaned and dried. The weights of the crucibles were taken and recorded as W1. The dry samples were separately chopped into small pieces that could easily fit into crucibles.

The dry samples were separately chopped into small pieces that could easily fit into crucibles. Between, 1.5gm and 2.0 gm of the samples were weighed into the crucibles which were also labeled accordingly using graphite pencils. Just before this was done, the weights of the clean dry crucibles were taken and recorded as $W_{1.}$ The weights of the samples with the crucible were recorded as W_2. The weights of the sample were therefore calculated as $W_s = W_2 - W_1$ and recorded accordingly. The crucibles were placed on wire gauze on hot coils under a fume hood and the temperature was increased gradually until smoking ceased and sampled completely charred. The charred sample were transferred to a muffle furnace and then the temperature adjusted to 250^{o}C and allowed to heat for 1 hour. The temperature was then re-adjusted to 550^{0}C and left to run for 6 hours. At the end of the six hours, the temperature was lowered to 300^{0}C. The processes were conducted in duplicates (Skoog, & Douglas 2008).

Plate 3.2: Desmoking and Charing Respectively

3.5.1 Soil sample Digestion Objective I

The samples were then dried at 160°C for six days in a muffle furnace. The samples were ground to pass 2mm sieve and packaged in a well-labelled self-sealing PVC paper bags and stored at 4°C. 2gm of dried soil sample were placed in digestion tubes and 20ml of conc. HNO_3 were added to each sample and the mixture boiled at 140°C and the temperature maintained for two hours to remove fumes of the acid (Uddin et al., 2016; Tüzen, 2003). The mixture was then cooled to room temperature and 10ml of H_2O_2 added and the mixture heated again for one hour. The mixture was cooled and filtered through a quantitative acid washed filter paper into a 50ml volumetric flask and the digestions tubes rinsed with 0.1% HNO_3 to volume (Walsh, 2006). The soil samples were also digested in duplicates. It is important to note that there was a total of four soil samples namely, the soil contaminated (with petroleum sludge (**S_1**)), virgin soil (**S_2**), soil blended by mixing the contaminated soil and the virgin soil (**$S_3=S_1+S_2$**) at start of experiment, and **S_4** at end of the experiment. S_4 at end of experiment was sampled from the containers 1 of the end of the 12^{th}, months for both the plants. The samples were mixed to homogeneous mixture. All the soils including petroleum sludge were prepared for analysis using the same method as described above.

3.5.2 Plant Sample digestion- Objective II and III

The ashes were quantitatively transferred to 25ml beakers using 10ml, of 1N $HNO_{3.}$ The samples were heated at 90^0C on a hot plate for 5 minutes. The sample was then transferred to a 50ml volumetric flask and filled up to the mark with 1N HNO_3. It was shaken thoroughly to mix well. The mixture was filtered into clean dry polyethylene sample bottle and labeled according to the name of sample digested (Uddin et al., 2016; Tüzen, 2003).

3.6 Preparation of Standards to Test for Concentration of the Heavy Metals

Three standards namely, copper, lead and Chromium were prepared from analytical stocks of 1000ppm. The research focused on three heavy metals namely lead, copper and chromium. These metals form part of heavy metal contaminants commonly found in petroleum sludge. Secondly these metals pose the highest risk to the environment and food chains in the ecosystem. According to Battikhi and Mohammed, (2014), these metals are carcinogenic and mutagenic. Additionally, the standards for carrying out the concentration analysis of the contaminants from the samples were readily available at the time of the experiment albeit expensive. The conventional formula of Concentration C1 x Volume V1 = Concentration C2 x Volume V2 was used to calculate the volume of the stalk which would be taken to make 2ppm, 1.5 ppm, 1ppm, 0.5ppm, 0.2ppm and 0.1ppm (FAO of the UN, & WHO, 2013)

3.6.1 Copper standard for calibrating AAS machine for Copper Analysis

The AAS machine used was the AA-700 model with specification of detection limits indicated in Table 3.1.

Analytical stock of 1000 ppm was bought, and from it 100 ppm was prepared by pipetting 10 ml into 100ml volumetric flask and toped to the mark with 1 N nitric acid (HNO_3) (Equation 3.1):

$$C_1 * V_1 = C_2 * V_2 \qquad 3.1$$

$$V_2 = C_1 * \frac{V_1}{C_2} \qquad 3.2$$

C1* V1 = C2*V2, ⇒ V2 = C1*V1/C2

V1 = 10ml

C1 = 1000ppm

C2 = 100ppm

10 ppm was prepared from 100ppm by pipetting 10 ml in 100mlvolumetric flask and toped to mark with 1 N nitric acid (HNO_3) as shown:

C1* V1=C2*V2

∴V2 = 1000ppm*10ml/100ppm = 100ml

Series of 2ppm, 1.5ppm, 1.0 ppm 0.5 ppm 0.2 ppm and 0.1 ppm were prepared by using the same method as shown above. Example 2ppm was prepared by pipetting 2ml from 10 ppm stock in 10 ml volumetric flask and topped to mark with nitric 1 N nitric acid.

The same method applied as above for Lead (Pb) and Chromium (Cr). These series were used to calibrate AAS machine so that standard curves for all the three elements could be established.

3.6.2 Precautions

In the processes, various precautions were taken to mitigate on errors that could cause significant effects on the research project. These precautions included hygienic handling of samples to prevent cross contamination, correct measurement quantities of mixtures for the plant growth medium, measurement of correct quantities of water for irrigating the plants to prevent flooding and over leaching.

3.7 Atomic Absorption Spectroscopic Analysis

All the samples were analyzed from three elements namely, Copper, Lead and Chromium . The machine was turned on and the lamp allowed warming up for 15 minutes. During this period, align the instrument, position the monochromator at the correct wavelength, select the proper monochromator slit width, and adjust the hollow cathode current according to the manufacturer’s recommendation. Subsequently, light the flame and regulate the flow of fuel and oxidant, adjust the burner and nebulizer flow rate for maximum percent absorption and stability, and balance the photometer. Run a series of standards of the element under analysis and construct a calibration curve by plotting the concentrations of the standards against the absorbance. Aspirate the samples and determine the concentrations either directly or from the calibration curve. Standards must be run each time a sample or series of samples are run (McCarthy, 2012). The data collected from the analysis were recorded as shown in Tables 3.1. The data were taken directly from the readings of AAS machines. Plate 3.4 shows AAS Analysis in process

Plate 3.3: AAS Analysis Process

3.8 Test of Leaching of Heavy Metals

To test for the heavy metals which would be washed away through leaching, the study calculated the difference between the ambient concentration of the heavy metals in the soil and the sum of residual concentration of the same metals in the soil at the end of the experiment and the amount of the metals accumulated by the plants. In other words, the amount of metals leached equaled to the Amount of the heavy metal is the soils medium at start of experiments (S_3) less amount of the metals in the medium at end of experiment (S_4), less the amount of the metals accumulated by the plants (B_{Acc}) or P_{Acc}). Where B_{Acc} represents average accumulation by bamboo and P_{Acc} represent average accumulation by papyrus.

3.9 Measurement of Performance of Bamboo and Papyrus

Heavy metals accumulation capacity of plants is calculated in terms of bioconcentration factor (BCF), and translocation factor (TF), bioenrichment factor (EF) and bioconversion factor (CF). Bioconcetration factor (BCF) is the ratio of the amount of heavy metal accumulated at various parts of a plant to the amount of the same metal in the soil where the plant (Liu et al., 2009). The value of the ratio tells whether the plant absorbs and accumulates the metal or just absorb. For example, if BCF ≤ 1.00 indicate that the plant only absorbs the metal but do not accumulate it. On the hand, if the BCF > 1.00 it indicates that the plant absorbs and accumulates the metal in question (Liu et al. 2009).

BCF is calculated using the following formula:

$$\text{BCF}= \frac{C_{plant}}{C_{soil}}$$

(Sulaiman, & Hamzah, 2018)

Where C_{plant} is the concentration of metal in plant part and C_{soil} is the concentration of metal in soil

Apart from BCF, the translocation factor (TF) can also be used to determine the bioremediation performance of a plant. The translocating factor measures the capacity from the root to the shoot including both stem and leaves. TF is calculated using the formula:

$$\text{TF} = \frac{C_{Stem\ or\ Leaf}}{C_{Root\ or\ Stem}}$$

(Sulaiman, & Hamzah, 2018)

Where C_{stem} or leaves is the concentration of metal in stem or leaves and C_{root} or stem is the concentration of metal in root or stem.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

The results represent tests done of the contaminated soil, a raw sludge sample from KPC and virgin soil on which plants were grown. It also presents the results of testing for the presence and concertation of the heavy metals targeted from a soil blended by mixing the contaminated soil and the virgin soil. The blending was done to dilute any possible high concentration which could cause stress to the plants. A final test was conducted on the blended soils in which bamboo and papyrus were grown.

4.2 Concentration of Heavy Metals

The unblended contaminated soil contained the highest concentration of the metals while the blended soil at the end of the experiment contained the least (Table 4.1). However, pure metal concentration levels for raw sludge were within the range obtained by Akpoveta *et al*. (2014). There was a significance difference (0.0019ppm) between the levels of Lead as found out in this research compared to the findings of Akpoveta *et al*. (2014). The significant difference can mainly be attributed to the fact that previously some petroleum products were leaded unlike presently when the process was banned. The concentrations of the other metals (Copper and Chromium) were also within range obtained by Akpoveta *et al.* (2014). Although the level detected in the petroleum sludge in this study were generally lower, as this was expected due to the improved refinery technologies in the distillation processes. The results of the analysis were also in line with the results of Nkeng *et al*., (2012), who studied bioremediation of soil contaminated with oily sludge in Buea, Cameroon.

A comparison of the concentration of the metals in the soil blend at start and end of experiment indicated a significant reduction in the concentration of all the three metals is shown in Table 4.1. This implies that appreciable amounts of the heavy metals were either absorbed by the plants or leached out with water through the holes at the bottom of the containers.

Notably, the concentration of the metal in polluted soil S_2 is higher than that of the blended soil S_3. This implies that the virgin soil lowered the concentration of the heavy metals in the contaminated soil, which coincided with the aim of the blending of the virgin soil and the polluted soil. The blending was to modify the polluted soil to encourage the growth of the target plants.

4.3 Mean Accumulation of Heavy Metals by Moso Bamboo (*Phyllostachys pubescens*)

The trend of uptake of the heavy metals by bamboo after 3rd, 6th, 9th and 12th month of planting are indicated in Tables 4.2, 4.3, 4.4 and 4.5 respectively. The tables present the amounts of the heavy metals accumulated by the various parts of the plants under the various treatments. The total accumulation in the plants for every season and every treatment has been established from the R+S+L columns each of the tables. The mean accumulation was calculated by summing the accumulations in the roots, stems and leaves.

Notably, there was a significant drop in amount of Cu^{2+} accumulated at end of the sixth month compared to the third month under the 0ml extra water treatment and significant rise in uptake of lead in the third month under 2000ml extra water treatment. This observation was not expected and could have been caused by either machine error, or human error, during handling and preparation of the samples for example during extraction or auto sampling.

Figure 4.1 presents the total mean accumulation of heavy metals with varied season and treatment for the whole plant. From the results, it is evident that accumulation of the heavy metals is highest with the least extra amount of water added (0ml), through all the season. Copper was the most readily accumulated by the plants. This could be because it was the most abundant in the soil contaminated with petroleum sludge or because of plant physiology. Also, notably is the consistent reduction in accumulation with more extra amount of water added for all the seasons and all the metals except for Lead in season 2. Additionally, throughout the experiment, Lead is the least bio-accumulated by bamboo.

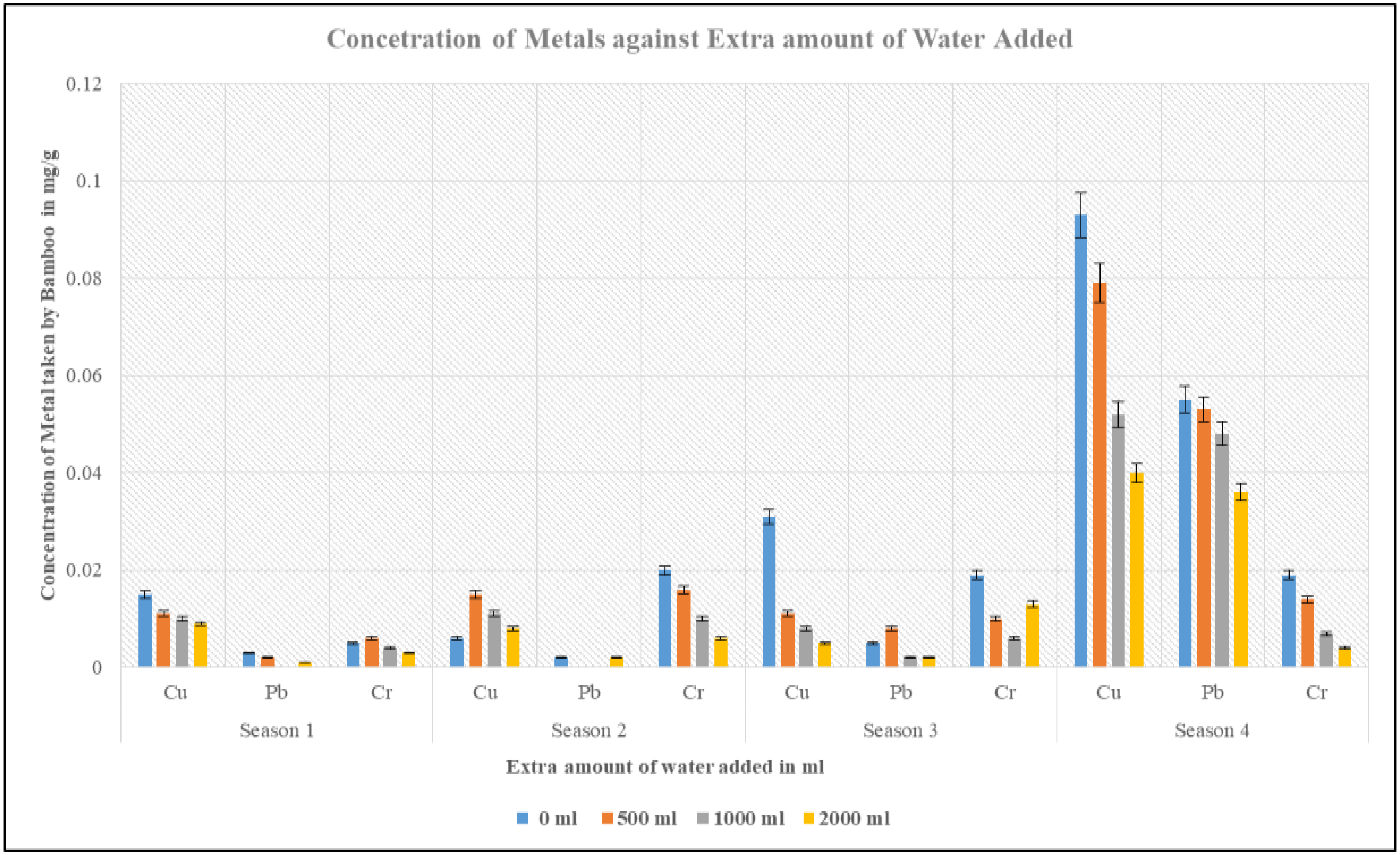


Figure 4.1: Cumulative Uptake of the Heavy Metals by Bamboo

From the data, Copper is more readily absorbed and accumulated by bamboo at the least amount of extra water added. Figure 4.1 also indicates that on adding more extra amount of water it minimizes the amount of heavy metals taken up by Bamboo. This is consistent with the findings of Liu et al (2015), who found that increased amount of water in soil, multiplies the rate of leaching. Leaching was found to reduce the availability of materials that can be adsorbed and absorbed by the roots of plants.

At the end of 6 months, Chromium was the most readily absorbed metal at 0ml of extra additional water than both copper and lead while Lead was the least absorbed. Further, the absorption of Chromium experienced the highest reduction based on treatments. The percentage reduction was 70% (0.02mg/g under 0ml extra water and 0.006mg/g under 2000ml) (Figure 4.2(a) and (b)).

Figure 4:1 shows an overall observation for the Experiment with Bamboo plant (*Phyllostachys pubescens*). It combines all the seasons and treatments through the seasons as well all the metals. It is important to note that it does not capture the trends of bioaccumulation of heavy metals in various parts of the plant but rather the mean total accumulation in the whole plant from season one to season four. The accumulation of the metals varies with season and treatment. From the bar graphs, it is evident that accumulation of the heavy metals is highest with the least extra amount of water added (0ml), through all the season. It is also evident that copper is the heavy metal most readily accumulated by the plant. This could be because it was the most abundant in the soil contaminated with petroleum sludge or because of plant physiology which is beyond the scope of this research. Also, notably is the consistent reduction in accumulation with more extra amount of water added for all the seasons and all the metals except for lead in season 2. Additionally, throughout the experiment, lead is the least absorbed by bamboo.

To get a better picture of the trends of bioaccumulation for all the season and the treatments, the graphs for every season have been extracted from the graph in Figure 4.1. The subsequent section presents the independent graphs for all the four seasons.

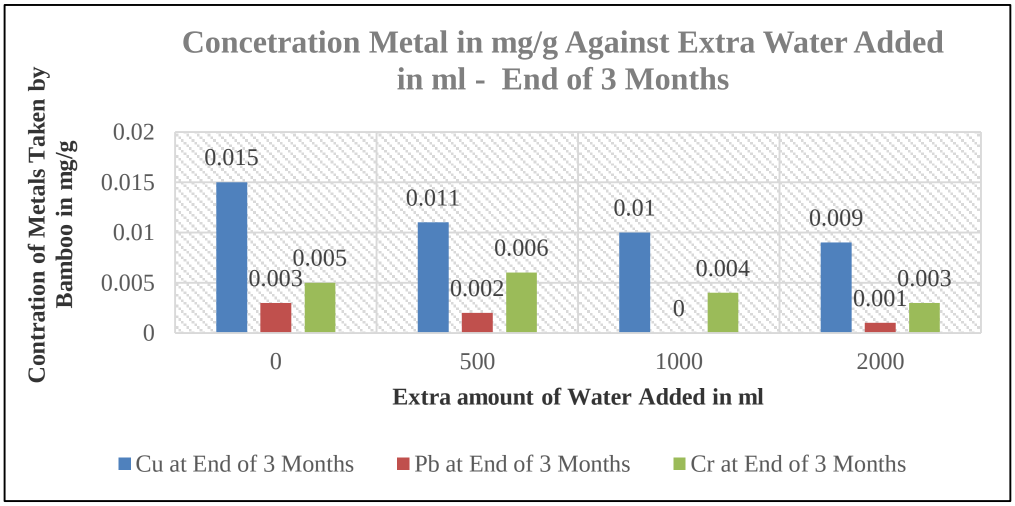


Figure 4.2: Accumulation of the Heavy Metals by Bamboo in 3rd Month

As the chart indicates, Copper is more readily absorbed and accumulated by bamboo at the least amount of extra water added. The chart also shows that more extra amount of water added directly minimizes the amount, of metals taken up by Bamboo. Considering Copper for example, the amount absorbed when 0ml extra amount of water is added is 0.015mg/g while with 2,000ml of extra additional water, the amount of copper absorbed was 0.009mg/g. The trend is consistent with all the metals as can be seen from the graph. This is consistent to the claims of Liu et al., (2015) that increased amount of water in the soil, multiplies the rate of leaching. Leaching on the other hand reduced bioavailability of materials that can be absorbed by the roots of plants. The trends in season two and the rests of the subsequent season are almost similar as can be seen in Figures 4.3, Figure 4.4, and Figure 4.5 below.

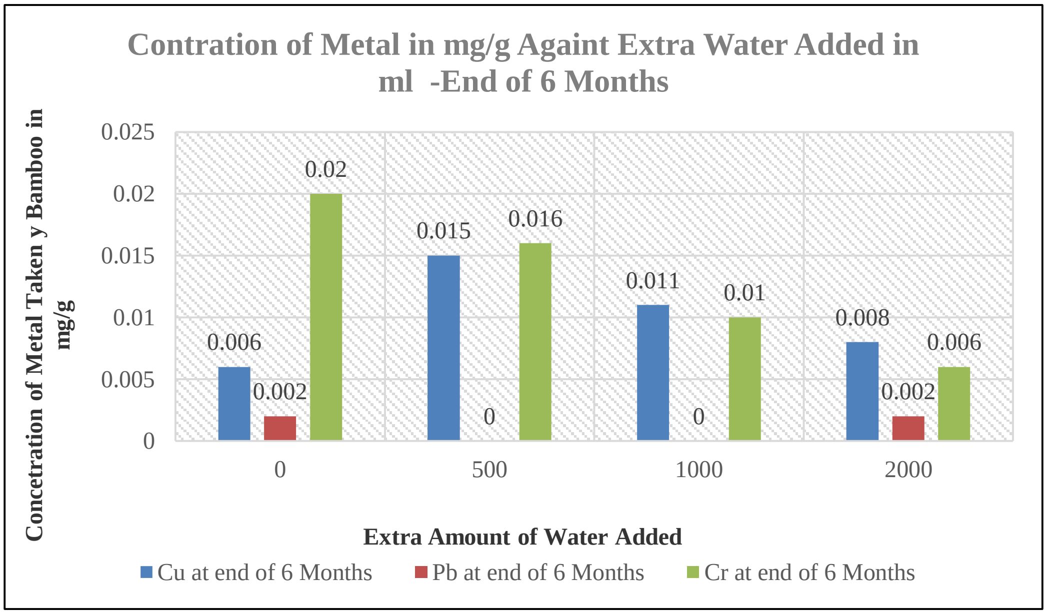


Figure 4.3: Accumulation of the Heavy Metals by Bamboo in the 6th Month

Comparing season 1 and 2, there is a slight difference. Unlike in season 1, Chromium is the most readily bioaccumulated at 0ml of extra additional water than both copper and lead. Lead, on the other hand is the least absorbed. In fact, at 500ml, and 1000ml of extra amount of water added, bamboo absorbed undetectable amount of lead. Notable again in season two, the trend of absorption is steady for copper and chromium from 500ml, to 2000ml of extra amount of water. Notable is the possible error with the Copper sample for treatment 1 fed into the auto sampler. Nonetheless, the rest of the results were consistent with the results of the other seasons. Notably, the absorption of Chromium experienced the highest reduction based on treatments. The percentage reduction was 70% (0.02mg/g under 0ml extra water and 0.006mg/g under 2000ml). Figure 4.4 and 4.5 shows a graphical representation of the figures 4.2 and 4.3 respectively.

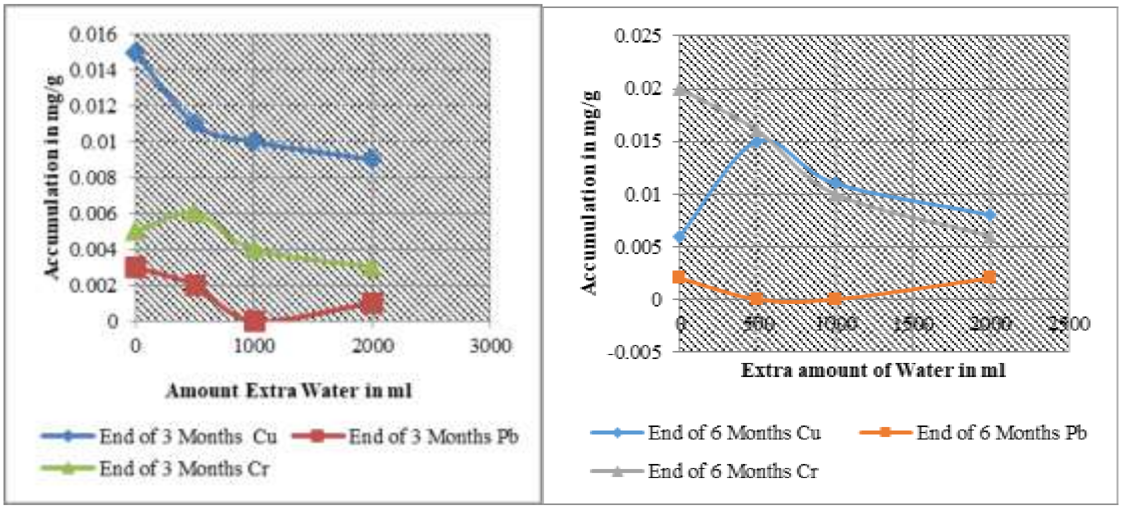


Figure 4.4: Accumulation trends by Bamboo at end of 3rd and 6th Months

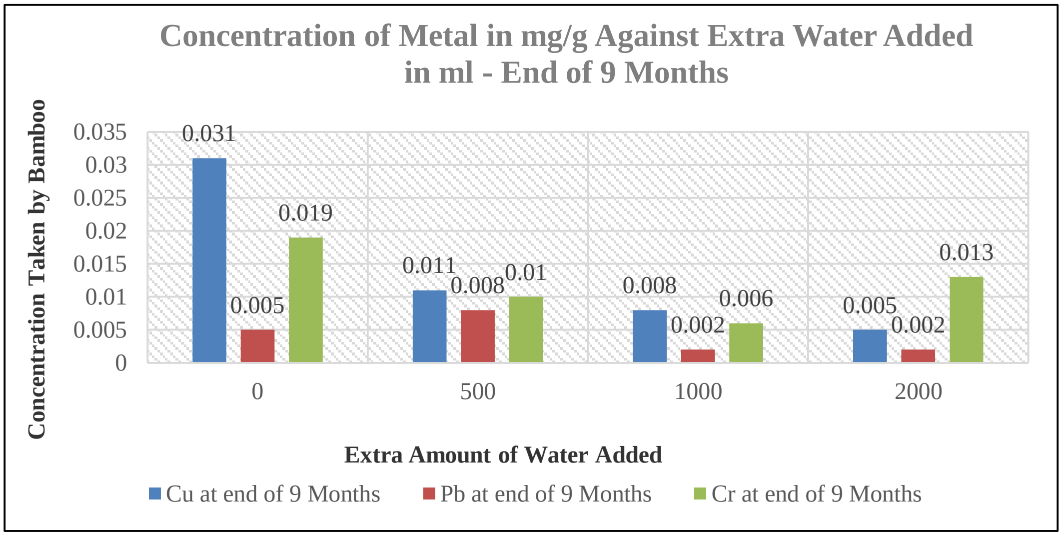


Figure 4.5: Accumulation of the Heavy Metals by Bamboo in the 9th Month

The storyline in season three is almost very similar to that of season one except that the plant was able to absorb lead in under all the treatment. The absorption for Copper steadily reduces with additional extra water. The same applies to Chromium for the first three treatments. The constancy of absorption of lead is observable from the second treatment through to the last. Absorption of Chromium in the last treatment is notably strange considering the previous trends. This could be due to technical error in handling the reading or machine error. From a general view, the uptake of copper is the highest under all the treatments. Under 0ml extra additional water, 0.031mg/g of copper was absorbed by bamboo, while under 2000ml extra additional water, only 0.005mg/g was accumulated by the same plant. The difference represents 83.87% reduction in the absorption due to increased amount of extra water added. The absorption of lead reduced by 60% (from 0.005mg/g to 0.002mg/g) under 0ml extra water and 2000ml extra water treatment. On the other hand, absorption of chromium fell by 68.42%, considering the same treatment (0.019mg/g under 0ml extra water and 0.006mg/g under 2000ml of extra water).

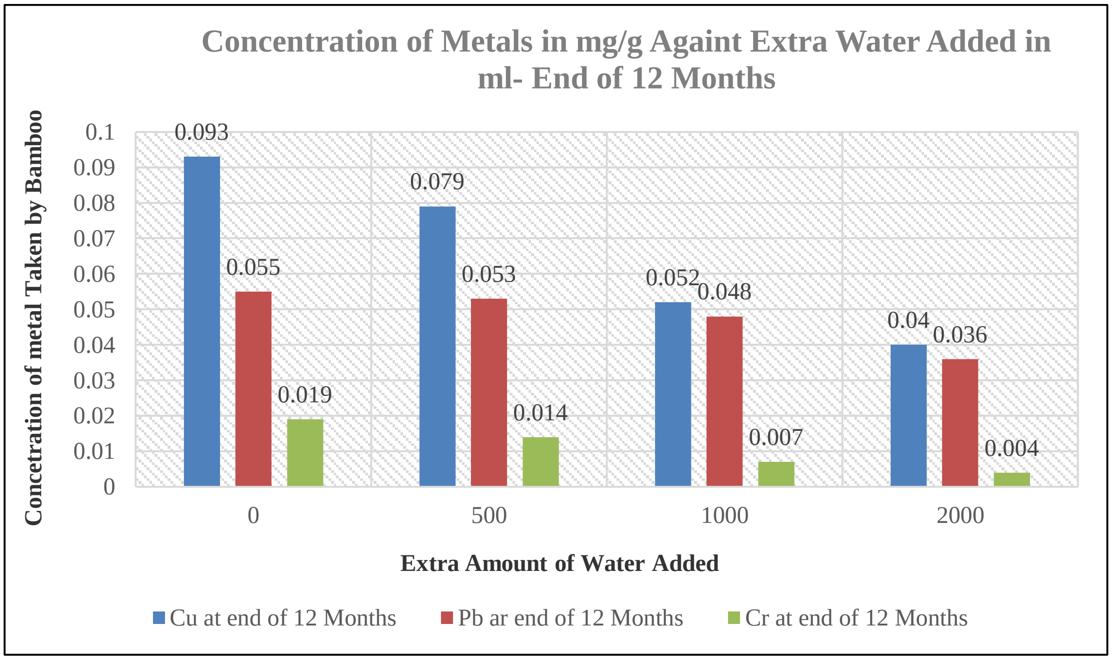


Figure 4.6: Accumulation of the Heavy Metals by Bamboo in the 12th Month

Figure 4.5 shows that season four recorded the highest uptake of heavy metals by Bamboo. Considering that season 4 took 12 months, it is evident that bioaccumulation needs times to occur. More amounts of the heavy metals were absorbed by bamboo towards the end of the study period. This implies that the longer the growth time in soil contaminated with petroleum sludge, the higher the level of heavy metals bio-accumulated by the plant. Taking copper for example, accumulation in the whole plant under the first treatment was 0.093mg/g, while in season one under the same treatment, the accumulation was 0.015mg/g (83.87% rise in accumulation). On the other hand, the moso bamboo of season 4 accumulated 0.055mg/g of lead, while season 1 under the same treatment accumulated 0.005mg/g of the same metal (90.91% rise in accumulation). The Accumulation of chromium in season 4 under the first treatment was 0.019mg/g while in season 1 it was 0.005mg/g (73.68% rise in accumulation). Similar trends were observed in the three other seasons under similar conditions of treatment. From the three examples given, it is evident that bioaccumulation increases with longer time of growth of the bio-accumulators in the contaminated medium. According to Audet & Charest (2007), the more moso bamboo grows in contaminated soil, the more it takes up the contaminant until such a level when the concentration of the contaminant in that soil is too low to allow natural absorption. The graphs in Figures 4.8 and 4.9 show graphical presentation of the trends of accumulation of the three metals. The accumulation in the 9th and the 12th months have been summarized in the graphs in Figure 4.7

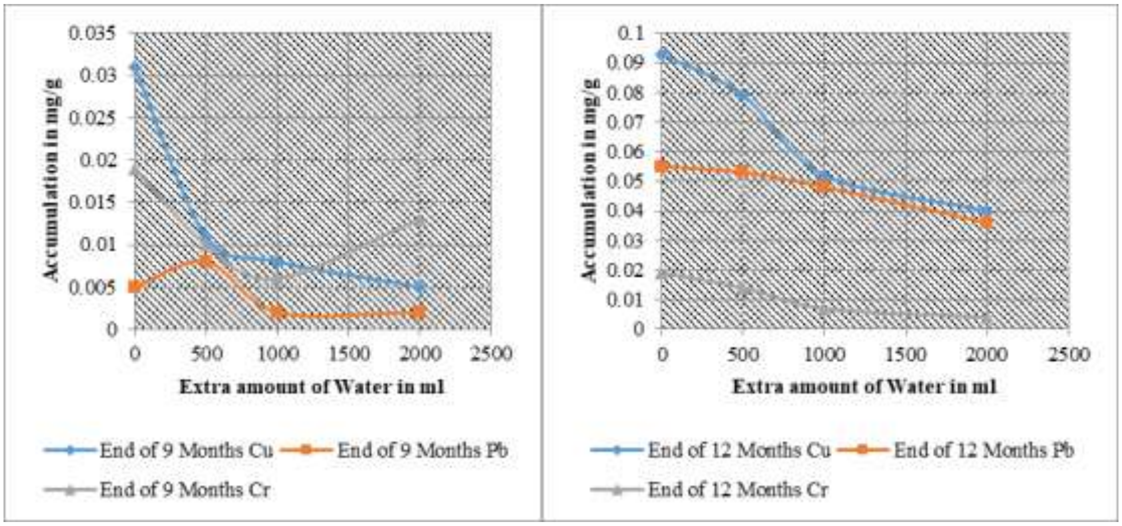


Figure 4.7: Summary of Uptake of the Metals by Bamboo in the 9th and 12th Months

4.4 Mean Accumulation of the Metals in Papyrus

The trend of uptake of the heavy metals by *Papyrus cyperus* was also established as presented in Tables 4.6 to 4.9. The tables present the amounts of the heavy metals accumulated by the various parts of the plants under the various treatments and for all the seasons. These tables also present the totals of the accumulation in the roots, stems and leaves of the plants.

It was observed that with more extra additional water, the amount of heavy metals absorbed after three months reduced significantly as shown in Table 4.6. There was 81.25% fall of total bio-accumulated copper on adding extra water from 0ml to 2000ml after three months. On the contrary, the mean accumulation of chromium was steady on the addition of extra water with 40% accumulation noted.

There was no discernable trend of Lead accumulation by Papyrus on the 3rd month. It was noted that there was accumulation of Lead under 2000ml extra added water. This could have been contributed by the extremely insignificant uptake of water when more water is present in the soil. This result augured well with the findings of Bentum *et al*. (2011).

Table 4.7 shows the concentration of accumulated metals after 6 months of planting Papyrus plant in contaminated soil. There was a high loss of accumulation of metal in root and stems than in the leaves. This finding was consistent with results of Verbruggen *et al.* (2009) who found that due to the molecular nature of metal substances dissolved or suspended in water accelerates their flow with gravity. This therefore makes them to be carried away by water, further away from the plant roots, during the leaching process and may thus, go further away from the reach of the plant roots.

Considering lead for example, the percentage reduction in absorption is 83.33% between 0.081mg/g at 0ml water and 0.003mg/g at 2000ml water. The percentage reduction in the absorption of chromium on the other hand is 69.23% between 0.013mg/g at 0ml of water treatment and 0.004mg/g at 2000ml water treatment.

The trends of accumulation of Lead, Copper and Chromium as demonstrated in Tables 4.2 to 4.5 and tables shows a reverse association between uptake of the metals and leaching. Leaching is a direct function of gravitational water, and the gravitational water is direct function of the amount of rainfall or irrigation water, with more gravitational water, the metals which are either suspended or dissolved in the soils water are drained with the gravitational water (Gabriel et al., 2018). Lead, Chromium and Copper are capable of moving in a soil water solution or in the surface run offs and leaching into the underground water because of their dissolution from soil minerals by acidic water in the soil. Under intense drainage of the gravitational water, more of the contaminants are drained far away from the root mass and therefore are said to be biologically less available (Bieby, et al., 2011). The plants could take up some of the metals but the uptake was notably so smaller in containers where less leaching occurred. Consequently, bioremediation could be very minimal with extensive leaching or condition which encourage leaching. It is therefore certain that environmental condition like heavy rainfall in clay in on soils with high drainage properties can lower the ability of the plants to remediate such soils in the even that they are contaminated with heavy metals.

The uptake of copper reduced continuously with additional of extra water on the 9th month after planting (Table 4.8). It reached its highest in papyrus at 0.088mg/g in season three. The percentage difference between the highest absorption of copper and the lowest however grew to 64.29% compared to season 2 which was 47.62% between 0ml extra water treatment and 2000ml extra water treatment. The absorption and the percentage reduction of Chromium however remained constant. The absorption of chromium also reduced significantly from 0.013mg/g to 0.004mg/g (69.23%) between 0ml of extra water and 2000ml of extra water. Absorption of lead also reduced significantly with extra additional water. At 500ml additional water, papyrus accumulated 0.006mg/g while under 2000ml of extra water, 0.001mg/g (83.33% reduction in mean accumulation).

The uptake of copper under 0ml extra water treatment remained constant after 12th month in comparison to the previous season, with a significant reduction in the amount absorbed under 2000ml extra water treatment (Table 4.9). In comparison to season 3 of harvesting, there was a further reduction of accumulated copper from 64.29 to 67.86%. This result indicated a possibility of further increment of lead accumulation by Papyrus over a longer period of growth.

The trend in lead also exhibited the significant reduction in the amount taken up by papyrus under increasing amount of extra water. Under 0ml extra water treatment, the uptake was 0.009mg/g while under 2000ml extra water, the absorption was 0.001mg/g. The difference represents 88.89% reduction in uptake of lead by papyrus.

Figure 4.3 presents the cumulative absorption the heavy metals by Papyrus over the four seasons. Copper is the highly absorbed metal with its absorbed quantity increasing with time. However, on increasing the amount of extra added water, the accumulated quantity reduces due to leaching process.

The amount of extra amount of water added did not have a great influence on the amount of absorbed chromium in the first session of harvesting. On the other hand, undetectable traces of lead were absorbed on added 2000ml of extra water during the first session. Figure 4.6 below shows a graph developed from the data in Table 4.10. From the graph, the effect of time of growth in contaminated soil and the treatments effected are evident.

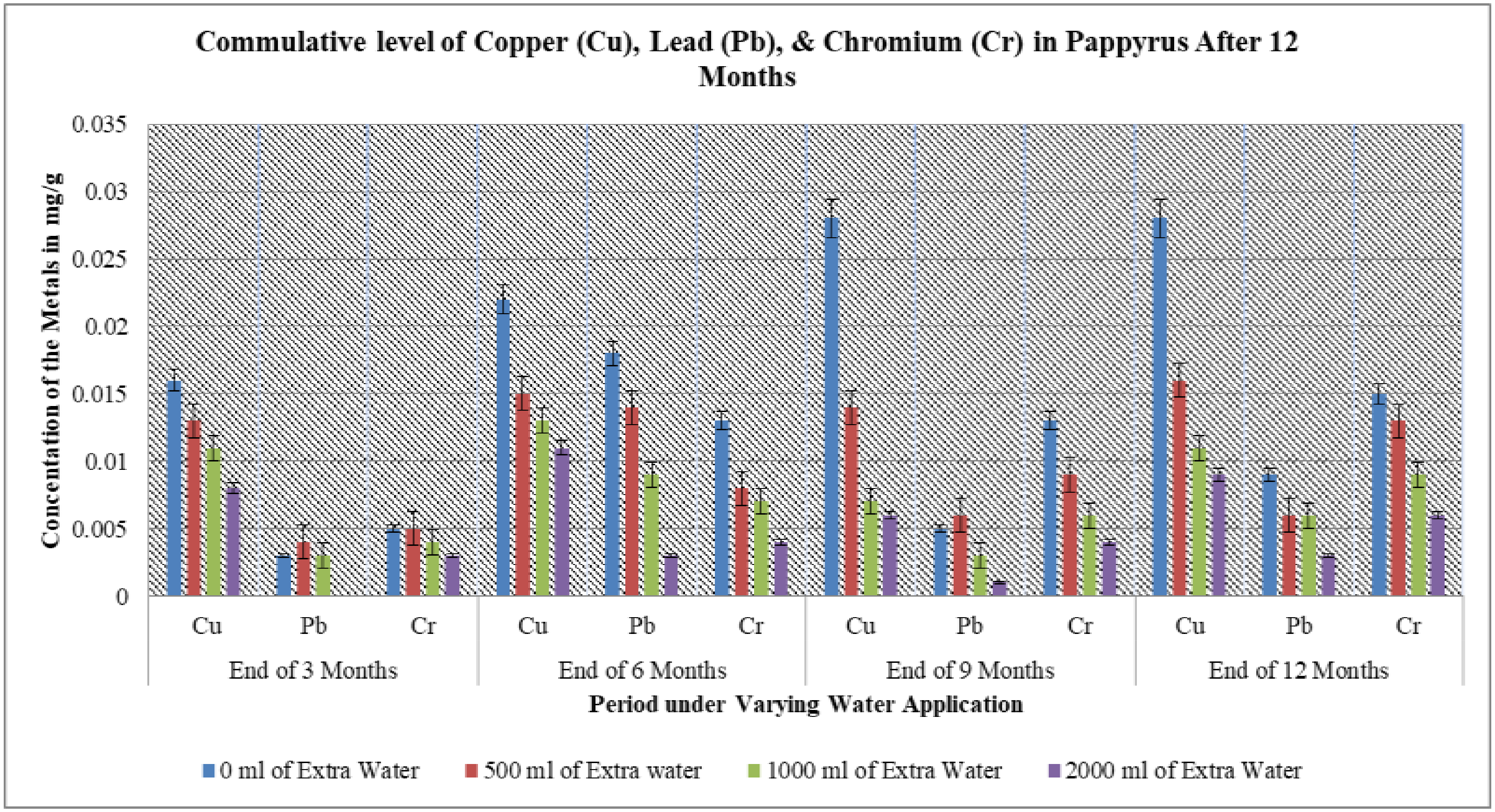


Figure 4.8: Cumulative Absorption of the Heavy Metals by Papyrus

From the figure, a consistent trend in uptake of the heavy metals is observable for all the metals in all the seasons except for lead in the first season. Leaching significantly influence uptake of the heavy metals. This is because, with a lot of extra amount of water added, the metals are leached far away from the roots of the plants and thus reduction in bioavailability of the elements for the plant roots. This observation coincides with the establishments of Verbruggen *et al*., (2009) who found that the molecular nature of the elements in the soils makes them to be carried by water in leaching process. Also observable from the graph is that copper tops in absorption followed by Chromium except for first season where more lead than chromium was taken by papyrus. The congestion in Figure 4.6 is solved by Figures 4.7 all through to Figure 4.10. The figures have all been extracted from Figure 4.6 purposefully to paint a better view per season.

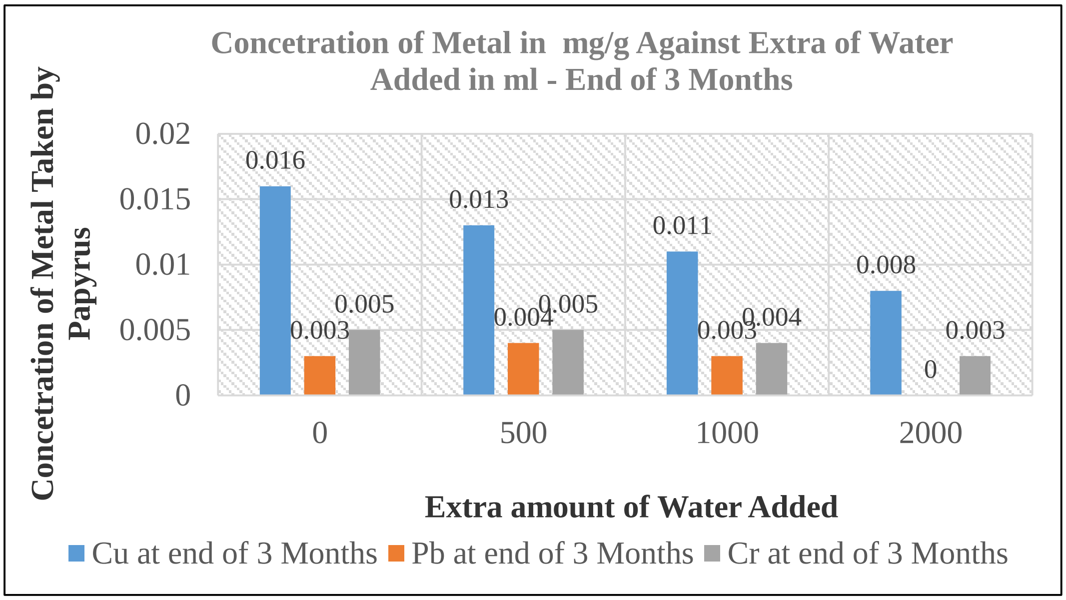


Figure 4.9: Accumulation of the Heavy Metals by Papyrus in the 3rd Month

The consistency in the trend of uptake of heavy metals is evident. With more extra additional water, the amount of heavy metals taken reduces significantly. For example, 0.016mg/g of copper was accumulated under 0ml extra water added; while under

2000ml extra water added, *Papyrus cyperus* only accumulated 0.003mg/g. This change represents 81.25% fall in the level of copper bio-accumulated with additional extra water. The mean accumulation of chromium was equally steady from first treatment to the last. The uptake also decreased with increase in extra additional water. The overall percentage reduction in the absorption is 40.0%. Although on the other hand, papyrus accumulated the least mean concentration of Lead, the uptake was not steady, and this could imply the uptake may not be directly affected by plant available water (Bentum *et al*., 2011). The percentage reduction of absorption of lead and chromium were not as significant as the reduction of uptake of copper. Also noted is undetected bio-accumulation of Lead under 2000ml extra water added. This could be due to extremely insignificant uptake of lead under this extra additional of water.

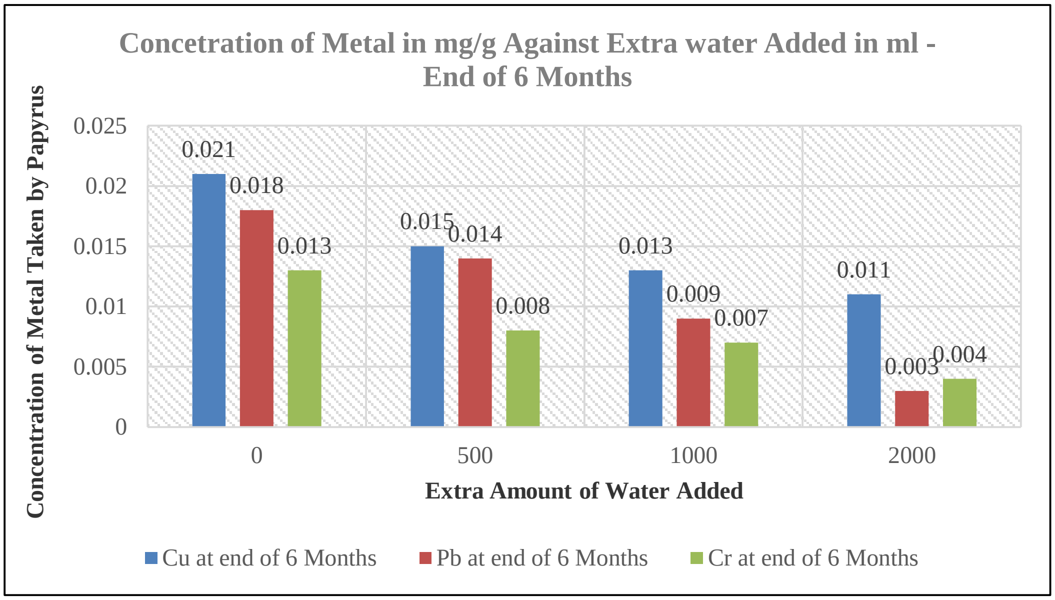


Figure 4.10: Accumulation of the Heavy Metals by Papyrus at end of 6th Month

Figure 4.8 is a perfect demonstration of the direct impacts of plant available water, and the potential of *Papyrus cyperus* to absorb lead, chromium and copper from soil contaminated with petroleum sludge. All the metals were absorbed, and the absorption reduced consistently with the extra amount of water added. The uptake of the three metals by *Papyrus cyperus* is consistent with the findings of Verbruggen *et al*., (2009), which state that the molecular nature of the substances dissolved or suspended accelerates their flow with gravity and may thus, go further away from the reach of the plat roots. Considering lead for example, the percentage reduction in absorption is 83.33% between 0.081mg/g at 0ml water and 0.003mg/g at 2000ml water. The percentage reduction in the absorption of chromium on the other hand is 69.23% between 0.013mg/g at 0ml of water treatment and 0.004mg/g at 2000ml water treatment. Figure 4.11 and 4.12 are further represented in the graphs in Figure 4.13 and 4.14.

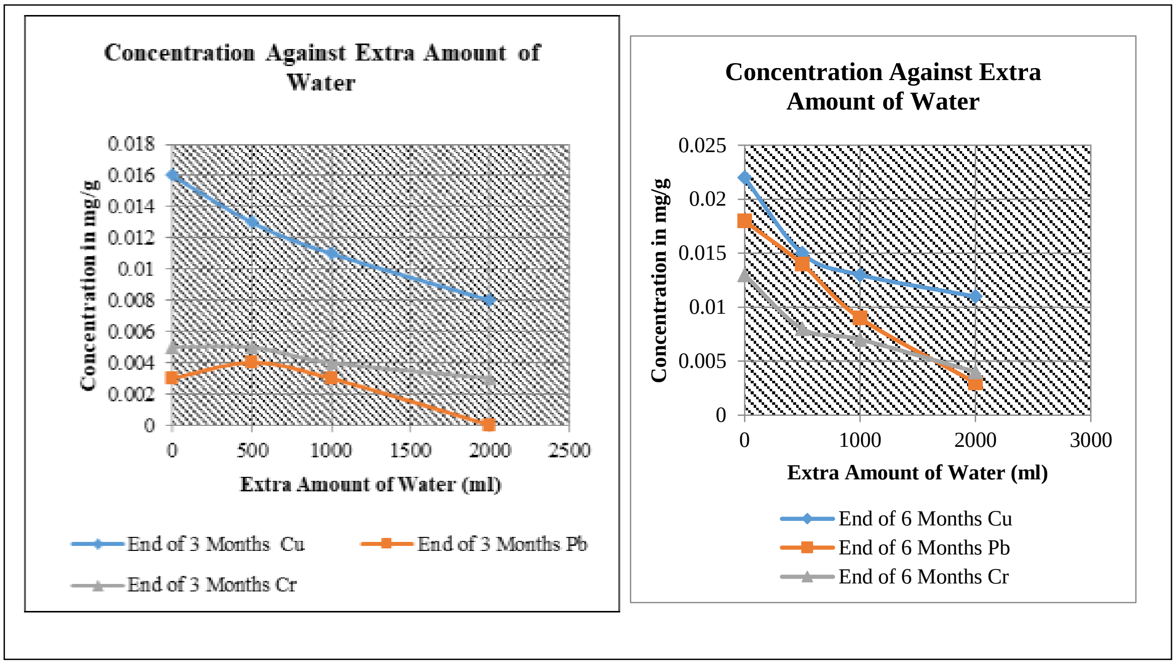


Figure 4.11: Trends of Uptake of the Metals by Papyrus in the 3rd and 6th Months

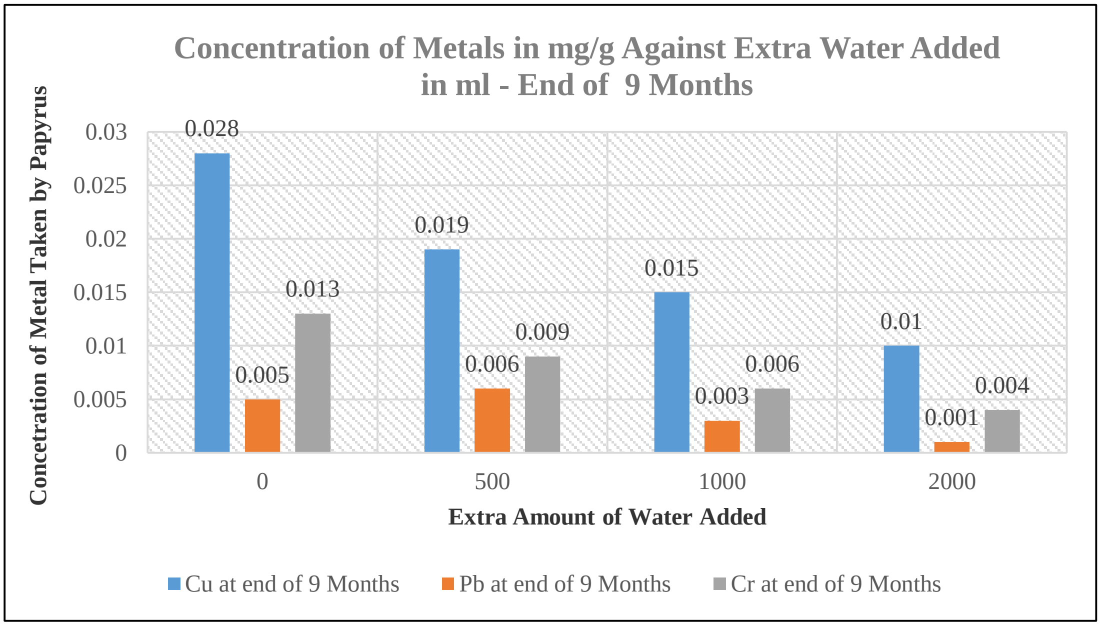


Figure 4.12: Accumulation of the Heavy Metals by Papyrus at end of 6th Months

The absorption of copper reduced continuously with additional of extra water. It reached its highest in papyrus at 0.028mg/g in season three. The percentage difference between the highest absorption of copper and the lowest however grew to 64.29% compared to season 2 which was 47.62% between 0ml extra water treatment and 2000ml extra water treatment. The absorption and the percentage reduction of Chromium however remained constant. The absorption of chromium also reduced significantly from 0.013mg/g to 0.004mg/g (69.23%) between 0ml of extra water and 2000ml of extra water. Absorption of lead also reduced significantly with extra additional water. At 500ml additional water, papyrus accumulated 0.006mg/g while under 2000ml of extra water, 0.001mg/g (83.33% reduction in mean accumulation).

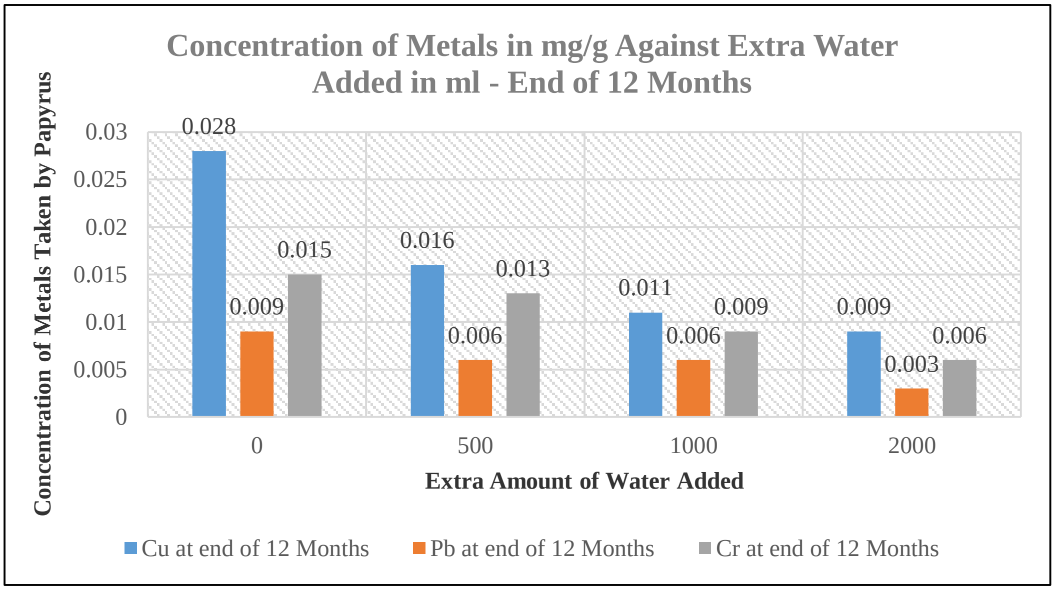


Figure 4.13: Accumulation of the Heavy Metals by the end of 12th Month

The uptake of Copper under 0ml extra water treatment remained constant in comparison to the previous season, but there was a significant reduction in the amount absorbed under 2000ml extra water treatment. The percentage reduction in absorption was significant. The previous season recorded 64.29% reduction in the amount of copper taken up by papyrus, while in this season, (Season 4) the percentage reduction in absorption reached 67.86%. This predicts that there is a possibility of zero absorption and some level of water which is the focal point of this research. The trend in lead also exhibited the significant reduction in the amount taken up by papyrus under increasing amount of extra water. Under 0ml extra water treatment, the uptake was 0.009mg/g while under 2000ml extra water, the absorption was 0.001mg/g. The difference represents 88.89% reduction in uptake of lead by papyrus. The trends in the changes in the amount of the metals accumulated by papyrus in season 3 and 4 are summarized in Figure 4. 13.

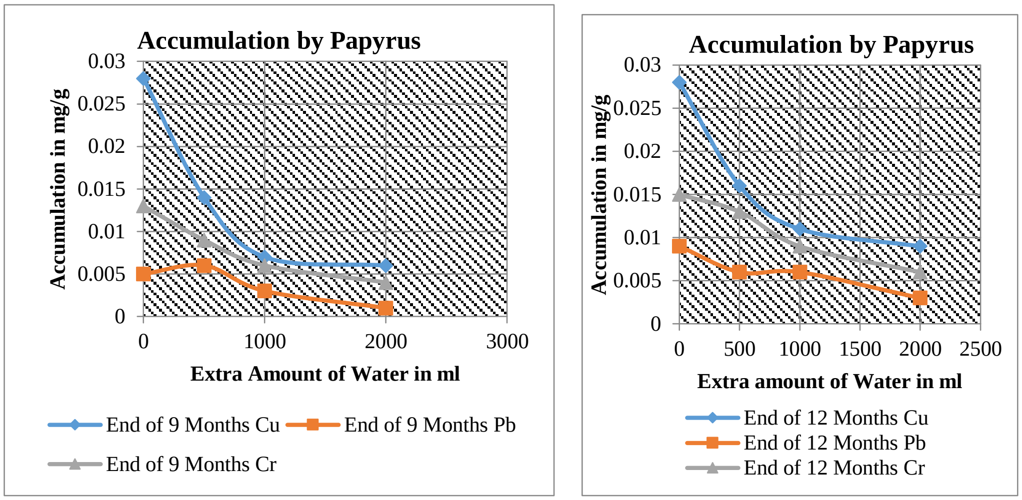


Figure 4.14: Summary of Trend of Uptake of the Heavy Metals by Papyrus in 9th and 12th Months

4.4.1 Summary of Removal of the Heavy Metals by the two Plant Species

Table 4.10 presents a comparative case of the bio-accumulation of the heavy metals by the bamboo and papyrus plant species. The accumulation has been compared to the ambient concentration of the same metals in the growth medium. From the comparison, based on extra amount of water added, bamboo could be better accumulator of the metals than papyrus. This comparison assumes that all factors are constant which not the case is.

The trends of accumulation of Lead, Copper and Chromium as demonstrated in Tables 4.2 to 4.5 shows a reverse association between uptake of the metals and leaching. Leaching is a direct function of gravitational water, and the gravitational water is direct function of the amount of rainfall or irrigation water, with more gravitational water, the metals which are either suspended or dissolved in the soils water are drained with the gravitational water (Gabriel et al., 2018). Lead, Chromium and Copper are capable of moving in a soil water solution or in the surface run offs and leaching into the underground water because of their dissolution from soil minerals by acidic water in the soil. Under intense drainage of the gravitational water, more of the contaminants are drained far away from the root mass and therefore are said to be biologically less available (Bieby, et al., 2011). The plants could take up some of the metals but the uptake was notably so smaller in containers where less leaching occurred. Consequently, bioremediation could be very minimal with extensive leaching or condition which encourage leaching. It is therefore certain that environmental condition like heavy rainfall in clay in on soils with high drainage properties can lower the ability of the plants to remediate such soils in the even that they are contaminated with heavy metals. Notably, the heavy metals mostly occur in their nitrates forms which are highly soluble in water. Therefore, the more the soil water, the more the NO_3^- leaching occurs. Additionally, NO_3^- anions readily experience repulsion by negatively charged soil particles and consequently making them more mobile. The high level of mobility of the metal nitrates increases leaching of the metals when soil moisture conditions increasingly allow for downward movement of soil water (Wakida, & Lerner, 2005). This further explains why under more of the extra additional water, less of the heavy metals were biologically available around the roots mass.

Table 4.1: Percentage Removal of the Heavy Metals by the Two Plants Species

	Concentration in mg/kg				
	Ambient Concentration in Contaminated soil (S_3)	**Highest Accumulation by Bamboo**	**Highest Accumulation by Papyrus**	**% Removal by Bamboo**	**% Removal by Papyrus**
Lead (Pb)	101	55	18	54.45	17.82
Copper (Cu)	174	93	28	53.45	16.09
Chromium (Cr)	137	47	15	14.60	10.95

4.5 Measurement of Bioremediation Performance of Bamboo and Papyrus

The performance of the two plants by bio-concentration factor and translocation factors were as calculated below

Table 4.2: Bio-concentration Factors

Plant	BCF Formula	BCF for the Three Metals		
		Pb	Cu	Cr
Bamboo	BCF= $\frac{C_{Metal\ in\ bamboo}}{C_{Metal\ soil}}$	BCF= $\frac{0.055}{0.101}$ = 0.545	BCF= $\frac{0.093}{0.174}$ = 0.534	BCF= $\frac{0.047}{0.137}$ = 0.343
Papyrus	BCF= $\frac{C_{Metal\ in\ Papyrus}}{C_{Metal\ soil}}$	BCF= $\frac{0.018}{0.101}$ = 0.178	BCF= $\frac{0.028}{0.174}$ =0.161	BCF= $\frac{0.015}{0.137}$ = 0.109

From the above data, it is evident that bamboo exhibits better bio concentration of the three metals than papyrus in the conditions they were used. The possible explanation is based on the rate of leaching which was definitely much higher in the case of papyrus because the condition was kept water-logged all through the growth seasons. This condition caused less bioavailability of the heavy metals for papyrus.

To measure the translocation performance of the plants, data on accumulation on the roots and stems under the 0 ml extra additional water were considered at the end of the 12 months. The formula used, as initially identified was as:

$$TF = \frac{C_{Stem\ or\ Leaf}}{C_{Root\ or\ Stem}}$$

Since only data from accumulation in the roots and stems were considered, the formula thus becomes:

$$\text{TF} = \frac{C_{Stem}}{C_{Root}}$$

The TFs were calculated as shown in Table 4.12

Table 4.3: Bio-Translocation Factors

Plant	BTF Formula	BTF for the Three Metals		
Bamboo		Pb	Cu	Cr
	BTF= $\frac{C_{stem}}{C_{Root}}$	BTF= $\frac{0.021}{0.024}$	BTF= $\frac{0.032}{0.034}$	BTF= $\frac{0.008}{0.005}$
		= 0.875	= 0.941	= 1.6.
Papyrus	BTF= $\frac{C_{Stem}}{C_{Root}}$	BTF= $\frac{0.004}{0.003}$	BTF= $\frac{0.005}{0.007}$	BTF= $\frac{0.007}{0.005}$
		= 1.333	=0.714	= 1.400

*Refer to Tables 4.5 for bamboo and 4.9 for Papyrus BTFs

It is evident from the only BTF from root to stem for Chromium in the two plants and Lead in papyrus were greater than 1 which implies that chromium has tendency to be translocated more strongly from root to stem than it is retained at the root of the two plants while lead has stronger tendency to be translocated from root to stem of papyrus only. Copper in the root zone was translocated weakly to the stem in both the two plants just like lead in bamboo. The findings are closely related to those of Liu et al., (2009).

4.6 Measurement of Amount of Metals Leached

The amount of metals leached equaled to the Amount of the heavy metal is the soils medium at start of experiments (S_3) less amount of the metals in the medium at end of experiment (S_4), less the amount of the metals accumulated by the plants (B_{Acc}) or P_{Acc}). Where B_{Acc} represents average accumulation by bamboo and P_{Acc} represent average accumulation by papyrus. In Reference to Table 4.10-15 (Appendices XVI – XXI**)**, the study assumed that negligible amounts of the metals were adsorbed on soils surface and affected by microbial activity in the soil. Consequently, the amount of the metals leached were the difference between. The ambient concentration of the heavy metals in the blended soils at the beginning of the experiment, less the amount of the metals that were taken up by the metals at the end of the experiment under all the extra water treatments, less the amount of the heavy metals which remained in the soil at end of the experiment. The results show that significant amounts of the metals leached and thus explaining the effects of leaching on uptake of the three metals by the two plant species. To determine the amount of the metals leached therefore, the study considered the residual amount of the metals in the soils at the end of the experiment, compared with the ambient concentration of the metals in the soil at the start of the experiment relative to, the cumulative amount of the metals taken up by the plants. Figure 4.16 and 4.17 shows the variation the leaching rates between two plants under the various water treatments.

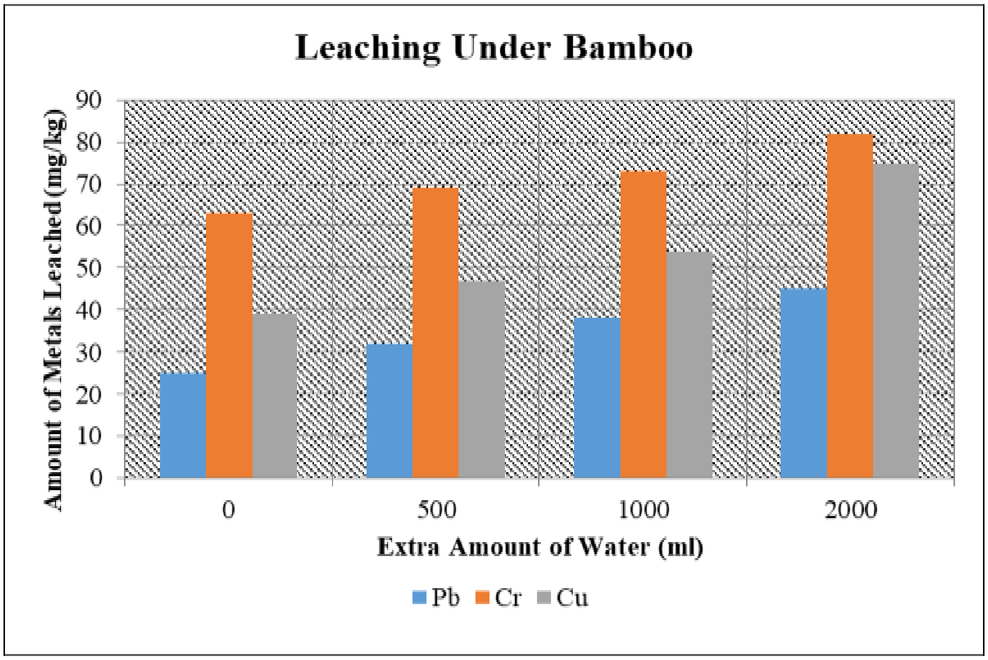


Figure 4.15: Comparison of Leaching under the Various Amounts of Extra Water Added under Bamboo

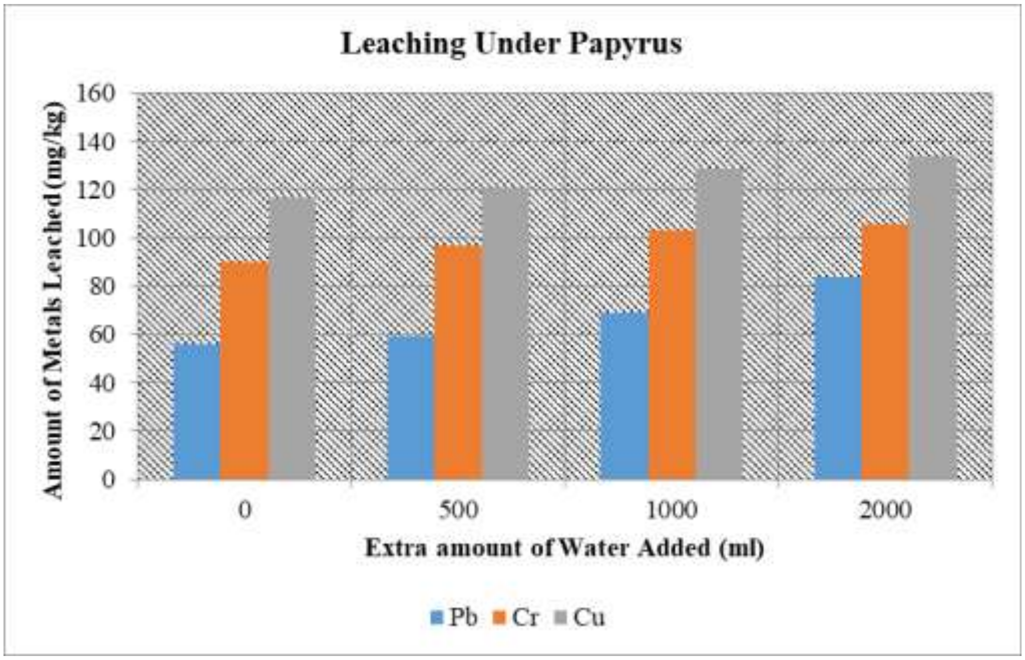


Figure 4.16: Comparison of Leaching under the various amounts of Extra water added under papyrus

According to O'Connor et al., (2018) heavy metals are more likely to leach into the soils because most plants do not have ready mechanisms of uptake, owing to the fact that they are not very useful to the plants. In other words, it takes plants more time to take up heavy metals and non-essential elements as compared to nutrients and essential elements. The longer time encourages leaching and therefore with increased water drainage in the soils, more leaching occurs. Schoumans, (2015), notes that the main factor of leaching is water content in the soil and soil structure. In the study, the soil structure was uniform and therefore, the variable of leaching to consider is water. This is because; the water content of the plants was varied consistently through the growth period. Pérez-Lucas, et al., (2019), also adds that leaching occurs due to gravity which directly depends on weight but weight is itself a function of volume and density. Since density of water is constant (the same source of water was used), the focus shifts to volume. More water in the soils implies higher weight of the water and therefore more force of gravity which pulls the water down and far from the roots of the plant. As correctly noted by O'Connor et al., (2018), heavy metals mostly occurs in their nitrate forms which are soluble in water. The heavy metals are therefore carried with the ground water, as it percolates into the soil under the influence of gravity, and adhesive and cohesive forces. More water implies more solvent and therefore, more leaching.

4.7 Validation of Statistical Significance of the Experimental Results

The mean levels of the three heavy metals recovered from the two plants were calculated separately and then compared to the standards specified by NEMA/WSRB and USEPA for the respective metals for disposal into the environment or water bodies. This comparison was used to calculate the statistical significance of the metal in comparison to the allowable standards by these regulatory bodies. The data was analysed using SPSS one way ANOVA at 95% confidence. A high statistical significance in comparison to the allowable levels indicates efficiency of phytoremediation technology using *Phyllostachys pubescens* and papyrus (*Papyrus cyperus*).

4.4.2 Methods Validation by SPSS Results and Analysis

This section presents a discussion of the data as analysed by SPSS, one-Way ANOVA at SNK, $\alpha = 0.05$. The p-values have been used to validate or invalidate the claims made in the null hypothesis. The mean concentration of values or the three heavy metals were adopted in the analysis.

Table 4:4 show that only four (25%) out of the twelve sets of data produced a *p*-value of less than 0.05. Consequently, the null hypothesis can only be rejected for those four sets of data concentration of chromium taken up by bamboo. These sets of data include leaves in the first season (*p*-values of 0.001), roots in the second season (*p*-values of 0.00), roots in the third season (*p*-value of 0.001) and leaves in the fourth season (*p*-values of 0.0001)

Table 4.4: Concentration of Chromium in mg/g in Bamboo (*Phyllostachys pubescens*)

		Treatment (Extra Amount of Water Added in ml)				
Harvesting time	**Part of plant**	**0**	**500**	**1000**	**2000**	**P value**
Season 1	Roots	0.0035±0.00059^{b}	0.0029±0.00020^{b}	0.0031±0.00015^{b}	0.0024±0.00076^{a}	0.106
	Stem	0.0096±0.01635^{c}	0.0023±0.00020^{b}	0.0024±0.00038^{b}	0.0008±0.00025^{a}	0.610
	leaves	0.0010±0.00017^{b}	0.0024±0.00017^{c}	0.0025±0.00025^{c}	0.0001±0.00006^{a}	0.0001
Season 2	Roots	0.0059±0.00035^{c}	0.0021±0.00056^{a}	0.0043±0.00025^{b}	0.0080±0.00030^{d}	0.0001
	Stem	0.0095±0.01642^{d}	0.0047±0.00312^{c}	0.0039±0.00026^{b}	0.0028±0.00017^{a}	0.795
	leaves	0.0020±0.00031^{a}	0.0016±0.00046^{a}	0.0016±0.00026^{a}	0.0022±0.00031^{a}	0.139
Season 3	Roots	0.0088±0.00246^{b}	0.0042±0.00038^{a}	0.0031±0.00015^{a}	0.0021±0.00030^{a}	0.001
	Stem	0.0113±0.01520^{c}	0.0034±0.00021^{a}	0.0037±0.00361^{a}	0.0053±0.00060^{b}	0.620
	leaves	0.0031±0.00021^{a}	0.0031±0.00020^{a}	0.0012±0.00025^{a}	0.0030±0.00254^{a}	0.258
Season 4	Roots	0.0125±0.01440^{d}	0.0074±0.00082^{c}	0.0033±0.00015^{a}	0.0051±0.00111^{b}	0.515
	Stem	0.0147±0.01297^{d}	0.0015±0.00024^{a}	0.0033±0.00025^{b}	0.0041±0.00025^{c}	0.145

Mean values followed by the same small letter within the same row for the different treatments do not differ significantly from one another (**One-way ANOVA, SNK-test, α =0.05)**

Table 4.11 75% of the data shows that the null hypothesis is correct and thus the independent variable do not have significant effect on the dependent variable. Only four (25%) out of the twelve sets of data produces a *p*–Value of less than 0.05. Consequently, the null hypothesis can only be rejected for those four sets of data namely the concentration of chromium taken by leaves in the first season, roots in the second season, roots of the third season and leaves in the fourth season. A p-value of less than 0.05 implies that the null hypothesis is rejected. However, 75% (8/12) of the data in the table shows that the null hypothesis is correct and thus the independent variables do not have significant effects on the dependent variables. According to Davies, (2017), *p*-value is a measure of validity or invalidity of the claims made in the null hypothesis. Accumulation of 0.0147 ± 0.01297^{d} mg/g was the highest absorption recorded in stem of season 4 under first treatment (0ml extra water) while 0.0001 ± 0.00006^{a} mg/g was the least recorded in the leaves in season 1 under fourth treatment (2000 ml extra water). With the accumulation of chromium going as high as 0.0147 ± 0.01297^{d} mg/g within 12 months, sustainable bioremediation of chromium pollution can be achieved.

Table 4.5: Concentration of Lead in Bamboo in mg/g

Harvesting time	Part of plant	Treatment (Extra Amount of Water Added in ml)				P value
		0	500	1000	2000	
Season 1	Roots	0.0022±0.00026^{b}	0.0023±0.00032^{c}	0.0003±0.00036^{a}	0.0023±0.00078^{b}	0.002
	Stem	0.0096±0.01635^{d}	0.0007±0.00036^{b}	0.0010±0.00076^{c}	0.0003±0.00049^{a}	0.514
	leaves	0.0002±0.00012^{a}	0.004±0.00044^{a}	0.0005±0.00044^{a}	0.0012±0.00026^{b}	0.042
Season 2	Roots	0.0011±0.00024^{a}	0.0019±0.00057^{b}	0.0070±0.00020^{c}	0.0010±0.00031^{a}	0.0001
	Stem	0.0095±0.01639^{c}	0.0005±0.00038^{a}	0.0001±0.00017^{a}	0.0011±0.00031^{b}	0.509
	leaves	0.0007±0.00061^{a}	0.0033±0.00111^{b}	0.0002±0.00015^{a}	0.0003±0.00026^{a}	0.001
Season 3	Roots	0.0091±0.01015^{b}	0.0004±0.00045^{a}	0.0003±0.00036^{a}	0.0001±0.00032^{a}	0.171
	Stem	0.0102±0.01590^{c}	0.0001±0.00006^{a}	0.0002±0.00010^{a}	0.0010±0.00031^{b}	0.413
	leaves	0.0213±0.00520^{b}	0.0011±0.00021^{a}	0.0021±0.00020^{a}	0.0031±0.00017^{a}	0.0001
Season 4	Roots	0.0269±0.00486^{b}	0.0372±0.00263^{c}	0.0210±0.00210^{b}	0.0139±0.00209^{a}	0.0001
	Stem	0.0246±0.00968^{c}	0.0091±0.00252^{ab}	0.0164±0.00056^{bc}	0.0022±0.00015^{a}	0.004
	leaves	0.0134±0.00238^{b}	0.0013±0.00021^{a}	0.0124±0.00161^{b}	0.0130±0.00069^{b}	0.0001

Mean values followed by the same small letter within the same row for the different treatments do not differ significantly from one another (One-way ANOVA, SNK-test, α =0.05

From Table 4.5, it can be observed 75% of the 12 data sets produced *p*-value of less than 0.05 (<0.05) which therefore nullifies the null hypothesis. This implies that the claims of the null hypothesis do not apply. In other words, the independent variables (Presence and Concentration of Heavy Metals in the Soil Contaminated with Petroleum Sludge and Extra amount of water added) significantly affect the results of the dependent variable (amount of heavy metals taken up by bamboo (*Phyllostachys pubescens*) and papyrus (*Papyrus cyperus*) grown in Soil contaminated with Petroleum Sludge and amount of heavy metals remaining in Soil contaminated with petroleum sludge after uptake of Heavy Metal by Bamboo and Papyrus).

With specific focus to this research, extra addition of water to the plants influenced the rate and quantity of heavy metals taken up by bamboo (*Phyllostachys pubescens*) and papyrus (*Papyrus cyperus).* The findings auger well with conclusions of Khan et al., (2015) who also established that the rate of uptake of any soluble or suspended materials in soil water film is directly affected by the rate of leaching which is on the other hand depends on type of soil and water saturation. Werner, (2002), claims that moisture content is a determining factor on roots response to substances suspended and dissolved in the soil.

Table 4.6: Concentration of Copper in mg/g in Bamboo

Harvesting time	Part of plant	Treatment (Extra Amount of Water Added in ml)				P value
		0	**500**	**1000**	**2000**	
Season 1	Roots	0.0061 ± 0.00030^{b}	0.0061 ± 0.00026^{b}	0.0041 ± 0.00030^{a}	0.0046 ± 0.00030^{a}	≤0.0001
	Stem	0.0125 ± 0.01440^{c}	0.0037 ± 0.00118^{a}	0.0042 ± 0.00031^{b}	0.0032 ± 0.00036^{a}	0.422
	Leaves	0.0042 ± 0.00010^{b}	0.0023 ± 0.00032^{a}	0.0027 ± 0.00079^{a}	0.0021 ± 0.00030^{a}	0.002
Season 2	Roots	0.0022 ± 0.00031^{a}	0.0044 ± 0.00010^{c}	0.0033 ± 0.00045^{b}	0.0063 ± 0.00035^{a}	≤0.0001
	Stem	0.0131 ± 0.01397^{d}	0.0084 ± 0.00030^{c}	0.0045 ± 0.00057^{b}	0.0034 ± 0.00035^{a}	0.415
	Leaves	0.0071 ± 0.00025^{b}	0.0034 ± 0.00035^{a}	0.0031 ± 0.00180^{a}	0.0030 ± 0.00040^{a}	0.002
Season 3	Roots	0.0184 ± 0.01050^{c}	0.0047 ± 0.0051^{b}	0.0041 ± 0.0030^{b}	0.0022 ± 0.00032^{a}	0.020
	Stem	0.0185 ± 0.01044^{c}	0.0042 ± 0.00032^{b}	0.0041 ± 0.00015^{b}	0.0013 ± 0.00015^{a}	0.015
	Leaves	0.0212 ± 0.00026^{c}	0.0040 ± 0.00026^{b}	0.0043 ± 0.00045^{b}	0.0022 ± 0.00026^{a}	≤0.0001
Season 4	Roots	0.0343 ± 0.00044^{b}	0.0463 ± 0.00380^{c}	0.0199 ± 0.00311^{a}	0.0164 ± 0.00050^{a}	≤0.0001
	Stem	0.0329 ± 0.00067^{c}	0.0312 ± 0.03014^{c}	0.0192 ± 0.000150^{b}	0.0133 ± 0.00026^{a}	0.322
	Leaves	0.0272 ± 0.00025^{c}	0.0162 ± 0.00023^{b}	0.0123 ± 0.00271^{a}	0.0114 ± 0.00120^{a}	≤0.0001

Mean values followed by the same small letter within the same row for the different treatments do not differ significantly from one another (One-way ANOVA, SNK-test, α =0.05

From Table 4.6, 9 out of 12 data sets produced p-values of less than 0.05. That translates to 75% of all the data analyzed disapproved the null hypothesis. The null hypothesis on the relationship is rejected and therefore, leaching as a function of soil water content and gravity has significant impact on the amount of copper accumulated by bamboo. Also considering the amount of copper accumulated for example in the stem in the first season under first treatment and the corresponding treatment in season four, a significant variation can be witnessed. The accumulation of copper in the stem in the first season under first treatment is 0.0125 ± 0.01440^{c} while the accumulation of the same under the same treatment in season four is 0.0329 ± 0.00067^{c}. There is a 163.2% increase in accumulation.

Table 4.7: Concentration of Chromium in Papyrus in mg/g

Harvesting time	**Part of plant**	**Treatment (Extra Amount of Water Added in ml)**				P value
		0	**500**	**1000**	**2000**	
Season 1	Roots	0.0049±0.00118^{b}	0.0013±0.00006^{a}	0.0007±0.00006^{a}	0.0030±0.00010^{a}	$\leq$0.0001
	Stem	0.0014±0.00151^{c}	0.0023±0.00015^{b}	0.0010±0.00021^{a}	0.0010±0.00020^{a}	$\leq$0.0001
	leaves	0.0105±0.01577^{b}	0.0020±0.00031^{a}	0.0017±0.00015^{a}	0.0020±0.00026^{a}	0.575
Season 2	Roots	0.0069±0.00048^{a}	0.0054±0.00035^{c}	0.0024±0.00025^{b}	0.0012±0.00015^{a}	$\leq$0.0001
	Stem	0.0041±0.00020^{c}	0.0032±0.00035^{b}	0.0032±0.00031^{b}	0.0023±0.00040^{a}	0.001
	leaves	0.0103±0.01588^{c}	0.0023±0.00010^{b}	0.0021±0.00021^{b}	0.0016±0.00026^{a}	0.548
Season 3	Roots	0.0068±0.00062^{c}	0.0045±0.00046^{b}	0.0021±0.00021^{a}	0.0063±0.00040^{c}	$\leq$0.0001
	Stem	0.0063±0.00028^{c}	0.0021±0.00025^{b}	0.0019±0.00030^{b}	0.0011±0.00020^{a}	0.0001
	leaves	0.0117±0.01495^{d}	0.0051±0.00444^{c}	0.0021±0.00010^{b}	0.0008±0.00015^{a}	0.384
Season 4	Roots	0.0022±0.00254^{a}	0.0042±0.00280^{b}	0.0022±0.00038^{a}	0.0023±0.00040^{a}	0.517
	Stem	0.0049±0.00015^{c}	0.0009±0.00015^{a}	0.0033±0.00025^{b}	0.0032±0.00012^{b}	$\leq$0.0001
	leaves	0.0121±0.01467^{c}	0.0048±0.00025^{b}	0.0022±0.00010^{a}	0.0008±0.00012^{a}	0.339

Mean values followed by the same small letter within the same row for the different treatments do not differ significantly from one another (One-way ANOVA, SNK-test, α =0.05)

From Table 4.7 above, it is observed that 7 out of 12 data sets produced p-values of less than 0.05. This translates to 58.33% rejection of the null hypothesis. The rejection of the null hypothesis means that leaching had substantial effect on the uptake of chromium. Besides leaching, time is also a significant factor with direct implication on accumulation. If for instance accumulation of chromium in the leaves in the second and fourth seasons under second treatment are considered, the accumulations are 0.0020 ± 0.00031^{a} and 0.0048 ± 0.00025^{b}, a significant difference of 140% increase is observed. The longer the time therefore, the higher the accumulation of chromium in papyrus. The rest of the data also show that time has a great impact on amount of chromium that papyrus can accumulate within its biomass.

Table 4.8: Concentration of Lead in Papyrus in mg/g

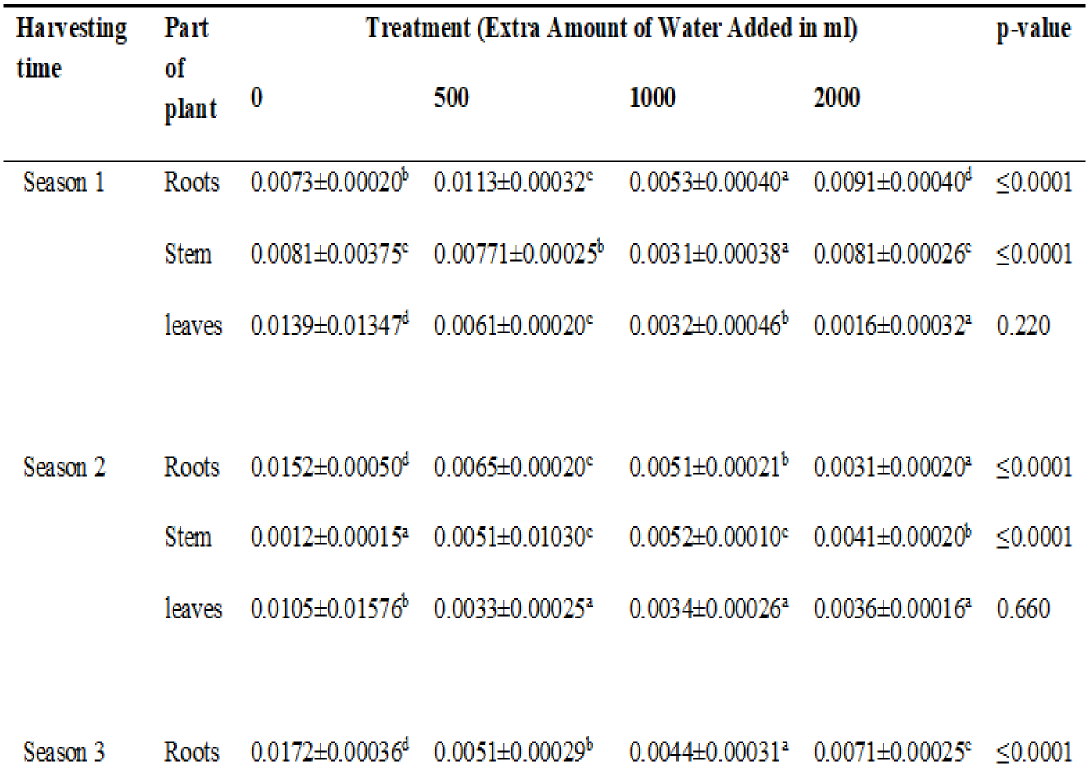
Harvesting time	**Part of plant**	**Treatment (Extra Amount of Water Added in ml)**				P value
		0	**500**	**1000**	**2000**	
Season 1	Roots	0.0038±0.00096^{b}	0.0012±0.00006^{a}	0.0011±0.00003^{a}	0.0003±0.00010^{a}	≤0.0001
	Stem	0.0023±0.00120^{c}	0.0024±0.00031^{c}	0.0016±0.00015^{b}	0.0003±0.00020^{a}	0.0021
	leaves	0.0104±0.01579^{d}	0.0022±0.01579^{d}	0.0032±0.00419^{c}	0.0001±0.00015^{a}	0.507
Season 2	Roots	0.0091±0.00025^{c}	0.0017±0.00015^{b}	0.0014±0.00025^{ab}	0.0010±0.00026^{a}	≤0.0001
	Stem	0.0044±0.00030^{c}	0.0034±0.00031^{b}	0.0021±0.00026^{a}	0.0020±0.00010^{a}	0.0001
	leaves	0.0123±0.01452^{c}	0.0032±0.00416^{b}	0.0032±0.00026^{b}	0.0004±0.00051^{a}	0.326
Season 3	Roots	0.0173±0.00006^{d}	0.0023±0.00035^{a}	0.0042±0.00045^{b}	0.0052±0.00036^{c}	≤0.0001
	Stem	0.0001±0.00010^{a}	0.0033±0.00040^{d}	0.0011±0.00020^{b}	0.0022±0.00023^{c}	≤0.0001
	leaves	0.0095±0.01643^{c}	0.0024±0.00030^{b}	0.0014±0.00006^{a}	0.0016±0.00023^{a}	0.625
Season 4	Roots	0.0018±0.00115^{a}	0.0038±0.00232^{b}	0.0041±0.00035^{b}	0.0020±0.00015^{a}	0.138
	Stem	0.0042±0.00046^{b}	0.0040±0.00081^{b}	0.0021±0.00015^{a}	0.0013±0.00010^{a}	≤0.0001
	leaves	0.0105±0.01574^{d}	0.0021±0.00115^{b}	0.0031±0.00025^{c}	0.0012±0.00015^{a}	0.526

Mean values followed by the same small letter within the same row for the different treatments do not differ significantly from one another (One-way ANOVA, SNK-test, α =0.05)

In Table 4.8 above, seven out of twelve data sets produced p-values of less than 0.05 meaning that leaching had significant effect on uptake of the heavy metals. The null hypothesis should be rejected by 58.33%. The rest of 42.37% of the data provided room for the adoption of the null hypothesis. This could be possible due to human or mechanical and experimental error. Nevertheless, considering Bosire (2009), the accumulation observed were within the ranges he established. In his study where he used Yellow, water, giant and green bamboos (species not specified), Bosire found between 0.0056±0.0007mg/g and 0.0210±0.0031mg/g of lead in leaves of yellow and giant bamboo grown in soils harvested from riverine of Nairobi River. The same plants accumulated between 0.0056±0.002mg/g and 0.0094±0.004 of lead in their stems. Their roots accumulated even slightly higher amounts of lead within the range of 0.0122±0.0015mg/g and 0.0410±0.0008mg/g. The accumulation of copper in the leaves, stems and roots of Moso bamboo grown in soil contaminated with petroleum sludge as established in this study also reflect what Bosire (2009) found out.

The interrelationship between the variable in concentration of copper in Papyrus are not well balanced as indicated in Table 4.16. However, 75% (9/12) of the data show that the null hypothesis is correct and thus the independent variables do not have significant effects on the dependent variables.

Table 4.9: Concentration of Copper in Papyrus in mg/g



Harvesting time	Part of plant	Treatment (Extra Amount of Water Added in ml)				p-value
		0	500	1000	2000	
Season 1	Roots	0.0073 ± 0.00020^{b}	0.0113 ± 0.00032^{c}	0.0053 ± 0.00040^{a}	0.0091 ± 0.00040^{d}	≤0.0001
	Stem	0.0081 ± 0.00375^{c}	0.00771 ± 0.00025^{b}	0.0031 ± 0.00038^{a}	0.0081 ± 0.00026^{c}	≤0.0001
	leaves	0.0139 ± 0.01347^{d}	0.0061 ± 0.00020^{c}	0.0032 ± 0.00046^{b}	0.0016 ± 0.00032^{a}	0.220
Season 2	Roots	0.0152 ± 0.00050^{d}	0.0065 ± 0.00020^{c}	0.0051 ± 0.00021^{b}	0.0031 ± 0.00020^{a}	≤0.0001
	Stem	0.0012 ± 0.00015^{a}	0.0051 ± 0.01030^{c}	0.0052 ± 0.00010^{c}	0.0041 ± 0.00020^{b}	≤0.0001
	leaves	0.0105 ± 0.01576^{b}	0.0033 ± 0.00025^{a}	0.0034 ± 0.00026^{a}	0.0036 ± 0.00016^{a}	0.660
Season 3	Roots	0.0172 ± 0.00036^{d}	0.0051 ± 0.00029^{b}	0.0044 ± 0.00031^{a}	0.0071 ± 0.00025^{c}	≤0.0001

Table 4.9 Shows test of hypothesis of the data. The interrelationship between the variable are not well balanced according to the analysis above. Eight out of 12 sets of data produce a p –Value of less than 0.05 which implies that leaching has significant effect on uptake copper by papyrus. That is equivalent to rejecting the null hypothesis by 66.67%. A summary of the trends of the p-Values and the implication on the null hypothesis are given in Table 4.10. According to Davies, (2017), p-values is a measure of the possible validity or invalidity of the claims made in the null hypothesis.

Table 4.10: Summary of Trends of p-Values and the Implication on Null Hypothesis

Order of Table	p-Values above 0.05	Percentage (%)	Remarks on H_01	p-Values below 0.05	Percentage (%)	Remarks on H_01
4.4	8	66.67	Invalid	4	33.33	Valid
4.5	4	33.33	Valid	8	66.67	Invalid
4.6	3	25.00	Valid	9	75.00	Invalid
4.7	5	41.67	Valid	7	58.33	Invalid
4.8	5	41.67	Valid	7	58.33	Invalid
4.9	3	25.00	Valid	9	75.00	Invalid

NOTE

H_01 *There is no significant impact of leaching on the uptake of heavy metals by Bamboo and Papyrus from soil contaminated with petroleum sludge (Section 1.3.4).*

From the summary of the p-value analysis in Table 4.10, the null hypothesis was only sustained in in the case of chromium concentration in bamboo. In all the other cases the null hypothesis was nullified. This indicated that leaching has a significant impact on the potential of bamboo and papyrus on sustainable phytoremediation of soil contaminated with petroleum sludge. It can therefore be established that leaching significantly affects the amount of heavy metals that can be absorbed by Bamboo and Papyrus from soil contaminated with petroleum sludge. This was demonstrated by the significant differences between the amounts of the heavy metals that the plants accumulated under the treatment with 0ml of extra additional water through and the same metals accumulated under the treatment of the plants with 2000ml extra additional water.

4.7 Effectiveness of Bioremediation by Bamboo and Papyrus

For most accurate effectiveness of bioremediation by both the two plants, the study considered the results of accumulation and leaching under the 0ml extra water treatment. Table 4.18 shows a comparing of the residual concentration of the three heavy metals in the contaminated soil at the end of the different seasons. From data in Table 4:18 above, the two plant species lead to the achievement of reduction of the contamination by lead, chromium and copper to the range of allowable limits by WHO/FAO. Nerveless minimum allowable limits were not achieved by either of the plants. Notably, significant amount of the metals were taken up by the plants in the one year period, although the minimum time for effective bioremediation is 5 years (Bian *et al*., 2018). Within the one year however, there were significant accumulation of the metals in the biomass of the plants. The results suggest therefore that if the study would be run for 5 years, with the aid of chelating agents, there would be higher accumulation of the heavy metals which would reduce the levels of the heavy metals in the soil to within the minimum allowable limits.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter presents a brief conclusion based on the results on the observation made and the discussion of the data collected. It also presents the recommendation of the study.

5.2 Summary

The main objective of the study was to establish the effectiveness of *Phyllostachys pubescens* (Moso Bamboo) and *Papyrus cyperus* (Papyrus) in remediation of soil contaminated with petroleum sludge. More specifically the study focused on remediation of the soils against pollution caused by heavy metals namely, lead, Chromium and Copper. The study therefore established the presence and concentration of the heavy metals in the contaminated soil before growing the two plant species in the soils for a period of 12 months. The plants were grown in green houses where bet agronomic practices could be implemented during the 12 months. The study also focused on how leaching could affect the effectiveness of the plants on uptake of the heavy metals from the contaminated soil. To test for the presence and concentration of the heavy metals in the contaminated soil as well as the amounts of the heavy metals taken up by the plants, Atomic Absorption Spectrometry was used. The study established that there were significant amounts of the three metals (204ppm of Cu, 107ppm of Pb, and 151ppm od Cr) in the soil contaminated with petroleum sludge and that the plants are able to take up significant amounts of the metals (93ppm of Cu, 55 ppm of Pb and 47ppm of Cr by Bamboo and 28ppm of Cu, 18ppm of Pb and 15ppm of Cr by Papyrus) although the uptake directly depends of the rate of leaching in the soils where the plants are grown. The study established that the plants were able to contribute to reduction of the contaminants in the soil to the recommended level by WHO/FAO. The study further found that Bamboo would be a better phytoremediator than papyrus under the same condition as was the case in the study.

5.3 Conclusions

On the first objective, there was significant amount of lead (0.107mg/g), copper (0.204mg/g) and chromium (0.151mg/g) in the soil contaminated with petroleum sludge. The objective was thus realized because the methodology was able to detect the presence and the amounts of the heavy metals targeted from the soil sample in question. The same methodology was also able to ascertain the levels of the heavy metals in the sample was been shown. From the analysis conducted, it is only possible that petroleum sludge was the source of the heavy metals detected in the contaminated oil. This is because, virgin soil, harvested from a different site was tested, there was very low mount of the metals (2ppm of Pb, 5ppm of Cr and 21ppm of Cu). According to what other studies have revealed, heavy metals can find their way into petroleum products from the migration of metals in the mother rocks to the reservoir rock, and introduction of drilling mud fluids into the oil well during extraction of crude oil. Another possible source of heavy metal contamination includes storage and transport vessels (Pazoki, & Hasanidarabadi, 2017).

On the second objective, both *Phyllostachys pubescens* (Bamboo) and *Papyrus cyperus* (Papyrus) exhibited significant accumulation of lead, copper and chromium from soil contaminated with petroleum sludge. The trends of the uptake were almost similar with respect to the extra amount of water added and the time factor. The study discovered that the trend of uptake of the metals is such that the most accumulation occurs in the roots, while the least accumulations in the leaves. Among the reasons includes the storage mechanisms at the roots, and phytovolatilization which occurs at the roots. Longer time of growth of the plants in soil contaminated with petroleum sludge improve bioaccumulation. Bioaccumulation is directly dependent on the time of growth of the plants in the ambient concentration of the heavy metals. Longer duration of growth in the ambient concentration of the heavy metals translates to more bioaccumulation (Idodo-Umeh, & Ogbeibu, 2010). The accumulation is however directly dependent of the rate of leaching. The variation in concertation of the metals and their bioavailability was demonstrated by how much of the same metals were bio-accumulated by the plants with time, and variation in the amount of extra water were periodically added to the containers.

With regards to the third objective, the study concludes that:

- The highest amount of heavy metals was accumulated with 0ml of extra water with considerable reduction on adding more extra water. The overall percentage removal of the three heavy metals by bamboo was 54.45% lead, 53.45% copper and 34.31% of chromium. While the percentage removal of heavy metals by papyrus was 17.82% lead, 16.09% copper and 10.95 % of chromium.
- Bamboo exhibited better performance than papyrus because the Bio-concentration Factor (BCF) for all the three metals were higher, i.e, 0.545, 0.534 and 0.343 for lead, coper and chromium respectively in bamboo and 0.178, 0.161 and 0.109 of the same metals in papyrus. However, the BCFs shows that the plants perform based on absorption but not bio-accumulation because the BCFs were all ≤ 1.00. Notably, papyrus is most likely to perform better in terms of bio-translocation because the Bio-translocations factors were 1.333, 0.714 and 1.4 for lead, copper and chromium respectively as compared to bamboo's bio-translocation factors of 0.875, 0.714 and 1.6 of the same metals in the same order.
- The rate of leaching directly influences the trend of uptake of heavy metals by Bamboo and Papyrus. Higher rate of leaching lowers the potential of the roots to accumulate the metals due to carrying away of the metals in soil water. Leaching lowers bioavailability of the metals to the roots

On the fourth objective, the study concludes that significant amounts the metals were taken up by the plants during the 12 months' growth period. At the end of experiment, the amount of the metals in the contaminated soil had reduced consideratly. Bamboo accumulated 55mg/kg (54.46%) of lead, 93mg/kg (53.45%) of copper and 47mg/kg

(34.31%) of chromium. Papyrus on the other hand accumulated 18mg/kg (17.82%) of Lead, 28mg/kg (16.09%) of Copper and 15mg/kg (10.95%) of Chromium. From these findings, it can be concluded that Bamboo is a better phytoremediator of soil contaminated with petroleum sludge compared to Papyrus under the same conditions of the treatments. Both bamboo and papyrus are suitable for use in remediation of soils contaminated with petroleum sludge. More specifically, Bamboo is most suitable for remediating dry land while papyrus is suitable for remediating wetland (waterlogged land/site).

5.4 Recommendations

5.4.1 Adoption of Bamboo and Papyrus

The study recommends that:

- For sustainable management of remediation of petroleum sludge polluted soil, bamboo (*Phyllostachys pubescens*) and papyrus (*Papyrus cyperus*) should be adopted particularly in Kenya when and where the pollution occurs on dry land and wet land respectively.
- Sanitary landfills should be constructed and commissioned in Kenya so that petroleum sludge can safely be disposed without any alarm on pollution of soil of underground water.
- Based on the ability of the *Phyllostachys pubescens* and *Papyrus cyperus* to accumulated high level of lead, and other heavy metals, they can be planted in the land polluted with lead in Owino Uhuru, a suburb of Mombasa, where lead pollution has been established to cause tragic pollution. The plants can also be planted at Port Reitz, Mombasa where the soils are heavily polluted with Waste Oil.

5.4.2 Need for Further Studies Research

The study recommends that more research should be done on:

- The effects chelants/chelators, or sequestering agents for example ethylenediamine tetraacetic acid (EDTA), ethylene glycol tetra acetic acid (EGTA), and sodium dodecyl sulfate (SDS), ethylene glycol tetra acetic acid (EGTA), would have on the effectiveness of phytoremediation of soil contaminated with petroleum sludge using *Phyllostachys pubescens* and *Papyrus cyperus*.
- Full scope characterization of the soil contaminated petroleum sludge in terms of all the heavy metals present in the soil and the hydrocarbons which could pose threats to the soil.
- Sustainable bioremediation of soil due to pollution caused by hydrocarbons in petroleum sludge

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APPENDICES

Appendix I: Table 2.1 - Mean Concentration of Some Heavy Metals in Oily Sludge

Parameters	**Values**					
Oil-Soluble Component (wt.%)	25.70					
Moisture Content (%)	54.28					
Solid Content (%)	20.02					
pH	8.24					
Heavy Metals	Ba	Cu	Zn	Cr	Ni	Pb
Content (mg/L)	1.562	0.825	0.670	0.282	0136	0.333

Source: Xiao,Yao, & Zhang, (2019)

Appendix II: Table 2.2 - Mean Concertation (mg/L) of Some Heavy Metals in Petroleum Products

Sample	**Zn**	**Cu**	**Cr**	**Pb**	**Cd**
Gasoline	1.43	1.74	0.54	0.24	1.68
Kerosene	2.63	1.98	0.33	0.41	1.33
Diesel	2.87	1.77	0.86	1. 01	1.5
Standard limits	5. 00	0.10	1.00	0. 075	5 00

Appendix III: Table 2.3 - Concentration of Heavy Metals in Petroleum Sludge and at Bioremediation Site

Heavy metals	**Oily sludge**	**Pilot plot (Day zero)**		**Pilot plot (180 days)**	
		30cm	60cm	30cm	60cm
Cd	0.013± 0.00	0.018± 0.01	0.022 ± 0.01	0.028 ± 0.00	0.010 ± 0.01
Cr	0.035 ± 0.00	0.039 ± 0.00	0.044 ± 0.00	0.049 ± 0.01	0.028 ± 0.00
As	0.005 ± 0.00	0.007 ± 0.00	0.009 ± 0.00	0.004 ± 0.00	0.002 ± 0.00
Pb	36.4 ± 0.15	39.6 ± 0.2	35.1± 0.3	49.6 ± 0.2	14.3 ± 0.00
Ni	0.042 ± 0.10	0.044 ± 0.00	0.032 ± 0.01	0.056 ± 0.00	0.019 ± 0.00

The values are means standard deviations for 3 samples

Appendix IV: Table 2.4 - Allowable Levels of Heavy Metals by WHO/FAO

Element	Elements in Collected Soils mg/Kg (range)	World range of elements in non-polluted soil mg/Kg	Maximum allowable limits of elements in fruits and vegetables mg/Kg dry weight
Cd	0.014 – 0.721	0.07-1.1	0.3
Pb	0.629 – 11.59	10-70	0.2
Cu	0.948 – 7.865	6-60	40
Cr	0.016 - 2.847	5-121	2.3

Appendix V: Table 2.4 Maximum Permissible Levels by NEMA/WSRB

Item	Enforcing body	Heavy metal	Max. Conc.mg/Litre
1	NEMA/WSRB	Lead	0.01
2	NEMA/WSRB	Lead and its compounds	0.1
3	NEMA/WSRB	Chromium (vi)	0.05
4	NEMA/WSRB	Chromium (total)	2.0
5	NEMA/WSRB	Cadmium	0.01
6	NEMA/WSRB	Cadmium and its compounds	0.1
7	EPA(US)	Silver	0.14

Source: Nkonge et al., (2012)

NEMA: National Environmental Management Authority (Kenya)

WSRB: Water Services Regulatory Board (Kenya)

EPA (US): Environmental Protection Authority of the United States

Appendix VI: Table 3.1 - Detection Limits

	Lowest Detection Limits in ppm (mg/g)		
Model Type	Copper (Cu)	Lead (Pb)	Chromium (Cr)
Flame	<0.01	0.01 – 0.9	0.01 -0.9
Furnace	<0.01	0.01 – 0.9	0.01 – 0.09

Source

Appendix VII: Table 4.1 - Concentration of Heavy Metals in the Polluted Soil, Blended Soil and Final Concentration of the Metals in the Blended Soil

	Mean Concentration of heavy metals in mg/g (0ml Extra Water)					
Metal	**Virgin Soil (S_1)**	**Polluted Soil (S_2)**	**Raw Sludge**	**Blended Soil at Start of experiment ($S_3= S_1+S_2$)**	**Blended Soil at End of Experiment (Bamboo) ($S_4=S_1 +S_2$)**	**Blended Soil at End of Experiment (Papyrus) (S_5)**
Lead	2	107	121	101	25	27
Chromium	5	151	209	137	27	32
Copper	21	204	537	174	42	29

* S_1 represents virgin Soil, S_2 represents soil contaminated with petroleum sludge, S_3 represents a blend of S_1 and S_2, S_4 represent S_1+S_2 at end of experiment under bamboo, while S_5 represent S_1+S_2 and end of experiment under papyrus.

Appendix VIII: Table 4.2 - Heavy Metal Uptake by Bamboo – 3rd Month after Planting

Extra Added Water per Pot	**Plant Part**	**Mean Concentration of Heavy Metals in mg/g of Sample Analyzed**					
		Copper	R+S+L*	Lead	R+S+L	Chromium	R+S+L
0ml	Root (R)	0.006	0.015	0.002	0.003	0.003	0.005
	Stem (S)	0.005		0.001		0.001	
	Leaf (L)	0.004		0.000		0.001	
500ml	Root (R)	0.005	0.011	0.001	0.002	0.002	0.006
	Stem (S)	0.004		0.001		0.002	
	Leaf (L)	0.002		0.000		0.002	
1000ml	Root (R)	0.004	0.010	0.000	0.000	0.002	0.004
	Stem (S)	0.004		0.000		0.002	
	Leaf (L)	0.002		0.000		0.000	
2000ml	Root (R)	0.004	0.009	0.000	0.001	0.002	0.003
	Stem (S)	0.003		0.000		0.001	
	Leaf (L)	0.002		0.001		0.000	

NOTE: R+ S+L represent the summation of the accumulation of the metals in the Roots (R), the Stem (S) and the Leaves (L)

Appendix IX: Table 4.3: Heavy metal uptake by Bamboo – 6th month after planting

Extra Added Water per Pot	Plant Part	Mean Concentration of Heavy Metals in mg/g of Sample Analyzed					
		Copper	**R+S+L**	**Lead**	**R+S+L**	**Chromium**	**R+S+L**
0ml	Root (R)	0.002	0.006	0.001	0.002	0.006	0.020
	Stem (S)	0.001		0.001		0.006	
	Leaf (L)	0.003		0.000		0.008	
500ml	Root (R)	0.006	0.021	0.000	0.000	0.008	0.016
	Stem (S)	0.008		0.000		0.006	
	Leaf (L)	0.007		0.000		0.002	
1000ml	Root (R)	0.005	0.011	0.000	0.000	0.004	0.010
	Stem (S)	0.004		0.000		0.004	
	Leaf (L)	0.002		0.000		0.002	
2000ml	Root (R)	0.002	0.008	0.001	0.002	0.001	0.006
	Stem (S)	0.003		0.001		0.003	
	Leaf (L)	0.003		0.000		0.002	

NOTE: R+ S+L represent the summation of the accumulation of the metals in the Roots (R), the Stem (S) and the Leaves (L)

Appendix X: Table 4.4: Heavy metal uptake by Bamboo – 9th Month after Planting

Extra Added Water per Pot	Plant Part	Mean Concentration of Heavy Metals in mg/g of Sample Analyzed					
		Copper	R+S+L	Lead	R+S+L	Chromium	R+S+L
0ml	Root (R)	0.013	0.031	0.003	0.005	0.010	0.019
	Stem (S)	0.013		0.002		0.004	
	Leaf (L)	0.005		0.000		0.005	
500ml	Root (R)	0.003	0.011	0.007	0.008	0.004	0.010
	Stem (S)	0.004		0.000		0.003	
	Leaf (L)	0.004		0.001		0.003	
1000ml	Root (R)	0.003	0.011	0.000	0.002	0.003	0.006
	Stem (S)	0.004		0.000		0.002	
	Leaf (L)	0.004		0.002		0.001	
2000ml	Root (R)	0.002	0.005	0.000	0.004	0.006	0.013
	Stem (S)	0.001		0.001		0.005	
	Leaf (L)	0.002		0.003		0.002	

NOTE: R+ S+L represent the summation of the accumulation of the metals in the Roots (R), the Stem (S) and the Leaves (L)

Appendix 1: Table 4.5: Heavy Metal Uptake by Bamboo – 12th Month after Planting

Extra Added Water per Pot	Plant Part	Mean Concentration of Heavy Metals in mg/g of Sample Analyzed					
		Copper	**R+S+L**	**Lead**	**R+S+L**	**Chromium**	**R+S+L**
0ml	Root (R)	0.034	0.093	0.024	0.055	0.005	0.019
	Stem (S)	0.032		0.021		0.008	
	Leaf (L)	0.027		0.010		0.006	
500ml	Root (R)	0.021	0.079	0.035	0.053	0.007	0.014
	Stem (S)	0.017		0.017		0.004	
	Leaf (L)	0.016		0.001		0.003	
1000ml	Root (R)	0.021	0.052	0.021	0.048	0.003	0.007
	Stem (S)	0.019		0.016		0.003	
	Leaf (L)	0.012		0.011		0.001	
2000ml	Root (R)	0.016	0.040	0.015	0.036	0.002	0.004
	Stem (S)	0.013		0.009		0.001	
	Leaf (L)	0.011		0.012		0.001	

NOTE: R+ S+L represent the summation of the accumulation of the metals in the Roots (R), the Stem (S) and the Leaves (L)

Appendix XII: Table 4.6: Summary of the Cumulative Concentration of the Metals in Bamboo for all the 12 Months

Amount of Extra Water (ml) Added	Cumulative Concentration of Copper (Cu), Lead (Pb), Chromium (Cr) in Bamboo (mg/g (ppm)											
	3^{RD} Month			6^{TH} Month			9^{TH} Month			12^{TH} Month		
	Cu	Pb	Cr	Cu	Pb	Cr	Cu	Pb	Cr	Cu	Pb	Cr
0	0.015	0.003	0.005	0.006	0.002	0.020	0.031	0.005	0.019	0.093	0.055	0.047
	±0.007	±0.002	±0.001	±0.002	±0.000	±0.007	±0.006	±0.001	±0.005	±0.013	±0.014	±0.011
500	0.011	0.002	0.006	0.021	0.000	0.016	0.011	0.008	0.010	0.079	0.053	0.024
	±0.002	±0.000	±0.001	±0.003	±0.000	±0.007	±0.002	±0.001	±0.003	±0.019	±0.011	±0.006
1000	0.010	0.000	0.004	0.011	0.000	0.010	0.008	0.002	0.006	0.052	0.048	0.017
	±0.001	±0.000	±0.000	±0.002	±0.000	±0.001	±0.002	±0.000	±0.001	±0.013	±0.009	±0.000
2000	0.009	0.001	0.003	0.008	0.002	0.006	0.005	0.002	0.013	0.040	0.036	0.014
	±0.001	±0.000	±0.000	±0.001	±0.000	±0.000	±0.001	±0.000	±0.001	±0.004	±0.008	±0.000

Appendix XIII: Table 4.6: Trend of Heavy Metal Uptake by Papyrus-3rd Month after Planting

Extra Water Added per Pot	Plant Part	Mean Concentrations of Heavy Metals in mg/g of Sample Analyzed					
		Copper	R+S+L	Lead	R+S+L	Chromium	R+S+L
0ml	Root (R)	0.011	0.026	0.001	0.003	0.001	0.005
	Stem (S)	0.008		0.000		0.001	
	Leaf (L)	0.007		0.002		0.003	
500ml	Root (R)	0.011	0.024	0.001	0.004	0.001	0.005
	Stem (S)	0.007		0.002		0.002	
	Leaf (L)	0.006		0.001		0.002	
1000ml	Root (R)	0.005	0.011	0.001	0.003	0.001	0.004
	Stem (S)	0.003		0.001		0.001	
	Leaf (L)	0.003		0.001		0.002	
2000ml	Root (R)	0.009	0.018	0.000	0.000	0.000	0.003
	Stem (S)	0.008		0.000		0.001	
	Leaf (L)	0.001		0.000		0.002	

NOTE: R+ S+L represent the summation of the accumulation of the metals in the Roots (R), the Stem (S) and the Leaves (L)

Appendix XIV: Table 4.7: Trend of Heavy Metal Uptake by Papyrus – 6th Month after Planting

Extra Water Added per Pot	Plant Part	Mean Concentrations of Heavy Metals in mg/g of Sample Analyzed					
		Copper	R+S+L	Lead	R+S+L	Chromium	R+S+L
0ml	Root (R)	0.015	0.028	0.009	0.018	0.007	0.013
	Stem (S)	0.011		0.004		0.004	
	Leaf (L)	0.002		0.005		0.002	
500ml	Root (R)	0.007	0.015	0.006	0.014	0.003	0.008
	Stem (S)	0.005		0.003		0.003	
	Leaf (L)	0.003		0.005		0.002	
1000ml	Root (R)	0.005	0.013	0.004	0.009	0.002	0.007
	Stem (S)	0.005		0.002		0.003	
	Leaf (L)	0.003		0.003		0.002	
2000ml	Root (R)	0.003	0.011	0.001	0.003	0.001	0.004
	Stem (S)	0.004		0.002		0.002	
	Leaf (L)	0.003		0.000		0.001	

NOTE: R+ S+L represent the summation of the accumulation of the metals in the Roots (R), the Stem (S) and the Leaves (L)

Appendix XV: Table 4.8: Trend of Heavy Metal Uptake by Papyrus – 9th Month after Planting

Extra Water Added per Pot	Plant Part	Mean Concentration of Heavy Metals in mg/g of Sample Analyzed					
		Copper	R+S+L	Lead	R+S+L	Chromium	R+S+L
0ml	Root (R)	0.007	0.018	0.004	0.005	0.003	0.013
	Stem (S)	0.007		0.000		0.006	
	Leaf (L)	0.004		0.001		0.004	
500ml	Root (R)	0.005	0.014	0.002	0.006	0.004	0.009
	Stem (S)	0.006		0.003		0.002	
	Leaf (L)	0.003		0.001		0.003	
1000ml	Root (R)	0.004	0.007	0.004	0.006	0.002	0.006
	Stem (S)	0.002		0.001		0.002	
	Leaf (L)	0.001		0.001		0.002	
2000ml	Root (R)	0.004	0.006	0.000	0.003	0.002	0.004
	Stem (S)	0.001		0.002		0.001	
	Leaf (L)	0.001		0.001		0.001	

NOTE: R+ S+L represent the summation of the accumulation of the metals in the Roots (R), the Stem (S) and the Leaves (L)

Appendix XVI: Table 4.9: Trend of Heavy Metal Uptake by Papyrus - 12th month after Planting

Extra Water Added per Pot	Plant Part	Mean Concentration of Heavy Metals in mg/g of Sample Analyzed					
		Copper	R+S+L	Lead	R+S+L	Chromium	R+S+L
0ml	Root (R)	0.007	0.016	0.003	0.009	0.005	0.015
	Stem (S)	0.005		0.004		0.007	
	Leaf (L)	0.004		0.002		0.003	
500ml	Root (R)	0.007	0.016	0.005	0.006	0.006	0.013
	Stem (S)	0.004		0.000		0.002	
	Leaf (L)	0.005		0.001		0.005	
1000ml	Root (R)	0.003	0.011	0.003	0.008	0.004	0.009
	Stem (S)	0.004		0.002		0.003	
	Leaf (L)	0.003		0.003		0.002	
2000ml	Root (R)	0.004	0.009	0.002	0.003	0.002	0.006
	Stem (S)	0.003		0.001		0.003	
	Leaf (L)	0.002		0.000		0.001	

NOTE: R+ S+L represent the summation of the accumulation of the metals in the Roots (R), the Stem (S) and the Leaves (L)

Appendix XVII: Table 4.10: Summary of Cumulative Concentration of the Metals in Papyrus for the 12 Months

Extra Amount of Water added to containers (ml)	Cumulative Concentration of Copper (Cu), Lead (Pb), Chromium (Cr) in Papyrus (mg/g)											
	End of 3 Months			End of 6 Months			End of 9 Months			End of 12 Months		
	Cu	Pb	Cr	Cu	Pb	Cr	Cu	Pb	Cr	Cu	Pb	Cr
0	0.016 ±0.003	0.003 ±0.000	0.005 ±0.001	0.022 ±0.006	0.018 ±0.004	0.013 ±0.001	0.028 ±0.007	0.005 ±0.001	0.013 ±0.003	0.028 ±0.008	0.009 ±0.002	0.015 ±0.003
500	0.013 ±0.002	0.004 ±0.000	0.005 ±0.000	0.015 ±0.003	0.014 ±0.005	0.008 ±0.001	0.014 ±0.002	0.006 ±0.002	0.009 ±0.001	0.016 ±0.007	0.006 ±0.000	0.013 0.002
1000	0.011 ±0.003	0.003 ±0.000	0.004 ±0.001	0.013 ±0.001	0.009 ±0.000	0.007 ±0.002	0.007 ±0.001	0.003 ±0.000	0.006 ±0.000	0.011 ±0.003	0.006 ±0.001	0.009 ±0.002
2000	0.008 0.002	0.000 ±0.000	0.003 ±0.000	0.011 ±0.001	0.003 ±0.002	0.004 ±0.000	0.006 0.002	0.001 ±0.000	0.004 ±0.001	0.009 ±0.000	0.003 ±0.001	0.006 ±0.001

Appendix: Table 4.11 Leaching under 0ml Extra Water Treatment

	Concentration in mg/kg Under 0ml Extra Water						
	Ambient Concentration in Contaminated soil (S_3)	**B_{Acc}**	**P_{Acc}**	**Residual Concentration at end of experiment**		**Amount Leached under bamboo**	**Amount Leached under Papyrus**
				Bamboo	Papyrus		
Lead (Pb)	101	55	18	21	27	25	56
Chromium (Cr)	137	47	15	27	32	63	90
Copper (Cu)	174	93	28	42	29	39	117

Appendix XIX: Table 12: Leaching under 500ml Extra Water Treatment

	Concentration in mg/kg Under 500ml Extra Water						
	Ambient Concentration in Contaminated soil (S_3)	**B_{Acc}**	**P_{Acc}**	**Residual Concentration at end of experiment**		**Amount Leached under bamboo**	**Amount Leached under Papyrus**
				Bamboo	Papyrus		
Lead (Pb)	101	53	06	16	04	32	59
Chromium (Cr)	137	24	13	44	27	69	97
Copper (Cu)	174	79	16	48	37	47	121

Appendix XX: Table 4:13: Leaching under 1000ml Extra Water Treatment

		Concentration in mg/kg Under 1000ml Extra Water					
	Ambient Concentration in Contaminated soil (S_3)	**B_{Acc}**	**P_{Acc}**	**Residual Concentration at end of experiment**		**Amount Leached under bamboo**	**Amount Leached under Papyrus**
				Bamboo	Papyrus		
Lead (Pb)	101	48	06	15	34	38	61
Chromium (Cr)	137	17	09	42	25	73	103
Copper (Cu)	174	52	11	68	34	54	129

Table 4:14: Leaching under 2000ml Extra Water Treatment

		Concentration in mg/kg Under 2000ml Extra Water					
	Ambient Concentration in Contaminated soil (S_3)	**B_{Acc}**	**P_{Acc}**	**Residual Concentration at end of experiment**		**Amount Leached under bamboo**	**Amount Leached under Papyrus**
				Bamboo	Papyrus		
Lead (Pb)	101	36	03	20	53	45	84
Chromium (Cr)	137	14	06	41	25	82	106
Copper (Cu)	174	40	09	59	31	75	134

Appendix XXI: Table 4.15: Summary of Amount of the Metals Leached

Extra amount of Water (ml)	**Amount of Metals Leached (mg/kg)**					
	Under Bamboo			**Under Papyrus**		
	Lead	**Chromium**	**Copper**	**Lead**	**Chromium**	**Copper**
0	25	63	39	56	90	117
500	32	69	47	59	97	121
1000	38	73	54	69	103	129
2000	45	82	75	84	106	134

Appendix XXII: Table 4.18: Comparative Effectiveness

	Concentration of the Heavy Metals in mg/kg					
Metal	Concentration in soil	Recommended levels of Metals in soil WHO/FAO	Highest accumulation by Bamboo	Residual under bamboo	Highest accumulation by Papyrus	Residual under Papyrus
Lead (Pb)	101	10-70	55	21	18	27
Copper (Cu)	174	6-60	93	42	28	29
Chromium (Cr)	137	5-121	20	42	15	32

Appendix XXIII: Logical Framework

Objective	Activity	Requirement	Expected Outcome	Remarks
1 To find out the presence and concentration of lead, copper and chromium metals in soil that has been contaminated with petroleum sludge at Sultan Hamud dumping site	Harvesting of contaminated soil from the damp site. Digestion of the soil and AAS analysis for Copper, Lead and Chromium	AAS machine and its laboratory provisions, Standards for the three metals, and guidance of the Laboratory technician	Significant levels of the metals in the Contaminated Soil	Was successfully done with positive results
2. To investigate the trend of Bamboo and Papyrus plant species uptake lead, chromium and copper from the soil	Growing of the two plants separately in plastic containers with the contaminated soil. Periodic harvesting of roots, stems and leaves, digesting them and testing for the metals.	10L plastic containers, Soil contaminated with petroleum sludge, Greenhouse, Drip irrigation system, and caretaker	A consistency in uptake of the metals relative to length of growth time in the contaminated soil	Successfully achieved
To investigate the effect of leaching on the concentration/bioavailability of the heavy metals in the soil that has been	Additional of specific and constant amounts of	Drip irrigation system, caretaker, and constant	Significant influence of leaching on uptake of the Heavy	Bioremediation is dependent on leaching

Objective	**Activity**	**Requirement**	**Expected Outcome**	**Remarks**
contaminated with petroleum sludge	extra waters in the containers with the pants. Harvesting of the plants, digesting the samples and testing by AAS	supply of tap water	metals.	
4 To formulate an appropriate Bioremediation strategy for soil contaminated with petroleum sludge containing the heavy metals	Integration of the observation with existing literature to conduct a pilot which integrates both the plants on a polluted site	Further research of suitability of the approach in different environmental conditions	Successful bioremediation of site/soil contaminated with petroleum sludge	

Appendix XXIV: Table 4.6: Summary of the Cumulative Concentration of the Metals in Bamboo for all the Four Seasons

Amount of Water (ml)	**Cumulative Concentration of Copper (Cu), Lead (Pb), Chromium (Cr) in Bamboo (mg/g)**											
	Season 1			**Season 2**			**Season 3**			**Season 4**		
	Cu	**Pb**	**Cr**	**Cu**	**Pb**	**Cr**	**Cu**	**Pb**	**Cr**	**Cu**	**Pb**	**Cr**
0	0.015	0.003	0.005	0.006	0.002	0.020	0.031	0.005	0.019	0.093	0.055	0.019
500	0.011	0.002	0.006	0.021	0.000	0.016	0.011	0.008	0.010	0.079	0.053	0.014
1000	0.010	0.000	0.004	0.011	0.000	0.010	0.008	0.002	0.006	0.052	0.048	0.007
2000	0.009	0.001	0.003	0.008	0.002	0.006	0.005	0.002	0.013	0.040	0.036	0.004

Appendix XXV: Table 4.10: Summary of Cumulative Concentration of the Metals in Papyrus for the Four Seasons.

Amount of Water (ml)	**Cumulative Concentration of Copper (Cu), Lead (Pb), Chromium (Cr) in Papyrus (mg/g)**											
	Season 1			**Season 2**			**Season 3**			**Season 4**		
	Cu	**Pb**	**Cr**	**Cu**	**Pb**	**Cr**	**Cu**	**Pb**	**Cr**	**Cu**	**Pb**	**Cr**
0	0.016	0.003	0.005	0.022	0.018	0.013	0.028	0.005	0.013	0.028	0.009	0.015
500	0.013	0.004	0.005	0.015	0.014	0.008	0.014	0.006	0.009	0.016	0.006	0.013
1000	0.011	0.003	0.004	0.013	0.009	0.007	0.007	0.003	0.006	0.011	0.006	0.009
2000	0.008	0.000	0.003	0.011	0.003	0.004	0.006	0.001	0.004	0.009	0.003	0.006

https://www.gardenloversclub.com/ornamental/foliage/papyrus/growing-papyrus-plant/