KINETICS OF METAL POLLUTANTS BIOSORPTION IN LAKE VICTORIA AND ITS ENVIRONS USING *MORINGA OLEIFERA* SEED POWDER

BY

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DECLARATION

Declaration by the Candidate

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I dedicate this work to my lovely children: Arthur, Brian and Stella: You all mean a lot in my life: you give me a reason to solder on in life.

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ABSTRACT

In the last three decades attention has been paid to environmental management caused by hazardous materials such as heavy metals whose levels have risen due to increased anthropogenic activities. One of the techniques used to remove heavy metals is biosorption. The kinetics of biosorption of metal pollutants in Lake Victoria and its environs using *Moringa oleifera* seed powder was investigated with a view of using it as a low cost biosorbent for the removal of toxic heavy metals from waste water. FT-IR analysis was done for identifying the functional groups of *moringa oleifera* seeds powder. The FT-IR spectrum indicated the presence of –NH, -OH, -COOH in the biomass. Samples from eight sites on Winam Gulf were taken and analyzed for heavy metals (Cu, Cd, Cr, Mn, Pb and Zn) using AAS prior to digestion using HNO_3 and HCl. Measurements of electrical conductivity, salinity, dissolved oxygen and total dissolved solids were done at the sampling sites. Model polluted water sample was prepared for use in the batch biosorption experiments with respect to parameters such as contact time, pH, particle size, adsorbent dosage, presence of other metal ions, effect of adsorbent pretreatment, isotherms, kinetics and adsorbate concentration. The levels of trace heavy metals in most of the samples were above WHO standard; Cd (range; 0.013 - 0.037 mg/l) mean value 0.0263 mg/l ± 0.005, Pb (range; ND- 1.13 mg/1) mean value 0.545 mg/l ± 0.013, Zn (range; 0.3005- 3.467 mg/L) mean value 3.011 mg/l ± 0.0311, Cr (ND-0.459 mg/l) mean value 0.317 mg/l ± 0.0113, Mn (0.153-4.967 mg/l) mean 2.312 mg/l ± 0.0281, Cu (range ND- 1.543 mg/l) mean value 0.05 mg/l ± 0.005 was the only heavy metal below WHO standard of 1.0 mg/L The maximum biosorption capacity was observed at pH 5 for all six metals, particle size of 0.250 mm, and adsorbent dosage of 0.6 g. Increased initial metal concentration led to increase in the rate of biosorption. Biosorption was higher in single systems and lower in binary systems. The data fitted into both the Langmuir and Freundlich adsorption isotherms with coefficient of determination values ranging between 0.958 and 0.999. However, the data fitted better into the Langmuir isotherm for most of the experiments with ccoefficient of determination between 0.986 and 0.999. Pseudo second order kinetics fitted best into the experimental data with coorelation coefficient ranging between 0.913 and 0.999. The results suggest that *Moringa oleifera* seeds have the potential application in heavy metal ions decontamination from aqueous effluents. The results also demonstrated that pretreatments influenced the biosorption capacity of the biomass for most of the metals significantly, and the order of modification efficiency was NaOH > $CaCl_2$ > $KMnO_4$ > HCl. Hence pretreatment of *Moringa oleifera* further enhances biosorption.

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ACRONYMS AND SYMBOLS

AAS:	Atomic absorption spectroscopy
BOD:	Biochemical oxygen demand
C_0:	Initial metal concentration
C_e:	Final metal (or residual/equilibrium) concentration
CWB:	Citrus reculata waster
DO:	Dissolved oxygen
FAO:	Food and Agricultural Organization
FTIR:	Fourier Transform Infrared Spectroscopy
K_1:	Rate constant for pseudo-first order reaction
K_2:	Rate constant for pseudo-second order reaction
KEBS:	Kenya bureau of standards
KEMRI:	Kenya medical research institute
K_L:	Langmuir constant (empirical constant)
K_f :	Freundlich capacity factor
L:	Litre
m:	Dry weight of biomass (dose)
mL :	milli Litre (0.001 litre)
mm:	Millimetre (0.001 metre)
n:	Freundlich intensity parameter (dimensionless)
POL:	*Platanus orientalis* Leaves
PSU	Practical salinity units

PZC: Zero point charge

q_e : Equilibrium uptake of metal

q_m : Maximum metal uptake or maximum adsorption capacity

ROS: Reactive oxygen species

rpm: revolutions per minute

TDS: Total dissolved salts

V: Volume of liquid

WHO: World health organization

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Human activities such as industries and domestic sewage discharge their wastewaters containing pollutants including heavy metals into the environment. Since such metals are toxic, their removal from the environment is necessary. The commonly used procedures for removing metal ions from aqueous streams like ultra-filtration, reverse osmosis, ion exchange, solvent extraction and chemical precipitation are expensive and have disadvantages like incomplete metal removal, high treatment and energy requirements, and generation of toxic sludge or waste products that require careful disposal. Therefore, a cost effective treatment method that is capable of removing heavy metals from aqueous solutions (Congeevaram *et al*., 2007) is needed. Biosorption is one such a technique and has provided an alternative treatment of industrial effluents from convectional physico-chemical methods (Selatnia *et al*., 2004). The biological materials that have been investigated for heavy metal uptake include bacteria, fungi, yeast and algae (Ozturk, 2007).

The pollution of the environment increased considerably in the past century. Industrial expansion, the rapid growth of human population as well as the effects of released toxic compounds all had their impact on the pollution of the environment. The worldwide proportions of chemical pollution have become alarming. Dangers related to heavy metal, as the accumulation of microelements and toxic heavy metals can have decisive human health related, ecological and biological consequences. The emission of micro-elements and heavy metals considerably increased with industrial revolution, where beside traffic and industrial activities, the modernization of agriculture has also become a potential source of heavy metal pollution (Odada *et al*., 2006).

In the recent decades the levels of heavy metals in the soils, water and sediments have become critically higher as a result of the placement of sewage water and sewage sludge on agricultural fields, around industrial districts in cities and along main traffic roads. These contaminated areas represent basic environmental and health related problems. Soils are able to accumulate heavy metals for long periods of time without any sign of toxic effects yet in case these metals reach the nutrition chain (for instance by the consumption of plants grown on contaminated fields, and thus they enter the human body) they can accumulate there and cause acute or chronic damage, ultimately bringing about the destruction of living organisms. Over the past two decades the activities to assess and recognize the status of contaminated natural elements (such as geological media, waters under surface), and to clean them from pollutants have multiplied exponentially. As a result, environmental protection has become a specific industry, for which a cumulative social and economic demand emerges. Remediation technologies depend on local characteristics of contaminated fields; therefore, methods that rely on highly sophisticated processes of living organisms are used more often to achieve the best results (Kishe and Machiwa, 2003).

Heavy metals in effluents are a major concern for industries and domestic sewage treatment systems. Removal of the pollutants from waste waters is a major ecological problem. Most of the conventional treatment methods are expensive and potentially risky due to possibility of hazardous by product generation (Congeevaram *et al*., 2007). Environmentally friendly methods using biosorbents and modifiers that are fast and effective in the removal of heavy metals and the environmental parameters for their removal are gaining popularity. This study therefore, intended to come up with data on the best modifier for cleaning up different heavy metals and the best environmental parameters for optimum adsorption. The mechanism of metal ions uptake from solutions can be due to physical sorption, complexation with microbial cell surface groups or bioaccumulation. Biosorption could occur through interactions between metal ions and functional groups of the cell wall biopolymers of living dead organisms. The major functional groups of biopolymers which act as binding sites include carboxylate ($-COO^-$), amide ($-NH_2$), thiols (-SH), phosphate (PO_4^{3-}) and hydroxide ($-OH^-$) (Sud *et al*., 2008).

Moringa oleifera (Saihjan or drumstick), a cosmopolitan tropical, drought tolerant tree, available throughout the year, has been well documented for its various pharmacological importance, viz. its analgesic, antihypertensive and anti-inflammatory effects. The powdered seed of the plant *Moringa oleifera* has coagulating properties that have been used for various aspects of water treatment such as turbidity, alkalinity, total dissolved solids and hardness. However, its biosorption behaviour for the removal of toxic metals from water bodies has not been given adequate attention (Kumari *et al*., 2005). There are different approaches to biosorption whereby either raw biomass is used for direct sorption or the biomass is chemically pretreated to reinforce it for sorption process applications and enhance the sorption performance. Pretreatment may be in terms of hardening of the cell structure through cross linking reactions using glutaraldehyde ($CH_2(CH_2CHO)_2$), or increasing the negative charge on the cell surface by NaOH treatment, or opening of the available sites for the adsorption by acid treatment, and enhancing ion exchange by Ca^{2+} solution treatment. Other chemical treatments of biomass using epichlorohydrin and potassium permanganate have also been reported. The purpose is to produce pretreated biomass that has high potential as a biosorbent for the removal of heavy metals from waste waters. Equilibrium studies that give the capacity of the adsorbent and the equilibrium relationships between adsorbent and adsorbate are described by an isotherm is which usually the ratio between the quantities adsorbed and the amount remaining in solution at a fixed temperature at equilibrium. Freundlich and Langmuir isotherms are the earliest and simples known relationships describing the adsorption equation (Julali *et al.*, 2002).

1.2 The Study Area

Winam Gulf covers Kisumu county and Nyando sub-county. The Kisumu Railway Pier and fishing beaches (Kaloka, Usoma, Otonglo, Dunga, Tako, Ogal, Ogenya, Nyamware and Nduru) are within the Gulf area. Other important sites within the Gulf are the Yacht Club and the Police Pier, which have been adversely affected by water hyacinth infestations. The Dunga water supply intake point serving Kisumu Municipality is also within the Gulf area. Winam Gulf is a large inlet from Lake Victoria that extends into Kenya. It stretches about 100 km east to west and 50 km north-south and has a shoreline measuring about 550 km. The Gulf is comparatively shallow; having a recorded maximum and average depth of 68 m and 6 m, respectively. Surface water temperatures range between $23.5^{o}C$ and 29.0°C. Wind induced currents influence water mixing in the gulf. Secchi transparency ranges between 35 to 155 cm (Hughes and Hughes, 1992) and primary productivity is limited to a thin layer at the surface.

The Gulf has a narrow opening to the main lake, which was further reduced in 1980 when the causeway between Rusinga Island and the southern mainland was closed. It used to be the deeper opening to the main lake which considerably reduced water exchange between the gulf and the main lake. As a result there is occasional flooding during the rainy season on the mainland south of the opening. Four major rivers, Sondu-Miriu, R. Kibos, Nyando, and Kisat discharge an average of 231 m^3s^{-1} into the Gulf (Hughes and Hughes, 1992). Most of the beaches on Lake Victoria are used as car wash sites, for instance at Lwang'ni beach no fewer than 500 vehicles a day are soaped up and scrubbed down by some 300 car washers, the effluent all draining into the lake despite a ban on such activity.

According to a report by KEMRI (Hughes and Hughes, 1992) one hundred percent of the car washers are schistosomiasis infected, the report adds that it was rare to find somebody in good health among the people working at the lake. Schistosomiasis, bilharzia, cholera, pneumonia, diarrhoea and skin diseases are among the water-borne or abetted illnesses that afflict Lake Victoria residents with increasing frequency, health officials say. And the human excrement expelled into the lake from the Kisumu car washers is by far one of the least of the pollutants. "Millions of litres of untreated sewage sludge flow into the lake every day from major urban centres along the lake shore," (Odada *et al.*, 2006).

This contamination, coupled with chemical and fertilizer run-off from the lakeside industries and agriculture, has had a devastating effect, contributing to a disturbing rise in anoxia, lack of oxygen, in the lake water, it said. "Nearly half of the lake floor currently experiences prolonged anoxia for several months of the year, compared to the 1960s when anoxia was localised and sporadic," (Odada *et al*., 2006). The Winam Gulf catchment is the main water catchment and lies between 1000 to 2000 m above sea level it has a mean monthly air temperatures of between 21.9 and 24.3^{o}C. The area is characterised by an equatorial hot and humid climate modified by effects of altitude, relief and influence of the lake.

Relatively wet agro-ecological zones dominate the region except near the lake. The area is dominated by westerly winds from the lake which converge with the northeast passat (or trade) wind causing the air to rise and thus producing heavy showers over the gulf and its catchment. The yearly rainfall average is 1,000 mm. The area has two rainfall seasons, heavy and light rainfall periods. Heavy rainfall period is between March and May while light rainfall period is between September and November. Dry seasons are between December and February and June and September. Because of the cooling influence, which the lake exerts, temperatures are a little lower than a normal equatorial climate (Odada *et al*., 2006).

Relative humidity is high. Potential evaporation is between 1,800-2,200 mm per year and in most months, exceeds monthly rainfall. The area is extensively utilised for agriculture with notable increase in fertiliser application. Land for the main food crops is prepared from December-February and July-September. Land degradation, surface run-off and soil erosion have been on the increase. This has resulted in increase of sedimentation and nutrient enrichment (Muli, 1996). The enrichment of the Gulf has resulted in proliferation of macrophytes mainly water hyacinth, *Eicchornia crassipes*.

The lake is an important source of food for the people living in its vicinity. Total fish landings from the lake are estimated to be 500,000 m tons per year, generating over 200 million US$. The lake's fishery is driven by both national and international capital mainly in the processing industry. The lake supports a multi-species fishery. The main commercial species are Nile perch (*Lates niloticus)*, Tilapia (*Tilapia Niloticus)* and a cyprinid (*Rastrineobola argentea).* These are fished by trawling and by artisans using seines and lines from beaches and canoes (Muli, 1996).

1.3 Statement of the problem

Water is a resource that is essential for life and is required by every living organism. This resource is, however, becoming very limited in its pure state due to the many anthropogenic means of contamination which arise from the different advancements made over the years. Metal species released into the environment by technological activities tend to persist indefinitely, circulating and eventually accumulating throughout the food chain, thus becoming a serious threat to the environment, the metals include: Zn, Pb, Cd, Hg, Cu, Cr and Mn just to mention a few. Environment pollution by toxic metals occurs globally through military, industrial, and agricultural processes and waste disposal. This has led to a need for means of purifying / reclaiming of water for the various roles it plays in our lives.

Heavy metals have emerged as the main contaminants of water in recent years. They arise from different sources/activities carried out by industries. Industries which do not implement environmentally friendly industrial waste disposal protocols tend to be the main culprits for the contamination of water as they would just discharge the wastes with disregard to the future consequences thereof. Unsanitary landfills also contaminate ground water sources with heavy metals which would be from sources like electronics assembly and fabrication, battery manufacturing, paper and pulp industries, metal smelters, textiles, usage of fertilizers and pesticides, and mining activities. There is a need for developing a means of purification of water which is efficient and also cost effective since heavy metals are still toxic to humans even at very low concentrations. Other methods that have been employed are ultra-filtration, reverse osmosis, ion exchange, solvent extraction and chemical precipitation, to mention but a few (Obuseng, *et al*., 2012). The methods previously used are costly and generate huge amounts of toxic sludge which are difficult to dispose.

1.4 Justification of the Study

Agricultural and industrial sectors depend on fertilizers and pesticides and both of these activities are carried out in the Winam Gulf. The water courses draining into Lake Victoria also transverse this area. The pollutants are introduced into the water system from various sources such as industrial effluents, agricultural run off, chemical spills and municipal wastes (Wandiga and Onyari, 1987). Consequently there is need to monitor water quality in Lake Victoria continuously to check on the pollution load (Karanja, 2002). This is important because it causes massive kills of fish and contamination of aquatic animals, which are eventually consumed by human beings and enter their body tissues (Bhushan *et al.*, 1997).

There is lack of adequate information on the accumulation of these pollutants in the soils and their leaching into water courses that finally end up into Lake Victoria. Since environmentally friendly farming methods are replacing the old polluting ones, there is need to monitor the levels of pollutants in water, soils and sediments at designated sites in the Winam Gulf. The study, therefore, intended to come up with data on the levels of heavy metals in the Winam Gulf since such available information is not sufficient yet. The mechanisms of river pollution are complex, and whereas most effluent can be treated, they are seldom so innocuous as not to lead to some alterations in the receiving waters. The need for baseline data on pollution studies to the aquatic biota of Kenyan lakes and rivers is, therefore, of utmost importance. Baseline data is a prerequisite to pollution evaluation, since even natural streams may also faithfully reproduce the effect of the addition of industrial pollutants to water. The presence of heavy metals in water systems is a threat to biota as they accumulate in various organisms often reaching lethal concentrations. It is therefore impotant that we establish the levels of heavy metals in the water bodies and thereafter need to control pollution not only in water but also in sediment, suspended solids and organisms most of which are important in food chains with man at the top of the food chain (Turgut, 2003).

The use of *Moringa oleifera* for the removal of heavy metals from polluted water is a healthy and cost-effective venture due to the biodegradability and lower cost of *Moringa oliefera*. Biosorbents cost about US$ 4-7/kg while ion exchange resins cost about ten times more (US$ 30-50/kg). Reverse osmosis is even more costly. Precipitation is inefficient at low ion concentrations and generates toxic sludge which is difficult to dispose of. In addition, metal recovery from the sludge is difficult.*Moringa oleifera* is easily cultivated and has other uses in pharmacology and in the food industry; it can be regenerated for subsequent use and is biodegradable. The biosorption process is fairly rapid, with contact times below one hour for most biosorbents. Biosorption using *Moringa oleifera* is therefore a potential solution to heavy metal pollution (Sharma *et al*., 2007).

1.5 Objectives

1.5.1 Overall Objective

The main objective was to optimize the biosorption of heavy metal water pollutants by use of *Moringa oleifera* seed powder under various reactions and pre-treatment conditions in Lake Victoria and its environs.

1.5.2 Specific Objectives

i. To determine the levels of selected heavy metals (Pb, Zn, Cd, Cr, Cu and Mn) by AAS and some phytochemical parameters of water at various sites of the Winam of the Lake Victoria.

ii. To characteriz untreated *Moringa oleifera* seeds using Fourier transform infrared spectroscopy (FTIR).

iii. To study the sorption behaviuor of metal ions with untreated and chemically modified *Moringa oleifera* seeds.

iv. To study the effect of adsorbent dose, pH, contact time, mixed ions, particle size and initial effluent concentration on the efficiency of the biosorption process.

v. To determine the most applicable isotherm for the adsorotion process.

vi. To determine the order of metal adsorption on the biomass.

1.6 Research Questions

i. Is water from Lake Victoria and its environs contaminated with heavy metals such as Pb, Zn, Cd, Cr, Cu and Mn and what are their levels?

ii. What functional groups are found on *Moringa oleifera* that enhance the biosorption process?

iii. Does chemical modification enhance biosorption and which is the best modifier for *Moringa oleifera*?

iv. What is the effect of initial effluent concentration, pH, contact time, mixing ions, dosage of adsorbent, particle size and contact time on equilibrium?

v. Which isotherm best describes the biosorption process best?

vi. What is the order of reaction between the heavy metals and the adsorbent?

CHAPTER TWO

LITERATURE REVIEW

2.1 Pollution around Lake Victoria

Lake Victoria has experienced dramatic changes especially in the past century. These changes occurred in the drainage basin involving the vegetation, industrialization, agricultural developments, introduction and invasion of alien species and intensive non-selective fishing. These, among other factors, have led to the destruction of native and endemic components of the basin. Lake Victoria lost about 60% of its cichlid taxa in the last decade and faced deterioration in water quality from over exploitation of the fishes and human impacts on the ecosystem (Witte *et al.*, 1992; Kudhongania and Chitamwebwa, 1995; Gophen *et al.*, 1995; Ogutu-Ohwayo *et al.*, 1997). Other components of the aquatic system that changed included algae, macrophytes, invertebrates, birds, amphibians and reptiles that are important parts of the ecosystem.

There has been progressive build-up of physical and chemical changes in the lake. Increased chlorophyll “a” concentration and primary production, a decrease in silica and sulphur concentrations compared to values measured 30 years ago was noted (Hecky, 1993). Other ecological shifts include the dominance of blue-green algae and leading to increased algal blooms. A number of endemic species of fish and birds in the lake basin face various threats from a variety of human activities. In 1988, about one hundred native fish species endemic to Lake Victoria were entered in the World Conservation Union’s Red Book of endangered species. A number of studies report remarkable post perch structural changes directly impacting on the niche composition at all levels of biodiversity (Mbahinzireki, 1994; Mugidde, 1992; Gichuki and Odhiambo, 1994; Seehausen and Witte, 1995; Seehausen, 1995; Chapman *et al.*, 1995).

The wetland ecosystems in Lake Basin are rich with vertebrates. Other than fish most of these are not well studied. It is reported that Kingfisher (*Ceryle rudis*) diet changed from haplochromines to Dagaa (Omena) following the explosion in Nile perch population (Goudswaard and Wanink, 1994). The ecosystems are quite diverse and provide different ecological niches or habitats for different species. The rivers and their associated wetlands provide *refugia* for fish species endangered from Nile perch and other predators (Chapman *et al.*, 1995). Some species, thought to have disappeared following the perch introduction in the Lake, have been found in satellite lakes and other *refugia*. Some of these species or populations could recover under effective ecosystem management. The Lake basin is estimated to have a population of 30 million people which is growing at > 3% per annum.

The Lake supports one of the most productive freshwater fisheries in the world with annual fish yields in excess of 500,000 tonnes annually. Other economic activities in the Lake basin include agriculture, mining, hydropower generation and transport. Three major cities (Kampala, Kisumu and Mwanza) with a combined population of at least six million people depend on the lake for domestic and municipal water supply and waste disposal. The challenge on Lake Victoria is to sustain the lucrative fishery that emerged out of the Nile perch introductions, and at the same time restore and conserve the lost fish diversity (Chapman *et al.*, 1995).

2.2 Physical Characteristics of Water in Winam Gulf

2.2.1 Temperature

Temperature affects physical, chemical and biological processes in water bodies and therefore the concentration of many parameters. As temperature increases the rate of chemical reaction generally increases together with evaporation and volatilization of substances from water. Increased temperature also decreases the solubility of gases such as oxygen, carbon dioxide, nitrogen and methane in water. With increased water temperatures, the rate of micro-organisms growth also increases leading to; increased turbidity, algae blooms and macrophyte growth. The metabolic rate of aquatic organisms is also related to temperature and in warm waters respiration rates increase leading to increased oxygen consumption and increased organic matter decomposition (Chek, 1999). Haiyan *et al.* (2013) noted that at higher temperature the release rates of metal increased with the temperature increasing, the release rate followed in the order: Zn > Cu > Pb > Cr > Cd at the same temperature which implied that biosorption is also temperature dependend. If temperature increasing, it usually enhances biosorptive removal of pollutant by increasing surface activity and kinetic energy of the adsorbate, but may damage physical structure of biosorbent (Park *et al.*, 2010)

2.2.2 Dissolved Oxygen

Dissolved oxygen (DO) standard establishes lower limits to protect propagation of fish and other aquatic life; enhances recreation and reduces the possibility of odours resulting from decomposition of organic matter and maintain a suitable quality for water treatment. The primary pollutant associated with depletion of dissolved oxygen is carbonaceous biochemical oxygen demand (BOD) (Greenberg *et al.*, 1992). Osumo (2001), observed dissolved oxygen levels of 1 – 8 mg/l during the sampling period. These were observed to decrease with depth. The deep water over the deep part of the section was characterised by low dissolved O_2 levels of 3-4 mg/l throughout the sampling period. The relatively low dissolved O_2 concentration observed in March may have been because of little algae growth (Osumo, 2001). Water hyacinth cover impedes algae growth and increases water clarity. Dissolved oxygen was comparatively lower in July than it was in March. It could have been as a result of the decomposition of water hyacinth organic matter by bacteria. In July, water hyacinth organic matter was being dumped in the lake by the mechanical shredding of water hyacinth. Haiyan *et al*. (2013) observed an increase in the release of Zn, Cu, Cr, and Pb from sediments under aerobic condition during the first 150 minutes and then the concentration kept stable, while adsorption of the same metals and the release of Cd occurred in the anaerobic condition, this implies that the amount of dissolved oxygen in wastewater affects the rate of biosorption of heavy metals.

2.2.3 Salinity

Salinity is a measure of the concentration of total dissolved salts in soil or water, mainly sodium chloride (common table salt). Primary salinity occurs naturally and is the result of rainfall interacting with geographical features over thousands of years. Secondary salinity is the result of human land use and either produces more salt or causes primary salinity to rise to the surface of the land. High concentrations of salt pose hazards for the environment as well as affecting agriculture and infrastructure and therefore, the wider economy. High levels of salinity in water and soil may cause native vegetation to become unhealthy or die and lead to a decline in biodiversity through dominance of salt-resistant species, potentially altering ecosystem structures.

2.3 Heavy Metals and Toxicicity

The term heavy metal refers to metallic elements with relatively high densities that are toxic at low concentrations. They are considered to be chemical elements with an atomic mass greater than 22 and a density greater than 5 g/mL. This definition includes 69 elements, of which 16 are synthetic. Some of them are extremely toxic to human beings, even at very low concentrations (Roane and Pepper, 2000; Wang and Chen, 2006). The main heavy metals associated with environmental contamination, and which offer potential danger to the ecosystem, are copper (Cu), zinc (Zn), silver (Ag), lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), strontium (Sr), ceasium (Cs), cobalt (Co), nickel (Ni), thallium (Tl), tin (Sn) and vanadium (V) (Wang and Chen, 2006).

In general, metal ions can be classified as: 1) Essential and important for metabolism (Na, K, Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo and W). 2) Toxic heavy metals (Hg, Cr, Pb, Cd, As, Sr, Ag, Si, Al, Tl), which have no biological function (in ecotoxicology terms, hexavalent forms of Cr ions are the most dangerous). 3) Radionuclides (U, Rn, Th, Ra, Am, Tc), which are radioactive isotopes and, although toxic to cells, they are nonetheless important in nuclear medicine procedures. 4) Semi-metals or metalloids (B, Si, Ge, As, Sb, Te, Po, At, Se), which exert distinct biological effects on metals. However, metals are predominantly present in the environment in cationic and anionic forms in semimetals, and as is often classified as a heavy metal (Roane and Pepper, 2000; Ahluwalia and Goyal, 2007). In the environment, metals can be divided into two categories: 1) bioavailable (soluble, non-absorbed, and mobile) and 2) nonbioavailable (precipitated, complexed, sorbed, non-mobile). The ionic form (speciation) of a metal determines its bioavailability and its destination. Most heavy metals are cations and this determines their sorption to negatively charged functional groups that are present in: cell surfaces, which are generally anionic at a pH of between 4 and 8; surfaces with residual hydroxides (OH^-) or thiol (SH^-) and anionic salts, such as PO_4^{3-} and SO_4^{2-}, humic acid, and clay minerals (Roane and Pepper, 2000). Heavy metal ions possess great electrostatic attraction and high binding affinities to the same sites that essential metal ions normally bind to various cellular structures, causing destabilization of the structures and biomolecules (cell-wall enzymes, DNA and RNA), thus inducing replication defects and consequent mutagenesis, hereditary genetic disorders and cancer. This occurs, for example, with arsenate, which competes with phosphate, and cadmium, which competes with zinc (Roane and Pepper, 2000).

By employing microarray technology, Kawata *et al.* (2007), found that six heavy metals (arsenic, cadmium, nickel, antimony, mercury and chromium) induce gene expression patterns that are very similar to the pattern induced by DMNQ (2.3-dimethoxy-1, 4-naphthoquinone), the reactive oxygen species (ROS) chemical generating agent, which causes "oxidative stress", leading to deleterious effects (membrane damage or other cellular lipid structures, modification of proteins, fragmentation and cross-links, changes in DNA that can induce mutations or be repaired by repair mechanisms). Therefore, the ions of heavy metals cause oxidative damage, both directly, by producing ROS, and indirectly, by inactivating the cellular antioxidant system, thus leading to cell damage (Liu *et al.*, 2009; Mannazzu *et al.*, 2000).

2.3.1 Lead

Elemental Pb is dense (11.3 g/cm^3), blue-grey in colour, melts at 327 ^{O}C and boils at 1744 ^{O}C. Soil is a sink for anthropogenic Pb and there are several well recognized major sources, namely mining and smelting activities, manures, sewage sludge usage in agriculture and contamination from vehicle exhausts. Lead arsenate ($PbHAsO_4$) has been applied to the orchard trees to control insects, pests and orchard soils may, therefore, contain elevated concentration of Pb (Matheickal and Yu, 1999). However, commercial use of these sprays is now infrequent since lead Arsenate ($PbHAsO_4$) has been replaced by organic pesticides.

In the early 1920s, it was discovered that Pb alkyls (tetraethyl and tetra methyl Pb) when added to petrol helped overcome the problem of knocking or pinking. In 1923 the first leaded petrol was sold and it had a higher octane rating, hence, became standard. It was later reported that soils and vegetation samples collected near roads contained unusually high Pb contents, hence it was remarked that contributions of Pb from petrol fumes merited attention (Matheickal and Yu, 1999) and levels of Pb in soil and grass decreased with distance from the road (Mannazzu *et al*., 2000). Consumption of food contaminated with Pb poses a general risk to the population. The health risks include; low rate of development in the pre-natal and post-natal stages in human and nervous system disorders, inhibition of enzyme systems necessary for formation of haemoglobin.

Pb is also known to cause mitochondria damage, brain damage, insomnia, and lack of ambitions, frequent colds, exhaustion, infertility, still births, premature senility, convulsion, coma, blindness, paralysis and even death. The symptoms of acute Pb poisoning in children include brain and nervous system damage among other organs (Matheickal and Yu, 1999).

High concentration of lead in the body can cause death or permanent damage to the central nervous system, the brain and kidney (Jenning and Sneed, 1996). The damage commonly results in behaviour and learning problems. Pb crosses the placenta easily throughout gestation and is usually associated with poor pregnancy and neonatal outcomes. Lead interferes with a variety of body processes and is toxic to many organs and tissues in the body. Pb interferes with a variety of body processes and is toxic to many organs and tis-sues including the heart, bones, intestines, kidneys, reproduc-tive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning behaviour disorders. One of the routes of exposure to lead is water. Lead also interferes with heme synthesis and leading to hematological damage. Pb inhibits several important enzymes involved in the overall process of heme synthesis. Lead finds entry into the water through the discharge of waste waters emanating from printing, dyeing industries and oil refineries (Matheickal and Yu, 1999).

2.3.2 Zinc

Zinc imparts an undesirable astringent taste to water at a taste threshold concentration of about 4 mg/litre (as zinc sulfate. Water containing zinc at concentrations in excess of 3–5 mg/litre may appear opalescent and develop a greasy film on boiling (WHO, 2008). The most exploited zinc ore is sphalerite, a zinc sulfide. Zinc belongs to the group of trace metals potentially most hazardous to the biosphere. Most of the concern about excessive zinc concentrations in soils relates to its possible uptake by crops and consequent adverse effects on the crops themselves and on livestock and human diets. Together with Cu, Ni and Cr, Zn is principally phytotoxic, so the concern about the metal is mainly directed at effects on crop yield and soil fertility. Inhalation of zinc fumes causes fever, depression, vomiting, excess salivation and headache. Zinc unlike lead, cadmium, arsenic and antimony does not accumulate in the biological systems including man (Alloway, 1995).

2.3.3 Cadmium

Cadmium is a highly toxic non-essential metal which affects the uptake of zinc. It accumulates in the kidneys of mammals causing kidney dysfunction. It is used increasingly in corrosion prevention, polymer stabilization, and electronics and pigments applications. It tends to be less strongly adsorbed than many other divalent metals and is, therefore, more labile in soils and sediments and more bioavailable (Alloway and Ayres, 1997). There is more danger from this metal moving through the food chain from contaminated water than most other metals. Its excess causes kidney damage and skeletal deformities.

The average concentration of cadmium in the Earth's crust is between 0.1 and 0.5 parts per million (ppm) (Alloway, 1995). Cd is introduced into the water bodies from smelting, metal plating, Cd-Ni batteries, phosphate fertilizer, mining, pigments, stabilizers, alloy industries and sewage sludge. The harmful effects of Cd include a number of acute and chronic disorders, such as “itai-itai” disease, renal damage, emphysema, hypertension, and testicular atrophy (Leyva-Ramos *et al.*, 2005). Cd contamination is a non-reversible accumulation process, with the estimated half-life in soil varying between 15 and 1100 years (Kabata Pendias and Pendias 1992), and high plant-soil mobility to be easily accumulated in plant tissues, while high accumulation of Cd in plants not only badly affects crop yield and quality, but also gives rise to threat to safe food production and human health. It is, therefore, a long term problem and so, pollution needs to be prevented and minimized wherever possible. The major hazard to human health from Cd is its chronic accumulation in the kidneys where it can cause dysfunction if the concentration in the kidney cortex exceeds 200 mg/kg (Alloway, 1995). Food is the main route by which Cd enters the body but tobacco smoking and occupational exposures to CdO fumes are also important sources of the metal.

FAO/WHO recommends maximum tolerable intake of Cd to be 400 to 500 µg/per week, which is equivalent to about 70 µg/day. Average dietary intake of Cd around the world ranges between 25 and 75 µg/day and there is a problem where the intake is near the top of the range. People who smoke can add an extra 20 to 35 µg/day to their intake (Alloway, 1995). Cd interacts or competes with other metals such as Cu, Zn, Fe, Mn and Ca for ligand sites in bimolecular systems thereby interfering with their homeostasis.

Inhalation of fumes or dust containing Cd and its compounds primarily affects the respiratory track. Long term occupational exposure results in severe chronic effects in lungs and kidneys.

2.3.4 Copper

Copper metal and its alloys have been used for thousands of years. Its compounds are commonly encountered as Cu (II) salts, which often impart blue or green colours to minerals such as turquoise and have been widely used historically as pigments. Architectural structures built with Cu corrode to give green verdigris (or patina). Decorative art prominently features Cu, both by itself and as part of pigments (Alloway, 1995). Cu (II) ions are water-soluble, where they function at low concentration as bacteriostatic substances, fungicides, and wood preservatives. In sufficient amounts, they are poisonous to higher organisms; at lower concentrations it is an essential trace nutrient to all higher plant and animal life. The main areas where copper is found in animals are tissues, liver, muscle and bone. Concentration of Cu in water from wells and other sources for human consumption is always below the 1mg/kg interim Cu limit, which is based on taste considerations. Because of Cu contamination of domestic water supplies by plumbing in developed countries, several approaches are used by various countries to establish standards for Cu in drinking water. Daily intake of Cu by humans is almost always above the recommended daily requirement of 2 mg/day (Alloway, 1995).

Hereditary Cu toxicosis is known as Wilson’s disease. Cu accumulates in the liver and affects infant growth, and evidence of histological damage can be seen in early infancy.

However, clinical illness is usually not observed before the age of 5 years. Wilson’s disease is rare and can be arrested and prevented by drug therapy. For most individuals haemostatic mechanism for Cu prevents excessive accumulations (Baker and Bowers, 1998). An excessive amount of Cu and Zn interferes with certain enzyme systems, for example; it interferes with membrane adenosine triphosphate and pyruvic acid -pyruvate oxidative systems.

2.3.5 Manganese

Mn is a metal with important industrial metal alloy uses, particularly in stainless steels (Baker and Bowers, 1998). Soils derive all their Mn content from the parent material, and the composition found in the mineral soils reflects the composition in the parent material. In soils, Mn commonly occurs as the oxides mineral birnessites and vernadite (Sharma and Forester, 1995). If a baby formula that already contains Mn is prepared with water containing Mn, the infant may get higher dose than the rest of the family. Further more young children appear to absorb more and excrete less Mn than older age groups. This adds up to a greater potential to exposure in very young children. Some studies suggest that early childhood and parenthood exposure to Mn can have effects on behaviour and learning. Thus, it is important to know what the Mn level in drinking water is when using it to make baby formula.

Occupational exposure to Mn, can lead to a disease similar to Parkinson’s disease. Pneumonia and coughs have been observed in workers exposed to Mn and recently in Japan where respiratory illnesses were observed in students near a factory emitting Mn. It has also been observed that, men exposed to Mn have impaired fertility as well as impotence and loss of libido. Other problems associated with exposure include; harm to lungs, neurological damage and harm to the reproductive system (Sharma and Forester, 1995). Excess manganese interferes with the absorption of dietary iron. Long-term exposure to excess levels may result in iron deficiency anemia. Increased manganese intake impairs the activity of copper metallo-enzymes.

A major development was the discovery that steel could be made highly resistant to corrosion and discoloration by adding Cr to form stainless steel. This application, along with chrome plating (electroplating with Cr) is currently the highest-volume uses of the metal. Cr and ferrochromium are produced from the single commercially viable ore, chromite, by silicothermic or aluminothermic reaction or by roasting and leaching processes (Alloway, 1995). There are two major sources of Cr contamination, wastewater metal finishing industries (hexavalent Cr) and tanneries (trivalent Cr). Cr occurs most frequently as Cr (VI) or Cr (III) in aqueous solutions. Both valences of Cr are potentially harmful but hexavalent Cr has a greater risk due to its carcinogenic properties (Dakikiy *et al.*, 2002). Hexavalent chromium, which is primarily present in form of chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$), poses significantly higher levels of toxicity in comparision with other valence states (Sharma and Forester, 1995). Toxicity of hexavalent chromium, even in small concentrations, has been well documented. Since the addition of Cr ions through industrial waste effluents into natural bodies of water causes serious environmental disruption, strict wastewater standards have been setup in many countries.

In Japan, the standard on wastewater quality states that the maximum level permitted in wastewater is 2 mg/dm^3 for total Cr and 0.05mg/dm^3 for Cr (VI), while the FAO/WHO maximum permitted level of Cr in drinking water is 0.05 mg/dm^3(Masakazu, 2003). Cr (VI) compounds are absorbed through the digestive tract, skin or alveoli of the lungs. Body depots of Cr are principally the lungs, muscles, fat and skin. Cell fraction studies reveal that about half of the body chromium is within the cell nucleus. Inhalation of dust or mist of hexavalent chromium is irritating to upper respiratory parts and causes sneezing, nasal discharge and vascular congestion. Bronchospasm resembling an asthmatic attack may occur and could cause death if it is prolonged. Cr is a chemical carcinogen connected with long-term occupation exposure that produces bronchogenic carcinomas (Masakazu, 2003).

2.4 Biosorption

In the 1970's increasing environmental awareness and concern led to a search for new techniques capable of inexpensive treatment of polluted wastewaters with metals.

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on binding capacities of various biological materials. To date, research in the area of biosorption suggests it to be an ideal alternative for decontamination of metal containing effluents. Biosorbents are attractive since naturally occurring biomass/adsorbents or spent biomass can be effectively used.

Biosorption is a rapid phenomenon of passive metal sequestration by the non-growing biomass/adsorbents (Ahalya *et al.*, 2006). Results are convincing and binding capacities of certain biomass/adsorbents are comparable with the commercial synthetic cation exchange resins. The biosorption process involves a solid phase (sorbent or biosorbent; adsorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (adsorbate, metal).

Due to the higher affinity of the adsorbent for the adsorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound adsorbate species and its portion remaining in the solution. The degree of adsorbent affinity for the adsorbate determines its distribution between the solid and liquid phases. There are many types of adsorbents; Earth's forests and plants, ocean and freshwater plankton, algae and fish scales, all living creatures, including animals are all "biomass/ adsorbents". The renewable character of biomass that grows, fuelled directly or indirectly by sunshine, makes it an inexhaustible pool of chemicals of all kinds. Biosorbents intended for environmental bioremediation applications are waste biomass of crops, algae, fungi, bacteria, among others, which are naturally abundant. Numerous chemical groups have been suggested to contribute to biosorption. Biosorption by microorganisms have various disadvantages, and hence many low cost adsorbents (industrial/agricultural waste products/byproducts) are increasingly used as biosorbents (Ahalya *et al*., 2006).

2.4.1 Importance of Biosorbents in Water Treatment

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Mahvi, 2008). Algae, bacteria and fungi and yeasts have proven to be potential metal biosorbents (Volesky and Holan, 1995). The major advantages of biosorption over conventional treatment methods include (Plazinski and Rudzinski, 2010):

- Low cost;
- High efficiency;
- Minimisation of chemical and /or biological sludge;
- No additional nutrient requirement;
- Regeneration of biosorbents;
- Possibility of metal recovery.
- Use of naturally abundant renewable biomaterials that can be cheaply produced; Biosorbents cost about US$ 4-7/kg while ion exchange resins cost about ten times more (US$ 30-50/kg). Reverse osmosis is even more costly. Precipitation is inefficient at low ion concentrations and generates toxic sludge which is difficult to dispose of.
- Ability to treat large volumes of wastewater due to rapid kinetics;
- High selectivity in terms of removal and recovery of specific heavy metals;
- Ability to handle multiple heavy metals and mixed wastes;
- High affinity, reducing residual metals to below 1 ppb in many cases;
- Less need for additional expensive reagents;
- Operation over a wide range of physiochemical conditions including temperature, pH, and presence of other ions (including Ca (II) and Mg (II));

- Relatively low capital investment and low operational cost;
- Greatly improved recovery of bound heavy metals from the biomass;
- Greatly reduced volume of hazardous waste produced.

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbates, metal ions). Due to the higher affinity of the sorbent for the sorbates species, the latter is attracted and bound there by different mechanisms. The process continues until equilibrium is established between the amount of solid-bound sorbates species and its portion remaining in the solution (Igwe *et al*., 2008; Tarley and Arruda, 2004; Volesky, 1999; Volesky and Holan, 1995). The degree of sorbent affinity for the sorbates determines its distribution between the solid and liquid phases

2.5 *Moringa oleifera*

Plate 1, 2 and 3 show the pictures of *Moringa oleifera* tree, pods and seeds, respectively. *Moringa oleifera* a medium sized tree species (plate 1) has gained importance due to its multipurpose usage and well adaptability to dry and hot climates of north-western plains, central India and dry regions of peninsular India (Pandey *et al*., 2011). The *Moringa Oleifera* tree is a perennial plant that grows very fast, with flowers and fruits appearing within 12 months after planting. The tree grows up to a height of 5–12 meters with branches extending between 30 and 120 cm (Arnoldsson *et al*., 2008). Knowledge that *Moringa oleifera* seeds can purify water is not new; the seeds have been used for water treatment for generations in countries like India and Sudan. For example, the women of

Sudan have used the seeds from the *Moringa oleifera* tree for water treatment since the beginning of the 20th century through a technique that comprehended the swirling of seeds in cloth bags with water for a few minutes and let it settle for an hour. The value of *Moringa oleifera* for edible pods (plate 2), seed oil, fodder, and for medicinal use was well known to Indians from time immemorial. An exceptionally nutritious vegetable tree

Plate 1: Tree of *Moringa oleifera* species
(SOURCE: AUTHOR, 2015)

Plate 2: Pods of *Moringa oleifera*
(SOURCE: AUTHOR, 2015)

Plate 3: Seeds of *Moringa Oleifera*
(SOURCE: AUTHOR, 2015)

with a variety of potential uses was grown as backyard species originally by the Dravidians (south Indians) and later by the Aryans (north Indians).

The genetic resource value of the species finds its entry in the earliest records in Ain-i-Akbari (400 years old) listing of plants used as favourite pickles by the north Indians. Only in the last two centuries it was introduced to other parts of the world mainly as an ornamental and multipurpose species (Pandey *et al*., 2011). This species has been principally utilized for fruit and leaves as vegetable, and to some extent for edible flowers and seed oil particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa. *Moringa Oleifera* leaves also posses significant antimicrobial activity against gram positive and negative fungal species (Dahot, 1998).

The oil from the seeds of *Moringa oleifera* is used as edible oil, an excellent salad oil, illuminant, and lubricant, as biofuel and in cosmetic industry. The seeds yield 38–40% of non-drying, sweet, odourless and clear oil that resembles the olive oil. Other multipurpose uses of the species are met from- plant (as hedge and agro/social forestry), leaves (fodder), seeds (seed cake as fertilizer), roots (especially from seedlings; pickle with vinegar), fuel wood (soft, porous and yellowish), bark gum (used for food seasoning and in calico printing), flowers (good source of nectar) and coarse fibre. The seed powder is used in treatment of rheumatism, venomous bites, fever, cardiac and circulatory diseases, abdominal tumours, counter-irritant, external stimulant of skin, purgative, expectorant, mild diuretic, epilepsy and hysteria. The coagulating ability of the seed powder has been used to purify water to make it suitable for drinking in arid regions. It is ten times cheaper than ion exchange resins for removal of heavy metals (Sharma *et al*. 2007).

Moringa oleifera seed shows good coagulating properties, and has many advantages as it does not affect the pH, alkalinity or conductivity of the water and it can be produced locally at low cost (Arnoldsson *et al*., 2008). An indigenous water treatment method uses *Moringa oleifera* seeds in the form of a water-soluble extract in suspension, resulting in an effective natural clarification agent for highly turbid and untreated pathogenic surface water. Efficient reduction (80.0% to 99.5%) of high turbidity produces an aesthetically clear supernatant, concurrently accompanied by 90.00% to 99.99% bacterial reduction (Lea, 2010).

Moringa oleifera seed (plate 3) has not only been studied for its coagulating properties but also its removal of heavy metals from aqueous solutions. One such study showed that with an initial Cd concentration of 7 mg/l, *Moringa oleifera* seed powder, at a dose of 2.50 g/100 mL, reduced the concentration of Cd by 58%. The increase in initial Cd (II) concentration resulted in a decrease of percent removal of the Cd (II). For a fixed dose of the biomass the total available binding sites are limited, thereby binding the same amount of metal ions. This resulted in a decrease in percentage removal of the metal ions as the initial metal ion concentration increased (Mataka *et al*., 2006).

In a similar study, *Moringa stenopetala* (a different species of moringa) was investigated for lead removal by means of jar tests. With an initial Pb concentration of 7 ppm, *Moringa stenopetala* seed powder, at doses of 0.50, 1.00, 1.50, 2.00 and 2.50 g/100 mL, reduced the concentration of Pb by 20.00 ± 0.00, 46.19 ± 2.06, 71.19 ± 2.06 and 89.43 ± 0.60 and 96.23 ± 0.12 %, respectively. The study thus showed that *Moringa stenopetala* seed powder can be used as an effective heavy metal purifier in water (Mataka, 2005). *Moringa oleifera* sorption of As has also been studied and showed that maximum sorption for As (III) and As (V) species is 60.21 and 85.6%, respectively. Protein/amino acid–Arsenic interactions were found to play an important role in the biosorption process using plant biomass of *Moringa oleifera* (Kumari *et al*., 2005).

Moringa oleifera wood, a solid waste has been used and studied for the preparation of activated carbon for the removal of Cu, Ni and Zn from synthetic wastewater. Effects of various operating variables namely solution pH, contact time, carbon dose, adsorbate concentration and temperature on the removal of metal ions were studied. The *Moringa oleifera* wood charcoal was found to be a promising adsorbent for the removal of Cu, Ni and Zn from aqueous solution. The data from the study showed that the *Moringa oleifera* wood was an inexpensive, indigenous, easily available material and *Moringa oleifera* wood charcoal had good potential in treating metal laden industrial effluents (Kalavathy and Miranda, 2010). In a relatively similar study, the bark of *Moringa oleifera* was used to study the biosorption of Pb^{2+} in aqueous solution. Likewise, parameters that influence the biosorption such as pH, biosorbent dose, contact time and concentration of metal ion were investigated. The experimental equilibrium adsorption data were tested by four widely used two-parameter equations, the Langmuir, Freundlich, Dubinin–Radushkevich (D–R) and Temkin isotherms. It was concluded that *Moringa oleifera* bark was an effective and efficient biosorbent for removal of Pb^{2+} ions from aqueous solution. *Moringa oleifera* bark was considered a viable alternative to activated carbon, ion-exchange-resin and other synthetic adsorbents used for this purpose (Reddy *et al*., 2010).

2.5.1 *Moringa oleifera* for Water purification

Moringa oleifera seeds contain between 30-42 % oil and the press cake obtained as a by-product of the oil extraction process contains a very high level of proteins especially lectins and glycoproteins. Approximately 1% of the proteins are active cationic polyelectrolytes having molecular weights between 7-17 kDa. The cationic polyelectrolytes neutralize the colloids in muddy or dirty water since the majority of these colloids have a negative electrical charge. The proteins can therefore be used as a non-toxic natural polypeptide for sedimenting mineral particles and organics in the purification of drinking water, for cleaning vegetable oil, or for sedimenting fibers in the juice and beer industries. It thus works as a primary coagulant as natural bridges are continuously formed between the colloid particles. In contrast, industrial coagulants such as alumina can be toxic. Their proper use requires qualified personnel and the majority of underdeveloped countries don’t have the means of producing them. In addition, these industrial coagulants are expensive and represent a considerable drain on the hard currency reserves of developing countries (Muyibi *et al*., 2002b).

The properties of the natural polypeptides produced from the seeds of *Moringa oleifera* have been known for many centuries in China. With the colonization of India by the British, this knowledge was effectively dispersed to the rest of the world. It has been employed with particular effectiveness in both Egypt and Sudan for cleaning water from the Nile specifically for human consumption. The wings are removed from the dry seeds and then the seeds are ground to powder. The powder is mixed with water, agitated for approximately five minutes and after about an hour it is filtered through a piece of woven fabric to obtain pure water. Alternatively, a cloth containing the seed powder is suspended in water, generally overnight, to coagulate impurities. The cloth containing the seeds is then removed, and the purified water is decanted leaving behind the coagulated particles on the bottom. Up to 99 % of colloids can be removed. Only one seed is required per litre for slightly contaminated water and two seeds for very dirty water (Odee, 1998).

2.6 Applications of natural adsorbents in biosorption in the recent past

Biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals from even very little aqueous solutions. Biomass exhibits this property, acting just as a chemical substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi, bacteria and even higher plants which was found responsible for this phenomenon. Pioneering research on biosorption of heavy metals at McGill University in Montreal led to identification of a number of microbial biomass types which are extremely effective in concentrating metals. Some of the biomass types come as a waste by-product of large-scale industrial fermentation (the mold Rhizopus or the bacterium *Bacillus subtilis*). Other metal-binding biomass types, certain abundant seaweeds (particularly brown algae for example, Sargassum, Ecklonia), can be readily collected from the oceans (Davis *et al*., 2003).

These biomass types, serving as a basis for metal biosorption process, can accumulate in excess of 25% of their dry weight in deposited heavy metals. Research on biosorption has revealed that it is a complex phenomenon where the metallic species could be deposited in the solid biosorbent through different sorption processes of ion exchange, complexation, chelation, microprecipitation, among others (Davis *et al*., 2003). A whole new family of suitably ‘formulated’ biosorbents can be used in the process of metal removal and detoxification of industrial metal-bearing effluents. Recovery of the deposited metals from saturated biosorbents can be accomplished because they can be easily released from the biosorbent in a concentrated wash solution which also regenerates the biosorbent for subsequent multiple use. Different types of discipline, from engineering to biochemistry, can make a significant contribution in elucidating the biosorption phenomenon (Davis *et al*., 2003).

Many industrial effluents contain toxic metals that must be removed. Removal can be accomplished with biosorption techniques. It is an alternative to using man-made ion-exchange resins, which cost ten times more than biosorbents (Abdelkrim *et al*., 2011). The cost is so much less, because the biosorbents used are often wastes from farms or they are very easy to regenerate, as is the case with seaweed and other unharvested biomass. Industrious biosorption is often done by using sorption columns, where effluent containing heavy metal ions is fed into a column from the top. The biosorbents adsorb the contaminants and let the ion-free effluent to exit the column at the bottom. The process can be reversed to collect a highly concentrated solution of metal contaminants. The biosorbents can then be re-used or discarded and replaced. In recent years, a lot of work has been done on to the removal of heavy metals from waste water by using adsorption techniques with different low-cost materials. Commercial activated carbon (Davis *et al*., 2003), various cellulosic materials such as nuts and walnuts shells (Al-Asheh *et al*., 2000), tree barks (Lopez *et al*., 1995), rice hulls (Mahvi *et al*., 2007), and industrial wastes such as fly ash (Dadhich *et al*., 2004), animal bones (Al-Asheh *et al*., 2000) are just a few examples of low-cost materials used in the removal of Cu(II) and Cd(II).

Other biosorption studies have utilized biomass types such as seaweed, bacteria, fungus (Yun *et al*., 2001), and even human hair for copper removal ((Mahvi *et al*., 2005). Cationic metals are effectively removed by brown algae (Davis *et al*., 2003), with sargassum species probably the most widely studied because of their high metal uptake capacity and both mechanical and chemical resistances. Crab shells have been discovered to bind anionic metal complexes (Volesky, 2001). Macroalgae, especially brown algae, have been found to be very effective biosorbents in removing heavy metals from wastewater because of their high uptake capacities, similar to commercial ion-exchange resins and their availability in nearly unlimited amounts from the ocean. In China, *Laminaria japonica*, an abundant waste product is economically used as a potential biosorbent for heavy metals removal from aqueous solutions. The raw alga is chemically pretreated in order to reinforce it for sorption process applications and also to enhance the sorption performance (Davis *et al*., 2003).

In Japan, activated carbon from cheap and readily available sources such as coal, coke, peat wood, rice husk and tree leaves may be successfully employed for the removal of Cd and other toxic heavy metals from aqueous solution (Yun *et al*., 2001; Dadhich *et al*., 2004). Other adsorbents such as a wood charcoal, red mud, sun flower stalks, petioles felt-sheath and rice husks have also been used for the adsorption of Cd (Lopez *et al*., 1995). The feasibility of *Platanus orientalis* leaf and its ash, as adsorbent for removing Cd from aqueous solution, was investigated. The results indicated that adsorption of Cd increased with increasing contact time and became almost constant after 60 minutes.

The results also indicated that the sorption process could be considered very fast because of the largest amount of Cd attached to sorbent within the first 60 minutes of adsorption. Experiments, concerning the effect of pH on the sorption, were also carried out with the range of pH that was not influenced by the metal precipitation as metal hydroxide. The suitable pH ranges for cadmium was performed for the pH range variations of 3 to 9. Adsorption of Cd on POL and its ash increased with increasing initial concentration of Cd. The results could be explained by an increase in the number of metal ions competing for the available binding sites in the adsorbent for complexation of Cd ions at higher concentration levels. The percentage adsorption of Cd on rice husks and its ash increased as the pH of the solution increased and reached a maximum value at pH 9 (Mahvi *et al*., 2005).

Adsorption of metal cations on adsorbent depends upon the nature of adsorbent surface and species distribution of the metal cation. Surface distribution mainly depends on pH of the system (Namasivayam and Ranganthan, 1995). Percent adsorption of metal ion decreased with the decrease in pH, because H^+ compete with metal ion for sorption sites on the adsorbent surface as well as the concomitant decrease of negative charge of the same surface (Mahvi *et al.*, 2005). It has been reported that precipitation of Cd starts at pH 8.3 (Ajmal *et al.*, 2005). The results indicated that adsorption of Cd increased with increase in contact time and became almost constant after 45 min for rice husks and 30 min for its ash (Mahvi *et al.*, 2005).

These results also indicate that the sorption process can be considered very fast because of the largest amount of Cd attached to the sorbent within the first 30 min of adsorption. Similar results have been reported by Ajmal *et al.* (2005) and Namasivayam and Ranganathan (1995). The percentage adsorption increased from 95 to 97.8% for rice husks and from 96 to 99.4% for rice husks ash when adsorbents doses were increased from 0.5 to 10 g/L for both sorbents, but at the same time, adsorption density decreased from 38.02 to 1.95 mg/g for rice husk and from 38.4 to 1.99 mg/g for its ash (Mahvi *et al.*, 2005). Similar results were reported by other researchers such as Namasivayam and Ranganthan (1995). The efficiency of tea waste has been determined in processing heavy metal removal from both single metal solutions and various mixtures. Metals of interest were Cd and Pb. They were chosen based on their industrial applications and potential pollution impact on the environment. Cd is an inessential and useless element to plants and animals. Pb is a hazardous waste and is highly toxic to humans, plants and animals (Low *et al.*, 2000). In addition, Ni is also one of the important toxic metals to humans, plants and animals (Ajmal *et al.*, 1998). Matheickal and Yu (1999) observed that the maximum uptake capacities of *Durvillaea potatorum* and *Ecklonia radiata* for Pb^{2+} were 1.6 and 1.3 mmol/g, respectively.

Biosorption of Pb (II) and Cd (II) from aqueous environments by brown algae sargassum spp. biomass was studied by Nabizadeh *et al*. (2005). Biosorption of Cd (II) and Pb (II) from aqueous solutions using various biomasses has also been studied. Cd (II) biosorption on Asppergillus oryzae reached equilibrium in 1 h with 90% biosorption taking place in the initial 10 minutes (Ahalya, 2003). Kinetic data of cadmium (II) biosorption by chitin presented high correlation with the pseudo-second order rate equation (Benguella and Benaissa, 2002). Scott and Karanjkar (1995) studied Cd (up to 25 ppm) adsorption on to biofilm covered granular activated carbon. Feasibility of rice husk and its ash as an adsorbent for the removal of Cd from aqueous solution was investigated by Mahvi *et al*. (2005). The results of this study indicated that the adsorption efficiency is maximum for Pb and minimum for Cd.

Ayodele and Okeoghene (2014) studied the adsorption capacity of eggshell in removing Pb^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+} and Co^{2+} from standard solution and consolidated brewery effluent. The result indicated that the affinity of metal ion sorption was in the order of $Co^{2+} > Pb^{2+} > Ni^{2+} > Zn^{2+} > Mn^{2+}$ and the percentage adsorptions of Mn^{2+} and Zn^{2+} from the brewery effluent were 95.86% and 44.29%, respectively, while the corresponding percentage desorption of Mn^{2+} and Zn^{2+} were 19.94% and 35.48%, respectively. Their results also indicated that the sorption process dependent on pH, contact time, initial metal ion concentration and sorbent dosage with pH 7.0 being the optimum value, and maximum sorption was attained within the first 60 minutes. The equilibrium sorption data followed the Langmuir and Freundlich isotherms with R^2 ranges of 0.834 – 0.993 and 0.939 – 0.998, respectively. The kinetic data were best described with pseudo second order kinetic.

Emine *et al*. (2010) studied the behaviour of Cu^{2+} and Zn^{2+} biosorption onto raw and modified *Roccella phycopsis* from aqueous solutions. Modification process was applied by autoclaving at 121°C for 30 minutes. The effects of pH, initial metal concentration and biosorbent dosage were investigated. The maximum Cu^{2+} biosorption was achieved at pH 5.0 and the maximum biosorption capacities of 31.5 and 37.8 mg/g were recorded for raw and modified biosorbent, respectively. In the case of Zn^{2+} biosorption, maximum biosorption capacities were obtained at pH 4.0 as 29.1 and 35.3 mg/g for raw and modified biosorbent, respectively. Biosorption of Zn^{2+} and Cu^{2+} on all form of *R. phycopsis* increased much quickly with increasing initial metal concentrations from 10 to 100 mg/L.

Abdelkrim *et al*. (2011) studied Cu ions biosorption properties of biomass derived from Algerian Sahara plants. The results indicated that the process of biosorption reached equilibrium in 04 hours and the biosorption of metals was pH and temperature dependent, respectively optimal pH was 5 and temperature was 25-30ºC. Better results were obtained with *Acacia raddiana* bark, *Retama reatam* and *Tamarix galliga* with percentage of copper cations removal for the same initial concentration (100 mg l^{-1}), being;: 86%, 79%

and 81%, respectively. Their result demonstrates the possibility of usage of inexpensive biosorbent, as suitable alternatives for the removal of copper ions from wastewater.

Agricultural by-products have been widely studied for metal removal from water. Peat, wood, pine bark, banana pith, soybean and cotton hulls, rice bran, saw dust, wool, orange peel have been demonstrated to remove heavy metals from wastewater (Ozer and Ozer, 2004). These agricultural waste biosorbents are inexhaustible, cheap and non-hazardous materials, which are specifically selective for heavy metals and can be easily disposed by incineration. The feasibility of using bio-waste obtained from fruit juice industry for the removal of toxic heavy metals, Hg (II), Pb (II), Cd (II), Cu (II), Zn (II), and Ni (II), from wastewaters was explored by Senthilkumaar *et al*. (2000). Fruit residues and phosphate fruit residues were separately used as biosorbents in which the latter showed higher biosorption capacity.

The pH of solution was identified as the most important variable influencing metal adsorption on biosorbents. Sorption of six metal ions (Pb^{2+}, Ni^{2+}, Cd^{2+} , Cr^{3+} and Zn^{2+}) from contaminated water by petiolar felt-sheath of palm was studied (Iqbal *et al*., 2005). The uptake was rapid, with more than 70% completed within 15 min. This biosorbent was found to remove all toxic metal ions efficiently with selectivity order of $Pb^{2+} > Cd^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Cr^{3+}$. Adsorption behaviour of Ni (II), Zn (II), Cd (II) and Cr (III) on untreated and phosphate-treated rice husk was studied by Ajmal *et al.* (2003). The adsorption of Ni (II) and Cd (II) was found to be greater when phosphate treated rice husk was used as an adsorbent.

Sorption of Cd (II) was dependent on contact time, concentration, temperature, adsorbent doses and pH of the solution. Basci *et al*. (2004) reported the adsorption capacity of wheat shell for Cu (II) from aqueous solutions. The maximum biosorption efficiency was at pH 5. An increase in Cu concentration had a negative effect on the efficiency of biosorption. Sorption of Cr(III) ions from aqueous solutions by hen eggshells was investigated (Katarzyna, 2006). Effect of process parameters [pH, temperature, and initial concentration of Cr(III) ions] was studied. The potential of papaya wood waste to treat wastewaters contaminated with heavy metals Cd(III), Cu(II) and Zn(II)) was studied by Saeed *et al*. (2005a). Sorption was most efficient at pH 5 during contact time of 60 min. Metal ion biosorption was found to increase as the ratio of metal solution to the biomass quantity decreased. The affinity of papaya wood to biosorb metals was in the order of Cu (II) > Cd(II) > Zn(II). Removal and recovery of Pb(II) from single and multi metal (Cd, Cu, Ni, and Zn) solutions by crop milling waste (black gram husk) was reported by Saeed *et al*. (2005b).

Selectivity order of the biosorbent for metals was Pb> Cd > Zn > Cu > Ni. Complete desorption of Pb and other metals in single and multi metal solutions was achieved with 0.1 M HCl in both shake flask and fixed bed column studies. Biosorption characteristics of dried sugar beet pulp for removing Cu (II) from aqueous solutions revealed that at 250 mg L^{-1} initial Cu (II) concentration and pH 4, highest Cu (II) uptake capacity (28.5 mg g^{-1}) at 25°C was observed (Aksu and Isoglu, 2005). The capacity of raw rice bran to remove Cr and Ni from aqueous solutions was investigated by Oliveira *et al*. (2005). The adsorption equilibrium was modeled using the Langmuir and Freundlich isotherm and the experimental data were well fitted to the Freundlich equation. The possibility of utilization of the rice bran for sorption of Cu ions from aqueous solutions was also studied by Wang and Qin (2005). The experimental results were fitted to the Langmuir, Freundlich, Temkin and Redlich-Peterson isotherms to obtain the characteristic parameters of each model. Except Freundlich, the sorption data fitted well in other three isotherm models. Hossain and Kumita (2005) studied the dynamic characteristics of Cr (VI) sorption using black tea leaves as biosorbent. Batch experiments were conducted to evaluate the effects of Cr (VI). Experimental and calculated kinetics data for equilibrium were expressed by Langmuir effect on the adsorption rate.

2.7 Parameters affecting Sorption

The investigation of factors affecting the efficiency of heavy metal sorption is of great interest for the industrial community. The efficiency is strongly influenced by the physico-chemical characteristics of the solutions, such as pH, temperature, initial metal concentration, presence of other ions and sorbent dosage (Arief *et al.*, 2008). These factors are important in evaluating the maximal sorption performance of any sorbent.

2.7.1 pH

pH has a very significant effect on biosorption of metal ions from solutions. It is known that the pH of a solution affects the surface charge of the biomass, the specification of the functional groups such as carboxylate, phosphate, hydroxyl and amino groups of the cell wall (Vilar *et al.*, 2006). The pH of the solution is the most important parameter that in the biosorption process can significantly influence the removal of heavy metals. The solution pH is a crucial factor in heavy metal sorption. The pH value significantly influences the dissociation of the sorbent and the solution chemistry of the heavy metals, that is, metal speciation, hydrolysis, complexation by organic and/or inorganic ligands, redox reactions and precipitation are all pH dependent (Arief *et al*., 2008). Competition between cations and protons for binding sites means that sorption of metals like Cu, Cd, Ni, Co and Zn is often reduced at low pH values (Gadd, 2004). Due to the importance of pH in sorption; many studies have been conducted on its effect on the removal of various heavy metals. Nuhoglu and Oguz (2003) reported that the removal of Cu(II) from aqueous solution increased as pH was increased, and that the maximum adsorption occurred at a pH of 7.7. Pino *et al. (2006)* noted that the removal of Cd and As by green coconut shell increased from 69% at pH 4 to 98% at pH 7.

Cu^{2+} adsorption onto activated rubber and wood sawdust was found to be pH-dependent and maximum removal was observed at pH 6 (Kalavathy *et al*., 2005). The optimum pH for As^{3+} removal (96.2%) by activated alumina was reported to be 7.6. Yu *et al. (2000)* showed that the greatest increase in the sorption rate of Cu^{2+} on sawdust was observed in the pH range 2 to 8. During the biosorption of Cu^{2+}, Pb^{2+}, Zn^{2+}, and Cd^{2+} by Nile rose plant, Abdul-Ghani and Elchaghaby (2007) found out that the sorption efficiencies were pH-dependent, increasing with increasing pH from 2.5 to 8.5, except for Pb^{2+}. Other researchers, including Anurag *et al.* (2007), Pehlivan *et al.* (2009), Sari and Tuzen (2009) and Rahaman *et al.* (2008) also reported similar trends in pH influence on biosorption of heavy metals such as Cu, As and Pb.

Abdelkrim *et al*. (2011) reported that the greatest increase in the biosorption rate of Cu^{2+} ions on the plant biomass (*Acacia raddiana*, *Retama raetem*, *Anabasis aretioides* and *Tamarix gallica*) was observed at pH 5. At lower pH, H^+ ions compete with copper cation for the exchange sites in the system. The heavy metal cations are completely released under circumstances of extreme acidic conditions. It was also observed that at pH (3 - 7) there are three species present in solution, Cu^{2+}, $CuOH^+$ and $Cu(OH)_2$. The species are adsorbed by electrostatic interaction at the surface of the biomass, and as the pH decreases, the surface of the *A. raddiana*, *T. gallica* and *R. raetam* plants exhibits an increasing positive character. H^+ ions are present at a higher concentration in the reaction mixture and compete with Cu^{2+} ions for the biosorption sites resulting in the reduced uptake of Cu cation.

At higher pH values, precipitation of $Cu(OH)_2$ occurs and both sorption and precipitation would be the effective mechanisms to remove the Cu ions in aqueous solution. At around pH 5, Cu cations, mainly Cu^{2+}, would be expected to interact more strongly with the negatively charged binding sites in the sorbent (Abdelkrim *et al*., 2011). Similar effects of pH on cadmium and lead binding have also been observed for bacteria such as *Lactobacillus rhamnosus* LC705 (Ibrahim *et al*., 2006), *Lactobacillus plantarum* (Seki *et al*., 2006), *Micrococcus luteus* (Seki *et al*., 2006), *Bacillus subtilis* (Fein *et al*., 1997), *Pseudomonas putida* (Pardo *et al*., 2003), *Pseudomonas aeruginosa* (Chang *et al*., 1997), *Sphaerotilus natans* (Esposito *et al*., 2002) and a Citrobacter strain (Puranik and Paknikar, 1999).

Many studies have also shown that pH is an important factor affecting absorption of heavy metals (Huang *et al.*, 1990; Sanchez *et al*, 2006). The dependence is closely related to the acid-base properties of various functional groups on the adsorbent surfaces (Pereira and Arruda, 2003). The change in initial pH affects the adsorptive process through dissociation of functional groups on the active sites on the surface of the adsorbent.

2.7.2 Temperature

Depending on the structure and surface functional groups of a sorbent, temperature has an impact on the adsorption capacity within the range 20–35 °C (Aksu *at al.*, 1992). It is well known that a temperature change alters the adsorption equilibrium in a specific way determined by the exothermic or endothermic nature of a process (Arief *et al.*, 2008). Higher temperatures usually enhance sorption due to the increased surface activities and kinetic energy of the solute. However, physical damage can be expected at higher temperatures (Vijayaraghavan and Yun, 2007). It is always desirable to evaluate the sorption performance at room temperature, as this condition is easy to replicate. The impact of temperature on the adsorption isotherm of Cu^{2+} and Cd^{2+} by corncob particles at a certain pH was explored by Shen and Duvnjak (2004). They found that the uptake of metal ions increased at higher temperature. Igwe and Abia (2006) noted that temperature and particle size are very crucial parameters in biosorption reactions. They investigated the effects of these two parameters on the biosorption of As(III) from aqueous solution using modified and unmodified coconut fibres and found that the most suitable temperature was 30 °C.

2.7.3 Presence of other ions

Real industrial effluent usually contains various ionic components, including metal cations and anions. Some studies indicated that cations and anions additional to the ions of interest have a generally detrimental impact on metal accumulation (Suh and Kim, 2000). Usually, the biosorption capacity of one metal ion is interfered and reduced by co-ions, including other metal ions and anions present in solution-: however the gross uptake capacity of all metals in solutions remains almost unchangeable. Competitive effect occurred in a mixed solution containing Pb and Cr during the biosorption process by flocculating brewer's yeast (Ferraz and Teixeira, 1999). The yeast of *S. cerevisiae* seems to have more affinity, higher selectivity and biosorption capacity to Pb (II) than to Cr(III) in aqueous solutions. The decrease of metal uptake in competitive conditions was thought to be a response to increased competition between same charged species for binding sites of the yeast cells.

The presence of other ions (co-ions) can affect the sorption of metal ions (primary ion) to the biomass and it is called competitive adsorption (Mishra *et al*., 1998). In reality, wastewater is a mixture of different metals and competition among the co-ions are common. This interaction can be synergistic, antagonistic or non-interactive, and cannot be predicted on the basis of single metal studies (Ting and Teo, 1994; Tsezos *et al*., 1996). During biosorption on biosorbents, metal ions often compete with each other for surface binding sites (Kolodynska and Hubicki, 2008; Satiroglu *et al*., 1998). For example, mutual inhibition was observed during the sorption of $Cd^{2+,}$ Cu^{2+} and Zn^{2+} on brown algae *Ascophyllum nodosum* (Leusch *et al*., 1995), Zn^{2+} being the weakest bound ion and Cu^{2+} the most strongly bound ion (Chong and Volesky, 1996). Similarly, Pb^{2+}, Cd^{2+} and Cu^{2+} sorption on the fungus *Aspergillus niger* was found to be lower when the metal ions were present in a mixture than when they were present individually in the solution (Kapoor and Viraraghavan, 1997). In addition, another study showed the 2–57 about these figures influence of Cd^{2+}, Cu^{2+}, Co^{2+} and Ni^{2+} on Pb(II) and Zn(II) sorption by *Streptovercillium cinnamoneum* and *Penicillium chrysogenum* (Puranik and Paknikar, 1999).

Mutual inhibition was observed in binary systems containing Zn^{2+}, whereas systems including lead displayed unequal inhibition. Furthermore recent studies have shown that light metals such as Na^{+}, Ca^{2+}, Mg^{2+}, and K^{+} competed with heavy metals for ionic sorption sites and diminished heavy metal sorption (Matheickal and Yu, 1999; Saha *et al.*, 2001). However, the inhibition of the metal ion binding to microbial biomass by Na^{+}, and K^{+} was shown to be lower than the uranium or radium binding inhibition by heavy metals such as Zn^{2+}, $Cu^{2+}$and Fe^{2+} (Tsezos and Georgousis, 1997). The binding strength to the biomass depends on metal chemistry (including parameters such as the charge, the ionic radius, and the Pauling electronegativity), affinity for binding sites, and the type of metal binding (electrostatic or covalent) (Fourest and Roux, 1992; Veglio and Beolchini, 1997).

Lead binding by *B. longum* 46 followed a similar pattern, but a slight reduction in binding was observed in the presence of iron and cadmium. Similarly, only a minor decrease in Pb removal was observed by *Citrobacter* MCM B-181 (Puranik and

Paknikar, 1999) and *Rhodotorula glutinis* (Cho and Kim, 2003) from binary metal solutions with zinc, nickel, cobalt and copper, and potassium, sodium, calcium and magnesium, respectively. Many explanations have been suggested for the selectivity of some metals over others. Salehzadeh and Shojasadati (2003) studied the removal of cationic metals (Na, Ca, Mn, Ni, Sr, Zn and Mg) by both native and chemically modified extracted cell walls of *Bacillus firmus,* and observed that both the affinity and number of binding sites for each metal were different.

Okoronkwo and Anwasi (2010) studied the effect of cation interference on Cu and Zn binding by *Tithonia diversifolia* biomass, and observed that the presence of other ions impacted negatively on the removal efficiency of the biomass for Cu and Zn. Copper which hitherto without interfernce recorded near 100% adsorbtion to *Tithonia diversifolia* biomass had a significant reduction to about 63.57, 61.52 and 62.54% at 1M concentration of the interfering magnesium, calcium and mixed magnesium and calcium ions respectively. Similar impact was observed for zinc as its removal was reduced from 75% to 44, 46 and 43%, respectively. In all cases, the percentage metal removed decreased dramatically with increasing concentration of the interfering ions.

Fourest and Volesky (1997) found a correlation between metal uptake and the electronegativity of metals in four different brown seaweeds. Pardo *et al.* (2003) reported a positive linear correlation between metal adsorption constant and stability constant of first metal-hydroxy complex (such as, $PbOH^+$). Pavasant *et al*, (2006) classified metal ions into three groups (a, b and borderline) based on their preference for nitrogen, oxygen and sulphur containing ligands. This classification is also known as Pearson's Hard and Soft Acid Base (HSAB) theory (Atkins *et al.*, 2006). Pavasant *et al*, (2006) have classified magnesium and calcium to group 'a', Pb to group 'b' and zinc, iron and cadmium to the 'borderline' group. According to them, the metal ions belonging to the same group prefer the same types of ligand donor atoms, and therefore should exert the highest interference on each other.

2.7.4 Initial metal ion concentration

Removal of heavy metals by biosorbents critically depends upon the initial concentration of metals in the solution. Reports available in the literature indicate that the metal removal efficiency of algal biomass increased with increase in the initial metal concentration (Kiran *et al.*, 2005; Liu *et al*, 2010; Mehta and Gaur, 2005; Singh *et al.*, 2007; Ozturk, 2007). Ozer and Ozer (2004) also reported that Cu removal by *C. crispate* increased with increase in metal concentration and saturation plateau reached at 200 mg Cu L^{-1} and did not change with further increase in metal concentration, suggesting that available sites on the surface of *C. crispata* were the limiting factor for Cu biosorption. In contrast to total sorption, per cent removal of a metal generally decreases with its increasing concentration in solution (Mehta and Gaur, 2005).

The authors observed that *C. vulgaris* removed 70 and 80% of Ni and Cu, respectively, from their 2.5 mg L^{-1} solutions; however, only 37 and 42% of Ni and Cu, respectively, were removed from their 10 mgL^{-1} solution. If metal removal is to be explained in terms of percent (%) removal then it is desired that it should be explained as a percentage percent of the original amount. The increase in initial concentration of chromium resulted in the increased uptake capacity and decreased percent metal removal, since at high initial concentrations, the numbers of moles of chromium available to the surface area are high and adsorption depends upon initial concentration (Zubair *et al*., 2008).

The initial ion concentration can alter the metal removal efficiency through a combination of factors, that is: availability of functional groups on the specific surface and the ability of surface functional groups to bind metal ions (especially at higher concentrations). Higher initial concentration of heavy metal ions results in a high solute uptake (Ho and McKay, 1999). According to Arief *et al.* (2008), the initial concentration acts as a driving force to overcome mass transfer resistance to metal transport between the solution and the surface of the biomass.

The initial concentration of metal solution strongly influences the biosorption of metals ions (Fourest and Roux, 1992). As long as the binding sites are not saturated, the higher the initial concentration of the metal ion, the larger the metal uptake (Reddad *et al*., 2002). However, it is apparent that increasing the amount of the available metal results in decreasing the fraction of metal bound (Blanco *et al*., 1999). The relationship between variable initial metal concentrations and metal uptake, at a fixed temperature and fixed biomass concentration is commonly represented by sorption isotherms (Astier *et al*., 2012). In the study by Bhatti *et al*. (2010) biosorption of Pb (II) and Co (II) by *Citrus reticulata* waste (CWB) increased with increasing metal concentration in the solution and the percentage removal of both metals decreased with increase in concentration.

Maximum biosorption capacity of Pb^{2+} and Co^{2+} was observed in a solution with initial metal concentration of 800 mg/L. This was probably due to the fact that at high initial concentration the number of metal ions available to surface area was high, so biosorption became dependent on initial concentration. This biosorption characteristic indicated that surface saturation was dependent on the initial metal ion concentration.

A number of studies on the effect of the initial metal ion concentration have been undertaken in the past. Ajmal *et al*. (2005) found out that when the initial Cu^{2+} concentration was increased from 5 to 50 mg L^{-1}, the amount adsorbed increased from 8.8 to 96 mg L^{-1}, showing that adsorption of Cu^{2+} depends upon the initial concentration because the amount of Cu^{2+} adsorbed increased with increasing initial concentration. Gulnaz *et al.* (2005) also investigated the effect of initial Cu^{2+} ion concentrations between 100 and 400 mg L^{-1} at 20 °C and pH 4. They determined the biosorption capacity of dried activated sludge for the < 0.063 mm particle size was 76, 256 and 243 mg g^{-1} for 100, 200 and 400 mg L^{-1} initial Cu^{2+} concentrations, respectively. This indicated that the initial Cu^{2+} concentration is an important parameter for biosorption of Cu^{2+} by dried activated alumina. Anurag *et al*. (2007) also investigated the effect of initial concentration (25–200 mg L^{-1}) on biosorption of Cu^{2+} by agarose gels at 35 °C and reported that the removal was highest (70%) at an initial Cu^{2+} concentration of 25 mg L^{-1}. They explained that because at higher concentrations, more ions compete for limited binding sites on the agarose gels, the rate of adsorption decreases, resulting in a lower adsorption percentage. In a study by Mataka *et al*. (2010) the increase in initial Cd^{2+}

concentration decreased Cd percentage removals and increased the amount of Cd^{2+} uptake per unit mass of the *Moringa oleifera* powders (mg/g).

The removal of Cd(II) ions decreased from 12.9% (0.65 mg/g) to 6.2% (0.092 mg/g) for *Moringa oleifera* and 13.1% (0.65 mg/g) to 7.6 (0.113 mg/g) for *Moringa stenopetala*. Further, the results indicated that at several initial concentrations tested, *Moringa stenopetala* was more effective than *Moringa oleifera* for cadmium ion sorption ($p < 0.05$). Effect of initial metal ion concentration is an important parameter, which determines the maximum uptake capacity of the chelating biomass (Reddy and Reddy, 2003). For a fixed dose of the biomass the total available binding sites are limited, thereby binding the same amount of metal ions. This results in a decrease in percentage removal of the metal ions as the initial metal ion concentration increases because the number of sites remains the same. The enhanced metal uptake with an increase in initial metal ion concentration might be due to the increase in the ratio of initial number of moles of Cd^{2+} to the available surface area of the powders. These results agree with those reported in literature on other metal ion-sorption using wild cocoyam and steam activated sulphurised activated carbon prepared from sugarcane bagasse pith (Horsefall and Spiff, 2004; Krishnan and Anirudhan, 2003).

Various steps are involved in the biosorption mechanism, which deal with the transfer of metal ions from the solution to the surface of biosorbent, for instance, bulk transport of metal ions in solution phase, film transport involving diffusion of metal ions and the actual adsorption of the metal ions by the active site of the biosorbent (Bhatti *et al.*, 2010). Thus initial concentration provides an important driving force to overcome all mass transfer resistance of metal ions between the aqueous and solid phases.

2.7.5 Adsorbent dosage

It is commonly reported in the literature that the amount of metal bound per biomass decreases as the biomass concentration increases. The amount of biomass in the solution also affects the specific metal uptake. For lower values of biomass concentrations, there is an increase in the specific uptake (Fourest and Roux, 1992). Gadd (2004) suggested that an increase in biomass concentration leads to interference between the binding sites. By increasing the adsorbent dosage, the adsorption efficiency increases even though the amount adsorbed per unit mass decreases. In principle, with more adsorbent present, the available adsorption sites or functional groups also increase. In turn, the amount of adsorbed heavy metal ions increases and this results in improved adsorption efficiency (Dundar *et al.*, 2008). With metals biosorption at favourable pH, the increase of biomass concentration causes a diminution of the maximum specific metal uptake due probably to metal aggregation phenomena, whereas at acidic pH values the previous trend is inverted perhaps because of the effect of partial hydrolysis of the bacterial cell wall constituents.

For instance, this trend has been observed for the biosorption of Ni, Cd and Zn on mycelial wastes of Rhizopus arrhizus (Fourest and Roux, 1992), the biosorption of Cu^{2+}, Fe^{2+}, Ni^{2+} and Zn^{2+} on immobilized *Phormidium laminosum* (Blanco *et al.*, 1999) biomass, the biosorption of Cu^{2+} , Ni^{2+} and Cr^{6+} on dried algae Aksu and Dönmez, 2003).

Contributing factors that have been suggested for such biomass concentration dependency include electrostatic interactions between cells (Gadd, 2004; Lodeiro *et al*., 2006), interference between binding sites, and reduced mixing (Akhtar *et al*., 2003; Gadd, 2004; Gadd, 2009) at high cell densities.

2.7.6 Contact time

As reported in the literature, the metal sorption is often rapid, and usually complete in less than one hour. For metabolism independent uptake by algae, sorption is generally achieved in 5-10 minutes (Gadd, 2004). Binding of metal ions to aquatic particulates is considered to be a fast chemical reaction, with an equilibrium time depending only on the mass transfer resistance (Waite *et al*., 1994). To establish an appropriate contact time between the biosorbents and metallic ions solution, adsorption capacities of metal ion need to be measured as a function of time. Normally the removal is higher in the beginning and gradually drops with time. That is probably due to the larger surface area of the biosorbents being available at the beginning for the adsorption of metals. As the surface adsorption sites become exhausted, the uptake rate is controlled by the rate at which the adsorbate is transported from the exterior to the interior sites of the adsorbent particles (Yang *et al*., 2009).

2.7.7 Adsorbent size

The size of the biosorbent also plays a vital role in biosorption. Smaller sized particles have a higher surface to volume ratio, which in turns favours biosorption and results in a shorter equilibrium time. The effect of leaf grinding on removal of Cu was investigated by Al-Subu *et al*. (2001), and it was found that Cu removal was increased by grinding and crushing, that is, metal removal by the three forms of leaves was in the order; ground leaves (83%) > crushed leaves (75%) and whole leaves (70%). Simultaneously, a particle for biosorption should be sufficiently resilient to withstand the application of pressure and extreme conditions applied during regeneration cycles (Volesky, 2001). Therefore, preliminary experiments are necessary to determine a suitable size for a biosorbent.

Surface to volume ration of a particle is a function of the particle's size. Increase in particle size decreases metal adsorption while increase in pore size increases the metal adsorption. This may be due to availability of more surface area for adsorption in case of small size particles (Kumar *et al*., 2010). The removal efficiency and sorption capacity increases with the decrease of the particle size. This indicates that the smaller the sorbent particle size, then for a given mass, more surface area is made available and therefore the number of sites increases (Ho *et al*., 2002). The rate constant (k) of kinetic adsorption by the clay adsorbent decreased with increasing particle size (Tsai *et al*., 2003). The solid phase diffusion coefficient estimated from batch reactor analysis, is found to be invariant with particle diameter (Kahraman *et al*., 2008). Biosorption generally decreases with an increase in biosorbent size, but there is an optimum particle size required for effective metal uptake.

Too small or too large particle size decreases interaction of biomass with adsorbate, which results in reduction in biosorption capacity of a biomass (Bhatti *et al*., 2007). Secondly, there is a definite relationship between hydrated metal ion radius and biomass particle size as shown by Iftikhar *et al*. (2009). In a study done by Javed *et al*. (2007) the optimum biosorption of Pb (II) and Co (II) using rose waste biomass was with a particle size of 0.25 mm. Different biomasses contain different types of compounds having a variety of functional groups which are generally exposed during biosorption. A particular size of biomass provides particular number/types of functional groups and hence binding sites for the metal ions.

2.8 Effect of Pretreatment on biomass

Physical and chemical pretreatments either increase or decrease the uptake capacity of biosorbents. Physical modification of biomass removes mineral matter from biomass and introduces more sorption sites on the biomass surface. Tarley and Arruda (2004) found that adsorption of Cd increased by almost double when rice husk was treated with NaOH. The reported adsorption capacities for Cd were 7 and 4 mg g^{-1} for NaOH treated and unmodified rice husk, respectively. Meanwhile, most of the acids used for treatment of plant wastes such as sulfuric acid, hydrochloric acid and nitric acid were in dilute form. According to Esteghlalian *et al*. (1997), dilute acid pretreatment using sulfuric acid can achieve high reaction rates and improve cellulose hydrolysis. Concentrated acids are powerful agents for cellulose hydrolysis but they are toxic, corrosive and must be recovered (Galbe and Zacchi, 2007).

However, in some cases, hydrochloric acid treated rice husk showed lower adsorption capacity of cadmium than the untreated rice husk (Chowdhury *et al*., 2009). Wong *et al*. (2003) carried out an adsorption study of copper and lead on modified rice husk by various kinds of carboxylic acids (citric acid, salicylic acid, tartaric acid, oxalic acid, mandelic acid, malic and nitrilotriacetic acid) and it was reported that the highest adsorption capacity was achieved by tartaric acid modified rice husk. Esterified tartaric acid modified rice husk, however, significantly reduced the uptake of Cu and Pb. Heating of biomass results in decomposition of organic matter, while boiling removes mineral matter by dissolution, resulting in the introduction of more sorption sites on biomass surface, so biosorption capacity increases (Nadeem *et al*., 2008; Shafqat *et al*., 2008). Studies carried out by Pal *et al.* (2006) showed that sorption capacity of *Citrus reticulata* waste (CWB) increased after pretreatment with EDTA which is a strong complexing agent and could assist in the elution of metal ion sequestration already present on CWB, however, it decreased after pretreatment with $(NH_4)_2SO_4$, $CaCl_2$ and Na-alginate.

Acid pretreatment removes previously adsorbed cations (ions present in the nutrient medium), thus freeing the binding sites for metal binding. Acid treatment also causes hydrolysis of functional groups which may generate more anionic sites for metal removal (Fourest and Roux, 1992). Acid treatment also reduces the positive charge on cell surface and this may also be the reason for increase in metal removal (Ting *et al*., 1995). Generally, acid treatment has been used for cleaning the cell wall and replacing the natural mixture of ionic species bound on the cell wall with protons and sulphates (Davis *et al*. 2003; Yun, 2004). Mehta and Gaur (2005) observed that pretreatment of the biomass with dilute HCl increased sorption of Cu and Ni by *C. vulgaris*. Rao *et al*. (2010) demonstrated enhanced Ni sorption capacity of *Sargassum* after acid pretreatment.

Mehta *et al*. (2011) demonstrated that acid treatment of *Moringa Oleiferas* not only increased metal sorption but also alleviated the inhibitory effect of other metal ions on sorption of the metal of interest. Arica *et al.* (2004) explained that acid pretreatment of biomass results in physical cleaning of the biomass surface from impurities, thus producing additional available binding sites. They also added that denaturation of proteins and degradation of polysaccharides of the cell wall components also produces additional available binding sites. Kapoor and Viraraghavan (1998) reported that acetic acid pretreatment significantly reduced biosorption of cadmium but caused improvement in copper biosorption by *Aspergillus niger*.

Favourable effects of alkali treatment of biomass on metal removal have been reported by many workers. The pretreatment of cells with KOH/NaOH strongly enhances the metal removal by test biomass. The reason for this may be that alkali treatment may rupture the cell membranes resulting in the exposure of certain internal cellular binding sites making them available for adsorption, which enhanced the binding of cationic metal ion species. Generally alkali treatments decrease protonation of functional groups. NaOH treatment substitutes sodium ions on functional groups, increasing the electrostatic attraction by positive metal cations and facilitating ion exchange (Yan and Viraraghavan, 2003). Alkali pretreatment increased the Cu removal capacity of C. vulgaris, may be due to the removal of extraneous materials from the cell surface that might have otherwise impeded the binding of metal ions (Zhou *et al*., 2012; Mehta and Gaur, 2005). Nagase *et al*. (2005) demonstrated that alkali treatment is useful in improving the Cd^{2+} adsorption ability of T. tenuis cells.

Thus, due to damage of cell surface, Cd^{2+} ions permeated in to the cells. Gupta *et al.* (2000) observed that metal sorption remained unchanged after alkali pretreatment. It was argued that NaOH treatment perhaps caused contraction of cells thus making sites less accessible to metal ions and decreased metal removal. Tunali *et al*. (2005) suggested that increase in metal removal after alkali pretreatment could be due to the release of certain biopolymers from the cell wall having high affinities towards heavy metal ions. Alkali-pretreated biomass of Thiobacillus thiooxidance showed higher metal adsorption capacity may be due to an increase in the availability of binding sites or due to the removal of polysaccharides that presumably blocked the accessibility of metals to the binding sites (Liu *et al*., 2010). Rao *et al*. (2010) suggested that alkali pretreatment probably destroys autolytic enzymes that cause putrefaction of biomass and remove lipids and proteins that mask reactive sites.

2.9 Sorption Mechanisms

Biosorption is made possible by the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical uptake pathways. Due to the interaction of several factors on specific biosorbents, it is almost impossible to propose a general mechanism. Several metal-binding mechanisms have been put forward, such as chemisorption by ion exchange, coordination and/or chelation, complexation, physical adsorption, among others (Arief *et al.*, 2008). An important factor in metal uptake is the electrostatic attraction binding between metal cations and negatively charged sites at the cell surface (Deng *et al*., 2007). Metal ion biosorption could take place due to an electrostatic attraction between metal cations and negatively charged sites on the biosorption surface such as phosphoryl, phosphate, carboxyl, sulphate, amino and hydroxyl groups (Tunali *et al*., 2006).

Possible biosorption mechanisms are ion-exchange, physical adsorption, chemisorptions, and complexation. Ion-exchange refers to a class of mechanism in which adsorbing metal ions replace other species already associated with the biosorbent surface. Physical adsorption is due to weak Van der Waal forces between the adsorbate and the adsorbent. The forces involves in chemical adsorption are much stronger and involve electron exchange and the formation of chemical bonds between the adsorbate and the adsorbent (Tunali *et al*., 2006). As a result, chemisorption is highly specific and the adsorption energies are generally substantially greater than those for physical adsorption. Chemical adsorption is by its very nature limited to less than a monolayer coverage of the surface, while in physical adsorption, multilayer adsorption is common. Biosorption takes place in three steps: (1) bulk solution transport which moves the adsorbate through the bulk liquid by means of advection and dispersion, to the fixed film boundary layer surrounding the biosorbent media; (2) diffusive transport which moves the adsorbate across the fixed film boundary layer; and (3) bonding processes which act to attach the adsorbate to the media surface. Metal ion biosorption could take place due to an electrostatic attraction between metal cations and negatively charged sites on the biosorbent surface such as phosphoryl, phosphate, carboxyl, sulphate, amino and hydroxyl groups (Tunali *et al*., 2006).

2.9.1 Chemisorption Mechanism

Ion-exchange is an important concept in biosorption, because it explains many observations made during heavy metal uptake experiments (Davis *et al*., 2003). Ion-exchange is a reversible chemical reaction where an ion within a solution is replaced by a similarly charged ion attached onto an immobile solid particle (Han *et al*., 2006). Romero-Gonzalez *et al*. (2001) claimed that Cd biosorption on Saccharomyces cerevisiae followed an ion-exchange mechanism. Tan and Cheng (2003) examined the mechanism involved in the removal of five heavy metals, namely, Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+} and Cr^{3+} by *Penicillium chrysogenum*, and concluded that ion-exchange was the dominant mechanism.

Ahmady-Asbchin *et al*. (2008) noticed that in the biosorption of copper ions on Fucus serratus, the bond between the Cu ions and the surface functional groups of the biomass formed as soon as Ca ions were released from the surface. This ion-exchange process is one of the well-known surface reactions, which represents a great degree of complexity, primarily due to its multi-species nature. Volesky and Holan (1995) reported that ion-exchange plays a role in the biosorption of cobalt by marine algae (Ascophyllum nodosum). In bacterial biosorption, the bacterial cell wall is the first component that comes into contact with the metal, where the solute can be deposited on the surface or within the cell wall structure (Vijayaraghavan and Yun, 2008). Since the mode of solute uptake by dead or inactive cells is extracellular, the chemical functional groups of the cell wall play a vital role in biosorption. It is not only the surface area of the biomass that matters in the biosorption process but other factors like ion exchange, complexation, electrostatic attraction and micro-precipitation are also involved in heavy metal biosorption (Davis *et al*., 2003; Mehta and Gaur, 2005; Volesky and Holan, 1995). Metal removal by microorganisms is mainly due to their adsorption on to the cell surface and binding to cytoplasmic ligands, phytochelatins, metallothioneins and other intracellular molecules.

The algal cell wall may have many functional groups, such as hydroxyl, phosphoryl, amino, carboxyl, sulphydryl, among others, which confer negative charge to the cell surface. Most of heavy metal ions in water are in cationic form, thus they get adsorbed on to the cell surface (Skowronski *et al*., 2001; Mehta and Gaur, 2005). Since biosorption of heavy metals cations involves binding of metal ions to the anionic groups present on the biomass surface, thus number and ionic state of functional groups present on the biosorption surface play a significant role in heavy metal removal. The nature and presence of functional groups on the biomass surface can be easily ascertained through FTIR analysis. In the FTIR spectrum, one gets peaks at different wave numbers and these peaks result from different characteristics of functional groups. Biosorbents consist of complex organic and inorganic materials such as proteins, lipids, carbohydrate polymers and sometimes metals. Chemisorptions and ions exchange mostly depend on the available functional groups in a particular biosorbent and eventually metals biosorption depend on it. Carbon-oxygen and carbon bonds are the attracting and stimulating bond of metals biosorption (Ricordel *et al*., 2001).

2.9.2 Involvement of functional groups in Metal Binding

Another frequently encountered metal binding mechanism is chelation, which can be defined as a firm binding of metal ions with an organic molecule (ligand) to form a ring structure (Arief *et al*., 2008). Various functional groups including carboxylic (COOH), hydroxyl (−OH), sulphate (SO_4^{2-}), phosphate (PO_4^{3-}), organic amides (RC(O)NR'R) and amino ($-NH_2$) groups can be considered for possible sorption. Among these groups, the amino group is the most effective for removing heavy metals, since it does not merely chelate cationic metal ions but also absorbs anionic species through the electrostatic interaction or hydrogen bonding. As they are negatively charged and abundantly available, carboxyl groups actively participate in the binding of metal cations (Deng and Ting, 2005). According to Ahalya *et al*. (2006) in the biosorption of Fe (III) by the husk of Cider arientinum, carboxyls as well as amino groups were involved in the metal uptake. Deng and Ting (2005) indicated that carboxyl groups on the cell wall peptidoglycan of Streptomyces pilosus were responsible for the binding of Cu^{2+}. The contribution of amine groups in the Cu (II) adsorption by *Mucor rouxii* was verified by Majumder (2008). Altun and Pehlivan (2007) revealed that chelation and ion-exchange were mainly behind Cu (II) adsorption from aqueous solution by walnut, hazelnut and almond shells.

Agricultural and plant waste materials, such as coconut husk and shell, are usually composed of lignin and cellulose as the main constituents. Other components present in the metal binding process are hemicellulose, lipids, proteins, simple sugars, starches, water, hydrocarbons and many more compounds that contain a variety of functional groups (Sud *et al*., 2008). The presence of these functional groups and their complexation with heavy metals during biosorption has been reported by different researchers using spectroscopic techniques (Tarley and Arruda, 2004).

2.9.3. Sorption Isotherms and Models

Among all phenomena governing the mobility of substances in aqueous, porous and aquatic environments, the transfer of substances from a mobile phase (liquid or gas) to a solid phase is a universal phenomenon. That is the reason why the 'isotherm', a curve describing the retention of a substance on a solid at various concentrations, is a major tool to describe and predict the mobility of this substance in the environment. These retention/release phenomena are sometimes strongly kinetically controlled, so that time-dependence of the sorption isotherm must be specified. Models have an important role in technology transfer from a laboratory scale to industrial scale (Limousin *et al.*, 2007).

The equilibrium of the sorption process is often described by fitting the experimental points with models, usually used for the representation of isotherm sorption equilibrium. Appropriate models can help in understanding process mechanisms, analyze experimental data, predict answers to operational conditions and process optimization (Vijayaraghavan and Yun, 2008). As an effective quantitative means to compare binding strengths and design sorption processes, employing mathematical models for the prediction of the binding capacity can be useful (Limousin *et al.*, 2007). Examination and preliminary testing of the solid–liquid sorption system are based on two types of investigations: (a) equilibrium batch sorption tests and (b) dynamic continuous flow sorption studies. The two widely accepted and linearised equilibrium adsorption isotherm models for a single solute system are the Langmuir and the Freundlich isotherms.

When any biosorption system reaches a state of equilibrium, there is a defined distribution of adsorbate molecules at the solid-liquid interface and also in the bulk at a particular temperature. The maximum possible accumulation of the adsorbate at the solid surface is a function of its concentration at a constant temperature. Adsorption isotherms are used to describe equilibrium data and are important for developing equations that can be used to compare different biosorbents under different operational conditions. Several equilibrium models have been used to describe the metal transfer between the solution and solid phase during the biosorption process (Martin *et al.*, 2011). The simplest forms of these isotherms are Freundlich and Langmuir isotherms which in most cases are used to obtain maximum biosorption capacity of the biosorbent.

2.9.4 Langmuir Isotherm Model

The Langmuir model suggests that a monomolecular layer is formed when the biosorption occurs on a homogenous surface without any interaction between the adsorbed species (Aksu, 2005). It assumes that every adsorption site is equivalent and the ability of sorbate to get bound is independent on whether or not the neighbouring sites are occupied. The Langmuir equation may be written as

$$q_e = \frac{q_m K_L C_e}{1+K_L C_e} \qquad \text{………………………… (1)}$$

Eq. (1) can be expressed in its linear form as:

$$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m} \quad \text{…………………………(2)}$$

where q_e is the amount adsorbed (mg/g), C_e is the equilibrium concentration of the metal ion (mg/L), q_m (mg/g) is the maximum amount of adsorbed metal ion per unit mass of sorbent corresponding to complete coverage of the adsorptive sites, K_L (L/mg) is the constant related to the free energy of adsorption (mg/L).

2.9.5 Freundlich Isotherm Model

The Freundlich isotherm model describes the absorption of a solute from a liquid to a solid surface under the assumption that the stronger binding sites are occupied first and that the binding strength decreases with an increasing degree of site occupation. The Freundlich model proposes a monolayer adsorption with a heterogeneous energetic distribution of active sites, and/or interactions between adsorbed species, i.e. multilayer absorption. This model is usually chosen to estimate the biosorption intensity of the absorption towards the adsorbate.

The Freundlich isotherm is represented by the equation:

$q_e = K_f \, C_{eq}^{1/n.}$ ………………………………………………………………………… (3)

where C_{eq} is the equilibrium concentration (mg/L), q is the amount adsorbed (mg/g) and K_f and n are constants incorporating all parameters affecting the adsorption process, such as adsorption capacity (K_f) and intensity respectively (n). The linearised form of Freundlich adsorption isotherm was used to evaluate the sorption data and is represented as:

$\text{Log } q_e = \log K_f + 1/n \log C_{eq}$ …………………………… (4)

A graph of log q_e against log C_e was plotted; n and K_f were calculated from the slope and intercept of the Freundlich plots, respectively. The Freundlich isotherm basically indicates whether the adsorption proceeds with ease or difficulty. Log K_f is equivalent to log q_e when C_{eq} equals unity. However, in other cases when $1/n = 1$, the K_f value depends on the units upon which q_e and C_{eq} are expressed.

On average, a favourable adsorption tends to have Freundlich constant n between 1 and 10. Larger value of n (smaller value of $1/n$) implies stronger interaction between biosorbent and adsorbate while $1/n$ equals to 1 indicates linear adsorption leading to identical adsorption energies for all sites. This isotherm attempts to incorporate the role of substrate-substrate interactions on the surface (Febrianto *et al.*, 2009; Reddy *et al.*, 2010). The n value indicates the degree of non-linearity between solution concentration and adsorption as follows: if $n = 1$, then adsorption is linear; if $n < 1$, then adsorption is a chemical process; if $n > 1$, then adsorption is a physical process. The Freundlich isotherm model is the well-known earliest relationship describing the adsorption process. Application of the Freundlich equation also suggests that sorption energy decreases exponentially until completion of the sorption centres of an adsorbent. Freundlich isotherm is an empirical equation and it is among the most widely used isotherms for the description of adsorption equilibrium. Freundlich isotherm is capable of describing the adsorption of organic and inorganic compounds on a wide variety of adsorbents including biosorbent. The Freundlich model is well-fitted for metal adsorption by biomass because of the heterogenous nature of sites present on it (Pavasant *et al*., 2006). Jain and Ram (1997) showed that the order based on K_f values was different from that based on 1/n values for Pb and Zn sorption by sediments hence it seemed both models described metal sorption by the wood ash satisfactorily as reflected by the relatively high fitting coefficients of determination values ($R^2 > 0.91$) of the sorption data.

2.10 Kinetics of Metal Adsorption

The dependence of a chemical reaction on initial reactant concentration can be shown by a rate equation. The rate equation once integrated gives expressions for the variation of concentration of a reactant with time. The order of reaction is deduced from the integrated rate equation. The pseudo-first-order kinetic model assumes that the uptake rate of metal ions with time is directly proportional to the amount of available active site on the adsorbent surface.The first order rate equation for metal adsorption is;

$$\frac{\partial q_t}{\partial t} = k_1 (q_e - q_t) \text{ ....} (5)$$

where q_t and q_e are the masses of metal adsorbed by the biosorbent in (mg/g) at any time t and at equilibrium, respectively, k_1 is the rate constant for the adsorption. On integration, equation 5 gives the solution;

$$k_1 t = \ln q_e - \ln (q_e - q_t) \text{ ....} (6)$$

which indicates that a plot of $\ln(q_e - q_t)$ against time t should give a straight line with a slope of -k

If a plot of t against $ln\ (q_e - q_t)$ gives a straight line with a coefficient of determination value (R^2) tending to unity then the experimental data agrees with model and the reaction is pseudo is first order.

The second order rate equation can be represented as;

$$\frac{\partial q_t}{\partial t} = k_2 \left(q_e - q_t\right)^2 \text{...} (7)$$

where k_2 is the second order rate constant, q_e and q_t as defined above. Upon integration, equation 7 gives the solution;

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 {q_e}^2} \text{...} (8)$$

Equation 8 predicts that for a second order process plotting t/q_t against t should give a straight line with $1/\ q_e$ as the gradient from which k_2 can be calculated since $1/k_2 q_e^2$ is the y- intercept. The sorption data of Cr (III) and Cr (VI) uptake by Mentha biomass were fitted to pseudo-first-order and pseudo-second-order kinetic models. The Cr (III) and Cr(VI) sorption by Mentha biomass fitted well the pseudo- second-order kinetic. The pseudo-second-order kinetics model is also based on the assumption that the sorption rate is controlled by a chemical sorption mechanism involving electron sharing or electron transfer between the adsorbent and adsorbate (Ding *et al.,* 2012).

2.11 FTIR Spectroscopy

Fourier transform infra-red technique (Figure 2.1) is based on the excitation of molecular vibrations by light absorption. It is widely used in the determination of structure and identification of both organic and inorganic compounds. It is mainly used in the identification of functional groups present in a given sample. In infrared spectroscopy, infrared radiation is passed through a sample. Some of the infrared radiation spectra is absorbed by the sample and some is transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint, no two unique molecular structures produce the same infrared spectrum. This makes FTIR spectroscopy useful for several types of analyses, including: identification of an unknown material, quality control of samples and the determination of amounts of components in a mixture (Pavia *et al*., 2001).

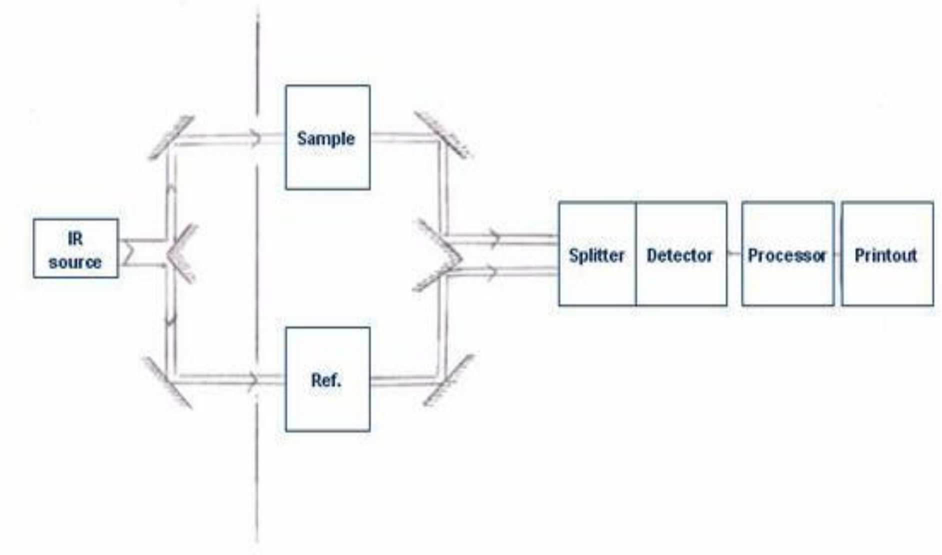


Figure 2.1 A schematic diagram of the FTIR system

The sample analysis process involves the emission of infrared radiation from a black body source. The beam of radiation passes through an aperture which controls the amount of energy presented to the sample and ultimately to the detector. The beam enters the interferometer where the ‘spectral encoding’ takes place. The resulting interferogram signal then exits the interferometer. The beam enters the sample compartment where it is transmitted through or reflected off the surface of the sample, depending on the type of analysis being carried out. This is where specific frequencies of energy, uniquely characteristic of the sample, are absorbed. The beam finally passes through to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal (Pavia *et al.*, 2001).

CHAPTER THREE

MATERIALS AND EXPERIMENTAL METHODS

3.1 Chemical Reagents

In all cases, analytical grade reagents were used unless otherwise stated; anhydrous Sodium hydoxide (NaOH), Cadmium chloride ($CdCl_2$), Lead(II) nitrate ($Pb(NO_3)_2$ Chromium(II) nitrate ($Cr(NO_3)_2$, Copper nitrate ($Cu(NO_3)_2$ Manganese (IV) oxide (MnO_2), Zinc chloride ($ZnCl_2$), Calcium chloride ($CaCl_2$), Potassium permanganate ($KMnO_4$), Potassium bromide (KBr), liquid buffer of pH 4.00, liquid buffer of pH 7.00, Hydrochloric acid (HCl), Sulphuric acid (H_2SO_4), Sodium carbonate (Na_2CO_3), Sodium acetate, Acetic acid, hexamethylene tetraamine, Ethanol, Sodium chloride. The reagents were manufactured by Aldrich.

3.2 Equipment and Glassware

3.2.1 Glassware

The various glassware, which were used in the experimental methods included; separating funnels, measuring cylinders, filter funnels, round bottomed flasks, volumetric flasks, beakers, acid washed glassware, volumetric flasks: (various sizes), graduated pipette, tubes, pipettes (various sizes). All the glassware was cleaned thoroughly to avoid the possibility of contamination. This was done by soaking the glassware in detergent and hot water (75^oC) overnight. It was then rinsed in plenty of hot water, rinsed again in distilled water and afterwards rinsed in double distilled water. It was then dried in an oven for at least two hours. Clean glassware was stored in separate labeled cabinets away from dust and possible contaminants. Before use, all glassware was rinsed in double distilled water and finally rinsed with the samples to be used. An electronic analytical balance made by Sartorius Handy Company, Germany was used in all measurements (weighing to 4 decimal places).

3.2.2 pH meter

The pH measurements were done using pH meter, Hanna Model, which was calibrated using buffer solution at pH 4 and 7. The experiments were done in triplicate; control experiments were also done using de-ionized water.

3.2.3 Atomic Absorption Spectrophotometer

The atomic absorption spectrophotometer used was Chemtech Analytical 2000 (CTA2000) model interfaced with a computer. A deuterium lamp was used for the automatic background correction of the signal. The burner height was adjusted to heights ranging from 6.0-7.0 cm depending on the element to be analysed. Air-acetylene mixture flame was used during the analysis. Cylinders of compressed acetylene of analytical grade were obtained from the British Oxygen Company (BOC) Kenya Limited. Air was pumped by automatic air pumps to mix with acetylene during the analysis. The analysis was done at the University of Eldoret, school of environmental Sciences.

3.2.4 FT-IR

A Fourier Transform IR Spectrophotometer (Shimadzu prestige – 21, FTIR – 8400) fitted with a pellet cell of KBr was used for characterization of the *Moringa oleifera* biomass.

A resolution of 2 cm^{-1} was selected. The analysis was done at Jomo Kenyatta University of Science and Technology.

3.2.5 Other apparatus and materials

A muffle furnace, refrigerator, electrical fume chamber and a water distiller, plastic reagent bottles, crucibles, spatulas, centrifuge cotton wool, stickers and whatman filter papers were also used. An electronic analytical balance made by Sartorius Handy Company, West Germany was used in all measurements (weighing to 4 decimal places).

3.3 Sampling and Study Area

Sampling was carried out in July, 2013 at 8 sites on Winam gulf, grab samples were collected randomly in triplicates. Water samples were collected from all the eight sites (3 Rivers and 5 Beaches) to analyze for heavy metals, three samples were taken from each site and mixed to form composite samples which were then used in the analysis. Water samples were collected in polyethylene bottles (Washed with detergent then with double-distilled water followed by 2 M nitric acid, then double-distilled water again and finally with sampled water). Water samples were acidified with 10% HNO_3, this was done on site because analytes in water may also form salts that precipitate. These precipitates may adhere to the sides of the collection bottle if the sample is not prepared properly and can prevent proper analysis. The most common occurrence is precipitation of metal oxides and hydroxides due to metal ions reacting with oxygen. This precipitation can be prevented by adding nitric acid; the combination of a low pH (less than 2) and nitrate ions keep most metal ions in solution. The acidified samples were then brought to the laboratory and kept refrigerated until needed for analysis. The above procedure also prevents microbial growth and flocculation.

Sampling was done on the shores of Lake Victoria (Winam Gulf) at five (5) sites that is, Yatch Club, Dunga Beach, Lwang'ni Beach Hotels, Kisumu Beach Resort and Tilapia Beach. Water was also sampled from three rivers that drain into the Lake Victoria, that is, Nyamasaria, Kibos and Kissat (Figure 3.1). The sites were selected based on their proximity to Kisumu and also the fact that there was no data on the levels of heavy metals in the past three years.

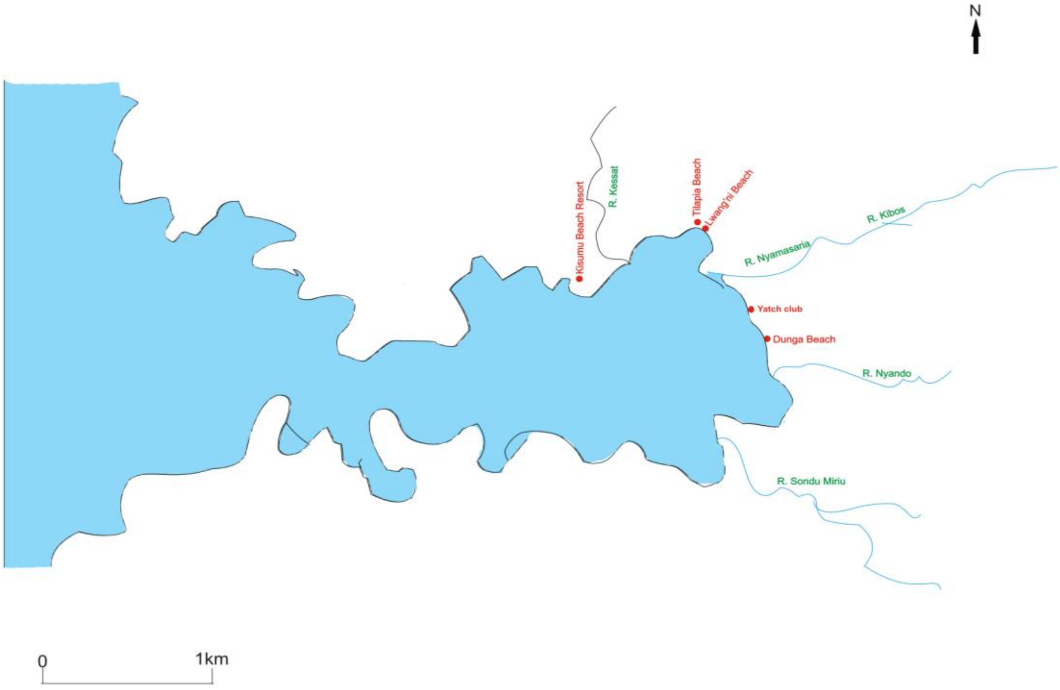


Figure 3.1: Map showing Winam Gulf

(SOURCE: AUTHOR, 2016)

3.4 Digestion of Water Samples

The digestion method described by Lalah *et al*. (2008) was adopted. Representative water samples of 5 mL of each were transferred into Pyrex beakers containing 5 mL of conc. HNO_3. The samples were boiled slowly and then evaporated on a hot plate for 30 minutes. The beakers were allowed to cool and another 5 mL of conc. HNO_3 acid was added followed by heating on a hot plate. Some 10 mL conc. HCl was added and digestion continued until the solution remained light brown or colourless. The volume was then adjusted to 25 mL with distilled water. These solutions were then used for the elemental analysis. The heavy metals analyzed were Zn, Pb, Cd, Mn, Cu and Cr.

3.5 Preparation of Stock Solutions

Stock solutions of Pb^{2+}, Cu^{2+}, Mn^{2+}, Cd^{2+}, Cr^{2+} and Zn^{2+} were prepared from the metal salts and later diluted to the required levels for preparation of artificial waste waters. The same solutions were used as standards for calibration curves.

3.5.1 Lead Stock Solution and Working Solution

A stock solution of Pb (II) was made by dissolving 1.599 g of Pb $(NO_3)_2$ in nitric acid and making it to 1000 cm^3. At 25 ^oC, the solution had a density of 1.02 g/cm^3 and contained 1.000 $\mu g/cm^3$ (1000 ppm) of Pb (II) ions. A stock solution of 100 ppm was prepared by pipetting 10.0 cm^3 from 1000 ppm stock solution, into 100.0 cm^3 volumetric flask and making it up to the mark with deionised water.

The 1000 ppm stock solutions of Cd(II), Cu(II), Cr(II), Zn (II) and Mn(II) were prepared by weighing 1.6875 g of $CdCl_2$ in 12% HCl acid, 3.802 g of $Cu(NO_3)_2$ in 12% HNO3 acid, 7.695 g $Cr(NO_3)_2$ in 12% HNO_3 acid, 2.085 $ZnCl_2$ in 12% HCl and 1.582 g MnO_2 in 12% HCl and making upto 1000 cm^3, respectively. Standard solutions of 2.5 ppm, 5.0 ppm, 7.5 ppm, 10.0 ppm were prepared from the stock solutions in a similar way.

3.6 Preparation of biomass, characterization and modification

3.6.1 *Moringa oleifera* seeds

Moringa Oleifera seeds were purchased from farms in Otonglo-Kisumu County. Good quality seeds were deshelled mechanically to remove the outer coat. The kernels were washed in de-ionised water to remove dust, and then soaked in 1 M HCl for 3 days, to remove the original content of heavy metals. The seeds were again washed in distilled water, dried in air then ground using a pestle and mortar. Defatting was done by adding ethanol/acetone mixture at a ratio of 1:1, followed by shaking in a centrifuge and decanting, it was repeated severally until no more fat was visible in the biomass. After centrifugation the wet moringa powder was dried in an oven at 30 ^{o}C for 24 hrs, it was crushed again and sieved through 750 and 250 µm sieves. Heavy metals were not detected in *Moringa oleifera* seeds at the point of use.

3.6.2 Adjustment of pH values of model polluted water

Synthetic polluted water was prepared from de-ionized water for Cu^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+} and Cr^{2+} with concentrations ranging between 1 ppm to 10 ppm. Each of the prepared solutions was divided into 6 (60 mL which was again divided into 3 of 20 mL, the experiments were done in triplicate) and the pH values adjusted to 3, 4, 5, 6, 7 and 8. Buffer solutions of pH 4 and 7 were used to adjust the solution pH. The pH was measured using Hanna pH meter at the beginning and at the end of the experiments. With 20 cm^3 of each of the heavy metal solutions in Erlenmeyer flasks arranged in order of pH values ranging from 3 to 8, exactly 0.2 g of moringa powder was added to each flask at the same time and immediately put on an electric shaker for 20 minutes to increase the rate of biosorption at various pH values. After shaking the solutions were filtered in a whatman and the filtrate analysed by AAS. A graph was then drawn by plotting the percentage removal of each metal ion against time to establish the optimum pH for metal biosorption.

3.6.3 Characterization of Biomass (FTIR)

The finely divided biomass was analyzed to determine the functional groups using a Fourier Transform Infrared Spectroscopy (Shimadzu IR Prestige - 21, FTIR - 8400) using KBr as a pellet cell, a resolution of 2 cm^{-1} was sellected. The finely ground biomass (on the pellet cell) was inserted in the path of the IR beam and held in position; the infrared radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some is transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample on the computer which can be printed.

3.6.4 Modification of Biomass

Powdered *Moringa oleifera* seeds were subjected to chemical treatments. About 100 mL of 0.5 M sodium hydroxide, hydrochloric acid and calcium chloride were used for pretreatment. In each pretreatment, the biomass was slowly stirred in the chemical solution for 1 hr. After each pretreatment the biomass was washed in double distilled water till the pH of the washing solution reached a neutral range (pH between 6.8 and 7.2). The pretreated biomass was dried in an oven at 60 ^{o}C for 25 hours and homogenised in a blender to break the aggregates into small fragments. It was then passed through 750 and 250 μm mesh sieves to obtain particle sizes of 750 and 250 μm diameters, respectively. The dried biomass was preserved in an air-tight jar to be used in biosorption tests.

Potassium permanganate modification was done using a modified procedure of Jeon *et al*. (2002) in which powdered biomass (10 g) was oxidized in 300 mL of 10 mM $KMnO_4$ solution at 30^{o}C for 30 min. The reacted mixture was separated by centrifiguration and washed thoroughly with distilled water and dried in an oven at 60 ^{o}C.

3.7 Biosorption Experiments

The samples were first analyzed for the levels of the various heavy metals (Cu(II), Zn(II), Mn(II), Cd(II), Pb(II) and Cr(II)) using AAS in triplicate. Synthetic wastewater was prepared from the stock solution prepared earlier. The synthetic water had almost the same level of heavy metals as the samples; binary systems were also prepared to check the effect of interfering ions. In order to adjust the environmental parameters, 0.1 M NaCl was used to control the ionic strength and hexamethylene tetramine buffer solution was used for keeping proton balance.

A set of 250 mL Erlenmeyer flasks containing 100 mL of metal solution (100 mg/L) were used, a 0.4 g biosorbent was contacted with the metal solution by incubating the flasks on a rotating shaker with constant shaking at 100 rpm for 1hr. The contents of the flask were filtered through filter paper (Whatman No. 4) and filtrate analysed for residual metal concentration using AAS. The experiments were done in triplicate, a control experiment was also done using de-ionized.

The amount of heavy metal ions adsorbed at equilibrium, q (mmol g^{-1}) which represents the metal uptake was calculated from the difference in metal concentration in the aqueous phase before and after biosorption, according to the following equation:

$$q = \frac{(C_o - C_f)V}{M} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

Where V is the volume of metal solution (mL), C_0 and C_f are the initial and equilibrium concentrations of heavy metal ion in solution (mmol L^{-1}), respectively, and M is the mass of dry biosorbent (mg). Experiments were done in triplicates and results were given as averages. The same procedure was used in model water with binary components to compare the effect of interfering ions and the same formular was used (Sharma *et al.*, 2007).

3.8 Batch adsorption experiment on raw and modified moringa

Batch experiments were carried out in triplicates to compare the effects of adsorbent dose, initial effluent concentration, contact time, solution pH, particle size and effect of co-ions. In each case, the flasks were incubated in a rotary shaker and after every 10 minutes filtered with a filter paper and the filtrate analysed for heavy metals using an AAS. De-ionized water was also used as control in each experiment..

3.8.1 Effect of Adsorbent Dose

Samples of 0.4 and 0.6 g of each adsorbent was equilibrated with 100 mL of synthetic wastewater containing heavy metals of different concentrations with stirring using a magnetic stirrer at 100 rpm on a 250 mL round bottomed volumetric flask. Adsorbent dosages of 0.4 and 0.6 g were selected based on dosages that had been used by other researchers in the past, which were either too smaller or too large. The mixture was filtered every 10 minutes and each time the level of heavy metal in the filtrate measured using an AAS, this went on for 70 minutes. The experiments were done in triplicates, control experiments were also done using de-ionized water.

3.8.2 Effect of initial Metal Concentration

The initial concentration which gave rise to the highest metal uptake was investigated. Samples of 0.4 g of each adsorbent were equilibrated with 50 mL of synthetic wastewater containing heavy metals (2 different concentrations in ppm), the concentrations varied from metal to metal. Stirring was done at 200 rpm in a 250 mL round bottom volumetric flask. The level of heavy metals in each solution was determined using an AAS at an interval of 10 minutes for 70 minutes, because 60 minutes was the optimum time from earlier studies. The experiments were done in triplicates, control experiments were also done using de-ionized water.

3.8.3 Effect of Contact Time

The rate of biosorption is important for designing batch biosorption experiments. Therefore, the effect of contact time on the biosorption of metals was investigated using 0.4 g/ 50 mL adsorbent dosage in a 250 mL flask. A 10 mL portion of the solution was withdrawn at time intervals of 10 minutes. The first 10 mL was withdrawn before addition of the moringa (t = 0). Subsequent withdrawals were done at intervals of 10 minutes for a period of 70 minutes. The procedure was repeated for all the metals under investigation and the pH for each solution was adjusted to 5 (optimum pH for biosorption of *Moringa oleifera* seeds from earlier experiments of the study). The difference between the initial and remaining metal concentration was assumed to be taken by the biosorbent. The effect of contact time was investigated for 0, 10, 20, 30, 40, 50, 60, and 70 minutes using 1 g/ 50 mL adsorbent dosage in a 250 mL round bottomed volumetric flask. 10 mL portions of this solution were withdrawn at time intervals of 10 minutes. The first 10 mL was withdrawn before addition of the moringa (t = 0). Subsequent withdrawals were done at intervals of 10 minutes for a period of 70 minutes. The withdrawn solutions were filtered and levels of heavy metal in the filtrate measured using an AAS. The experiments were done in triplicates, control experiments were also done using de-ionized water.

3.8.4 Effect of pH

Batch biosorption experiments were conducted on model solutions of Cd^{2+}, Cr^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Pb^{2+} to determine the optimum pH for metal uptake by *Moringa oleifera* seeds. The initial pH of 100 mL of synthetic wastewater was adjusted to 3, 5, 7 and 8 using 0.1 M NaOH and HCl and batch reactions run using 1 g adsorbent dosage at 10-70 minutes using Hanna pH meter.

3.8.5 Effect of particle size

Particle size of the adsorbent was varied by sieving them under mesh 0.25 and mesh 0.75 mm to determine the best particle size for the process. For purposes of accuracy, the experiments were done in triplicates and their average obtained, a control experiment was done using de-ionized water.

3.8.6 Effect of other ions

Each metal ion solution was added to an equal volume of competing metal ion, which was another heavy metal to investigate the effect of other ions. *Moringa oleifera* was then added to each solution and the levels of heavy metals remaining in solution determined after every 10 minutes. For purposes of control, the experiments were done alongside single ions of the same concentration. The experiments were done in triplicate and the average obtained.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Introduction

Below are results of analyses carried out to determine the levels of selected heavy metals (Pb, Zn, Cd, Cr, Cu and Mn) by AAS, phytochemical parameters of water at various sites of the Winam of the Lake Victoria, characterization of untreated *Moringa oleifera* seeds using Fourier transform infrared spectroscopy (FTIR), Others were; sorption behaviour of metal ions with untreated and chemically modified *Moringa oleifera* seeds, effect of adsorbent dose, pH, contact time, mixing ions, particle size and initial effluent concentration on the efficiency of the biosorption process, applicable isotherm for the adsorption process and kinetics of metal adsorption on the biomass

4.2 Parameters Measured Insitu

4.2.1 Total dissolved solids

Total dissolved solids (TDS) is a measure of the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized or micro granular suspended form. The permissible limit of TDS of drinking water is 500 ppm (Nzomo, 2005). The levels TDS at different sampling sites along Winam Gulf are shown in table 4.1. The observation shows that the TDS was within the permissible range as prescribed by WHO except for River Kissat with 799.5 ppm. Other sites that also recorded high values were; Yatch, Nyamasaria, Dunga, Tilapia beach and Kisumu beach Resort which had values of 436.5, 429, 429, 422 and 416 ppm, respectively. The remaining sites had relatively low values, that is, Lwang'ni and Kibos with corresponding values of 299 ppm and 241 ppm. The high values at River Kissat could be due to the raw sewage from the municipal council that is released into the river directly. Another reason could be due to the widespread erosion around the area leading to washing of substances into the river. The other sites which recorded high levels could be as a result of food kiosks and hotels and there is a likely hood that some of the left overs are directly thrown into the water. River Nyamasaria, Lwangni, Dunga, Kisumu beach Resort and Tilapia beach sites are also used for car washing and the high TDS could be due to oil and particulates from the cars. High TDS at Nyamasaria could be due to the bridge that was under construction and a lot of particulates could have been released into the water. Nzomo (2005) reported TDS values of 83 mg/L at Winam gulf and 113 mg/L at Kisumu bay, the values were lower than the values obtained in this study. It could be due to geology and soil in the area, because rock and soil release ions very easily when water flows over them; for example, if acidic water flows over rocks containing calcite ($CaCO_3$), such as calcareous shales, calcium (Ca^{2+}) and carbonate (CO_3^{2-}) ions will dissolve into the water. Therefore, TDS will increase. Another reason could be urban runoff which washes pollutants such as salts from streets, fertilizers from lawns, and other material into streams and rivers, because of the large amount of pavement in urban areas, natural settling areas have been removed, and dissolved solids are carried through storm drains to creeks and rivers. Another reason could be fertilizer runoff, since fertilizer can dissolve in stormwater and be carried to surface water during storms, and contribute to TDS (Murphy, 2007).

Table 4.1: Insitu measurement of water samples

Area/conditions	**DO (ppm)**	**Conductivity. (µS/cm)**	**TDS (ppm)**	**SALINITY(psu)**
Kibos	2.52	327.70	241.80	0.18
Nyamasaria	3.64	657.00	429.00	0.32
Lwang’ni	2.01	428.00	299.00	0.22
Tilapia Beach	1.61	646.00	422.00	0.30
Kissat	0.70	1239.00	799.50	0.60
Ksm B. Resort	2.40	638.00	416.00	0.32
Dunga Beach	1.93	666.00	429.00	0.32
Stream at Yatch	2.17	673.00	436.50	0.32
Yatch Club	1.86	674.00	436.50	0.32

4.2.2 Dissolved Oxygen

The levels of dissolved oxygen (DO) at different sampling sites along Winam Gulf are illustrated in the Table 4.1. The mean values of DO were lowest at R. Kissat (0.7) ppm. Other sites like Tilapia beach, Yatch and Dunga had also low DO values of 1.61, 1.86 and 1.93 ppm, respectively. The values were all lower than the WHO/FAO minimum DO acceptable limits of 2 ppm which could probably be due to the increase in temperature and escape of dissolved oxygen from the surface of the water. The low levels of DO may also be due to the high microbial activities in the municipal runoffs at River Kissat which is known to carry untreated effluent from the municipal treatment plant and also agricultural runoffs at the site. The high microbial activities could be due to high level of organic matter, hence, increased utilization of DO for decomposition process. The results are similar to those obtained by Osumo (2001), who observed dissolved oxygen levels of 1 – 8 ppm during the sampling period. The values obtained in this study were between 0.7 mg/L and 3.64 ppm, which were within the same range, implying that the Winam Gulf is getting more polluted since the amount of dissolved oxygen is reducing with time. Nzomo (2005) reported DO values of 7.3 ppm at Winam Gulf and 5.83 ppm at Kisumu Bay, both the values were much higher than the values obtained in this study, implying that the amount of oxygen in water within the gulf was reducing with time and later it may not sustain higher forms of aquartic life.

4.2.3 Electrical Conductivity (µS/cm)

Electrical conductivity indicates the amount of total dissolved salts. It is a tool to assess the purity of water. Electrical conductivity correlates with the concentration of dissolved minerals or TDS of water samples. The electrical conductivity values in the study area were found in the range of 372.3 at R. Kibos to 1239 µS/cm at R. Kissat. High electrical conductivity values at site River Kissat indicate the presence of high amount of dissolved inorganic substances in ionized form. The values of electrical conductivity depend upon temperature, concentration and types of ions present. At River Kissat raw sewage from Kisumu municipality is released and this could be the reason for the high values of electrical conductivity and TDS which are related to salinity of the water. Mdamo (2003) reported conductivity values of between 9.7 and 64.4 µS/cm in the Lake Victoria basin, the highest value was much lower than the value obtained in this study implying the conductivity values in the Winam Gulf have doubled over the years, which could be due to increased human activities which release electrolytes into the water bodies. Nzomo

(2005) reported conductivity values of 167μS/cm and 176 μS/cm, at Winam Gulf and Kisumu Bay, respectively, which were lower than what this study obtained.

4.2.4 Salinity

R. Kissat had the highest level of salinity (0.6 ppm), while R. Kibos had the lowest level (0.18 ppm). River Kissat had the highest salinity because of raw sewage that is released directly into the river. Nzomo (2005) reported values of 0.08 ppm and 0.06 ppm, respectively, at Kisumu bay and Winam Gulf, the values were much lower than the values obtained in this study, which means that salinity has been increasing over the years, especially at R. Kissat which is loaded with wastes from Kisumu sewage plant. Municipal untreated sewage, runoff, and storm water are the main causes of increasing salinity in the area. Direct discharge of municipal untreated effluent into rivers and the lake directly contributes to microbiological pollution which also reduces the amount of dissolved oxygen in water, which leads to degradation of river and lake-water quality for habitats and drinking use (Ntiba *et al*, 2001).

4.3 Levels of Heavy Metals in Sampled Water

Figure 4.1 and Appendix VI show the concentration of heavy metals at various sites in the Winam Gulf. The highest levels of Cu were at Dunga beach (1.543 ± 0.03ppm), followed by Tilapia beach (1.03 ± 0.011), while the other sites did not record any copper. The levels at Dunga were almost 2 times the FAO/WHO maximum acceptable limits of 1 ppm in drinking water. Mireji *et al*. (2008) reported values of 0.111 ppm in water of larval habitat in the Winam Gulf, their values were far much lower than the values obtained in this study. In another study, Matindi *et al*. (2014) reported mean Cu levels of 0.1603 ppm. Ongeri *et al*. (2009) reported values of between 1.53 ppm and 3.86 ppm in Winam gulf. The values in the three studies were all lower than the highest value in this study which implies that levels of Cu are increasing with time. This could be due to activities going on at the beach like fishing and car washing.

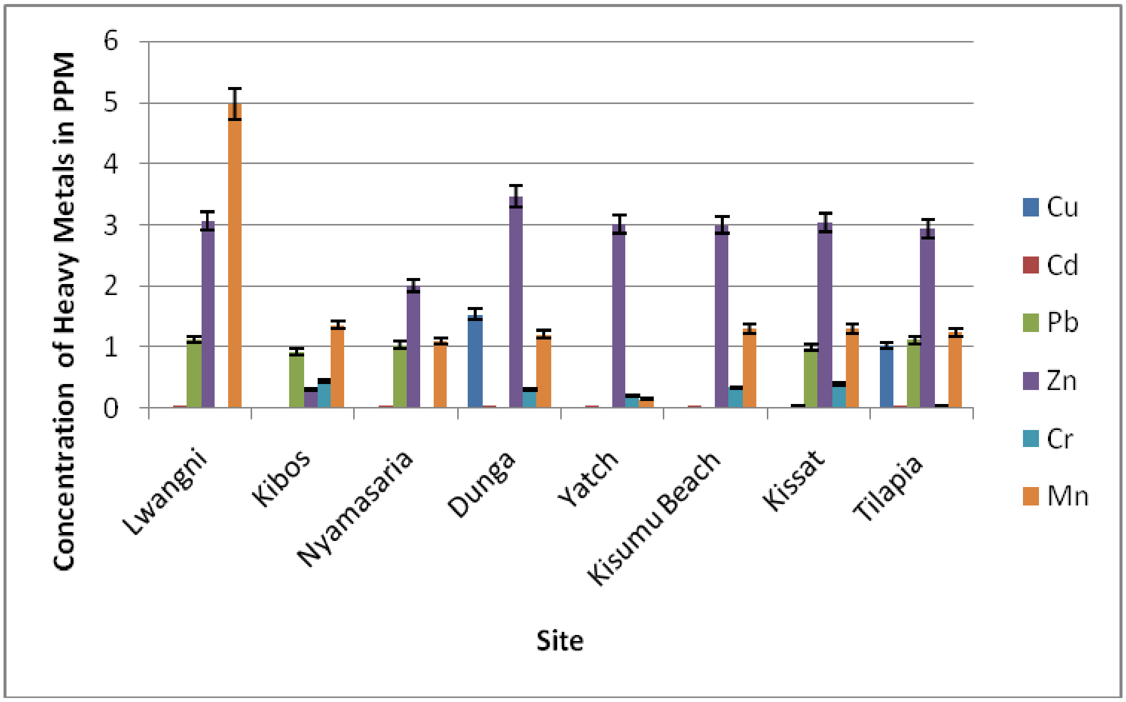


Figure 4.1: Levels of heavy metals at various sites

The highest level of Cd was at the Tilapia beach (0.037±0.007 ppm), followed by the river near Yatch club which had 0.0±36 ppm. However, the lowest levels were at R. Kibos (0.013 ± 0.001 ppm). All these values were more than ten times the WHO limits (0.003 ppm) in drinking water. A study by Muinde *et al*. (2013) reported Cd ranges from below detection limits to 0.02 ppm, while Ogoyi *et al*. (2011) did not detect any Cd in the water samples in the Winam Gulf which implies that the levels of Cd are increasing with time. The high levels could imply that cadmium compounds from agricultural, domestic and industrial sources are released into the water bodies around Lake Victoria and are carried by run off towards the lake. This could also imply that the Lake acts as a ‘sink’ of pollution loads from both point and diffuse sources. This means that pollution in the Winam Gulf is going up year after year, which could be due to increase in activities that bring about pollution in the area.

The highest levels of Zn were at Dunga beach (3.467± 0.032 ppm) followed by Lwang’ni beach (3.06±0.015 ppm) while the lowest levels were at R. Kibos (0.3005 ppm). All the values were, however, much lower than the FAO/WHO maximum acceptable limits of 5 ppm. Muinde *et al*. (2005) reported Zn levels of 0.14 ppm in the same area which was still lower than the FAO/WHO limits of 5 ppm. Ogoyi *et al*, (2011) obtained similar results, in their study they found Zn levels of between 0.36 ppm and 0.047 ppm with a mean value of 0.05 ppm. This implies that the area is still not polluted with respect to zinc, which could be due to the fact that activities around the Gulf don’t release Zn into the water bodies. The highest levels of lead were recorded at Lwang’ni beach (1.13 ± 0.011 ppm), Tilapia beach (1.12 ± 0.031ppm) and River Nyamasaria (1.04± 0.011 ppm), No lead was detected at Kisumu beach Resort, Dunga beach and the Yatch club. The other sites, that is, River Kibos and River Kissat had 0.92 ± 0.008 ppm and 0.99± 0.01 ppm, respectively.

The values of Pb at Lwangni, Tilapia Beach and Nyamasaria were ten times higher than the KEBS maximum acceptable limits of 0.1 ppm and more than 100 times higher than the FAO/WHO maximum acceptable limits of 0.01 ppm for drinking water. The levels at Kissat and Kibos were also more than 9 times the KEBS acceptable limits of 0.1 ppm and more than 90 times the FAO/WHO acceptable limits for drinking water. The high levels at the five sites could be due to the different activities practised. Lwangni beach is used as a car wash and the Pb could be from car engines. Raw sewerage is also released into River Kissat directly by the Kisumu Municipal council and this could lead to pollution of the river by Pb from domestic and industrial sources. River Nyamasaria is also used as a car wash and during the research period the area was highly eroded because the bridge was under construction, the high levels of Pb could be because of the vehicles being washed in the area, or from the soil parent material which releases Pb into the environment due to erosion as a result of the bridge construction. The study revealed that 66% of the sites had Pb levels above the WHO prescribed limit for drinking water.

A study by Ogoyi *et al*. (2011) reported mean Pb^{2+} levels were between 0 and 1.622 ppm, with a mean value of 0.823 ppm which was more than 80 times higher than the WHO/FAO maximum acceptable limits of 0.01 ppm, however, the value was still lower than most of the values reported in this research, which implies that the amount of Pb is increasing with time in the Winam Gulf. Makokha *et al*. (2011) reported values of 0.02 ppm and 0.015 ppm at Tilapia beach and Dunga beach, respectively, which were still lower than the value reported in this research. The highest levels of Mn were at Lwang'ni beach (4.967±0.04 ppm) while the lowest was at the Yatch club (0.153±0.003 ppm), R. Kibos had also a significant amount of Mn (1.365 ± 0.021 ppm). These levels were all more than 10 times the WHO maximum permissible limits of 0.1 ppm. The results obtained indicate that 20% of the sites had manganese levels above WHO allowable range for drinking water. Mireji *et al*. (2008) reported values of 8.270 ppm of Mn in water of larval habitat in the same area. The value was higher than the value obtained in this study implying that there could be dilution caused by runoff or a reduction in use Mn containing compounds in the area. In another study by Matindi *et al*. (2014) the mean value of Mn in water hyacinth was reported as 16.03 ppm, the value was again much higher than the mean reported in this study. Most of the manganese in the environment is due to burning of fossil fuels. Use of manganese bearing fertilizers also contributes to air and water pollution by Mn. The industrial processes are the major sources of manganese pollution.

The highest level of Cr was at River Kibos (0.459 ± 0.021 ppm), but not detected at River Nyamasaria and Lwang'ni beach. Tilapia beach had very low levels of Cr (0.033± 0.005 ppm). However, Cr levels at River Kibos (Figure 4.1) were almost 10 times the FAO/WHO maximum acceptable limits of 0.05 ppm. Other areas that had high leves were; R. Kissat (0.402± 0.02 ppm) and Kisumu beach resort (0.342 ± 0.02). The high levels of Cr at R. Kibos could be due to the fact that the river drains the sugar belt and therefore the chromium compounds used in the catchment area in fungicides, seed protectants and wood-preservatives are released and carried by run off to the water bodies. In a study done by Ogoyi *et al*. (2011) reported Cr levels of between 0.183 ppm and 0.298 ppm, with a mean of 0.178 ppm, while Muinde *et al*. (2013) reported Cr levels of 0.06 ppm. The values in the two studies were still lower than the values obtained in this study, which implies that pollution due to Cr is increasing with time. It was found that at Winam Gulf, concentrations of metallic elements were higher in water samples than what was found in microalgae samples while the opposite was true for water and microalgae samples from the Mwanza Gulf. All the levels of heavy metals in this study were higher than the values by Ogoyi *et al*. (2011).

4.4 Biosorption Studies

Various variables were investigated on their effects on biosorption of heavy metals by *Moringa oleifera* seeds, the variables were; pH, adsorbent size, adsorbent dosage, initial metal concentration, contact time, pretreatment, competition from other ions, kinetics models and sorption isotherms.

4.4.1 Effect of pH

Figure 4.2 shows the effect of pH on the removal of Cu^{2+}, Mn^{2+}, Pb^{2+}, Cd^{2+}, Cr^{2+} and Zn^{2+} onto adsorbents from aqueous solutions. As it can be depicted, biosorption of the metal ions under this study by *Moringa oleifera* seed powder was strongly affected by pH

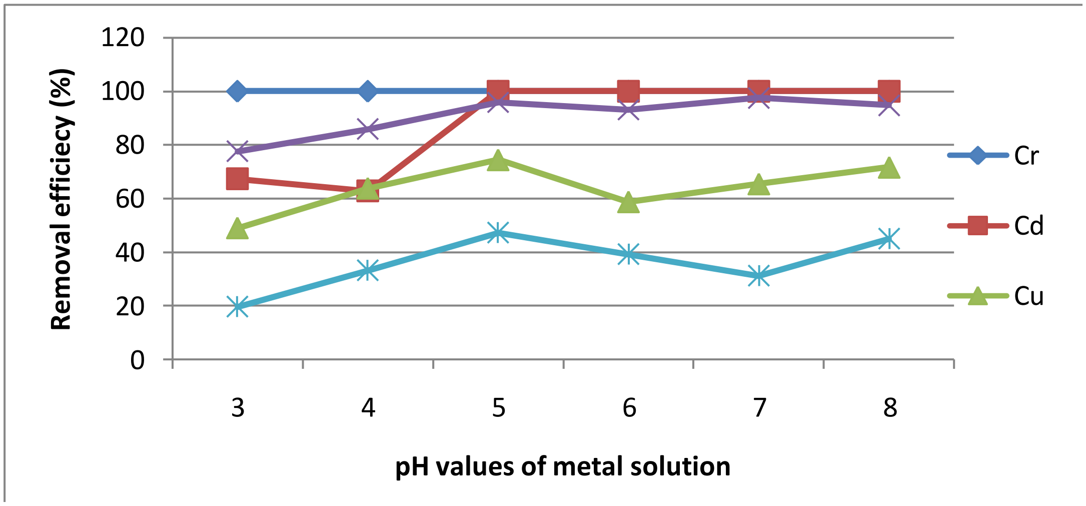


Figure 4.2: Effect of pH on metal biosorption using *M. oleifera* seed powder

A sharp increase in metal uptake was observed with increasing pH. It was observed that with the increase of the initial pH, the removal efficiency of the metal ions increased at first but generally decreased after attaining the pH of 5. The maximum removal was 100% for Cd^{2+} and Cr^{2+}, 95.85% for Zn^{2+}, 47.23% for Mn^{2+} and 74.4% for Cu^{2+}. The optimum pH for removal of most metals was 5. The binding of metal ion to adsorbent was therefore pH dependent. At pH values of 6 to 8 for most metals the metal uptake by moringa either remained constant or decreased, which could be due to precipitation of metal ions at high pH. For example, the uptake of Mn^{2+} by moringa increased steadily upto pH 5 (47%), and then started reducing so that at pH values of 7 and 8, the respective percentage removal was 31 and 45. Cd^{2+} uptake increased steadily between pH 3 (67.3%) and 5 (100%) the uptake did not change after pH 5. Most interesting biosorption was Cr^{2+} which had a biosorption percentage of 100 at all pH values. For the rest of the metals studied the uptake increased upto pH 5 and then decreased or remained constant.

The adsorbent had a biosorption capacity of 47% (Mn^{2+}) and above at pH 5. For Cu^{2+}, Zn^{2+} and Pb^{2+} biosorption increased upto pH 5 and then reduced as the pH increased. The pH dependence of metal uptake can be attributed to the nature of *Moringa oleifera*. Adsorption increased with increasing solution pH since more metal binding sites could be exposed with negative charges, hence subsequent attraction of metal ions with positive charges and absorption occurring onto the cell surface (Taffarel and Rubio, 2009). This phenomenon is also attributed to the fact that substantial hydrogen ions compete for vacant adsorption sites of adsorbents at lower pH values. A decrease in removal of metal ions by the adsorbent was noticed at pH values above 5. With the increase of initial pH above 5, there were more amounts of negative ions and the metal ions were surrounded by anions. It is difficult to combine with the adsorption sites on adsorbent's surface of negative charge. Experiments were carried out up to pH 8 because metal precipitation occurred at higher pH values and interfered with the accumulation or biomass deterioration.

Another reason for increasing biosorption of metal ions with increasing pH is the zero point charge (PZC) of the biomass (pH 2.9–3.0), is the pH at which the biomass surface electrical charge is zero (Abu Al-Ru *et al*., 2006). At pH values above the PZC, the biomass cells would have negative net charge and the electrostatic attractions between positively charged and negatively charged binding sites on the biomass surface is enhanced. However, adsorption of anionic species will be favoured at pH < PZC. The PZC of the *Moringa oleifera* seeds has been found to be between 6.0 and 7.0 (Alves *et al*., 2010) indicating that the surface of the biosorbent presents acid characteristics. The surface charge of the seeds is positive at pH < PZC, is neutral at pH = PZC and is negative at pH > PZC (Cleide *et al*., 2013). The variation in pH caused by protonation and deprotonation of the adsorbent reflects the presence of functional groups. All these reasons explain the significant increase of the binding efficiency by increasing the pH from 3 to 5. At pH below 3, biosorption capacity is negligible, probably due to the cation competition effects with hydroxonium H_3O^+ ion. Furthermore, at pH 6 biosorption of metals decreased probably because of chemical precipitation (Aksu and Tezer, 2005). *Moringa oleifera* seed powder contains a large number of functional groups in which carboxyl (from protein) is the most abundant acidic functional group. At low pH there is competition between H_3O^+ and metal ions, at low pH values the concentration of of H_3O^+ far exceeds that of metal ions and hence occupying all the binding sites, leaving metal ions unbound.

When the pH is increased the competing effect of H_3O^+ decreases and the positively charged metal ions occupy the free binding sites. Another reason could be that at low pH the ligands are closely associated with hydronium ions and hence the access of the metal ion to the ligand is restricted. With increasing pH carboxyl groups would be exposed leading to attraction between the negative charges and the metals and hence increase in biosorption. At much higher pH values, precipitation occurs and both sorption and precipitation would be the effective mechanisms to remove the metal ions in aqueous solution.

The findings are in agreement with those of Kannan and Veemaraj (2009) who studied the removal of Pb^{2+} by bamboo dust and commercial carbons. Other scientists who have produced similar results are; Adelaja *et al*, (2011) who studied the biosorption of Pb^{2+} from aqueous solution using *M. oleifera* pods. Paula *et al*. (2013) found that the highest level of metal removal was achieved at pH 5. A smaller degree of removal at pH levels of 6 and 7 can be explained by the fact that the mobility of some elements may be reduced, which can lead to precipitation of insoluble forms at pH values close to neutral. In solutions of low pH values, H^+ and metal ions compete for the active sites of adsorption (Adelaja *et al*., 2011).

However, in a study done using the pods of the *Moringa oleifera* tree for biosorption of heavy metals by Bhatti *et al*. (2007) the removal of Zn (II) ions from aqueous solution using pure and chemically pre-treated biomass of *Moringa oleifera* pods, the experimental results showed that the maximum pH (pHmax) for efficient sorption of Zn(II) was 7 ± 0.1 at which evaluated biosorbent dosage and biosorbent particle size, were 0.5 g/L, < 0.255 mm, respectively. The optimum metal removal pH compares well with Mataka *et al*. (2010), percentage removal of Cd by *Moringa oleifera* seed powder increased sharply to about 80% with increase in pH upto 5 and then gradually increasing slowly to maximum removal 0f 93.8%, that is, the optimum pH range for Cd removal is 5 and above. Similar results were obtained by Sharma *et al*. (2006) who got optimum pH of 6.5 using *Moringa oleifera* seed powder. Sajidu *et al*. (2013) reported an optimum pH of 7.8 on Cd (II) ion removal using water and sodium chloride extracts of *Moringa stenopetala* and *Moringa oleifera.* The reduced sorption efficiency of metal removal with a decrease in pH could be attributed to the presence of H^+ ions in the mixture, which compete with metal ions for the binding sites.

The results were similar to those obtained by Affonso *et al*. (2012) who set the optimum pH as 5 for biosorption of Cr, Cd and Pb, after obtaining the point of zero charge for *Moringa oleifera* in KCl to be about pH 4.4. Thus, the adsorption of cations in case of heavy metals is favoured at pH values above the pH_{PZC} (Sharma *et al*., 2007). The pH chosen was pH 5.0 for solutions in subsequent tests, because, in addition to the pH_{PZC} test, it was also possible to prove in the pH test that fortified solutions at pH 5.0 needed lower mass to achieve a similar efficiency to that obtained under conditions of pH 7.0. One important factor is that lower pH values keep the metal soluble and favour its mobility (Fetter, 1993). Sud *et al*. (2008), used Cd and Pb metals and found the optimum pH values for adsorption using agricultural residues to be that with acidic character.

Different pH values were obtained in another study by Sajidu *et al*. (2013), using *Moringa oleifera* seeds at initial metal concentration of about 4 ppm. The extracts showed complete sorption for Cd^{2+}, Zn^{2+} and Cr^{3+} ions at pH above 7.8, 4.0 and 4.0, respectively, at a dose of 1.0 mL of sorbent in 9.50 mL of metal solution. Cu^{2+} sorption increased slightly with pH to about 60% at pH 6 and then became constant up to pH 8 when sorption reached completion.

4.4.2 Effect of contact time and comparison of biosorption rates

The mass chosen for the procedure was 0.400 g. The rate and time taken for biosorption to get to completion is important for designing batch biosorption experiments. The biosorption of metal ions increased considerably until contact time reached 60 min at 25°C. Further increase in contact time did not enhance the biosorption process; so, the optimum contact time was taken to be 60 minutes for further experiments (Figure 4.3).

The concentration of all the metal ions studied decreased significantly in solution. The percentage removal was rather fast in the first 10 minutes, after which the removal rate significantly decreased and eventually reached a plateau after 30 minutes. The rate of removal, however, increased gradually and reached equilibrium at 60 minutes of contact between the adsorbent and the aqueous solution.

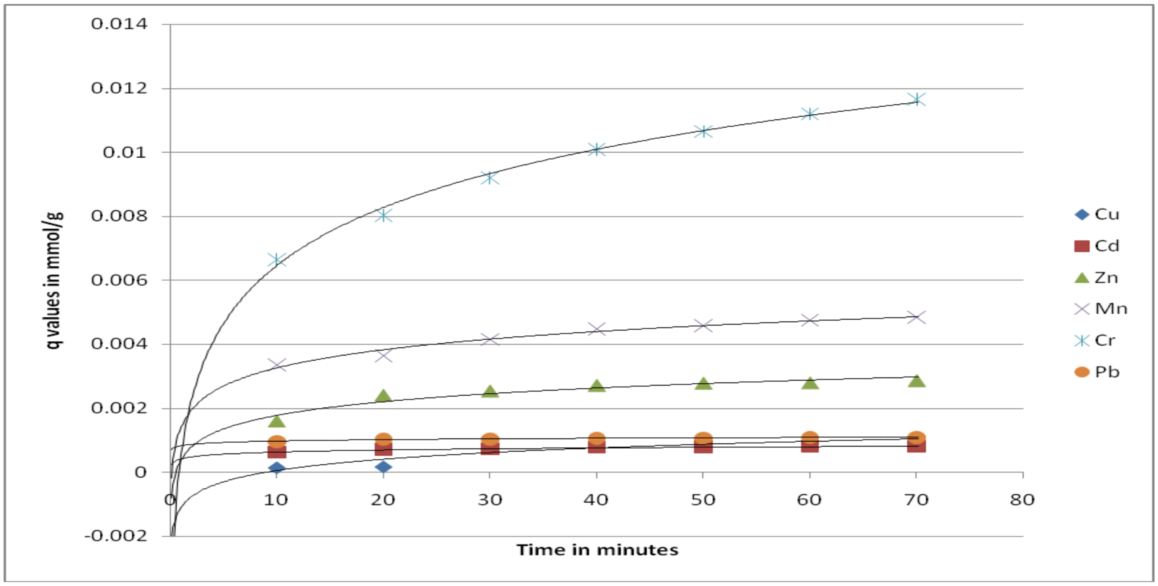


Figure 4.3: Effect of contact time on removal of the metals by *M. oliefera*

The initial fast phase occurs due to surface adsorption on the biomass. The subsequent slow phase occurs due to diffusion of the metal ions into the inner part of the biomass. Contact time required for a system to reach equilibrium depended on the nature of biosorbent and amount of available adsorption sites. This result is important, as equilibrium time is one of the important parameters for an economical wastewater treatment system (Bhatti *et al*., 2007).

According to Affonso *et al*. (2012) biosorption of Cd, Cr and Pb using *Moringa oleifera* seeds, the metal removal efficiency increased with the mass increase, a fact that could be attributed to the increased surface area for adsorption and to the availability of active adsorption sites. However, an excessive increase in the adsorbent amount could cause a reduction in the removal, may be due to the formation of aggregates during sorption, as described by Ekmekyapar *et al*. (2006). According to Oboh *et al*. (2009) biosorption of Pb using sour sop seeds, Kannan and Veemaraj (2009) biosorption using bamboo dust and Reddy *et al.* (2010) biosorption activated carbon as adsorbent, all reported that contact time was directly proportional to amount of Pb^{2+} ions adsorbed from aqueous solution. This phenomenon may be related to the vacant adsorption sites on the adsorbent surface. During the initial stage of sorption, a large number of vacant surface sites are available for adsorption. After a lapse of time, the remaining vacant surface sites can be occupied with a lot of difficulty due to repulsive forces between the solute molecules on adsorbent surface and the bulk phase (Wongjunda and Saueprasearsit, 2010). The biosorption of Cu (II) by the biomass was rapid for the first 30 min as a result of the free binding sites on the biomass.

The binding sites later became limited and the remaining vacant adsorbents surface sites were difficult to contain more copper ions due to the formation of repulsive forces between the metal ions on the solid surface of the adsorbent and the liquid phase (Anwar *et al*., 2010). This was in agreement with the effect of contact time presented by Dhabab (2011), on the removal of Fe^{2+}, Cu^{2+}, Zn^{2+}, and Pb^{2+} ions from aqueous solutions by duckweed. The difference in rate of biosorption to the surface of biosorbent could be attributed to the variation in ionic size because the charge is the same (+2) for all the ions. It was found that the order of biosorption efficiency was $Cr^{2+} > Mn^{2+} > Zn^{2+} > Pb^{2+} > Cd^{2+} > Cu^{2+}$. However, according to Vikashni *et al*. (2012) the percentage removal obtained by *Moringa oleifera* seeds showed adsorption of metals for which the percentage adsorption for copper was about 90 %, Pb was about 80 %, cadmium was 60 %, and chromium and zinc were about 50 % which were higher compared to other local seeds.

The trend for the first three metal ions could be attributed to the size of the ions which is related to the hydration radius. In aqueous solution, metal ions are hydrated and the larger ions are less hydrated (smaller hydration radius) hence the more attraction to the anions on the biosorbent. The order of the first three metal ions could be attributed to their decreasing size, where Cr^{2+} is the largest hence has the smallest hydration radius making it more attracted to the negative ligands and therefore the highest biosorberd, while Zn^{2+} being the smallest has the largest hydration radius making it the least biosorbed. In the case of Pb^{2+}, Cd^{2+} and Cu^{2+}, Cu^{2+} and Cd^{2+} are first and second series transition elements, respectively, while Pb is not a transition element. Pb^{2+} is the largest of the three, hence the smallest hydrated radius and more attracted to the biosorbent, followed by Cd^{2+} and the smallest is Cu^{2+} the largest hydrated radius and so the least biosorbed (Sag *et al*., 2002).

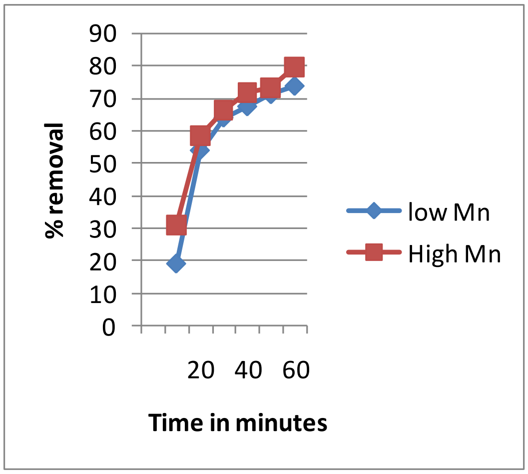
Also, factors related to electronegativity, hydrolysis constant and ionic radius can favour the adsorption of a metal species over another. Regarding eletronegativity, there is the following selectivity order: Pb > Cu > Cd > Cr > Zn > Mn, since the higher the electronegativity, the greater the affinity for sorption (Sag *et al.*, 2002). Regarding the ionic radius, Pb would be the most adsorbed, followed by Cd, Cr, Mn, Zn and Cu. Pb ions have the smallest hydrated radius compared to the other metals studied, thus, it has greater accessibility to the surface and pores of the adsorbent, resulting in higher adsorption capacity (Srivastava *et al.*, 2008). As reported by Liu *et al.* (2009), the weak bonding of Zn compared to Cr and Cu is attributed to the electron configuration of Zn^{2+} [$3d^{10}$ $4s^0$] compared to Cr^{2+} [$3d^4$ $4s^0$] and Cu^{2+} [$3d^9$ $4s^0$]. According to the electron configuration, the outer orbital of Zn^{2+} is full [$3d^{10}$] and there are no suitable empty orbitals to form a strong bond with surface functional groups. However, in Cr^{2+} [$3d^4$] and Cu^{2+} [$3d^9$], there are outer empty orbitals which have empty spaces (which can be filled up to [$3d^{10}$].

Transient metals such as Zn, Cr and Cu with small ionic radii and empty orbital easily complex with surface functional groups (Gunawardana *et al.*, 2012). Therefore, Zn forms weak bonds with charge sites compared to the transition metal elements studied. Cr is a divalent cation, which has a high electro negativity (hard acid) compared to other metal elements in this study. Therefore, Cr can compete with other metal elements for complexation sites and primarily forms strong bonds of chemisorption with solids particles (Gunawardana *et al.*, 2012). Cr association with solid particles through cation exchange is expected to be very low. This agrees with the findings of Covelo *et al*. (2007) who noted near-zero concentration of extractable Cr in batch adsorption studies using soil samples.

Ravikumar and Sheeja (2013) noted that the optimum dosage of *Moringa oleifera* aqueous extract for synthetic water samples containing heavy metal concentrations of 5 mg/L was 2 g/L and the removal efficiencies were 95%, 93%, 76% and 70% for copper, lead, cadmium and chromium, respectively. The results are also similar to those obtained by Kardam *et al*. (2010), who studied the biosorption of Cd by *Moringa oleifera* seed powder for durations of 10 – 60 minutes and found that the percent sorption of metal ions gradually increased within the first 30 minutes. This finally reached the optimum value at 40 minutes. Once equilibrium was attained, the percent-age sorption of Cd(II) ion did not change with further increase of time. According to Sharma *et al*. (2006), the aqueous solution containing the seeds by product of *Moringa oleifera* Lam. is a heterogeneous mixture containing various functional groups, especially low molecular weight amino acids. These amino acids can be considered an active group of binding agents, acting even at low concentrations due to the ability to interact with metal ions increasing their sorption.

4.4.3 Effect of initial metal concentration on biosorption of metal ions

The removal of metal ions (Mn^{2+}, Cu^{2+}, Cr^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+}) from solutions containing metal concentrations of 2 ppm and 3 ppm using 8 g/L *Moringa oleifera* seed powder was studied at pH 5 which had been found in an earlier experiment as the optimum pH for all metals under the study. The results (Figures 4.4 to 4.9) indicated that, increasing metal concentration of the solutions causes a corresponding increase in the rate of metal biosorption, that is, an increase in adsorbate concentration resulted in a corresponding increase in percentage adsorption. The range in percentage increase was from 73.8 – 79.9, 71.2 – 80.3, 70.2 – 79, 87.5 – 94.9, 92.2 – 98 and 96.1 – 98 for Mn^{2+}, Cu^{2+}, Cr^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+}, respectively. The order of biosorption for the lower concentration was; $Pb^{2+} > Zn^{2+} > Cd^{2+} > Mn^{2+} > Cu^{2+} > Cr^{2+}$, while that of the higher concentration was $Pb^{2+} > Zn^{2+} > Cd^{2+} > Cu^{2+} > Mn^{2+} > Cr^{2+}$. The two orders are similar and are close to the order of biosorption based on the electronegativities (Pb > Cu > Cd > Cr >Zn > Mn), that is, higher electronegativity implies higher affinity for sorption (Sag *et al*., 2002). This can be explained by a progressive increase in the electrostatic interactions, relative to covalent interactions, of the sites with lower affinity for metal ions when the initial concentration is increased (Salim *et al*., 1992). The trend could also suggest that increasing the adsorbate concentration results in increasing the number of available molecules per binding site of the adsorbent thus bringing about a higher probability of binding molecules to the adsorbent. The increase in metal uptake with increasing initial metal concentration is a phenomenon that has been studied.



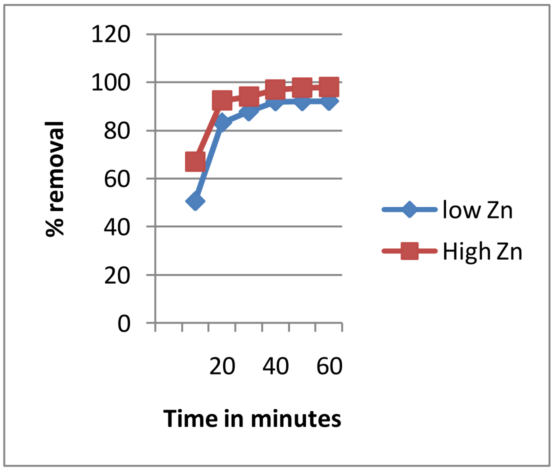
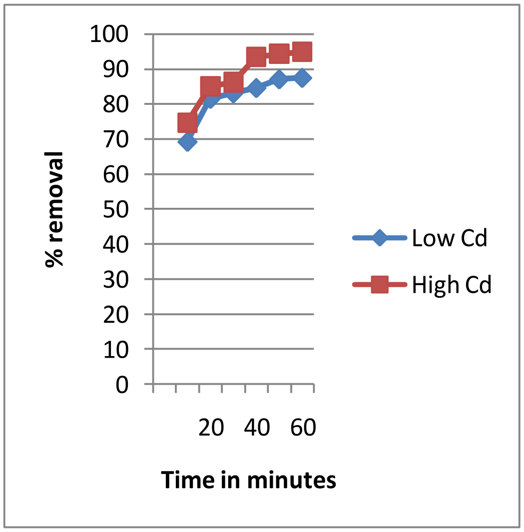


Figure 4.4: Effect of initial Mn^{2+} levels

Figure 4.5: Effect of initial Zn^{2+} levels



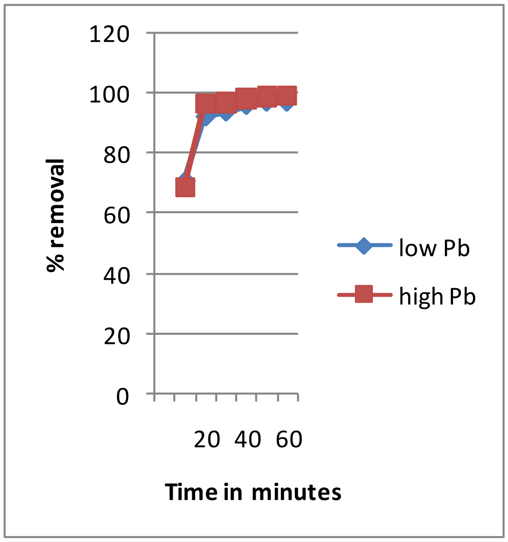
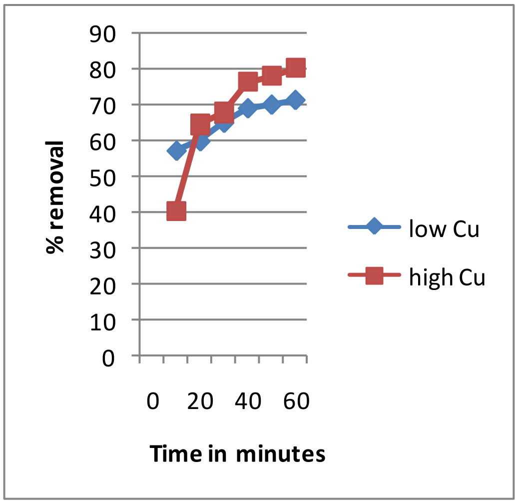


Figure 4.7: Effect of initial Cd^{2+} levels

Figure 4.7: Effect of initial Pb^{2+} levels



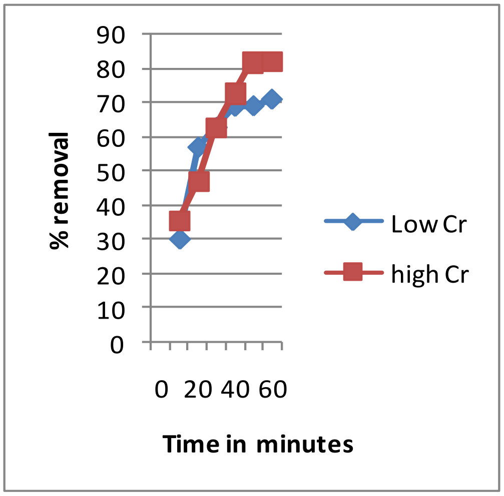


Figure 4.8: Effect of initial Cu^{2+} levels

Figure 4.9: Effect of initial Cr^{2+} levels

It was observed by Deshkar *et al*. (1990), Singh *et al*. 2007), AL-Asheh and Duvnjak (1998), AL-Subu *et al*. (2001), Elaigwu *et al.* (2009), Hashem (2007) and Karaca *et al*. (2010) using different kinds of adsorbents. The effect of initial metal ion concentration on the biosorption capacity shows that efficiency increased as the initial metal ion concentration increased. Similar results were obtained by Kumar *et al*. (2010) using *Moringa oleifera* for biosorption of Ni(II). According to them biosorption increased with increasing concentration of the metal ion reaching an optimal level (25 mg/L). Later, an increase in initial concentration decreased the percentage binding. This was explained as due to the fact that at medium concentrations, the ratio of sorptive surface area to metal ions available is high and thus, there is a greater chance for metal removal. When metal ion concentrations are increased, binding sites become more quickly saturated as the amount of biomass concentration remained constant (Yetilmezsoy and Demirel, 2008). Mataka *et al*. (2010) found that the increase in initial Cd(II) concentration decreased cadmium percentage removals and increased the amount of Cd(II) uptake per unit mass of the *Moringa oleifera* powders (mg/g). The removal of Cd(II) ions decreased from 12.9% (0.65 mg/g) to 6.2% (0.092 mg/g) for *Moringa oleifera* and 13.1% (0.65 mg/g) to 7.6 (0.113 mg/g) for *Moringa stenopetala*. Further, the results indicated that at several initial concentrations tested, *Moringa stenopetala* was more effective than *Moringa oleifera* for cadmium ion sorption ($p < 0.05$). Effect of initial metal ion concentration is an important parameter, which determines the maximum uptake capacity of the chelating biomass (Reddy and Reddy, 2003).

For a fixed dose of the biomass the total available binding sites are limited, thereby binding the same amount of metal ions. This results in a decrease in percentage removal of the metal ions as the initial metal ion concentration increases. The enhanced metal uptake with an increase in initial metal ion concentration could be due to the increase in the ratio of initial number of moles of Cd(II) to the available surface area of the *Moringa oleifera* seed powder. These results agree with those reported in literature on other metal ion-sorption using wild cocoyam and steam activated sulphurised activated carbon prepared from sugarcane bagasse pith (Horsefall and Spiff, 2004; Krishnan and Anirudhan, 2003).

4.4.4 Effect of biosorbent size

Mn^{2+}, Cu^{2+}, Cr^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+} biosorption capacities at two particle sizes of *Moringa oleifera* are shown in Figures 4.10 to 4.15. It was apparently observed that the percentage biosorption of Cu^{2+}, Pb^{2+}, Mn^{2+}, Cd^{2+}, Cr^{2+} and Zn^{2+} increased with decreasing particle size of *Moringa oleifera* biomass. The range percentage increase was

78-82, 80- 95, 92- 98, 85-91, 87-94 and 84 - 96 for Cr^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, Cd^{2+} and Zn^{2+}, respectively. It is evident from the figures that biosorption capacity of all the metal ions studied increased with a decrease in particle size of *Moringa oleifera* biomass. The effect of altering particle size on the Mn^{2+}, Cu^{2+}, Cr^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+} ions uptake by *Moringa oleifera* biomass showed that removal of metal ions was more dominated by smaller particle size, this could also be due to a change in surface morphology of the biomass caused by the decrease in size. In this study biosorption capacity of *Moringa oleifera* biomass was higher when the size of the particles was 0.25 mm than 0.75 mm. Thus high adsorption occurred with 0.25 mm biosorbent size.

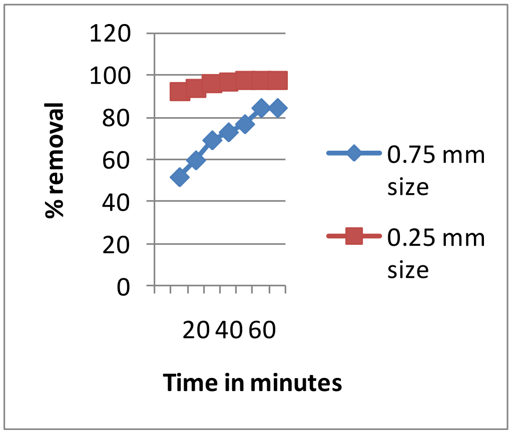


Fig 4.10: Effect of particle size on Zn^{2+}

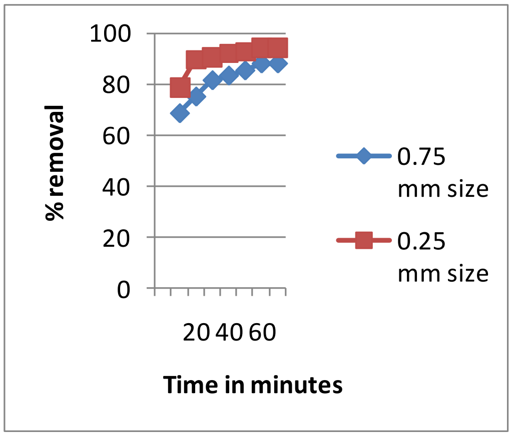
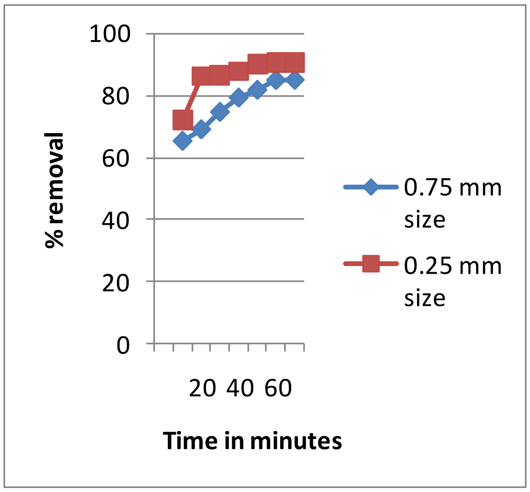


Fig 4.11: Effect of particle size on Cd^{2+}



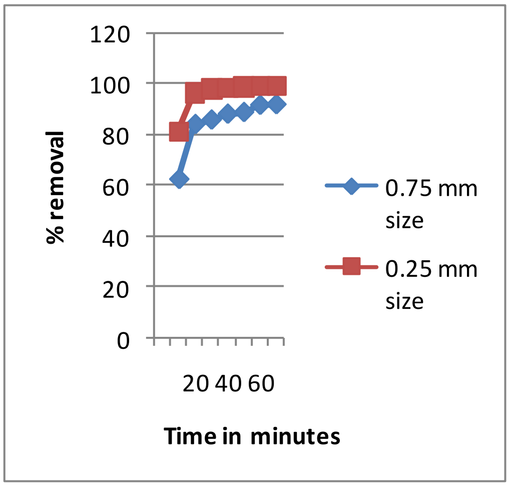
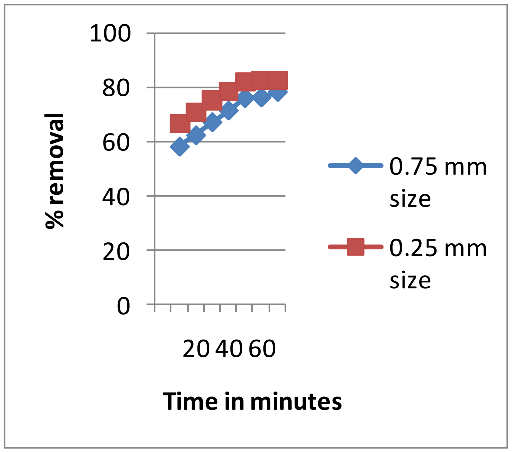


Fig 4.12: Effect of particle size on Mn^{2+}

Fig 4.13: Effect of particle size on Pb^{2+}



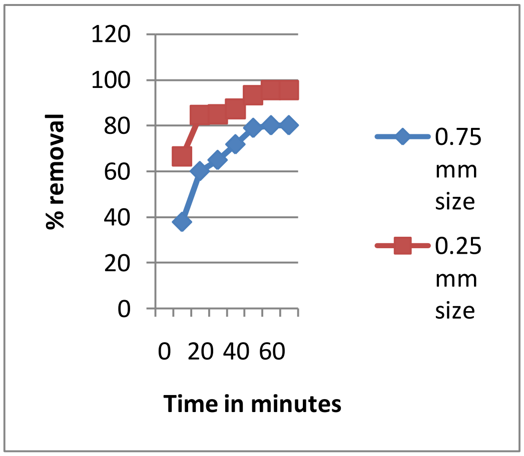


Fig 4.14: Effect of particle size on Cr^{2+}

Fig 4.15: Effect of particle size on Cu^{2+}

Similar results were reported by Eman *et al*. (2015), they used *Moringa oleifera* leaves of particle sizes; 2 mm, 1 mm, 500 µm, 250 µm and < 250 µm for the removal of Cd^{2+} from waste waters and reported that the 2 mm and 1 mm particle sizes had the lowest removal efficiency, while the particle size of < 250 µm had the highest removal efficiency (81%) which was due to the large surface area to volume ratio for the smallest particle sizes. This was most probably due to increase in the total surface area which provided more sorption sites for metal (Hanif *et al.*, 2007). Hosssein *et al*. (2013) who used *Moringa* *oleifera* seeds of particle sizes of < 0.125 mm, 0.125 mm – 0.42 mm and 0.42 mm – 1.18 mm, reported different results, they noted that the greater particle size, gave higher metal removal efficiency, which was due to very small sizes of sorbent used which could have passed through the filter and released the sorbed metal to the filtered solution. Neelima (2013) reported results similar to the ones of this study using particle sizes of 125 – 180, 180 – 250, 250 – 355 and 355 -500 µm for biosorption of Cr(VI) and found that the smaller particles sizes gave higher metal removal efficiencies.

4.4.5 Effect of adsorbent dosage on metal biosorption

The effect of the adsorbent dosage on the removal of Mn^{2+}, Cu^{2+}, Cr^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+} from aqueous solution was investigated by using a dosage of the adsorbent of 0.4 and 0.6 g in 50 mL of metal ion solution whose concentration varied for various metal ions due to adjustment of pH (Figures 4.16 to 4.21). It is expected that an increase in dosage of adsorbent should yield a corresponding increase in the amount of metal ion adsorbed onto the surface of the adsorbent since there are more sites for the adsorbate to be adsorbed. Therefore competition for bonding sites between molecules of the adsorbate should decrease with increase in dosage of the adsorbent. Increase in metal removal with increasing biomass concentration of both pretreated and raw biomass may be explained by a higher biomass/metal ratio (that is, higher number of binding sites available). A similar effect of biomass concentration has been observed in other studies (Esposito *et al*., 2002; Ngwenya *et al*., 2003). In some studies, reduced metal removal has been observed at high biomass concentrations. This may result from sorption of metals to dissolved organic acids that interfere with sorption to bacterial surface structures (Puranik and Paknikar, 1999) or formation of cell aggregates that reduce the surface area available for binding. Such a phenomenon was not observed in this work, probably because excessive *Moringa oleifera* biomass concentrations were not used.

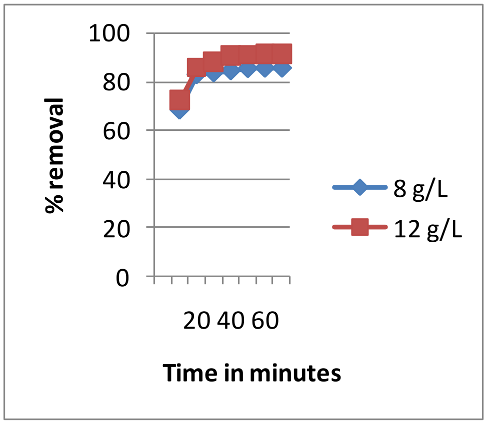


Fig 4.16: Effect of adsorbent dose on Mn^{2+}

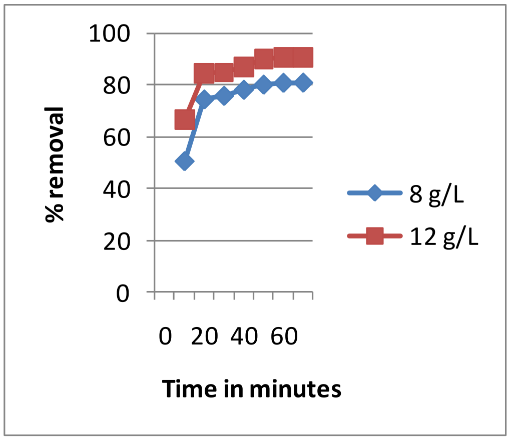


Fig 4.17: Effect of adsorbent dose on Cr^{2+}

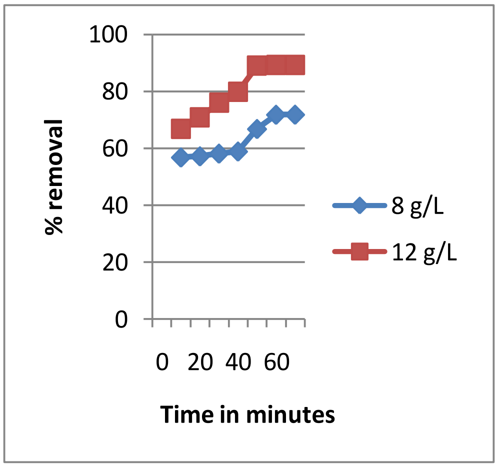


Fig 4.18: Effect of adsorbent dose on Cu^{2+}

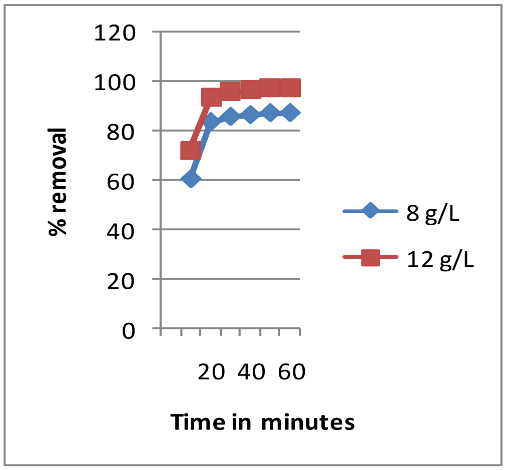
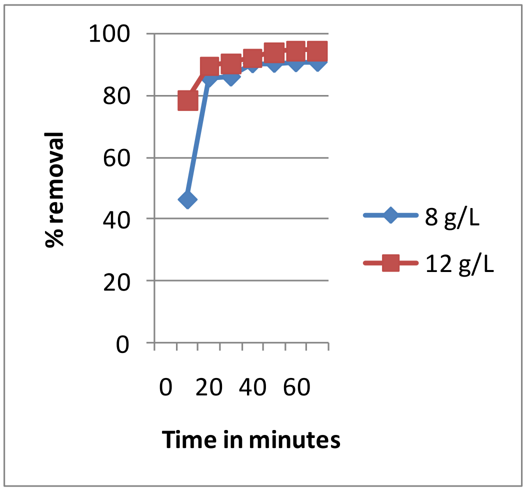


Fig 4.19: Effect of adsorbent dose on Zn^{2+}



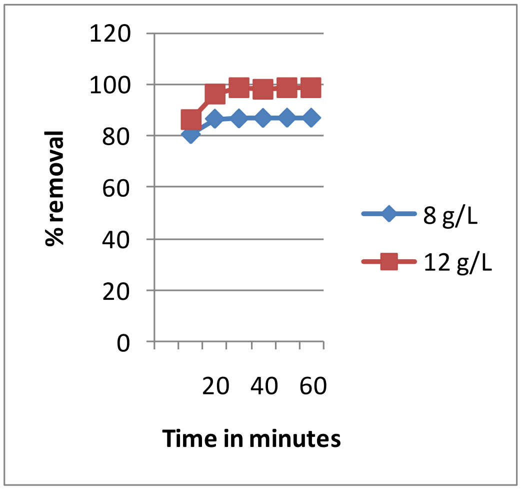


Fig 4.20: Effect of adsorbent dose on Cd^{2+} Fig 4.21: Effect of adsorbent dose on Pb^{2+}

This assertion is supported by Elaigwu *et al*. (2009). Other studies were conducted by Oboh *et al*. (2009) using sour sop seeds, Reddy *et al*. (2010) using *Moringa oleifera* bark and varying adsorbent dosage from 0.1 – 1 g. Tangjuank *et al*. (2009) used activated carbon prepared from cashew nut shells, and others reveal a direct proportional relationship between the adsorbent dosage and percentage adsorption.

Javed *et al*. (2007) reported that the biosorbent dose influences the Pb (II) and Co (II) uptake capacity of rose waste biomass, and a dose of 0.5 g/L was optimum for maximum uptake of these metals. Biosorbent dose is a significant factor to be considered for effective metal sorption from aqueous systems. It determines the sorbent/sorbate equilibrium of the system. Dose of biomass added in to the solution determines the number of binding sites available for adsorption (Hanif *et al*., 2007). At low dose level, all binding sites are exposed and adsorption on the surface is faster, but at higher biosorbent doses the availability of higher energy sites decreases with a larger fraction of low energy sites occupied, resulting in low q_e value since the ratio of biomass to adsorbate increase because the amount of biomass increase while the amount of adsorbate remains constant, Higher concentration of adsorbent sites could also show delayed or no saturation at all (Gupta and Rastogi, 2008).

Similar results were obtained by Vikashni *et al*. (2012), who found out that removal of metals increases with increasing dose of *Moringa* seed coagulants. The analysis was carried out using standard metal ion solutions and method of standard addition was used for the determination of metal ions after spiking the solution with varying *Moringa* seed dosages. This was then analysed using AAS and the concentrations were calculated using the standard calibration graphs of the desired metals. *Moringa* seeds were found to be more efficient in metal removal in water. The flocculation activities of *Moringa oleifera* seeds are based on the electrostatic patch charge mechanism, which is a descriptions that scientists use to account for the main effects of highly charged cationic polyelectrolytes having molecular masses in the approximate range of 100,000 to 2 million grams per mole and it appropriate in cases involving polyelectrolytes having a branched structure adsorbing onto surfaces of opposite charge (Muyibi *et al*. 2002a).

Studies have shown that the seeds have the capability to adsorb metal cations and attract highly toxic compounds (Muyibi *et al*. 2002a). A laboratory study showed that *Moringa oleifera* has the potential to adsorb heavy metals from leachate and from industrial wastewater (Muyibi *et al*. 2002a). The method can therefore be used for heavy metal removal from drinking water and wastewater. In the study by Vikashni *et al*. (2012) local

Moringa oleifera seeds did not significantly have toxic effects but aided in improving water quality for drinking purposes. The mechanism of coagulation with the seeds of *Moringa oleifera* consists of adsorption and neutralization of the colloidal positive charges that attract negatively charged impurities and metals in water. The results obtained in this study were comparable with the performance achieved in heavy metal removal by previous workers such as Muyibi *et al.* (2002a) using *Moringa oleifera* extracts, who found out that the rate of metal ion removal increased when the adsorbent dosage was increased from 10 g/l to 20g/l. Mataka *et al*. (2010) obtained similar results using *Moringa stenopetala* and *Moringa oleifera* seed powders, by increasing *Moringa stenopetala* powder dosage from 0.5 - 2.5 g/100 mL, increased the Cd^{2+} removal percentage from about 20% to about 58%, and by increasing *Moringa oleifera* dosage from 1.0 g/100 mL resulted in removal percentage of about 20% which increases to about 58% as the dosage increased to 2.5 g/100 mL. Similarly, in this study increasing the dosage of *Moringa oleifera* from 0.4 - 0.6 g/50 mL increased the metal ion removal from 85% - 92%, 80% - 92%, 72% - 90%, 82% - 100%, 90% - 98% and 85% - 98% for Mn^{2+}, Cr^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}, respectively. The percentage increase as a result of increased dosage was therefore between 7 and 18. This phenomenon is expected since the metal uptake capacity of the adsorbent increases with the increase in dosage as the number of active sites available.

4.4.6 Pretreatment of *Moringa oleifera* seeds

Various pretreatment methods have been tested with the aim of causing certain changes in the surface of biomass resulting in changed performance of the biomass in metal biosorption. Affinity of the biomass to metal can be manipulated by pre-treating the biomass with alkalies, acids, detergents and heat which may increase the amount of the metal sorbed (Kapoor and Viraraghavan, 1998). During the present study, biomass was treated chemically (with HCl, NaOH, $CaCl_2$,and $KMnO_4$) with the aim of enhancing the removal of Cd^{2+}, Cu^{2+}, Zn^{2+}, Cr^{2+}, Pb^{2+} and Mn^{2+} by *Moringa oleifera* seeds (Figures 4.22 to 4.27). The order of maximum metal uptake for Cu^{2+} and Zn^{2+} was NaOH > $CaCl_2$ > $KMnO_4$ > HCl. For Cd^{2+} and Mn^{2+} the order was NaOH > $KMnO_4$ > $CaCl_2$> HCl. For Pb^{2+} the order was $CaCl_2$ > NaOH > $KMnO_4$ > HCl and for Cr^{2+} the order was $KMnO_4$ > $CaCl_2$ > HCl > NaOH (Figures 4.22 to 4.27). In the study all the chemicals used for pretreatment improved the biosorption of Cu^{2+}, Zn^{2+}, Mn^{2+} and Cd^{2+}. However, it is only $CaCl_2$ pretreated biomass that improved the biosorption of Pb^{2+} ions. All the chemical pretreatments used improved the biosorption of Cr^{2+} except NaOH. The order of biosorption for each metal was different with different pretreatments; this could be because the mechanism of biosorption varies from metal to metal.

Specific pretreatment shows a specific behaviour that may be due to the specific interaction between the biomass and the chemical used for that treatment (Bhatti *et al.*, 2008). Acids can enhance uptake capacity of biomass by increasing surface area and porosity of original sample (Kapoor and Viraraghavan, 1997).

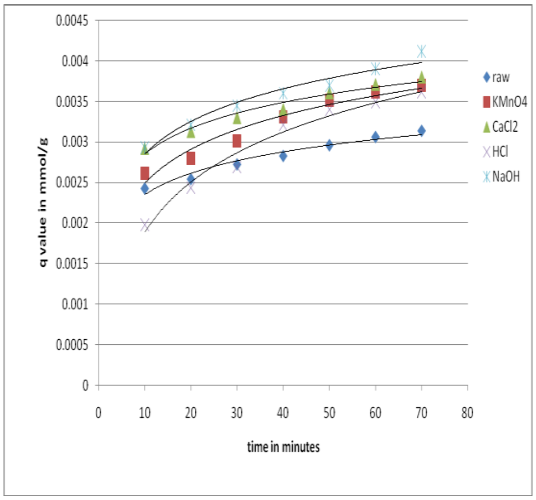


Fig 4.22: Biosorption of Cu^{2+} using treated *M. oleifera*

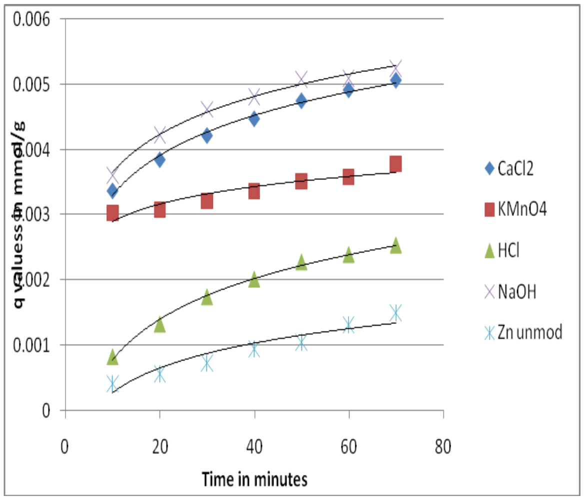


Fig 4.23: Biosorption of Zn^{2+} using treated *M. oleifera*

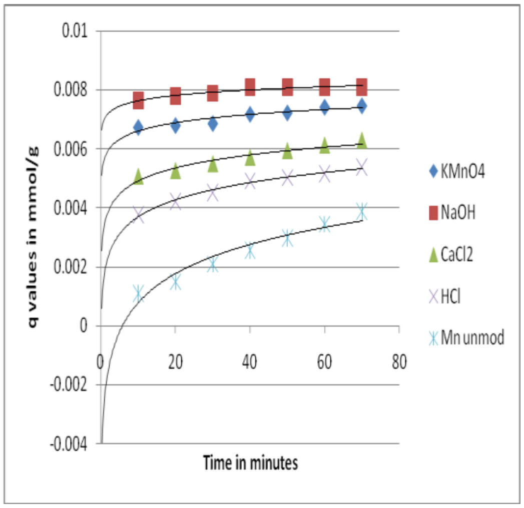


Fig 4.24: Biosorption of Mn^{2+} using treated *M.oleifera*

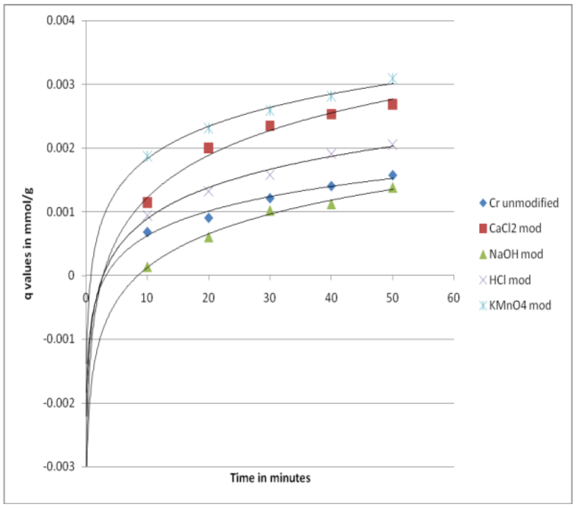


Fig 4.25: Biosorption of Cr^{2+} using treated *M.oleifera*

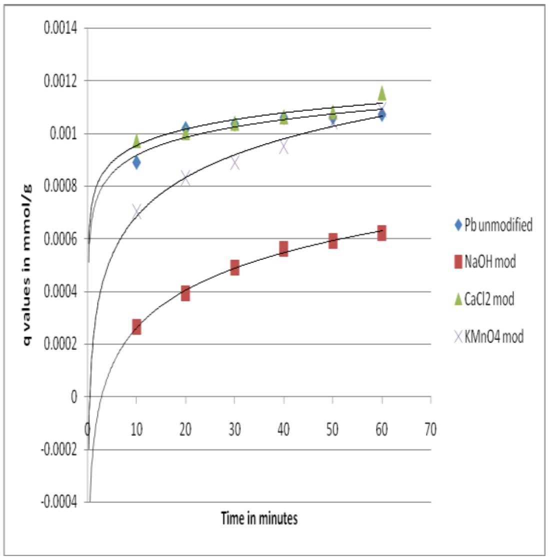


Fig 4.26: Biosorption of Pb^{2+} using treated *M.oleifera*

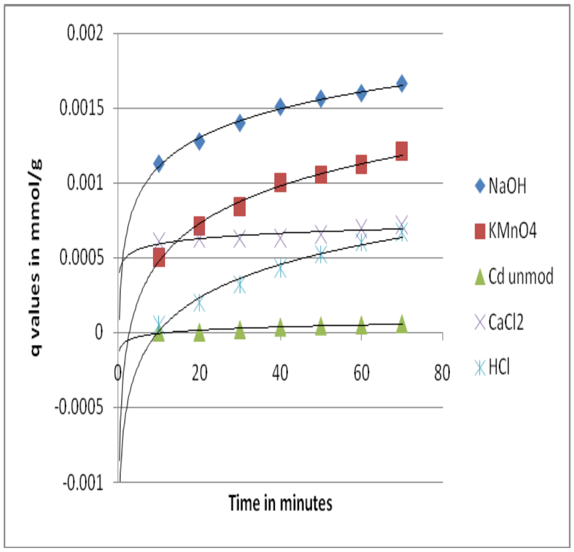


Fig 4.27: Biosorption of Cd^{2+} using treated *M.oleifera*

Alkali pretreatment can increase the biosorption of heavy metals because it may destroy autolytic enzymes (which drstroy the cell) causing putrefaction of biomass, remove lipids and proteins that mask binding sites and could release certain biopolymers from the cell wall that have a high affinity towards heavy metal ions ((Loace *et al.*, 1997; Rao *et al.*, 2010; Tunali *et al.*, 2005; Yan and Viraraghavan, 2003). Removal of surface impurities, rupture of cell membrane and exposure of available binding sites for metal biosorption after pretreatment might be responsible for the increase in metal biosorption. The residual alkalinity in biomass may also result in increased hydrolysis of certain metals and thereby enhance overall metal removal by the treated biomass (Brierley, 1990). Alkali pretreatment can also lower biosorption because the alkali may precipitate the metal as hydroxides and therefore it would remain the the mixture leading to a net decrease in biosorption efficiency (Mittelman and Geesey, 1995).

Therefore, if for example Pb^{2+} is to be removed from aqueous solution, the use of pretreatment with alkaline chemicals may improve removal efficiency. The results show that most of the pretreatments resulted in an increase in the metal up take capacity of biomass (Figures 4.22 to 4.27). The increase in uptake capacity depends on the nature of reagent used. Surfactants and acids can enhance uptake capacity of biomass by increasing the surface area and porosity of original sample (Bhatti *et al.*, 2007). Enhancement in metal removal potential of biomass after the pretreatment with dilute HCl was probably due to the removal of previously adsorbed cations (ions present in the nutrient medium), thus freeing the binding sites for metal binding. Acid treatment might have also caused hydrolysis of functional groups and may have generated more anionic sites for metal removal (Fourest and Roux, 1992). Acid treatment also reduces the positive charge on cell surface and this may also be the reason for increase in metal removal (Ting *et al*., 1995). Generally, acid treatment has been used for cleaning the cell wall and replacing the natural mixture of ionic species bound on the cell wall with protons and sulphates (Davis *et al*., 2003; Yun, 2004).

Mehta and Gaur (2005) observed that pretreatment of the biomass with dilute HCl increased sorption of Cu and Ni by C. vulgaris. Rao *et al*. (2010) demonstrated enhanced Ni sorption capacity of Sargassum after acid pretreatment. Mehta *et al*. (2011) demonstrated that acid treatment of *Moringa oleifera* not only increased metal sorption but also alleviated the inhibitory effect of other metal ions on sorption of the metal of interest. Arica *et al*. (2004) explained that acid pretreatment of biomass results in physical cleaning of the biomass surface from impurities, thus producing additional available binding sites. They also added that denaturation of proteins and degradation of polysaccharides of the cell wall components also produces additional available binding sites. Kapoor and Viraraghavan (1998) reported that acetic acid pretreatment significantly reduced biosorption of cadmium but caused improvement in copper biosorption by *Aspergillus niger*.

Similarly, organic solvents remove the lipids of biomass and hence more adsorption sites are available for the adsorbents. The obtained results are in accordance with the results reported earlier (Veglio *et al.*, 2008; Tsui *et al.*, 2006). As mentioned earlier, the difference in results after a specific chemical pretreatment might be due to the change in the nature of cell wall composition of biomass. NaOH was the best modifier of all the chemicals used in this study; this could be due to removal of surface impurities, rapture of cell membranes and exposure of available binding sites (Brierley, 1990). Some researchers found that alkali pretreatment of biomass may destroy autolytic enzymes that cause putrification of biomass and remove lipids and proteins that mask reactive sites. They found that cell walls could be ruptured using NaOH treatment. The explanation they offered is that the increase in the metal uptake after the protein removal steps is brought about by the unmasking of some of the cellular groups, which cannot participate in the sorption process without treatment with alkali (Ahluwalia and Goyal, 2006). In case of pretreatment with NaOH, hydrolysis reactions occurs causing high dissolution of organic substances from the biomass. The hydrolysis reactions can lead to the formation of more carboxylic (COOH), carboxylate (COO-) and alcohol groups (-OH), in the pretreated biomass, which enhances cationic biosorption (Loace *et al.*, 1997).

Favourable effects of alkali treatment of biomass on metal removal have been reported by many researchers. In the present study, treatment of *Moringa oleifera* with NaOH increased the metal removal efficiency of all the metals studied except Cr. The pretreatment of cells with NaOH strongly enhanced the Cd^{2+}, Cu^{2+}, Zn^{2+}, Cr^{2+}, Pb^{2+} and Mn^{2+} removal by *Moringa oleifera* biosorbent. The reason for this may be that alkali treatment may rupture the cell membranes resulting in the exposure of certain internal cellular binding sites making them available for adsorption, which enhanced the binding of cationic metal ion species. Generally alkali treatments decrease protonation of functional groups. NaOH treatment substitutes sodium ions on functional groups, increasing the electrostatic attraction by positive metal cations and facilitating ion exchange (Yan and Viraraghavan, 2003).

Alkali pretreatment increased the Cu removal capacity of *C. vulgaris*, may be due to the removal of foreign materials from the cell surface that might have otherwise impeded with the binding of metal ions (Zhou *et al*., 2012; Mehta and Gaur, 2005). Nagase *et al*. (2005) demonstrated that alkali treatment is useful in improving the metal adsorption ability; they observed that NaOH treatment destroyed the cell wall layers and the internal cellular structure was also disordered. Thus, due to damage of cell surface, metal ions permeated in to the cells. Ahuja *et al*. (1999) observed that metal sorption remained unchanged after alkali pretreatment. It was argued that NaOH treatment perhaps caused contraction of cells thus making sites less accessible to metal ions and decreased metal removal. It could also be due to an increase in the availability of binding sites or due to the removal of polysaccharides that presumably blocked the accessibility of metals to the binding sites (Liu *et al*. 2004).

$CaCl_2$ was next in efficiency of modification after NaOH; the treatment was performed so as to convert the active binding sites from H^+ to Ca^{2+} form. Such a substitution may favour the biosporption of metals, because of the size of the ions; it should be easier to exchange metal for calcium than for H^+. HCl pretreatment resulted in a reduction in adsorption capacity, which is also supported by several studies and researchers (Ahluwalia and Goyal, 2006). The H^+ ions binding to the biomass after acid treatment may be responsible for the reduction in adsorption of heavy metals. Thus, the remaining H^+ ions on the acidic pretreated that biomass may change the biomass electronegativity, resulting in a reduction in biosorption capacity. The polymer structure of biomass surface exhibits a negative charge due to the ionization of organic and inorganic groups. Researchers suggest that the higher the metal electronegativity, the greater the attraction and adsorption of heavy metal cations.

When acids are implicated in treatment of biomass, the H^+ ions can change the cross-linking bonds, which lead to the dissolution of organic matter (Ahluwalia and Goyal, 2006). Removal of impurities from surface and rupturing of cell membrane is reason behind the increase in metal uptake capacity of biomass after basic pretreatment (Loace *et al*, 1997). Similar studies were done by Chowdhury *et al*. (2009) using rice husks, they reported that rice husks treated with sodium hydroxide, sodium carbonate and epichlorohydrin enhanced the adsorption capacity of cadmium. The base treatment using

NaOH for instance appeared to remove base soluble materials on the rice husk surface that might interfere with its adsorption property. Pretreatments showed a significant effect on the uptake capacity of *Moringa oleifera* seeds. When *Moringa oleifera* seeds are treated with hydrochloric acid, adsorption sites on the surface of the seeds will be protonated, leaving the heavy metal ions in the aqueous phase rather than being adsorbed on the adsorbent surface. Thus, the results of present study are in agreement with the observations of other researchers, since pretreatment of the biomass using HCl also reduced the biosorption of metal ions.

4.4.7 Effect of competing metals

Unlike controlled laboratory conditions, industrial effluents contain various pollutants including the target and other ions not of interest. Thus there is need for investigation of the inhibitory effects of ionic strength and competing heavy metal ions. These factors influence biosorptive removal of a target pollutant by competing with the adsorbate for binding sites, by changing its activity, or by forming complexes with it (Vijayaraghavan and Yun, 2008). Industrial effluents can be contaminated by one or more metal ions.

Table 4.2: Percentage interference by co-ions

Metal ion/ co-ions	Cd (II)	Zn(II)	Pb (II)	Cu(II)	Mn (II)	Cr (II)
Cd (II)	-	41.0%	49.4%	17.7%	20.8%	33.0%
Zn (II)	50.0%	-	57.0%	40.0%	54.0%	46.0%
Pb (II)	23.5%	2.9%	-	14.7%	5.9%	11.7%
Cu (II)	64.3%	5.7%	68.4%	-	57.4%	47.1%

In this research it was considered important to compare the biosorption rates for single and mixed metal ions. The metal sorption performance based on q values was higher with individual metal solutions than with multi-metal solution (Figures 4.28 to 4.32).

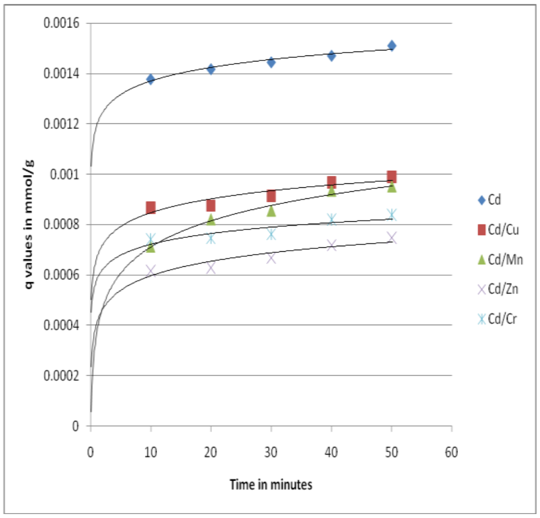


Figure 4.28: Cd^{2+} in binary systems

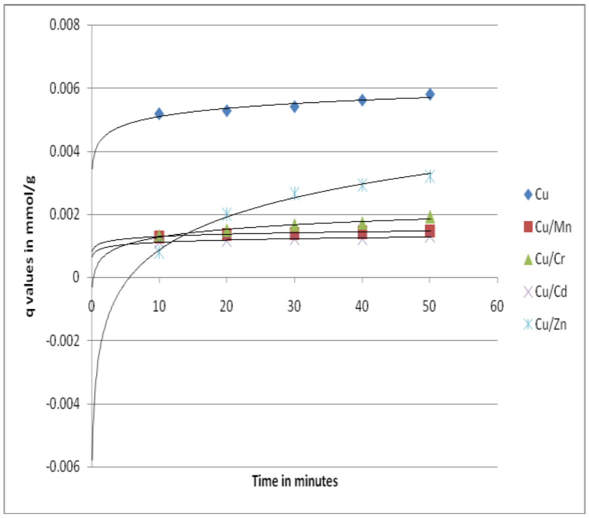


Figure 4.29: Cu^{2+} in binary systems

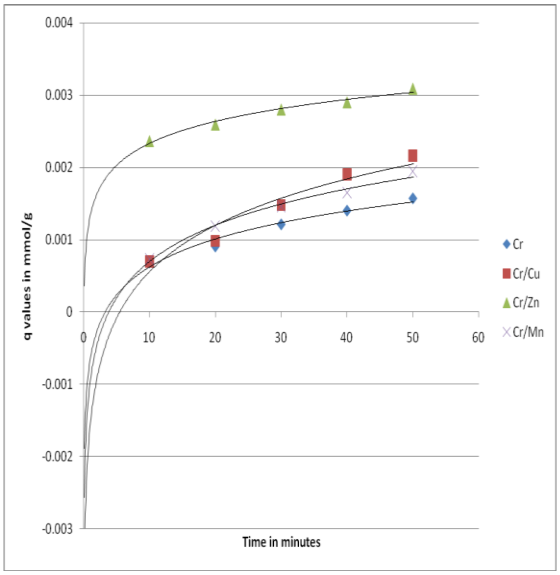


Figure 4.30: Cr^{2+} in binary systems

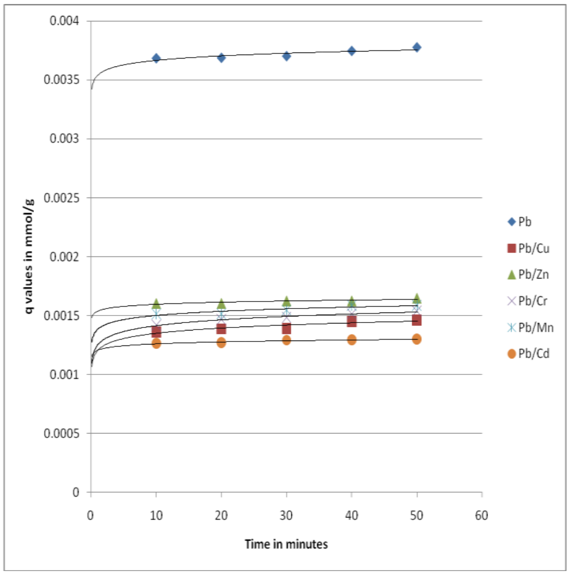


Figure 4.31: Pb^{2+} in binary systems

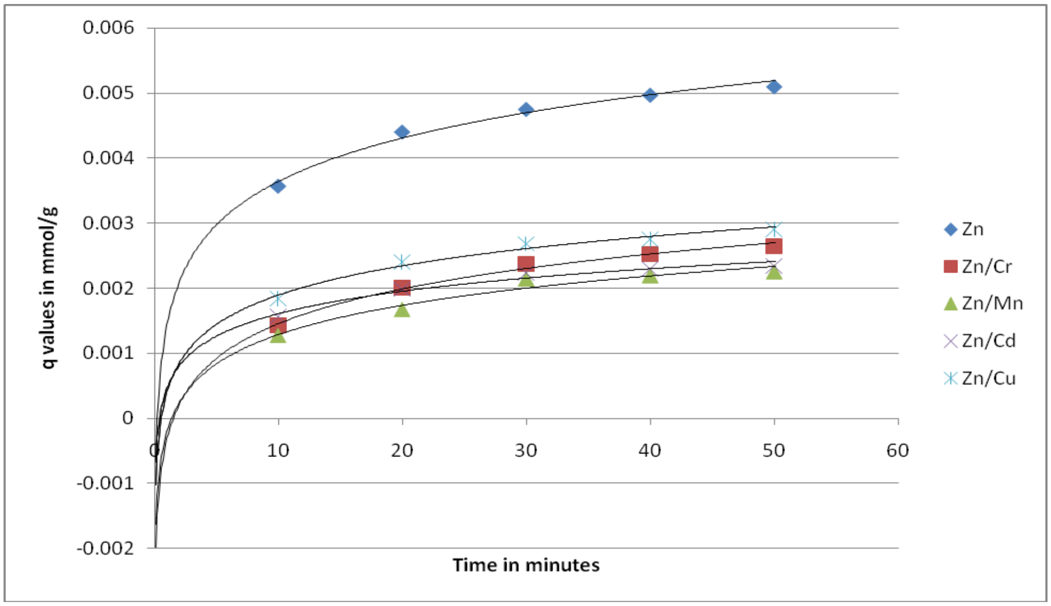


Figure 4.32: Zn^{2+} in binary systems

The presence of other cations significantly reduced metal ions binding by all metals The greatest interference with Cd^{2+} binding by *Moringa oleifera* seeds were observed with Pb^{2+} followed by Zn^{2+}, Cr^{2+}, Mn^{2+} and Cu^{2+} with percentages of 49.4, 41, 33, 20.83 and 16.66, respectively (Table 4.2). Cu^{2+} binding by *Moringa oleifera* seeds was most affected by the presence of Pb^{2+}, followed by Cd^{2+}, Mn^{2+}, Cr^{2+} and Zn^{2+} (Table 4.2). Pb^{2+} binding was most affected by the presence of Cd (23.52%), followed by Cu (14.70%), Cr (11.76%), Mn (5.88%) and Zn (2.94%) in that order. This means that Zn affected the binding of Pb least. However, when compared with the other metals the effect of other cations was much less for Pb^{2+} (Figure 4.31).

Interference of all the cations tested on Pb binding was less, whereas the interference of the borderline cations, Zn and Cr, was the largest on Cd, which belongs to the same group classification. The greatest interfence with zinc binding by *Moringa oleifera* seeds were observed with Pb followed by Mn, Cd and Cr, and Cu, which implies that Cu caused the least effect on Zn (Table 4.2).

Consequently, the *Moringa oleifera* seeds exhibited a net preference for Pb^{2+} over the other metal ions; this could be due to its high electronegativity and large ionic radius, which makes it less hydrated and therefore approaches binding sites easily. Pb^{2+} manifested the highest inhibitory effect on the biosorption of other metal ions, followed by Cd^{2+}, Mn^{2+}, Cu^{2+}, Cr^{2+} and Zn^{2+}. This again could be attributed to the metal chemistry (including parameters such as charge, ionic radius, and Pauling electronegativity), affinity for binding sites, and the type of metal binding (electrostatic or covalent) (Fourest and Roux, 1992; Veglio and Beolchini, 1997). The results clearly demonstrate that in absence of other heavy metal ions *Moringa oleifera* seeds showed good ability for Cu^{2+}, Cr^{2+}, Mn^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+} biosorption. On the basis of these results it can be concluded that the effect of ionic strength of different salts on the biosorption of Cu^{2+}, Cr^{2+}, Mn^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+} depends on experimental conditions. In multi-elemental solutions, there may be a decrease in the adsorption ability of a specific metal, compared to that of the same metal in mono-elemental solutions. This decrease can be attributed to fewer active sites available, that is, binding sites competitively are divided among metals present in the solution (Sharma *et al.*, 2007), which was observed by the same author in studies using seeds of *Moringa oleifera* Lam. in a ternary solution containing Cd, Cr and Ni, whose adsorption values decreased by about 10 to 20% compared to the adsorption using a mono-elemental solution.

4.4.8 Characterization of *Moringa oleifera* seeds

Functional groups of *Moringa oleifera* were characterized by FTIR spectra and showed in Figure 4.33. The FTIR of *Moringa oleifera* was prepared according to the procedure mentioned in Chapter 3, Section 3.6.3. This FTIR spectrum showed that there were several functional groups detected on the surface of *Moringa oleifera* seeds, indicating the complex nature of the material. The broadband centered at 3211 cm^{-1}may be attributed to the O-H stetching of the connection in proteins, carbohydrates and lignin units (Reddy *et al*., 2011). Due to the high protein content present in the seed there is also a contribution in this region from N-H stretching of the amide bond. The peaks present at 2925 cm^{-1} corresponds to asymmetric stretching of the C-H bond in $–CH_2$, while the one at 2860 cm^{-1} corresponds to symmetric stretching of the C-H bond of the CH_2 group. (The band at 2925 cm^{-1} characterizes the stretching vibration of the C–H bond of alkane groups). Due to the high intensity of these bands it is possible to assign them to the predominantly protein component of the seed, which is present in a high proportion (Reddy *et al*., 2012). In the region of 1800-1500 cm^{-1} a number of overlapping bands were observed and between 1741 and 1537 cm^{-1}, this can be attributed to C=O stretching.

Due to the heterogeneous nature of the seed, the carbonyl group may be bonded to different neighbourhoods as part of the fatty acids of the lipid portion or amides of the protein portion. Shoulders forming part of the main band that appears at 1649 cm^{-1} are attributed to the carbonyl amides present in the protein portion of the seeds.

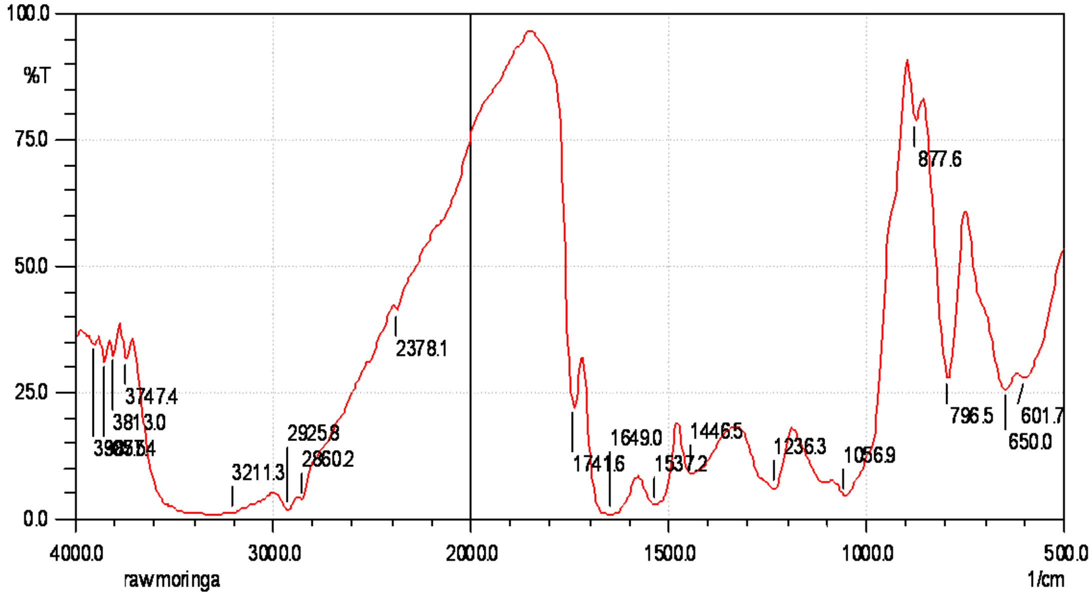


Figure 4.33: FT-IR spectrum of *Moringa oleifera* seeds

The peak observed at 1446 cm^{-1} may be attributed to stretching connecting C-N and also the deformation of the N-H linking the proteins present in the seeds (Reddy *et al*., 2011). The biosorption process includes the following mechanisms: transport across cell membrane, complexation, ion exchange, precipitation, and physical adsorption. From the FTIR spectra, the cell wall of *Moringa oleifera* seeds contains anionic groups such as –COO, –C–O,–OH and –NH whose adsorbent ability towards Pb^{2+}, Cd^{2+}, Cu^{2+}, Cr^{2+}, Mn^{2+} and Zn^{2+} oins was obtained to be fairly high. The fairly high adsorption can be attributed to the complexation reaction between the anionic groups on the biomass which form chelates with the metal ions. The groups act as ligands since they pocess lone pairs of electrons on their oxygen or nitrogen atoms which they donate to metal ions to form complex ions. This pH-dependent binding suggests that the binding of Cu(II), Cr(III), Mn (II), Pb(II), Cd(II) and Zn(II) by moringa oleifera is through carboxyl ligands (COO-). NaOH treated *moringa oleifera seeds* also showed enhancement in bioadsorption of Cd^{2+},

Cr^{2+}, Cu^{2+}, Mn^{2+}, Pb^{2+} and Zn^{2+}, the pretreatment using NaOH removes surface impurities, ruptures cell membrane and exposes binding sites for metal complexation with the functional groups, which further suggested that the metal ion binding was occurring through carboxyl ligands.

Acid pretreatment "obstructed" the carboxyl groups which led to a decrease in metal ion adsorption, which supports the hypothesis on the participation of *Moringa oleifera* carboxyl moieties in metal binding. In this study, the H^+ ions binding to the biomass after acid treatment may be responsible for the reduction in adsorption of heavy metals. The polymeric structure of biomass surface exhibits a negative charge due to the ionisation of organic groups and inorganic groups. Pretreatment using $KMnO_4$ oxidised all alkene functional groups to alchohols (ROH), it also oxidized primary alcohols (ROH) and aldehydes (RCOH) to carboxylic acids (RCO_2H) and secondary alcohols (RCHOHR) to ketones (RCOR) (Carey and Sundberg). Hence pretreatment with $KMnO_4$ increased the efficiency of biosorption since the carboxyl groups which are formed contain two binding sites unlike alcohols and aldehydes which contain only one. Pretrearment using $CaCl_2$ also increased the biosorption efficiency, which was due to replacement of H^+ in the functional groups by Ca^{2+} which could favour the mechanism of ion exchange since Ca^{2+} is closer in size to the ions being exchanged than H^+, hence it is easier to exchange the Ca^{2+} ions. $CaCl_2$ pretreatment also reduced the biosorption efficiency of some metals because it aoccupied the sites on the ligand which could have been occupied by the metal ions during complexation.

4.4.9 Adsorption isotherms

The Langmuir isotherm model was chosen for the estimation of maximum adsorption capacity corresponding to complete monolayer coverage on the *Moringa oleifera* seed powder. The specific plots of sorption (C_e/q_e) against the equilibrium concentration (C_e) for Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Cr^{2+} and Mn^{2+} are shown in Figure 4.34 and Appendix II and the linear isotherm parameters, q_m, K_L (b) and the coefficient of determinations (R^2) are presented in Table 4.3. The saturated monolayer sorption capacity, q_m, is a function of many parameters such as pH and temperature, it provides a good measure for comparing the efficiency of different sorbents in removing a given metal (Chang *et al*., 1997). Plotting graphs of C_e/q_e versus C_e for all the metal ions gave straight lines and from the slope and intercept, values of q_m and K_L were determined, respectively.

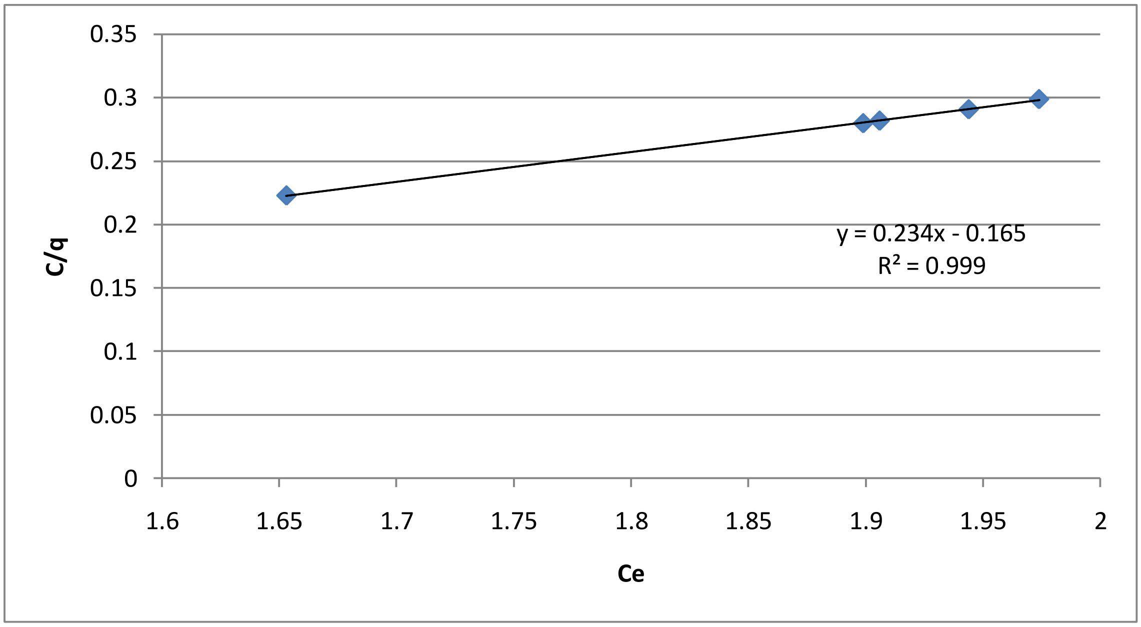


Figure 4.34: Langmuir isotherm for Cu^{2+}

Table 4.3 Sorption isotherm constants and coefficients of determination.

Langmuir equation				Freundlich equation		
Metal ion	q_{max}	b	R^2	K_F	n	R^2
Zn(II)	4.424	-20.550	0.999	4.709	-6.060	0.975
Pb(II)	15.38	0	1	14.791	-47.61	0.981
Cu(II)	4.273	-1.418	0.999	10.139	-1.584	0.998
Mn(II)	6.667	-16.667	0.999	6.918	-7.352	0.997
Cd(II)	2.70	-23.125	0.999	2.483	-5.07	0.991
Cr(II)	5.952	-2.947	0.986	9.268	-2.597	0.972

The Langmuir equation was used to describe the data derived from the solution by *Moringa oleifera* seeds show that experimental data fitted reasonably with the linearised equation of the Langmuir isotherm in the plot of C_e/q_e versus C_e. The coefficients of determination, R^2 values were close to unity (1) confirming the data for all metals fitted the Langmuir isotherm.

The sorption capacity, q_m, which is a measure of the maximum sorption capacity corresponding to complete monolayer coverage showed that the *Moringa oleifera* seeds had a mass capacity for Pb^{2+} (15.38 mg/g) which was the highest. The order of sorption for these metals for *Moringa oleifera* seeds was therefore: $Pb^{2+} > Mn^{2+} > Cr^{2+} > Zn^{2+} > Cu^{2+} > Cd^{2+}$. Theoretically the order of biosorption rate is $Pb^{2+} > Cd^{2+} > Cr^{2+} > Mn^{2+} > Zn^{2+} > Cu^{2+}$, which is attributed to the size of the ions according to Pauling, Mn^{2+} =

0.80Å , Pb^{2+} = 1.20 Å, Cu^{2+} = 0.72 Å, Zn^{2+} = 0.74 Å, Cd^{2+} = 0.97 Å , and Cr^{2+} = 0.83 Å. Binding strength to the biomass depends on metal chemistry (including parameters such as charge, ionic radius, and Pauling electronegativity), affinity for binding sites, and the type of metal binding (electrostatic or covalent) (Fourest and Roux, 1992; Veglio and Beolchini, 1997). Deviation from the theoretical expectation of biosorption that depends on ionic sizes could be attributed to other factors coming into play such as varying initial concentration. Therefore, initial concentration seems to be an influencing factor in the rate of biosorption, as reported by Kumar *et al*. (2010) using *Moringa oleifera* seeds for biosorption of Ni^{2+}.

Langmuir isotherm is the most important model of monolayer adsorption, on the basis of the assumption that there are a fixed number of adsorption sites, each capable of holding only one adsorbate molecule (the adsorbed layer is one molecule thick), all sites are equivalent and there is no interaction between adsorbed molecules. Therefore, Pb has the highest affinity for the sites of the adsorbent material, and the affinity of *Moringa oleifera* seeds for metals followed the order: $Pb^{2+} > Cu^{2+} > Cr^{2+} > Mn^{2+} > Cd^{2+} > Zn^{2+}$. This is supported by suggestion that preference of a biosorbent for metals is related to the physicochemical parameters of the metal (Sag *et al*., 2001). The diffusion of metals with higher atomic weight can generate higher momentum energy. This fact may facilitate the biosorption of the metal by increasing the probability of an effective collision between the metal and the solid surface. For this reason metals with higher atomic weights have more affinity for the biosorbent. Another factor to consider is the ionic radius. It has been reported that, in the ion-exchange process, larger multivalent ions are more effectively removed than smaller ones (Prasad and Saxima, 2004). On the other hand Mn^{2+}, Cr^{2+} and Cu^{2+} have unpaired electrons and could be attracted by a magnetic field probably originating in the biosorbent. Nevertheless, Pb^{2+}, Cd^{2+} and Zn^{2+} are very stable (absence of unpaired electrons) and could be repelled by a magnetic field. The higher the electronegativity of the atom, the more easily the ion is sorbed by the biosorbent (Chong and Volesky, 1996).

Freundlich isotherms were obtained by agitating the adsorbate solution of a fixed concentration and 0.4 g of the adsorbent for 70 minutes, which was greater than the equilibrium time (60 minutes). The plot of log q_e versus log C_e gives a slope with the value of 1/n and an intercept magnitude of log K_f. K_f and n were calculated from the slope and intercept of the Freundlich plots, where the gradient of the straight line was 1/n and the antilog of the intercept equal K_f. The Freundlich isotherm basically indicates whether the adsorption proceeds with ease or difficulty. Freundlich isotherm model was obeyed by all the adsorbates (Cu^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, Cd^{2+} and Cr^{2+}) under the studied conditions with R^2 values of between 0.972 and 0.998 (Figure 4.35, Appendix III and Table 4.3).

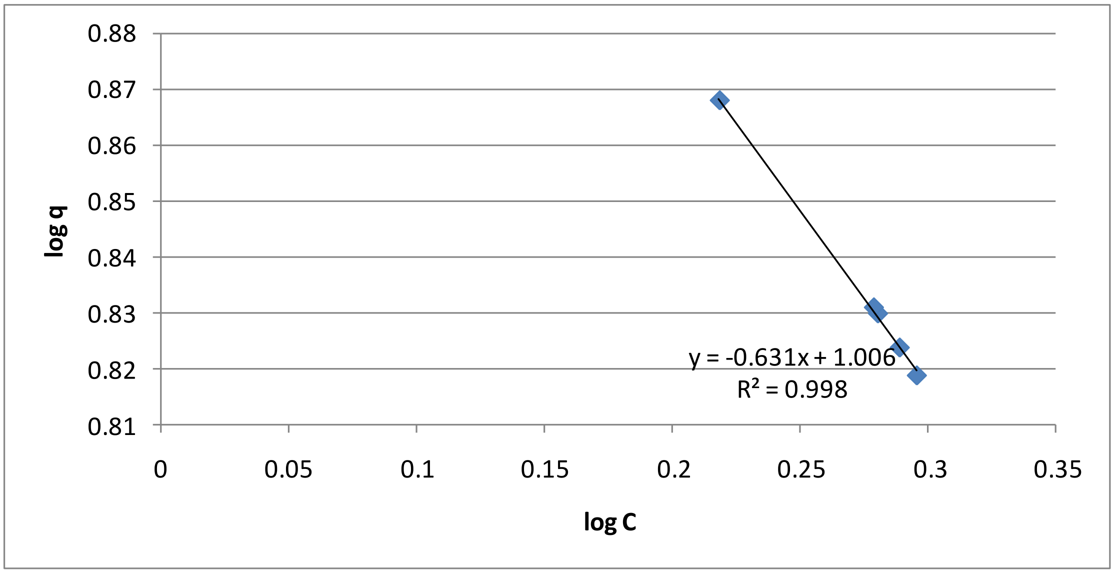


Figure 4.35: Freundlich isotherm for Cu^{2+}

These results may be explained if adsorbent surface sites have a spectrum of different binding energies as suggested by Anwar and Rashid (2007). The n value in Freundlich equation was found to be negative for all the metal ions shown in table 4.3. Since $n < 1$, this indicates that the biosorption of the metal ions under this study is a chemical process.

The experimental data fitted well into both Freundlich and Langmuir isotherm models as presented in Figures 4.34 and 4.35 and the parameters in Table 4.3. These isothermal biosorption parameters show that *Moringa oleifera* seeds are good biosorbents for the uptake of Cu^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, Cd^{2+} and Cr^{2+} from aqueous solution and therefore suitable for the uptake of the metals from waste waters. The parameters compare well with those of other sorbents that have been reported by Pavasant *et al.* (2006). Affonso *et al*. (2012) reported n values of 1.46, 0.57 and 0.54 for Cd^{2+}, Pb^{2+} and Cr^{2+}, they concluded that the biosorption of Pb^{2+} and Cr^{2+} was practically favourable while the biosorption of Cd on *Moringa oleifera* is unfavourable to heterogeneous surfaces. Anwar (2013) reported n values of 0.748, 2,582, 2.393 and 1.989 for biosorption of Cu^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+}, respectively on Garden grass and concluded that only the biosorption of Cu^{2+} was practically favourable since it had a value $n < 1$. Waghmare and Chaudhari (2014) reported a value of $n = 0.048$ for the biosorption of Mn^{2+} on *Moringa oleifera* bark and concluded that the bark was favourable for the adsorption of Mn^{2+} since was $n < 1$.

The Langmuir and Freundlich constants calculated from the isotherm equations are given in Tables 4.3 for the adsorption of metal ions. From table 4.3 the values of R^2 (approximately 0.99) indicate that the Langmuir model fits better and can be used to describe the biosorption of Cu, Cd, Pb, Mn, Cr and Zn on this biomass. The Freundlich R^2 values were 0.975, 0.981, 0.998, 0.997, 0.991 and 0.972 for Zn, Pb, Cu, Mn, Cd and Cr, respectively. The plots of log q_e versus log C_e were found to be linear indicating the applicability of the Freundlich model. However, Langmuir had values of R^2 much closer to unity than Freundlich for all the metals studied; hence data fitted better to Langmuir than Freundlich. Also the biosorption of the metals by *Moringa oleifera* seeds followed monolayer adsorption. Based on the K_f values of the Freundlich model, the sorption capacity followed the order: Pb > Cu > Cr > Mn > Zn > Cd (Table 4.3), which was consistent with the order based on the Langmuir model. However, a different order (Cu > Cr > Cd >Zn > Mn > Pb) was observed using the sorption intensity (1/n) of the Freundlich model. The magnitude of the exponent 'n' in the Freundlich equation gives the indication of favourability and K_f, the capacity of the adsorbent/adsorbate system. Table 4.3 shows that n values for metals were less than 1 under the studied conditions, indicating beneficial sorption- chemosorption (Yoshida, 2004).

Comparing the Langmuir and Freundlich models (Table 4.3), *Moringa oleifera* seeds demonstrated a good removal capacity for Cu^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, Cd^{2+} and Cr^{2+}. The variations in the removal percentage for metal ions can be explained by the different ionic radii of chemical species. In general, for the single metal solutions, ions with larger ionic radii are preferentially adsorbed. Among the metals tested, Pb (II) has the largest ionic radius and hence shows the highest adsorption percentage, whereas Cu (II) presents the lowest level of adsorption (Matos and Arruda, 2006). Thiago *et al.* (2013) observed that experimental data for Mn with *Moringa oleifera* provided a good fit for the two isotherm models, despite the fact that the value of the linear coefficient of determination (R^2) of the Freundlich model was higher than that of Langmuir model. Both Langmuir and Freundlich isotherm models fitted well to data of Zn^{2+} biosorption using *Moringa oleifera* in the study by Bhatti *et al*. (2007).

The preferential sorption behaviour of adsorbents for metal ions acquired in present investigations could also be explained in terms of ionic radii of the metal ions. Thus, the element with larger ionic radius will compete faster for exchange sites than those of smaller ionic radius because the ones with smaller ionic radius are more hydrated. Adsorption may be related to the loss of the entire hydration sphere that precedes hydrolysis. According to Horsefall and Spiff (2005), the smaller the ionic radius, the greater its tendency to hydrolyze leading to reduction of sorption. The observed order indicates that Pb^{2+} may have greater accessibility to the surface of certain pores than Cd^{2+}, Mn^{2+}, Cr^{2+}, $Zn^{2+}$and Cu^{2+} due to its larger ionic radius.

Desai and Desai (2013) working at an optimum pH of 8.7 and a contact time of 2.5 hrs obtained linear coefficient of determination (R^2) values of 0.979, 0.911 and 0.712 for Cu, Zn and Fe, respectively, for the Langmuir model and R^2 values of 0.854 and 0.884 for Cu and Zn for Freundlich model, which indicated favourable adsorption and followed both the Freundlich and Langmuir adsorption isotherms. For the Langmuir, the higher the value of adsorption capacity, that is q_{max} (mg/g), the greater the adsorption capacity of adsorbent and the higher the value of b(mg/l), the fastest rate of adsorption. For the Freundlich model the higher the value of adsorption capacity, that is, K_f (mg/g), the greater the adsorption capacity of adsorbent, and the higher the value of n (l/mg), the higher adsorption intensity. Higher adsorption capacity always follow slower rate of adsorption, while lower adsorption capacity is observed with higher rate of adsorption (Clair *et al*., 2005).

4.4.10 Kinetic Models

The kinetics of Cu^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Cr^{2+} and Zn^{2+} adsorption was evaluated by applying two common models: (1) the pseudo-first-order kinetic model (Bhattacharyya and Sharma, 2004) and (2) the pseudo-second-order kinetic model (Ho and McKay, 1999). The pseudo-first-order kinetic model assumes that the uptake rate of metal ions with time is directly proportional to the amount of available active sites on the adsorbent surface. Kinetic studies were carried out for initial metal concentration of between 2 ppm and 5 ppm and contact time of 70 minutes using *Moringa oleifera* seeds. The results are shown in Figures 4.36, Appendix IV and Table 4.4; the results indicate that about 90% of the adsorption occurred in the first 40 minutes.

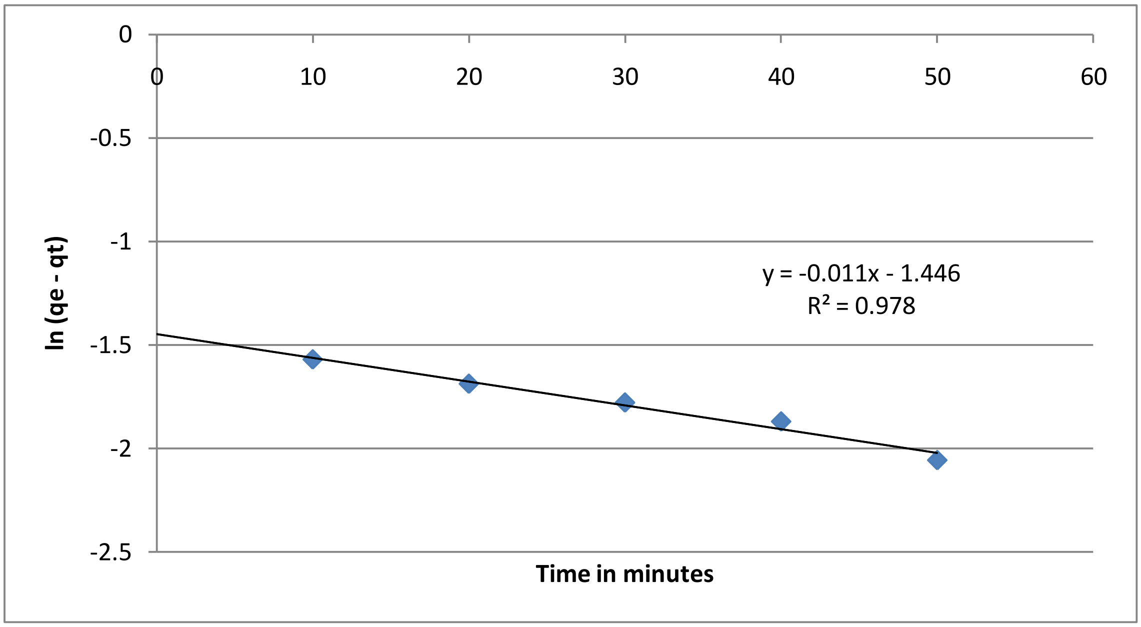


Figure 4.36: Pseudo First Order kinetic for Cd^{2+}

For all the metals, the rate of adsorption was relatively rapid in the first 30 minutes because of the high availability of adsorption sites in the solids particles. The maximum percentage adsorption was achieved within 50 minutes, with all the metal elements reaching an equilibrium state, confirming previous research studies (Polcaro *et al*., 2003). It was found that after 40 minutes, about 5% of the initial metal concentration remained in the supernatant confirming that there was sufficient metal solution available to achieve equilibrium. The calculated values and their corresponding linear regression coefficient of determination values are shown in Table 4.4. The linear regression coefficients of determination values show that this model cannot be applied to predict the adsorption kinetic model. Table 4.4 also presents the data of calculated q_e, pseudo-second order rate (K_2) and coefficient of determination (R^2). For most of the pseudo-second order kinetic models the calculated q_e values approach the experimental q_e values and the coefficients of determination are close to 1, indicating a good ability of this model to describe the kinetics of the metal ion adsorption process.

Table 4.4: Pseudo and pseudo second order kinetics for *Moringa oleifera* (data)

Metal ions	Pseudo first order			Pseudo second order		
	K_1	q_e	R^2	K_2	q_e	R^2
Pb (II)	0.022	0.046	0.740	0.1134	15.873	0.999
Zn (II)	0.044	0.296	0.740	0.0344	7.194	0.995
Cd (II)	0.011	0.2335	0.978	0.1048	3.636	0.994
Cu (II)	0.003	0.455	0.706	0.0588	7.462	0.992
Mn (II)	0.017	0.2233	0.875	0.0746	8.196	0.998
Cr (II)	0.038	0.922	0.96	0.0099	11.627	0.913

This observation suggests that the rate-limiting steps in the biosorption of metallic ions are chemisorption involving valence forces through the sharing or exchange of electrons between the sorbent and the sorbate, complexation, coordination and/or chelation, in which mass transfer in the solution was not involved.

The coefficient of determination values for *Moringa oleifera* seeds at various initial concentration of pseudo second order kinetic model were higher than those of pseudo first order kinetic model. This shows that kinetics of metal biosorption by *Moringa oleifera* seeds were better described by pseudo second order kinetic model (Figure 4.37

and Appendix V) which is supported by the findings of Puello *et al*. (2014); and Priyantha *et al*. (2013). Garcia-Fayos *et al*. (2012) reported similar results using *Moringa oleifera* shells, they obtained R^2 values for pseudo first order as 0.045, 0.274 and 0.301 for Ni^{2+}, Cu^{2+} and Cd^{2+}, respectively. They also obtained R^2 values of 0.957, 0.999 and 0.985 for Ni^{2+}, Cu^{2+} and Cd^{2+}, respectively for pseudo second order kinetic, and concluded that pseudo-second order model was the best fit for the experimental values for the three metals studied.

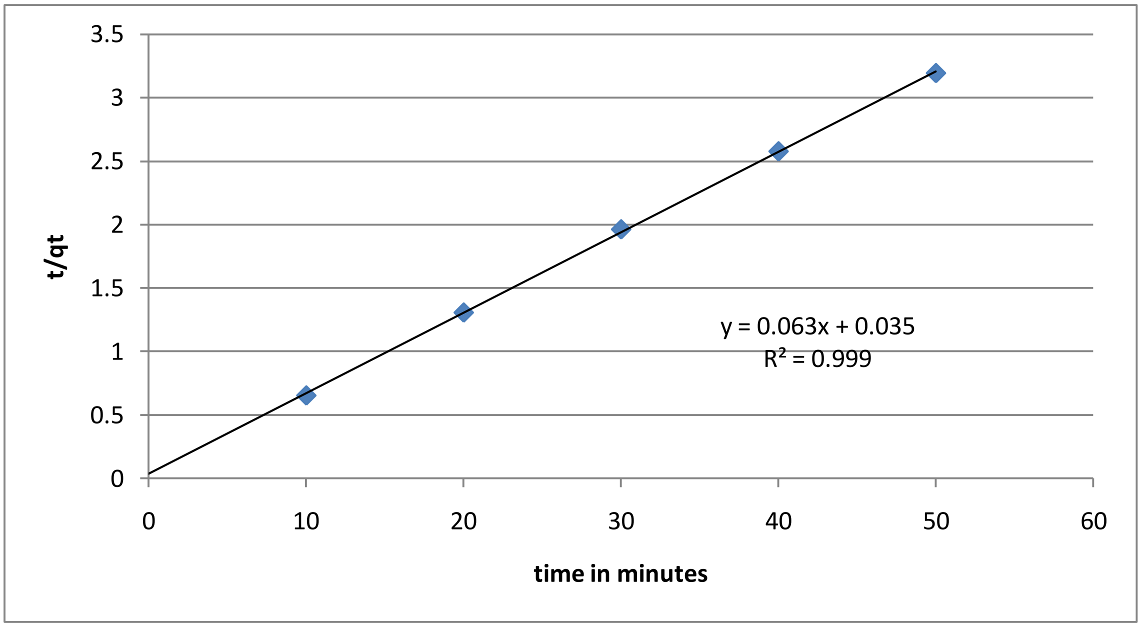


Figure 4.37: Pseudo second Order kinetic for Pb^{2+}

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study shows that some sites on the Winam gulf are polluted with heavy metals; the detected levels of Pb, Cr, Cu, Zn, Mn and Cd in water were higher than the WHO/FAO maximum limits. Pb at Lwang'ni beach was more than 10 times the KEBS maximum acceptable limits and more than 100 times the WHO/FAO maximum acceptable limits. Mn levels at Lwang'ni beach was about 50 times the FAO/WHO maximum limits. Cr values were 10 times higher (R. Kibos), while Cd levels at Tilapia beach were more than 12 times the WHO/FAO maximum permissible limits. However, the levels of Zn were below the maximum acceptable limits. It can be concluded that the heavy metals, used in farms, for domestic activities, industries and other human activities like car washing and shipping traffic in the Winam Gulf finally end-up in water.

Functional groups on *Moringa oleifera* seed biomass include: carbonyl, aldehyde, carboxylic, amine, carboxyl and hydroxyl groups. The absorption spectra revealed that –C-O, C-N and C=O bonds were predominant in the surface of the adsorbents and played a major role in the adsorption process.

Pretreatment of *Moringa oleifera* further enhances biosorption process and the general order of modification efficiency was NaOH > $CaCl_2$ > $KMnO_4$, pretreatment with HCl, however, decreased the capacity of biosorption by *Moringa oleifera* seed powder.

It was observed that the biosorption strongly depends on parameters such as pH of the solution, contact time, initial concentration of the metal ions, biosorbent dose, particle size and presence of co-ions. The uptake of metal ions increased with increase in the agitation time till the equilibrium was reached in 60 minutes. The best pH was 5, biosorption capacity increased with increase in adsorbent dosage and adsorbate concentration. It also increased with a decrease in particle size, the presence of other similar ions led to a decrease in the biosorption capacity.

The data fitted into both the Langmuir and Freundlich adsorption isotherms, but Langmuir isotherm fitted better for most of the experiments. Values of the equilibrium parameter (R_L) from Langmuir isotherm and n values from the Freundlich isotherm indicate that the adsorption process is favourable for all the metals.

Moringa Oleifera seed powder biosorbent has a great potential for the removal of Pb^{2+}, Cu^{2+}, Cd^{2+}, Mn^{2+}, Cr^{2+} and Zn^{2+} from contaminated water. The biosorption of all metals on the adsorbent was rapid as over 80 % of the metals were removed within the first 20 minutes of interaction. The pseudo first-order and pseudo second-order kinetic models were implemented for demonstrating the biosorption mechanism and, as a result, pseudo-second-order kinetics fitted best to the experimental data.

The results show that *Moringa oleifera* seeds may be used effectively in the removal of heavy metal from aqueous systems for environmental cleaning purposes because it is of low cost; its utility will be economical and can be viewed as a part of a feasible waste management strategy.

5.2 Recommendations

The following measures should also be undertaken around the lake to minimize pollution:

(i) Kisumu municipal council should include chemical treatment in the procedure at the main sewage works for the removal of nutrients especially heavy and alkaline metals to ensure that the final effluent discharged into the R. Kissat and other rivers is of internationally accepted quality. This can be done by starting a pilot-scale column with the developed biosorbent (*Moringa oleifera*), which can be conducted for real wastewater, it can be placed at strategic points along the wastewater pathway.

(ii) Creation of public awareness. Lack of knowledge and data has made millions of people throughout the world to suffer from diseases and contaminations, which could either be prevented or controlled. There is need to spread the knowledge available to the public to have them understand the impacts of pollution and how to prevent it in surface waters. This can be done through, provision of posters, brochures and manuals in local languages, television and radio programmes.

(iii) The enforcement of laws and regulations governing waste disposal should be made a priority by the relevant ministries. Although some laws exist to protect surface and groundwater from environmental degradation, enforcement of such laws and regulations are generally weak throughout the region. For instance washing of vehicles at the Lakeside should be discouraged and anybody found doing so should be prosecuted and fined heavily. National Environment Management Authority (NEMA) should be keen on monitoring pollution in the country.

(iv) Processes such as boiling, sand filtration, sedimentation and chemical sterilization will reduce or destroy most harmful organisms present in water but not heavy metals. There is need for the encouragement of small scale purification processes especially for people who use raw water for domestic purposes and drinking, by providing families in the affected areas with cartridges which have a lining of *Moringa oleifera* seed powder.

Further studies should be carried out to identify other shrubs and/or vegetation other than *Moringa oleifera* that take up heavy metals from water and soils as this can be planted in the water catchment areas to purify water by removing heavy metals. These can minimize chances of poisoning humans and animals through ingestion of heavy metals because clean environment means better health status for the people. Industrial effluents should be treated with *Moringa oleifera* seed biomass before being released to farms or water bodies; this would minimize pollution caused by heavy metals from industries.

Farmers in the area should be encouraged to practise organic farming, where no herbicides, pesticides and synthetic chemical products are used. To avoid the soil becoming impoverished and to achieve abundant harvests only, natural compost should be used, such as animal dung, ash, natural phosphates, and sweet cane residues, among others.

Due to increased urbanization and socio-economic activities, the threat of pollution is bound to increase within our aquatic systems and therefore it should be monitored regularly. Areas with high levels should be viewed with caution as it implies that the elements can be preferentially released making them bioavailable.

REFERENCES

Abdelkrim, C., Talhi, M. F., Belboukharia, N., Taleb, S.., and Roussel, C. (2011). Removal of copper from aqueous solution by Retama raetam Forssk growing in Algerian Sahara. *Desalination and Water Treatment* 10: 317–320.

Abdel-Ghani, N., and Elchaghaby, G. A. (2007). Influence of operating conditions on the removal of Cu, Zn, Cd and Pb ions from wastewater by adsorption. *International Journal of Environmental Science and Technology* 4 (4): 451-456.

Abu Al-Rub, F. A., El-Naas, M. H., Ashour, I., and Al-Marzouqi, M. (2006). Biosorption of Cu(II) from Aqueous Solutions by Pre-treated Biomass of Marine Algae Padina sp. *Biochem*. 41: 457–464.

Adelaja, O. A., Amoo, I. A., Aderibigbe, A. D. (2011). Biosorption of Lead (II) ions from aqueous solution using *Moringa oleifera* pod. *Scholar's research library Archives of Applied Science Research* 3(6): 50-60.

Affonso, C. G. J., Ana, P. M., Fernanda, R., Leonardo, S., Douglas, C. D. and Gustavo, C. F. (2012). Applicability of *Moringa oleifera* Lam. pie as an adsorbent for removal of heavy metals from waters. Revista Brasileira de Engenharia Agrícola e Ambiental 17(1): 94–99 UAEA/UFCG – http://www.agriambi.com.br. 17/5/2015.

Ahalya, N., Ramachandra, T. V., and Kanamadi R. D. (2003). Biosorption of heavy metals. *Res. J. Chem. Environ*. 7: 71-78.

Ahalya, A. Kanamad, R. D., *and* Ramachandra, T. V. *(*2006*)*. Biosorption of iron (III) from aqueous solution using the husk of Cicer arientinum. *Ind. J. Chem Technol* 13*:* 122–127.

Ahluwalia, S. S., and Goyal, D. (2007). Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour. Technol*. 98(12): 2243-2257.

Ahluwalia, S. S., and Goyal, D. (2006). "Microbial and plant derived biomass for removal of heavy metals from wastewater," *Bioresour. Technol*. 8: 2243-2257.

Ahmady-Asbchin, S., Andres, Y., Gerente, C., *and* Cloirec, P. L. *(*2008*)*. Biosorption of Cu(II) from aqueous solution by Fucus serratus: surface characterization and sorption mechanism. *Bioresource Technol* 99*:* 6150–6155.

Ahuja, P., Gupta, R., Saxena, R. K., and Mohapatra, H. (1999). Lead biosorption and desorption by intact and pretreated spirulina maxima biomass. *Bioresource technology* 69: 223-229.

Ajmal, M., Rao, R. A. K., *and* Khan, M.A. *(*2005*).* Adsorption of copper from aqueous solution on Brassica cumpestris (mustard oil cake). *J. Hazard mater* B122*:* 177–183.

Ajmal, M., Rao, R. A. K., Anwer, S., Ahmad, J., and Ahmad, R. (2003). Adsorption studies on rice husk: Removal and recovery of Cd (II) from wastewater, *Bioresource Technol* 86: 147- 149.

Ajmal, M., Hussain, K. A., Ahmad, S., and Ahmad, A. (1998). Role of sawdust in the removal of copper (II) from industrial wastes, *Wat. Res*. 32 (10): 3085-3091.

Akhtar, N., Saeed, A., and Iqbal, M. (2003). *Chlorella sorokiniana* immobilized on the biomatrix of vegetable sponge of Luffa cylindrica: a new system to remove cadmium from contaminated aqueous medium. *Bioresource Technology* 88(2): 163-165.

Aksu, Z., Sag, Y., *and* Kutsal, T. (1992). The biosorption of Cu by C. vulgaris and Z. ramigera. *Environmental Technol* 13*:* 579–586.

Aksu, Z., and Dönmez, G. (2003). Removal of chromium (VI) from saline wastewaters by Dunaliella species. *Process Biochemistry* 38: 751-762.

Aksu, Z., and Tezer, S. (2005). Biosorption of Cadmium (II) from Aqueous Solutions by Pre treated Biomass of Marine Algae Durvillaea potarotum. *Process Biochem* 40: 1347–1361.

Aksu, Z., and Isoglu, I. A. (2005). Removal of copper (II) ions from aqueous solution by biosorption onto agricultural waste sugar beet pulp, *Process Biochem* 40: 3031-3044.

Aksu, Z. (2005). Application of biosorption for the removal of organic pollutants: a review. *Process Biochemistry* 40(3–4): 997-1026.

Al-Asheh, S., Banat, F., Al-Omari, R., Duvnjak, Z. (2000). Predictions of binary sorption isotherms for the sorption of heavy metals by pine bark using single isotherm data, Chemosphere 41(5): 659-665.

Al-Asheh, S., and Duvnjak, Z. (1998). Binary metal sorption by pine bark: study of equilibria and mechanisms," *Separation Science and Technology* 33(9): 1303–1329.

Alloway, B. J. (1995). *Heavy metals in soils* 2nd edition. Blackie academic and professional.

Alloway, J., and Ayres, D. C. (1997). *Chemical Principles of Environmental Pollution*. (2nd edition). London: Chapman and Hall.

Al-Subu, M., Salim, R., Abu-Shqair, I., and Swaileh, K. (2001). Removal of dissolved copper from polluted water using plant leaves. *Revista Internacional de Cotaminacion Amiental* 17(91–96): 123–127.

Altun, T., *and* Pehlivan E. *(*2007*)*. Removal of copper (II) ions from aqueous solutions by walnut-, hazelnut- and almond-shells. *Clean* 35*:* 601–606.

Alves, V. N., Mosquetta, R., Coelho, N. M. M., Bianchin, J. N., Roux, K. C. P., Martendal, E., and Carasek, E. (2010). Determination of cadmium in alcohol fuel using *Moringa oleifera* seeds as a biosorbent in an on-line system coupled to FAAS. *Talanta* 80(3): 1133-38.

Anurag, P., Debarbrata, B., Anupam, S., *and* Lalitagauri, R. *(2007).* Potential of Agarose for biosorption of Cu(II) in aqueous system. *Am J. Biochem Biotechnol* 3*:* 55–59.

Anwar, F., and Rashid, U. (2007). Physico-chemical characteristics of *Moringa oleifera* seeds and seeds oil from a wild provenance of Pakistan. *Pakistan Journal of Botany* 39(5): 1443-53.

Anwar, J., Shafique, U., Waheeduz, Z., Salman, M., Dar, A., and Anwar, S. (2010). Removal of Pb(II) and Cd(II) from water by adsorption on peels of banana. *Bioresource Technology* 101(6): 1752-1755.

Anwar, H. (2013). Development of novel biosorbents in removing heavy metals from aqueous solution. D.Phil thesis in environmental engineering. *University of technology*, Sydney 4: 17-24.

Arica, M. Y., Bayramoglu, G., Yilmaz, M., Bektas, S., and Genc, O. (2004). Biosorption of Hg^{2+}, Cd^{2+}, and Zn^{2+} by Ca-alginate and immobilized woodrotting fungus Funalia trogii. *J Hazard Mater* 109:191-199.

Arief, V. O., Trilestari, K. Sunarso, J., Indraswati, N., *and* Ismadji, S. *(*2008*).* Recent progress on biosorption of heavy metals from liquids using low-cost biosorbents: characterization, biosorption parameters and mechanism studies. *Clean* 36*:* 937–962.

Arnoldsson, E., Bergman, M., Matsinhe, N., and Persson, K. M. (2008). Assessment of drinking water treatment using *Moringa oleifera* natural coagulant-Vatten Lund. 64: 137–150.

Astier, C., Chaleix, V., Faugeron, C., Ropartz, D., Krausz, P., and Gloaguen, V. (2012). Biosorption of lead (ii) on modified barks explained by the hard and soft acids and bases (HSAB) theory. *Bioresources* 7(1): 1100-1110.

Atkins, P., Overton, T., Rourke, J., Weller, M., and Armstrong, F. (2006). *Inorganic Chemistry,* 4th edition. Oxford, Oxford University Press.

Ayodele, R. I., and Okeoghene, G. T. (2014). Sorption and Desorption Studies on Toxic Metals From Brewery Effluent Using Eggshell as Adsorbent. *Advances in Natural Science* 7(2): 15-24.

Baker, D. E., and Bowers, M. E. (1998). Trace substances in environmental Health, part XXII ed. Hempill, D.D., University of Missouri, Columbia, M .O. 281 – 295.

Basci, N., Kocadagistan, E., and Kocadagistan, B. (2004). Biosorption of aqueous solutions by wheat shell, Desalination 164: 135-140.

Benguella, B., and Benaissa, H. (2002). Cadmium removal from aqueous solutions by chitin: kinetic and equilibrium studies, *Water Res*. 36(10): 2463-2474.

Bhatti, H. N., Mumtaz, B., Hanif, M. A., and Nadeem, R. (2007). Removal of Zn (II) ions fro aqueous solution using *Moringa oleifera* Lam. (Horseradish tree) biomass,” *Process Biochem*. 42: 547-553.

Bhatti, H. N., Khalid, R., and Hanif, M. A. (2008). Dynamic biosorption of Zn(II), Cu(II) using pretreated Rosa grass an teplitz (red rose) distillation sludge. *Chem. Eng. J*. 148: 434– 443.

Bhatti, N. H., Iqra, I. B., Muhammad, A. H., and Iftikhar, H. B. (2010). Removal of lead and cobalt using lignocellulosic fiber derived from Citrus reticulata waste biomass. *Korean J. Chem. Eng*. 27(1): 218-227.

Bhattacharyya, K. G., and Sharma, A. (2004). Azadirachta indica leaf powder as an effective biosorbent for dyes: a case study with aqueous Congo Red solutions," *Journal of Environmental Management* 71(3): 217–229.

Bhushan, R., Thapur, S., and Mathur, R. P. (1997). Accumulation pattern of pesticides in tropical fresh waters. *Bio Chroma*.11: 143 -150.

Blanco, A., Sanz, B., Llama, M. J., and Serra, J. L. (1999). Biosorption of heavy metals to immobilised Phormidium laminosum biomass. *Journal of Biotechnology* 69(2-3): 227-240.

Brierley, C. L. (1990). Metal immobilization using bacteria. In: *Microbial Mineral Recovery*. (Eds.): H. L. Ehrlich and C.L. Brierley. McGraw-Hill Publishing, New York.

Carey, F.A., Sundberg, R. A (2007). Advanced Organic Chemistry. 5th Edition. ISBN-13: 978-0-387-44897-8:678-704

Chang, J., Law, R., and Chang, C. (1997). Biosorption of Lead, Copper and Cadmium by Biomass of Pseudomonas acruginosa, *Water Research* 31(7): 1651-1658. doi:10.1016/S0043-1354(97)00008-0.

Chapman, L. J., Kaufman, L. S., Chapman, C. A., and MacKenzie, F. E. (1995). Hypoxia tolerance in twelve species of East African cichlids: Potential for low oxygen refugia in Lake Victoria. Conservation biology (Cambridge MA) 9 (5): 1274-1288.

Chek, A. (1999). Soil Analysis Methods.Kenya Agricultural Research Institute 24- 58.

Cho, D. H., and Kim, E. Y. (2003). Characterization of Pb^{2+} biosorption from aqueous solution by Rhodotorula glutinis. *Bioprocess Biosyst Eng*. 25: 271-277.

Chong, K.H., and Volesky, B. (1996). Description of 2-Metal Biosorption Equilibria by Langmuir-Type Models. *Biotechnology and Bioengineering* 47(4): 451-460.

Chowdhury, A. K., Sarkar, A. D., and Bandyopadhyay, A. (2009). Rice husk ash as a low cost adsorbent for the removal of methylene blue and Congo red in aqueous phases. *Clean - Soil, Air, Water* 37(7): 581-91.

Clair, N., Sawyer, P. L., and MacCarty, F. Parkin, G. (2005). Chemistry for nvironmental

Engineering and Science, 5th Edition. McGraw-Hill Edition, New York. 97-105.

Cleide, S. T., Araújo, D. C., Carvalho, H. C., Rezende, I. L. S., Almeida, S., Luciana, M., Coelho, N. M. M., Coelho, T. L., and Vanessa, N. A. (2013). Bioremediation of Waters Contaminated with Heavy Metals Using *Moringa oleifera* Seeds as Biosorbent. http://dx.doi.org/10.5772/56157.

Congeevaram, S., Dhanarani, J., Park, M., Dexilin, K., Thamaraiselvi, B. (2007). Biosorpion of Cr and Ni by heavy metal resistant fungal and bacterial isotopes, *J. Hazard. Master* 146: 270-277.

Covelo, E. F., Vega, F. A., Andrade, M. L., (2007). Competitive sorption and desorption of heavy metals by individual soil components. *Journal of Hazardous Materials* 140: 308-315.

Dadhich, A. S., Beebi, S. K., and Kavitha, G. V. (2004). Adsorption of Ni (II) using agrowaste, rice husk, *J. Environ. Sci. Eng*. 46 (3): 179-185.

Dahot, M. U. (1998). Antimicrobial activity of small portion of *Moringa oleifera* leaves. *Journal of Islamic Academy of Sciences* 11: 27-32.

Dakikiy, M., Khami, A., Manassara, A., and Mereb, M. (2002). Selective adsorption of Cr(VI) in industrial wastewater using low-cost abundantly available adsorbents, *Adv. Environ. Res*. 6 (4): 533-540.

Davis, T. A., Volesky, B., and Mucci, A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Wat. Res*. 37(18): 4311-4330.

Deng, S. B., *and* Ting, Y. P. *(*2005*)*. Polyethylenimine-modified fungal biomass as a high-capacity biosorbent for Cr(VI) anions: sorption capacity and uptake mechanisms. *Environ Sci Technol*. 39*:* 8490–8496.

Deng, L., Su, Y., Su, H., Wang, X., and Zhu, X., (2007). Sorption and desorption of lead (II) from wastewater by green algae Cladophora fascicularis. *J. Hazard Mater* 143: 220-225.

Desai, B., and Desai, H. (2013). Potential of *Moringa oleifera* (drum sticks) seeds and its application as natural adsorbent in removal of heavy metal ions. International *Journal of Environment, Ecology, Family and Urban Studies* (IJEEFUS) ISSN 2250-0065. 3(4): 9-22.

Deshkar, A. M., Bokade, S. S., and Dara S. S. (1990). Modified Hardwickia Binata bark for adsorption of mercury (II) from water. *Water Res*. 24(8), pp 1011-1016.

Dhabab, J. M. (2011). Removal of Fe(II), Cu(II), Zn(II), and Pb(II) ions from aqueous solutions by duckweed. *Journal of Oceanography and Marine Science*. 2(1): 17-22.

Ding, Y., Jing, D., Gong, H., Zhou, L., and Yang, X. (2012). Biosorption of aquatic Cd (II) by unmodified rice straw. *Bioresource Technology* 114: 20–5.

Dundar, M., Nuhoglu, C., and Nuhoglu, Y. (2008). Biosorption of Cu(II) ions onto the litter of natural trembling poplar forest. *Journal of Hazardous Materials* 151: 86-95.

Ekmekyapar, F., Aslan, A., Kemal, B. Y., and Cakici, A. (2006). Biosorption of copper(II) by non-living lichen biomass of Cladonia rangiformis hoffm. *Journal of Hazardous Materials* 137: 293-298.

Elaigwu, S. E., Usman, L. A., Awolola, G. V., Adebayo, G. B., Ajayi, R. M. K. (2009). Adsorption of Pb(II) from Aqueous Solution by Activated Carbon Prepared from Cow Dung. *Advances in Natural and Applied Sciences* 3(3): 442 – 446.

Eman, N., Ali, S. R., Alfarra, M. M. Y., and Lutfor, R. M. (2015). Environmentally friendly biosorbent from *Moringa oleifera* leaves for water treatment. *International Jounal of Environmental Science and dev*. 6: 3.

Emine, Y., Kultigin, C., and Kadir, K. (2010). Biosorption of Cu^{2+} and Zn^{2+} by raw and autoclaved Rocella phycopsis. *Journal of Environmental Sciences* 22(3): 367–373.

Esposito, A., Pagnanelli, F., and Veglió, F. (2002). pH-related equilibria models for biosorption in single metal systems. *Chem Eng Sci*. 57: 307-313.

Esteghlalian, A., Hashimoto, A. G., Fenske, J. J., and Penner, N. H. (1997). Modelling and optimization of the dilute sulphuric acid pretreatment of corn stover, poplar and switchgrass. *Bioresour, Technol*. 59: 129-136.

Febrianto, J., Kosasih, A. N., Sunarso, J., Ju, Y., Indraswati, N., and. Ismadji, S. (2009). "Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: a summary of recent studies," *Journal of Hazardous Materials* 162 (2-3): 616–645.

Fein, J., Daughney, C., Yee, N., and Davis, T. (1997). A chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochim Cosmochim Acta* 61: 3319-3328.

Ferraz, A. I., and Teixeira, J. A. (1999). The use of flocculating brewer's yeast for Cr (III) and Pb(III) removal from residual wastewaters. Bioprocess Eng. 21: 431–7.

Fetter, C. W. (1993). Contaminant hydrogeology. New York: *Macmillan Publishing Company*. USA, 1-458.

Fourest, E., and Roux, J. C. (1992). Heavy metal biosorption by fungal mycelial by-product; mechanisms and influence of pH. *Applied Microbiology and Biotechnology* 37(3): 399-403.

Fourest, E., and Volesky, B. (1997). Alginate properties and heavy metal biosorption by marine algae. *Appl Biochem Biotechnol*. 67: 215-226.

Gadd, G.M. (2004). Microbial influence on metal mobility and application for bioremediation. Geoderma 122(2-4): 109-119.

Gadd, G. M. (2009). Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. Journal of Chemical *Technology and Biotechnology* 84(1): 13-28.

Galbe, M., and Zacchi, G. (2007). Pretreatment of lignocellulosic materials for efficient bioethanol production. AdV. *Biochem. Eng./Biotechnol* 108: 41–65.

Garcia-Fayos, B., Miguel, J. A., and Alandia, R. S. (2012). Remediation study of liquid effluents with high concentration of heavy metals using *Moringa oleifera* biosorbent. Selected proceedings of the 18th international congress on project engineering. Valencia, Spain.

Gichuki, J., and Odhiambo, W. (1994). The role of macrophytes in the economy of the lower Sondu-Miriu River of Lake Victoria. *African Journal of Tropical Hydrobiology and Fisheries* (Uganda) [special issue] 5(2): 69-78.

Gophen, M., Ochumba, P. B. O., and Kaufman, L. S. (1995). Aspects of perturbation in the structure and biodiversity of the ecosystem of Lake Victoria (East Africa). *Aquatic Living Resources* (France) 8(1): 27-41.

Goudswaard, K. P. C., and Wanink, J. H. (1994). Anthropogenic perturbation in Lake Victoria: effects of fish introductions and fisheries on fish eating birds. Musee Royal de 1'Afrique Centrale Tervuren Belgique Annales serie IN-8 *Sciences Zoologiques*; 268: 312-318.

Greenberg, A., Clesceri, L., and Faton, A. (1992). Standard Methods for Examination of Water and Wastewater, *Langara Press*, Canada, 18th edition. pp 31-33.

Gulnaz, O., Saygideger, S., and Kusvuran, E. (2005). Study of Cu(II) biosorption by dried activated sludge: effect of physico-chemical environment and kinetics study. *Journal of Hazardous Materials* 120: 193-200.

Gunawardana, C., Goonetilleke, A., Egodawatta, P., Dawes, L., and Kokot, S. (2012). Role of solids in heavy metal pollution of receiving waters. *Journal of Environmental Engineering* 138(4): 490-498.

Gupta, R., Ahuja, P., Khan, S., Saxena, R. K., and Mohapatra, H. (2000). Microbial biosorbents: Meeting challenges of heavy metal pollution in aqueous solutions. Current Science 78: 967- 973.

Gupta, V. K., and Rastogi, A. (2008). Biosorption of lead(II) from aqueous solutions by nonliving algal biomass Oedogonium sp and Nostoc sp - A comparative study. *Colloids and Surfaces B-Biointerfaces* 64(2): 170-178.

Haiyan, L., Anbang, S., Mingyi, L., and Xiaoran, Z. (2013). Effect of pH, Temperature, Dissolved Oxygen, and Flow Rate of Overlying Water on Heavy Metals Release from Storm Sewer Sediments. *J of Chem*. 43412: 1-12.

Han, X., Wong, Y. S., *and* Tam, N. F. Y. (2006). Surface complexation mechanism and modeling in Cr(III) biosorption by a microalgal isolate, Chlorella miniata. *J Colloid Interface Sci* 303*:* 365–371.

Hanif, M.A., Nadeem, R., Bhatti, H. N., Ahmed, N. R., Ansari, T. M. (2007). "Ni(II) biosorption by Cassia fistula (Golden Shower) biomass," *J. Hazard. Mater* 139: 345-355.

Hashem, M. A. (2007). Adsorption of Lead Ion from Aqueous Solution by Okra Wastes. *International Journal of Physical Science* 2: 178-184.

Hecky, R. E. (1993). The eutrophication of Lake Victoria. Kilham Memorial Lecture, 25th congress of SIL. *Verandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie* 25: 39-48.

Ho, Y. S., and McKay, G. (1999). Pseudo-second order model for sorption processes, *Process Biochemistry* 34 (5): 451–465.

Ho, Y. S., Huang, C.T., and Huang, H. W. (2002). Equilibrium sorption isotherm for metal ions of tree fern. *Process Biochem*. 37: 1421–1430.

Horsefall, M. J., Spiff, I. F. (2004). Studies on the effect of pH on the sorption of Pb^{2+} and Cd^{2+} ions from aqueous solutions by Caladium bicolour (Wild Cocoyam) biomass. *Electro. J. Biotechnol.* [online] 7(3). Available from Internet: http://www.ejbiotechnology.info/content/vol7/issue3/full/8/index.ht ml. ISSN 0717 3458.

Horsefall, M. J., and Spiff, I. F. (2005). Effect of Metal ion concentration on Biosorption of Pb^{2+} and Cd^{2+} by Caladium Bicolor (Wild Cocoyam). *African J. Biotechnol*. 4: 191-196.

Hossain, M. A., and Kumita, M. (2005). Optimization of parameters for cr (VI) adsorption on used black tea leaves, *Adsorption* 11: 561-568.

Hossein, F., Tahari, E., Afshin, E., Ali. F., Marzieh, V. D and Bina, B. (2013). Effectiveness of *Moringa oleifera* powder in removal of heavy metals from aqueous solution. *Fresenius Environmental Bulletin*. 22: 5a.

Huang, C. P., Huang, C. N., and Morehart, A. L. (1990). The removal of Cu(II) from dilute aqueous solutions by Saccharomyces cerevisiae," *Water Research* 24(4): 433–439.

Hughes, R. H., and Hughes, J. R. (1992). Lake Victoria: Lake Description. *Directory of African Wildlife* [18/2/2015]. http://www.wcmc.org.uk/dynamic/lcdb/lakesheets/victoria.desc.html.

Ibrahim, F., Halttunen, T., Tahvonen, R., and Salminen, S. (2006). Probiotic bacteria as potential detoxification tools: assessing their heavy metal binding isotherms. *Can J. Microbiol* 52: 877-885.

Iftikhar, A. R., Bhatti, H. N., Hanif, M. A., and Nadeem, R. (2009). Kinetic and thermodynamic aspects of Cu(II) and Cr(III) removal from aqueous solutions using rose waste biomass. *Journal of Hazardous Materials* 161(2-3): 941-947.

Iqbal, M., Saeed, A., and Akhtar, N. (2005). Petiolar felt-sheath of palm: A new biosorbent for the removal of heavy metals from contaminated water, *Bioresour Technol*, 81, 151-153.

Igwe, J. C., and Abia, A. A. (2006). Review a bioseparation process for removing heavy metals from waste water using biosorbents, *African Journal of Biotechnology* 5 (12) , 1167-1179.

Igwe, J. C., Abia, A. A., and Ibeh, C. A. (2008). Adsorption kinetics and intraparticulate diffusivities of Hg(II), As(III) and Pb(II) ions on unmodified and thiolated coconut fiber. *International Journal of Environmental Science and Technology* : (IJEST), 5(1): 83-92.

Jain, C. K., and Ram, D. (1997). Adsorption of lead and zinc on bed sediments of the river kali. *Wat. Res*. 31(1): 154–162.

Javed, M. A., Bhatti, H. N., Hanif, M. A., and Nadeem, R. (2007). Sep. Sci. Technol., 42, 3641.

Jenning, G. D., and Sneed, R. E. (1996). St Clair M.B Metals in drinking water. *North Carolina Cooperative Extension service*. AG–473-1: 3.

Jeon, C. J. Y., Park, Y. J., and Yoo, S. (2002). Characteristics of metal removal using carboxylated alginic acid, *Water Res*. 36: 1814-1824.

Julali, R., Ghafourian, H., Asef, Y., Davarpanah, S. J., and Sepehr, S. (2002). Removal and recovery of lead using nonliving biomass of marine Algae. *Journal of Hazardous materials* 92(3): 253-262.

Kabata, P. A., and Pendias, H. (1992). Trace Elements in Soils and Plants. 2nd ed. *Baton Rouge*: CRC Press. Fl.

Kahraman, S., Dogan, N., and Erdemoglu, S. (2008). Use of various agricultural wastes for the removal of heavy metal ions. *International Journal of Environment and Pollution* 34(14): 275-284.

Kalavathy, M. H., Karthikeyan, T., Rajgopal, S., and Miranda, L. R. (2005). Kinetic and isotherm studies of Cu(II) adsorption onto H3PO4-activated rubber wood sawdust. *Journal of Colloid and Interface Science* 292: 354-362.

Kalavathy, H. M., and Miranda, L. R. (2010). *Moringa oleifera*—A solid phase extractant for the removal of copper, nickel and zinc from aqueous solutions, *Chemical Engineering Journal* 158: 188–199.

Kannan, N., and Veemaraj, T. (2009). Removal of Lead (II) Ions by Adsorption onto Bamboo Dust and Commercial Activated Carbons - a comparative Study. *E-Journal of Chemistry* 6(2): 247 – 56.

Kapoor, A., and Viraraghavan, T. (1997). Heavy metal biosorption sites in Aspergillus niger. *Bioresource Technology* 61(3): 221-227.

Kapoor, A., and Viraraghavan, T. (1998). An alternative treatment option for heavy metal bearing waste waters. *Bioresour. Technol*. 63: 109.

Karaca, H., Tay, T., Kıvanç, M. (2010). Kinetics of lead ion biosorption from aqueous solution onto lyophilized Aspergillus niveus.Water Practice and Technology, 5(1): 1- 10.

Karanja, D. M .S. (2002). Health and diseases: a case study of Lake Victoria basin. In: The Lake Victoria Training Project, Annual Report 2002. Pan-African START Secretariat, Nairobi.

Kardam, A., Raj, K. R., and Srivastava, S. (2013). Development of polyethylenimine modified Zea mays as a high capacity biosorbent for the removal of As (III) and As (V) from aqueous system. *International Journal of Mineral Processing* 122: 66-70.

Katarzyna, C. (2006). Biosorption of Cr (III) by eggshells, J. *Hazard Mater B*. 121: 167-173.

Kawata, K., Yokoo, H., Shimazaki, R., and Okabe, S. (2007). Classification of heavy-metal toxicity by human DNA microarray analysis. *Environ Sci Technol*. 15 (41): 3769-377.

Kiran, I., Akar, T., and Tunali, S. (2005). Biosorption of Pb(II) and Cu(II) from aqueous solutions by pretreated biomass of Neurospora crassa. *Process Biochemistry* 40: 3550-3558.

Kishe, M. A., and Machiwa, J. F. (2003). Distribution of heavy metals in sediments of Mwanza Gulf of Lake Victoria. *Tanzania Environment International* 28(7): 619-625.

Kolodynska, D., and Hubicki, Z. (2008). Comparison of chelating ion exchange resins in sorption of copper(II) and zinc(II) complexes with ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA). *Canadian Journal of Chemistry*-Revue Canadienne De Chimie 86(10): 958-969.

Krishnan, K. A., and Anirudhan, T. S. (2003). Removal of Cd(II) from aqueous solutions by steam-activated sulphurised carbon prepared from sugar-cane bagasse pith: Kinetics and equilibrium studies. *Water SA*. 29: 147-156.

Kudhongania, A. W., and Chitamwebwa, D. B. R. (1995). Impact of environmental change, species introductions and ecological interactions on the fish stocks of Lake Victoria. In Pitcher T. J., Hart P. J. B. (eds). The impact of species changes in African lakes. Chapman and Hall, *Fish and Fisheries Series* 18: 12-32.

Kumar, R. R., Abhishek, K., Jyoti, K. A., Man, M. S., and Shalini, S. (2010). Neutral Network Modeling for Ni(II) Removal fromAqueous System Using Shelled *Moringa Oleifera* Seed Powder as an Agricultural Waste. *J. Water Resource and Protection* 2: 331-338.

Kumari, P., Sharma, P., Shalini, S., and Srivastava, M. M. (2005). Arsenic removal from the aqueous system using plant biomass: a bioremedial approach, *J. Ind Microbiol Biotechnol*. 32: 521–526.

Lalah, J. O., Ochieng, E. Z., and Wandiga, S. O. (2008). Sources of Heavy Metal Input Into Winam Gulf, Kenya. *Bull. of Environ Contam and Toxicol*. 81(3): 277-284.

Lea, M. (2010). Bioremediation of Turbid Surface Water Using Seed Extract from *Moringa oleifera* Lam. (Drumstick) Tree, Curr. *Protoc.Microbiol*. 16:1G.2.1-1G.2.14. © John Wiley and Sons, Inc.

Leusch, A., Holan, Z. R., and Volesky, B. (1995). Biosorption of heavy metals (Cd, Cu, Ni, Pb, Zn) by chemically reinforced biomass of marine algae. *Journal of Chemical Technology and Biotechnology* 62(3): 279-288.

Leyva-Ramos, R., Bernal-Jacome, L. A., and Acosta-Rodriguez, I. (2005). Adsorption of cadmium(II) from aqueous solution on natural and oxidized corncob. *Sep. Purif. Technol.* 45: 41–49.

Limousin, G., Gaudet, J. P., Charlet, L., Szenknect, S., Barthès, V., *and* Krimissa, M. *(2007)*. Sorption isotherms: a review on physical bases, modeling and measurement. *Appl Geochem* 22*:* 249–275.

Liu, H. L., Chen, B. Y., Lan, Y. W., and Chen, Y. C. (2004). Biosorption of Zn(II) and Cu(II) by the indigenous Thiobacillus thiooxidans. *Chemical Engineering Journal* 97: 195-201.

Liu, C. C., Wang, M., Chiou, C., Li, Y., Yang, C., and Lin, Y. (2009). Biosorption of chromium, copper and zinc by wine-processing waste sludge: Single and multi-component system study. *Journal of Hazardous Materials* 171: 386-392.

Liu, Z. S., Lin, C. L., and Chou, J. D. (2010). Studies of Cd(II), Pb(II) and Cr(III) distribution characteristics in bottom ash following agglomeration/defluidization in a fluidized bed boiler incinerating artificial waste. *Fuel Processing Technology*, 91(6): 591-599.

Loace, M., Olier, R., Guezennec, J. (1997). Uptake of lead, cadmium and zinc by a novel bacterial expolysaccharide, *Water Res*. 31: 1171-1179.

Lodeiro, P., Barriada, J., Herrero, R., and Sastre de Vicente, M. (2006). The marine macroalga Cystoseira baccata as biosorbent for cadmium (II) and lead (II) removal: Kinetic and equilibrium studies. *Environmental Pollution* 142(2): 264-273.

Lopez, F. A., Perez, C., Sainz, E., and Alonso, M. (1995). Adsorption of Pb (II) on blast furnace sludge, *J. Chem. Tech. Biot*. 62 (2): 200-206.

Low, K. S., Lee, C. K., and Liew, S. C. (2000). Sorption of cadmium and lead from aqueous solution by spent grain, *Process Biochem*. 36 (1): 59-64.

Mahvi, A. H., Alavi, N., and Maleki, A. (2005). Application of rice husk and its ash in cadmium removal from aqueous solution, *Pakistan J. Biol. Sci*. 8 (5): 721-725.

Mahvi, A. H., Nouri, J., Omrani, G. A., and Gholami, F. (2007). Application of platanus orientalis leaves in removal of cadmium from aqueous solution, *World Appl. Sci. J.* 2 (3): 40-44.

Mahvi, A.H. (2008). Application of agricultural fibers in pollution removal from aqueous solution. *International Journal of Environmental Science and Technology* 5(2): 275-285.

Majumder, S. S. (2008). Adsorption behaviour of copper ions on Mucor rouxii biomass through microscopic and FTIR analysis. *Colloids Surf* 63*:* 138–145.

Makokha, A. O., Mghweno, L. R., Magoha, H. S., Nakajugo, A., and Wekesa, J. M. (2011). The effects of environmental Pb Pollution in Kisumu, Mwanza and Kampala. *The Open Environmental Engineering Journal* 4: 133-140

Mannazzu, I., Guerra, E., Ferretti, R., Pediconi, D., and Fatichenti, F. (2000). Vanadate and copper induce overlapping oxidative stress responses in the vanadate-tolerant yeast Hansenula polymorpha. *Biochem. Biophys. Acta* 1475: 151-156.

Martin, S. A., Rodriguez, M. G., Aguolar, R., and Soto, G. (2011). Removal of chromium hexavalent from rinsing chromating waters electrochemical reduction in a laboratory pilot plant. *Water Science and Technology* 49(1): 115-122.

Masakazu, A. (2003). Removal of Cr (VI) from aqueous solution London plane leaves, *J. Chem. Tech. Biotech*.78(5): 601-604.

Mataka, L. M. (2005). Lead remediation with *Moringa stenopetala* and *oleifera* seed powder, *WARFSA Conference paper* 1-19

Mataka, L. M., Henry, E. M. T., Masamba, W. R. L. and Sajidu, S. M. (2006). *Int. J. Environ. Sci. Technol.* 5: 26 - 35.

Mataka, L. M., Henry, E. M. T., Masamba, W. R. L., and Sajidu, S. M. (2010). Cadmium sorption by *Moringa stenopetala* and *Moringa oleifera* seed powders: *Int. J. Environ. Sci. Tech* 3: 131-139.

Matheickal, J. T., and Yu, Q. (1999). Biosorption of lead (II) and copper (II) from aqueous solutions by pre-treated biomass of Australian marine algae, *Bioresource Tech.* 69(3): 223 -229.

Matindi, C. N., Njogu, P. M., Kinyua, R., and Nemoto, Y. (2014). Analysis of heavy metals in water hyacinth from Lake Victoria, Kenya. Proceedings of 2014 *international conference on sustainable research and innovation Volume* 5, 7th-9th May 2014. JKUAT. Kenya. 2079-6226: 1-4. www.jkuat-sri.com/ojs/index.php/proceedings/article/view/133. 29/5/2015.

Matos, G. D., and Arruda, M. A. Z. (2006). Online preconcentration/determination of cadmium using grape bagasse in a flow system coupled to thermospray flame furnace atomic absorption spectrometry. *Spectroscopy Letters* 39: 1-14.

Mbahinzireki, G. (1994). Initial results of the benthic fauna studies in the northern Lake Victoria, *ICIPE Science* Nairobi (Kenya). 7-13. www.oceandocs.org/handle/.../browse?...**Mbahinzireki**%2C+**G**.B. 13

Mdamo, A. (2003). Nutrient removal by Wetlands: A preliminary study. In Ndaro S. G M and Kishimba m. proceedings of the LVEMP – Tanzania (2001), *Scientific Conference,* 6-10 August, 2001, Mwanza, Tanzania. 344-358.

Mehta, S. K., and Gaur, J. P. (2005). Use of algae for removing heavy metal ions from wastewater: progress and prospects. *Crit Rev Biotechnol*. 25: 113–152.

Mehta, J., Shukla, A., Bukhariya, V., and Charde, R. (2011). The magic remedy of *Moringa oleifera*: An overview. *International Journal of Biomedical and Advance Research* 02(5): 432-437.

Mireji, P. O., Keating, J., Hassanali, A., Kahindi, S., Beir, J. C., Mbogo, C. M., and Nyambaka, H. (2008). Heavy metals in mosquito larval habitats in urban Kisumu and Malindi, Kenya and their impact. *Ecotoxology and environmental safety* (70): 147-153.

Mugidde, R. (1992). Changes in Phytoplankton primary production and biomass in Lake Victoria (Uganda). M. Sc. thesis, Univ. Manitoba (Canada): 84.

Muli, R. J. (1996). Environmental problems in Lake Victoria (East Africa): What the international Community can do. Lakes and Reservoirs: *Research and Management* 2: 47-53. Nairobi. Kenya.

Mishra, S. P., Tiwari, D., Dubey, R., Mishra, M. (1998). Biosorptive behaviour of casein for Zn^{2+}, Hg^{2+} and Cr^{3+}: effects of physico-chemical treatments. *Bioresource Technology* 63(1): 1-5.

Mittelman, M. W., and Geesey, G. G. (1995). Appl. Environmental. Microbiology. 49: 846.

Muinde, V. M., Nguu, E. K., Ogoyi, D. O., and Shiundu, P. M. (2013). Effects of Heavy Metal Pollution on Omega-3 Polyunsaturated Fatty Acids Levels In Tilapia Fish from Winam Gulf of Lake Victoria. *The Open Environmental Engineering Journal* 6: 22-31.

Murphy, S. (2007). General Information on Solids. City of Boulder/USGS Water Quality Monitoring. 8/08/2016.

Muyibi, S. A., Ameen, E. S. M., Noor, J. M., and Ahmadum, F. R. (2002a). Bench Scale Studies for Pre-treatment of Sanitary Landfill Leachate with *Moringa oleifera* seed Extract. *International Journal of Environmental Studies* 59(5): 513-535.

Muyibi, S. A., Noor, J. M., Leong, T. K., and Loon, L. H. (2002b). Effects of oil extraction from *Moringa oleifera* seed on Coagulation of Turbid water. *International Journal of Environmental Studies* 59(2): 243-254.

Nabizadeh, R., Naddafi, K., Saeedi, R., Mahvi, A. H., Vaezi, F., Yaghmaeian, K., and Nazmara, S. (2005). Kinetic and equilibrium studies of lead and cadmium biosorption from aqueous solutions by sargassum spp. biomass, *Iran. J. Environ. Health Sci. Eng*. 2 (3): 159-168.

Nadeem, R., Hanif, M. A., Shaheen, F., Perveen, S., Zafar, M. N., and Iqbal, T. (2008). Biosorption of chromium (VI) by some algae. *J. Hazard. Mater* 150: 335.

Namasivayam, C., and Ranganthan, K. (1995). Revoval of Cd (II) from wastewater by adsorption on waste Fe (III)/ Cr (III) hydroxide, *Water. Res*. 29 (7): 1737-1744.

Nagase, H., D. Inthron, Y. Isaji, A., Oda, K., Hirata, K., and Miyamoto, K. (2005). Selective cadmium removal from hard water using NaOH--treated cells of the cyanobacterium Tolypothrix tenuis. *Journal of Fermentation and Bioengineering* 84: 151-154.

Neelima, C. (2013). Removal of Cr (VI) from aqueous solutions using *Moringa oleifera* as a biosorbent. *International journal of chemical, environmental and Pharmaceutical research.* 4(2): 17-19.

Ngwenya, B. T., Sutherland, I. A., and Kennedy, L. (2003). Comparison of the acid-base behaviour and metal adsorption characteristics of gram-negative bacterium with other strains. *Appl Geochem* 18: 527-538.

Ntiba, M. J., Kudoja, W. M., and Mukasa, C. T. (2001). Management issues in the Lake Victoria watershed. Lakes Reservoirs: *Res. Mgmt* 6: 211-216.

Nuhoglu, Y., Oguz, E. (2003). Removal of Cu(II) from aqueous solutions by biosorption on the cone biomass of Thuja orientalis. *Process Biochemistry* 38: 1627-1631.

Nzomo, R. (2005). Sustainable management of African Lakes -The case of Lake Victoria. Paper presented in the first living Lakes African regional conference held 27th October 2005 at Imperial Hotel, Kisumu. https://www.globalnature.org/.../RolinNzomo. 30/6/2015.

Oboh, I., Aluyor, E., and Audu, T. (2009). Biosorption of Heavy Metal Ions from Aqueous Solutions Using a Biomaterial Department of Chemical Engineering, University of Benin, *Leonardo Journal of Sciences* 14: 58-65.

Obuseng, V., Nareetsile, F., and Kwaambwa, M. H. (2012). A study of the removal of heavy metals from aqueous solutions by *Moringa oleifera* seeds and amine-based ligand 1,4-bis[N,N-bis(2-picoyl)amino]butane, *Analitica Chemica acta*. 730: 87-92.

Odada, E. O., Olago, D. O., and Ochola, W. (2006). Lake Victoria Environment outlook. *Environment and Development*: Nairobi. UNEP/PASS. apps.unep.org/publications/pmtdocuments/environment_development.pdf. 10/12/2014.

Odee, D. (1998). Forest biotechnology research in drylands of Kenya: the development of Moringa species. *Dryland Biodiversity* 2: 7 - 8.

Ogutu-Ohwayo, R., Hecky, R. E., Cohen, A. S., and Kaufman, L. (1997). Human impacts on African Great Lakes. *Environmental Biology of Fishes* 50: 117-131.

Ogoyi, D. O., Mwita, C. J., Nguu, E. K., and Shiundu, P. M (2011). Determination of Heavy Metal Content in Water, Sediment and Microalgae from Lake Victoria, East Africa. *The Open Environmental Engineering Journal* 4: 156-161.

Okoronkwo, E. A., and Anwasi, S. (2010). Modeling of copper and zinc adsorption from aqueous solution by tithonia diversifolia. Department of Chemistry, Federal University of Technology Pmb 704 Akure, Nigeria. *J. Chem. Soc. Nigeria* 3(1): 66-72.

Oliveira, E. A., Montanher, S. F., Andrade, A. D., Nobrega, J. A., and Rollemberg, M. C. (2005). Equilibrium studies for the sorption of chromium and nickel from aqueous solutions using raw rice bran, *Process Biochem*. 40: 3485-3490.

Ongeri, D. M. K., Lalah, J.O., Wandiga, S. O., Schramm, K. W., and Machalke, B. (2009). Levels of toxic metals in multisectoral samples fron Winam Gulf of Lake Victoria. Bull. Of environ. *Contam and toxicology* 21: 38-49.

Osumo, W. M. (2001). Effects of water hyacinth on water quality of winam gulf, Lake victoria kenya marine fisheries research institute university of akureyri and KEMFRI. 1-37.www.unuftp.is/static/fellows/document/osumoprf.pdf. 21/8/14.

Ozer, A., and Ozer, D. (2004). The adsorption of copper ions onto dehydrated wheat bran (DWB): Determination of equilibrium and thermodynamic parameters, *Process Biochem.* 39: 2183-2191.

Ozturk, A. (2007). Removal of nickel from aqueous solution by bacterium *Bacillus thuringiensis, J. Hazard. Master* 147: 518–523.

Pandey, A., Pradheep, K., and Gupta, R. (2011). Drumstick tree' (*Moringa oleifera* Lam.): a multipurpose potential species in India Genetic Resources and Crop Evolution 58: 453-460.

Pal, A., Ghosh, S., and Paul, A. K. (2006). Biosorption of cobalt by fungi from serpentile soil from Andaman. *Bioresource Technology* 97 (10): 1253-8.

Pardo, R., Herguedas, M., Barrado, E., and Vega, M. (2003). Biosorption of cadmium, copper, lead and zinc by inactive biomass of Pseudomonas putida. *Anal Bioanal Chem*. 376: 26-32.

Park, D., Yun, Y. S., and Moon, P. J. (2010). The Past, *Biotechnology and Bioprocess Eng*. 15: 86-93.

Paula, M. A., Gonçalves, J. R., Affonso, C., Teixeira, T., César, R., Stangarlin, J. R., Rubio, F., Nacke, H. (2013). Studies of Pb^{2+} adsorption by *Moringa oleifera* Lam. seeds from an aqueous medium in a batch system. *Water Science and Technology Academic Journal* 69(1): 163.

Pavasant, P. R., Apiratikul, V., Sungkhum, P., Suthiparinyanont, S., Wattanachira, T., and Marhaba, T. F. (2006). Biosorption of Cu^{2+}, Cd^{2+}, Pb^{2+}, and Zn^{2+}, using dried marine green macroalga caulerpa lentilligera: *Bioresour. Technol*. 97: 2321-2329.

Pavia, D. L., Lampman, G. M., and Kriz, G. S. (2001). Introduction to spectroscopy: A guide for for students of organic chemistry. *Thomson Learning*, Inc. 3rd edition, 24: 353-358.

Pehlivan, E., Altun, T., *and* Parlayici, S. *(2009)*. Utilization of barley straws as biosorbent for Cu^{2+} and Pb^{2+} ions. *J. Hazard Mater* 164*:* 982–986.

Pereira, G. M., and Arruda, M. A. Z. (2003). Trends in preconcentration procedures for metal determination using atomic spectrometry techniques. *Microchimica Acta* 141: 115-31.

Pino, G. H., de Mesquita, L. M. S., Torem, M. L., and Pinto, G. A. S. (2006). Biosorption of Heavy Metals by Powder of Green Coconut Shell. *Separation Science and Technology* 41: 3141-3153.

Plazinski, W., and Rudzinski, W. (2010). Binding stoichiometry in sorption of divalent metal ions: A theoretical analysis based on the ion-exchange model. *Journal of Colloid and Interface Science* 344(1): 165-170.

Polcaro, A. M., Mascia, M., Palmas, S., Vacca, A. and Tola, G. (2003). Competitive sorption of heavy metal ions by soils. Environmental Engineering Science 20(6): 607-616.

Prasad, M., and Saxima, S. (2004). Sorption mechanism of some divalent metal ions onto low-cost mineral adsorbent. *Ind. Eng. Chem. Res.* 43: 1512–1522.

Priyantha, N., Lim, L. B. L., Tennakoon, D. T. B., Mansor, N. H., Dahri, M. K., and Chieng, H. I. (2013). Breadfruit (*Artocamus altilis*) waste for bioremedation of Cu(II) and Cd(II) Ions from aqueous medium. *Ceylon Journal of Science (Physical Sciences)* 17: 19-29.

Puello, J., Silgado, K., Marrugo, G. D. (2014). Adsorption of Cr (VI) by activated carbon produced from oil palm endocarp. *Chemical Engineering Transaction* 37: 721-726.

Puranik, P. R., and Paknikar, K. M. (1999). Biosorption of Pb, Cd, and Zn by Citrobacter strain MCM B-181: Characterization studies. *Biotech Prog*. 15: 228-237.

Rahaman, M. S., Basu, A., *and* Iaslam, M. R. (2008). The removal of As(III) and As(V) from aqueous solutions by waste materials. *Bioresource Technol*. 99*:* 1815–2823.

Rao, H. J., Kalyani, G., Rao, K. V., Kumar, T. A., Mariadas, K., Kumar, Y. P., Vijetha, Pallavi, P., Sumalatha, B., and Kumaraswamy, K. (2010). Kinetic studies on biosorption of lead from aqueous solutions using eggshell powder. International *Journal of Biotechnology and Biochemistry* 6(6): 957 – 968.

Ravikumar, K. and Sheeja, A. K. (2013). Heavy Metal Removal from Water using *Moringa oleifera* Seed Coagulant and Double Filtration. *International Journal of Scientific and Engineering Research* 4: 2229-5518.

Reddad, Z., Gerente, C., Andres, Y., and Le Cloirec, P. (2002). Adsorption of several metal ions onto a low-cost biosorbent: kinetic and equilibrium studies. *Environmental Science and Technology* 36(9): 2067-2073.

Reddy, A. R., and Reddy, K. H. (2003). Heavy metal ion uptake properties of polystyrene-supported chelating polymer resins. *Process Indian Acad. Sci*. (Chemical Science) 115(3): 155-160.

Reddy, D.H.K., Harinatha, Y., Seshaiaha, K., and Reddy, A.V.R. (2010). Biosorption of Pb(II) from aqueous solutions using chemically modified *Moringa oleifera* tree leaves. *Chemical Engineering Journal* 162: 626–34.

Reddy, D.H.K., Ramana, D.K.V., Seshaiah, K., Reddy, A.V.R. (2011) Biosorption of Ni(II) from aqueous phase by *Moringa oleifera* bark, a low cost biosorbent. *Desalination* 268: 150–57.

Reddy, D. H. K., Seshaiaha, K., Reddy, A. V. R., and Leec, S. M. (2012). Optimization of Cd(II), Cu(II) and Ni(II) biosorption by chemically modified *Moringa oleifera* leaves powder. *Carbohydrate Polymers* 88: 1077–86.

Ricordel, S., Taha, S., Cisse, I., and Dorange, G. (2001). Heavy metals removal by adsorption onto peanut husks carbon: characterization, kinetic study and modeling. *Separation and Purification Technology* 24(3): 389-401.

Roane, T. M., and Pepper, I. L. (2000). Microbial responses to environmentally toxic cadmium, *Microbial Ecology* 38: 358–364.

Romero-Gonzalez, M. E., Williams, C. J., and Gardiner, P. H. E. *(2001)*. Study of the mechanisms of cadmium biosorption by dealginated seaweed waste. *Environ Sci Technol.* 35*:* 3025–3030.

Saeed, A. Akhtar, M. W., and Iqbal, M. (2005a). Removal and recovery of heavy metals from aqueous solution using papaya wood as a new biosorbent, *Sep Purif Technol.* 45: 25-31.

Saeed, A., Iqbal, M., Akhtar, M. W. (2005b). Removal and recovery of lead (II) from single and multimetal (Cd, Cu, Ni, Zn) solutions by crop milling waste (black gram husk), *J. Hazard Mater*, B. 117: 65-73.

Sag, Y., Akcael, B., Kutsal, T. (2001). Evaluation, interpretation, and representation of three-metal biosorption equilibria using a fungal biosorbent. *Process Biochem*. 37: 35–50.

Sag, Y., Akeael, B., Kutsal, T. (2002). Ternary biosorption equilibria of Cr (VI), Cu (II) and Cd (II), on Rhizopus arrihzus. *Separation Sci and Technology* 37: 279-309.

Saha, U., Taniguchi, S., and Sakurai, K. (2001). Adsorption behavior of cadmium, zinc, and lead on hydroxyaluminum–and hydroxyaluminosilicate–montmorillonite complexes. *Soil Science Society of America Journal* 65(3): 694-703.

Sajidu, S. M. I., Henry, E. T., Persson, I., Masamba, W. R. L., and Kayambazinthu, D. (2013). pH dependence of sorption of Cd $^{2+}$, Zn^{2+}, Cu^{2+} and Cr^{3+} on crude water and sodium chloride extracts of *Moringa stenopetala* and *Moringa oleifera*. *African journal of chemistry* 1(2): 063-066.

Salehzadeh, H., and Shojasadati, S. A. (2003). Removal of metal ions from aqueous solution by polysaccharide produced from Bacillus firmus. *Water Res*. 17: 4231–4235.

Salim, R., Al-Subu, M. M., and Sahrhage, E. (1992). Uptake of cadmium from water by beech leaves. *J. Environ. Sci. Heal*. A27(3): 603–627.

Sanchez, J. I., Martinez, B., Guillen, R., Jimenez-Diaz, R., and Rodriguez, A. (2006). Culture conditions determine the balance between two different exopolysaccharides produced by Lactobacillus pentosus LPS26. *Appl Environ Microbiol.* 72: 7495-7502.

Sarı, A., and Tuzen, M., (2009). Biosorption of As(III) and As(V) from aqueous solution by macrofungus (Inonotus hispidus) biomass: Equilibrium and kinetic studies. *Journal of Hazardous Material* 164: 1372-1378.

Satiroglu, N., Kesenci, K., Bektas, S., Genc, O., and Piskin, E. (1998). Competitive adsorption of heavy-metal ions on mono disperse polystyrene microspheres carrying dithiocarbamate groups. *Journal of Macromolecular Science-Pure and Applied Chemistry* A35 (1): 91-107.

Scott, J. A., and Karanjkar, A. M. (1995). Adsorp-tion isotherms and diffusion coefficients for metals biosorbed by biofilm coated granular activated carbon. *Biotech Letters* 17(11): 1267-70.

Seehausen, O., and Witte, F. (1995). Extinction of many, survival of some, and the current situation of the endemic cichlids in Lake Victoria. *Trop. Fish Hobbyist* 43(7): 96-105.

Seehausen, O. (1995). Cichlid rescue efforts in Southern Lake Victoria. *Bulletin of the American Cichlid Association* (Oxford, New York) 170: 1-8.

Seki, H., Noguchi, A., Suzuki, A., and Inoue, N. (2006). Biosorption of heavy metals onto Gram-positive bacteria, *Lactobacillus plantarum* and Micrococcus luteus. *Kagaku Kogaku Ronbunshu* 32: 352-355.

Selatnia, A., Bakhti, M. Z., Madani, A., Kertous, L., Mansouri, Y. (2004). Biosorption of Cd^{2+} from aqueous solution by a NaOH treated bacterial dead streptomyces rimosus biomass, *hydrometallurgy* 75: 11–24.

Senthilkumaar, S., Bharathi, S., Nithyanandhi, D., and Subburam, V. (2000). Biosorption of toxic heavy metals from aqueous solutions, *Bioresour Technol*. 75: 163-165.

Shafqat, F., Bhatti, H. N., Hanif, M. A., and Zubair, A. (2008). J. Chil. Chem. Society 54: 1565.

Sharma, D. C., and Forester, C. F. (1995). Column studies into the adsorption of Chromium using sphagnum moss peat. *Bioresource Tech* 52 (3): 261-267.

Sharma, P., Kumari, P., Srivastava, M. M., Srivastava, S. (2006). Removal of cadmium from aqueous system by shelled *Moringa oleifera* Lam. Seed powder. *Bioresource Technology* 97: 299-355.

Sharma, P., Kumari, P., Srivastava, M.M., Srivastava, S. (2007). Ternary biosorption studies of Cd(II), Cr(III) and Ni(II) on shelled *Moringa oleifera* seeds. *Bioresource Technology* 98 (2): 474-477.

Shen, J., and Duvnjak, Z., (2004). Effects of Temperature and pH on Adsorption Isotherms for Cupric and Cadmium Ions in Their Single and Binary Solutions Using Corncob Particles as Adsorbent. *Separation Science and Technology* 39: 3023-3041.

Singh, A., Mehta, S. K., and Gaur, J. P. (2007). Removal of heavy metals from aqueous solution by common freshwater filamentous algae. *World J. Microbiol. Biotechnol* 23: 1115–1120.

Skowronski, T., Pirszel, J., Pawlik-Skowronska, B. (2001). Heavy metal removal by the waste biomass of Penicillium chrysogenum. *Water Qual. Res. J. Can* 36:793–803.

Srivastava, V. C., Mall, I. D., Mishra, I. M. (2008). Antagonistic competitive equilibrium modeling for the adsorption of ternary metal ions mixtures onto bagasse fly ash. *Industrial and Engineer. Chemistry Research* 47: 3129–3137.

Sud, D., Mahajan, G., and Kaur, M. P. (2008). Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions—a review. *Bioresource Technol* 99*:* 6017–6027.

Suh, J. H., and Kim, D. S. (2000). Effects of Hg^{2+} and cell conditions on Pb^{2+} accumulation by Saccharomyces cerevisae. *Bioprocess Eng*. 23: 327–9.

Taffarel, S. R., and Rubio, J. (2009). "On the removal of Mn^{2+} ions by adsorption onto natural and activated Chilean zeolites," Minerals Engineering 22(4): 336–343.

Tan, T. W., and Cheng, P. (2003). Biosorption of metal ions with Penicillium chrysogenum. *Appl Biochem Biotechnol* 104*:* 119–128.

Tangjuank, S., Insuk, N., Udeye, V., Tontrakoon, J. (2009). Cr(III) sorption from aqueous solutions using activated carbon prepared from cashew nut shells. *Int J Phys Sci*. 4: 412-417.

Tarley, C. R. T., and Arruda, M. A. Z. (2004). Biosorption of heavy metals using rice milling byproducts: characterization and application for removal of metals from aqueous effluents. *Chemosphere* 54*:* 987–995.

Thiago, N. H., Coelho, J. S, Santos, N. D., Napoleão, T. H., Gomes, F. S., and Ferreira, R. S. (2013). Effect of *Moringa oleifera* lectin on development and mortality of Aedes aegypti larvae. *Chemosphere* 77: 934-938.

Ting, Y., and Teo, W. (1994). Uptake of cadmium and zinc by yeast: effects of co-metal ion and physical/chemical treatments. Bioresource Technology 50(2): 113-117.

Ting, V. P., Lawson, F., and Prince, I. G. (1995). *Biotechnol. Bioeng*. 3: 990–999.

Tsai, L. J., Yu, K. C., Chen, S. F., and Kung, P. Y. (2003). Effect of temperature on removal of heavy metals from contaminated river sediments via bioleaching. *Water Research* 37(10): 2449-2457.

Tsui, M., Cheung, K. C., Tam, N. F. Y., and Wong, M. H. (2006). *Bioresour. Technol*., 97: 232.

Tsezos, M., and Georgousis, Z. (1997). Mechanism of Aluminum Interference ön Uranium Biosorption by *Rhizopus arrhizus*. *International Biodeterioration and Biodegradation* 31(2): 13-22.

Tsezos, M., Remoudaki, E., and Angelatou, V. (1996). A study of the effects of competing ions on the biosorption of metals. *International Biodeterioration and Biodegradation* 38(1): 19-29.

Tunali, S., Kiran, I., and Akar, T. (2005). Chromium (VI) biosorption Characteristics of Neurospora crassa fungal biomass, *Mineral Eng*. 18: 681-689.

Tunali, S., Akar, T., Özcan, A. S., Kiran, I. and Özcan, A. (2006). Equilibrium and Kinetics of biosorption of Pb(II) from aqueous solutions by Cephalosporium aphidicola. Separati*on and Purification Technology* 47: 105-112.

Turgut, C. (2003). The contamination with organochlorine pesticides and heavy metals in Surface water in Küçük Menderes River in Turkey, 2000–2002. *Environment International* 29(1): 29-32.

Vikashni, N., Matakite, M., and Kanayathu, K. (2012). Water Purification using *Moringa oleifera* and Other Locally Available Seeds in Fiji for Heavy Metal Removal. *International Journal of Applied Science and Technology* 2: 5.

Veglio, F., Esposito, A., and Reverberi, A. P. (2008). Process Biochemistry 38, 953.

Veglio, F., and Beolchini, F. (1997). Removal of metals by biosorption: a review. *Hydrometallurgy* 44(3): 301-316.

Vijayaraghavan, K. *and* Yun, Y. S. (2008). Bacterial biosorbents and biosorption. *Biotechnol Adv* 26*:* 266–291.

Vijayaraghavan, K. *and* Yun, Y. S. (2007). Chemical modification and immobilization of Coryne bacterium glutamicum for biosorption of reactive black 5 from aqueous solution. *Ind Eng Chem Res* 46*:* 608–617.

Vilar, V. J. P., Botelho, C. M. S., Boaventura, R. A. R. (2006). Equilibrium and kinetic modelling of Cd(II) biosorption by algae Gelidium and agar extraction algal waste. *Water Res*. 40: 291–302.

Volesky, B., and Holan, Z. (1995). Biosorption of heavy metals. *Biotechnology Progress,* 11(3): 235-250.

Volesky, B. (1999). Biosorption for the next century, Biohydrometallurgy and the Environment toward the Mining of the 21st Century, Internat. Biohydrometallurgy Symposium Proceedings, 1999, volume B, Ballester, A. and Amils, R. (eds.) *Elsevier Sciences*, Amsterdam, The Netherlands 161-170.

Volesky, B. *(2001).* Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy* 59*:* 203–216.

Waghmare, V. H., and Chaudhari, U. E. (2014). Equilibrium uptake and sorption dynamics for the retrieval of divalent manganese from aqueous solution using *Moringa oleifera* bark. *Rasayan J. Chem* 7(3), 214 – 218.

Waite, T., Davis, J., Payne, T., Waychunas, G., and Xu, N. (1994). Uranium (VI) adsorption to ferrihydrite: Application of a surface complexation model. *Geochimica Et Cosmochimica Acta* 58(24): 5465-5478.

Wandiga, S., and Onyari, J. (1987). The concentration of heavy metals: Mn, Fe, Cu, Zn, Cd, and Pb in sediments and fish from the Winam Gulf of Lake Victoria and fish bought in Mombasa town markets. *Kenya J. Sci.* 8: 5-18.

Wang, X., and Qin, Y. (2005). Equilibrium sorption isotherms for Cu2+ on rice bran, *Process Biochem*. 40: 677-680.

Wang, J., and Chen, C. (2006). Biosorption of heavy metal by Saccharomyces cerevisiae. *Biotechnol. Adv*. 24: 427-451.

Witte, F., Goldschmidt, T., Goudswaard, P. C., Ligtvoet, W., van Oijen, M. J. P., and Wanink, J. H. (1992). Species extinction and concomitant ecological changes in Lake Victoria. *Neth. J. Zool*. 43(2-3): 214-232.

Wongjunda, J., and Saueprasearsit, T. (2010). Biosorption of chromium (VI) using rice husk ash and modified rice husk ash," *Environmental Research Journal* 4(3): 244–250.

Wong, K. K., Lee, C. K., Low, K. S., and Haron, M. J. (2003) Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial ffluents and municipal wastewater. *Chemosphere* 50: 23-34.

World Health Organization (WHO). (2008). Guidelines for drinking-water quality, *electronic resource*: incorporating 1st and 2nd addendum, Recommendations. 3rd ed. Geneva 1: 28.

Yan, G. Y., and Viraraghavan, T. (2003). Heavy-metal removal from aqueous solution by fungus Mucor rouxii. *Water Res*. 37: 4486-4496.

Yang, S., Li, J., Shao, D., Hu, J., and Wang, X. (2009). Adsorption of Ni (II) on oxidized multi-walled carbon nanotubes: effect of contact time, pH, foreign ions and PAA. *Journal of Hazardous Materials* 166(1): 109-116.

Yoshida, T., Ozaki, T., Ohnuki, T., and Francis, A. J. (2004). *Chem. Geol*, 212: 239.

Yetilmezsoy, K., and Demirel, S. (2008). Artificial Neural Net-work (ANN) Approach for Modeling of Pb (II) Adsorp-tion from Aqueous Solution by Antep Pistachio (Pistacia Vera L.) Shells," *Journal of Hazardous Materials* 153(3): 1288-1300.

Yu, B., Zhang, Y., Shukla, A., Shukla, S. S., and Dorris, K. L. (2000). The removal of heavy metal from aqueous solutions by sawdust adsorption - removal of copper. *Journal of Hazardous Materials* 80: 33-42.

Yun, Y. S. (2004). Characterization of functional groups of protonated Sargassum polycystum biomass capable of binding protons and metal ions. *J. Microbiol. Biotechnol.* 14: 29.

Yun, Y. S Park, D. Park, J. M. and Volesky, B. (2001). "Biosorption of trivalent chromium on the brown seaweed biomass," *Environ. Sci. Technol*. 35: 4353-4358.

Zhou, Y., Zhang, L., Fu, S., Zheng, L., and Zhan, H. (2012). Adsorption behavior of Cd^{2+}, Pb^{2+} and Ni^{2+} from aqueous solutions on cellulose-based hydrogels. *Bio. Resources* 7(3): 2752-2765.

Zubair, A. H., Bhatti, N., Hanif, M. A., and Shafqat, F. (2008). Kinetic and equilibrium modeling for Cr (III) and Cr (VI) removal from aqueous solutions by *Citrus reticulata* waste biomass. *Water, Air and Soil Pollut*. 191: 305-318.

APPENDICES

APPENDIX I: PLATES

Plate 1: A car wash at Lwangni Beach

(SOURCE: AUTHOR, 2015)

Plate 2: River Nyamasaria

(SOURCE: AUTHOR, 2015)

Plate 3: Lwang'ni beach car wash

(SOURCE: AUTHOR, 2015)

Plate 4: River Nyamasaria

(SOURCE: AUTHOR, 2015)

Plate 5: Lwang'ni beach sampling site

(SOURCE: AUTHOR, 2015)

Plate 6: River Nyamasaria (Women washing clothes)

(SOURCE: AUTHOR, 2015)

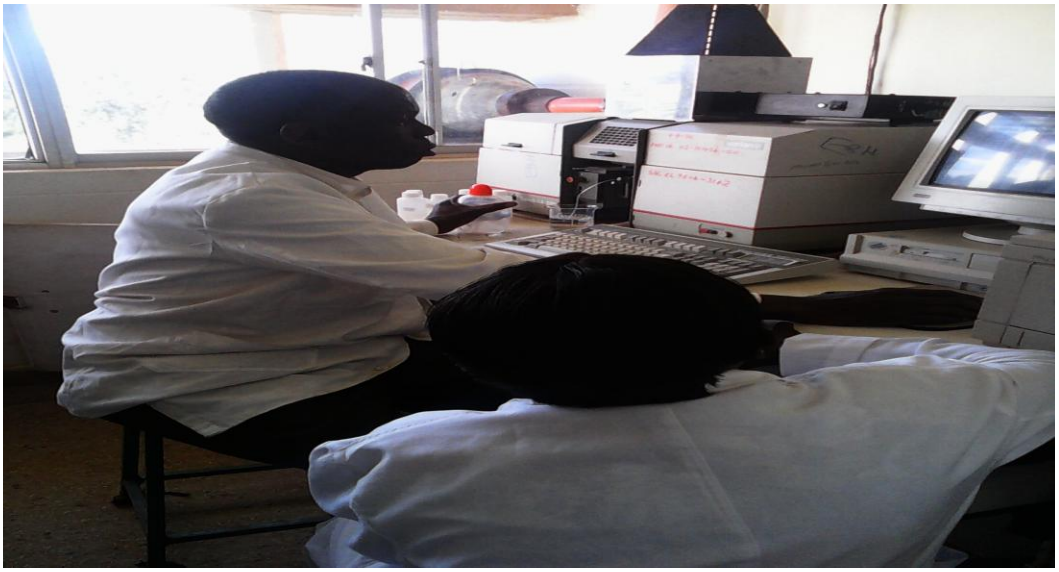


Plate 7: Analysing samples using AAS

(**SOURCE: AUTHOR, 2015)**

Plate 8: *Moringa oleifera* tree

(SOURCE: AUTHOR, 2015)

Plate 9: Analysing samples using AAS

(SOURCE: AUTHOR, 2015)

Appendix II: Langmuir Isotherms

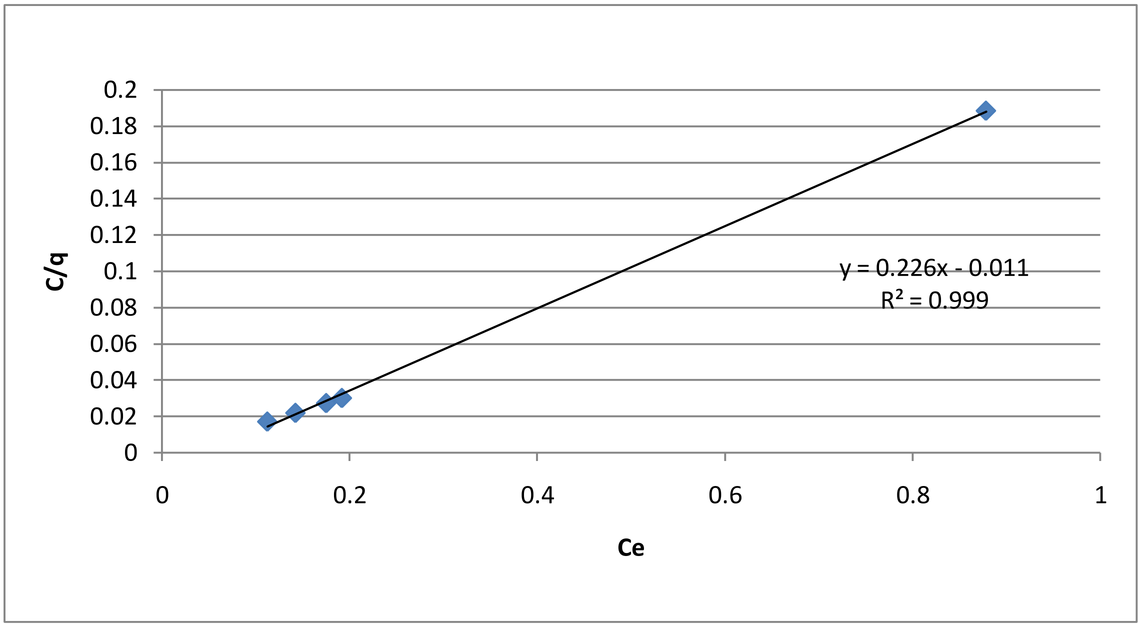


Figure 1: Langmuir isotherm for Zn^{2+}

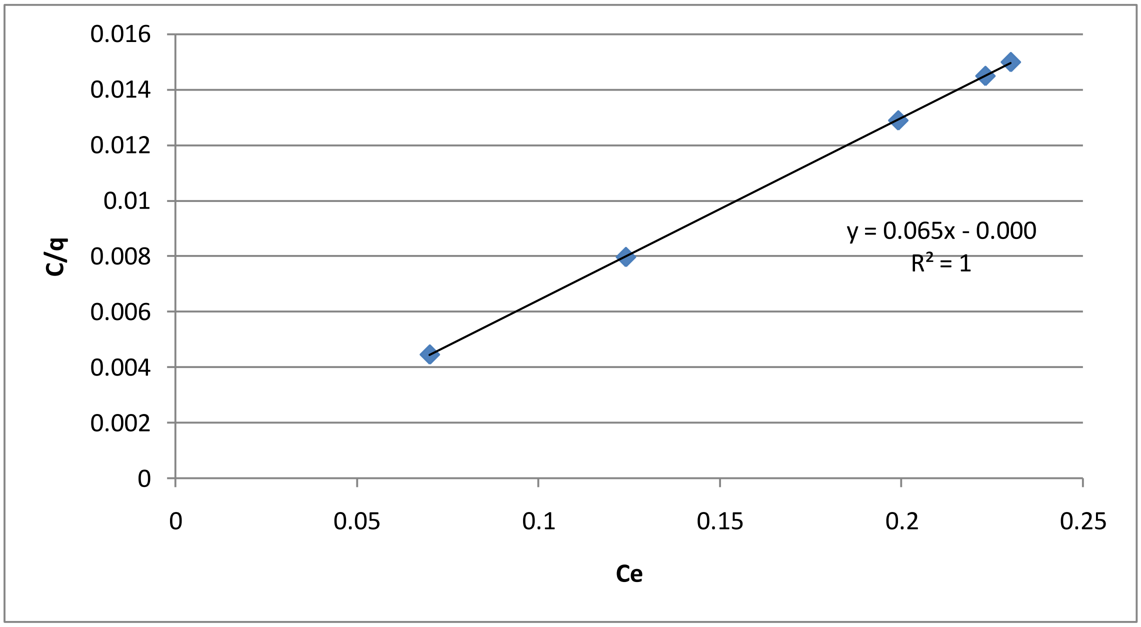


Figure 2: Langmuir isotherm for Pb^{2+}

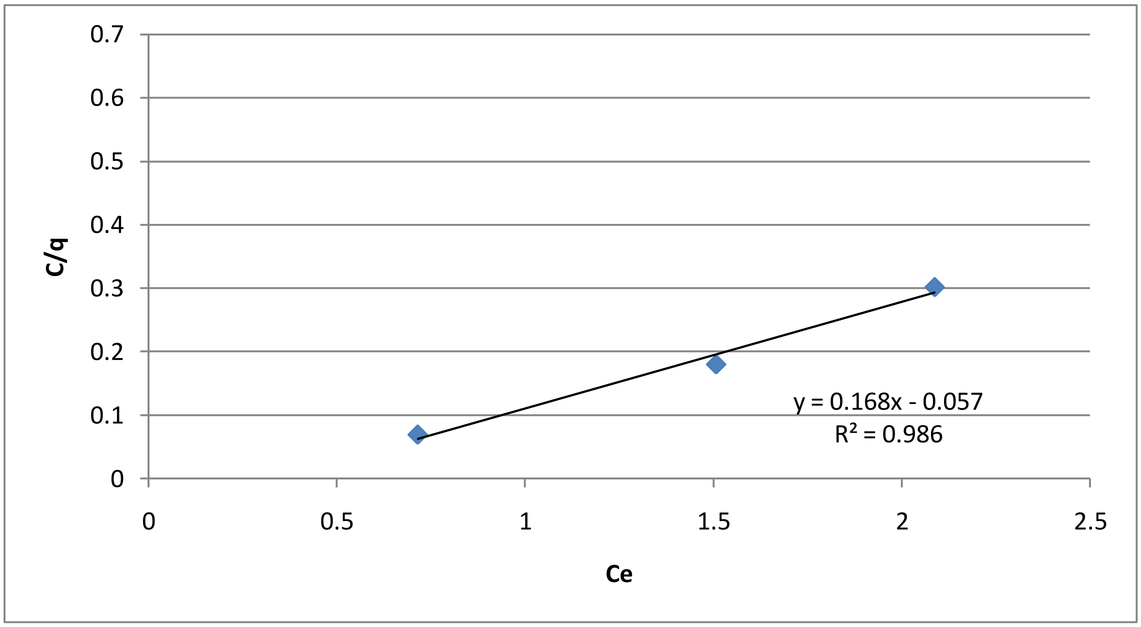


Figure 3: Langmuir isotherm for Cr^{2+}

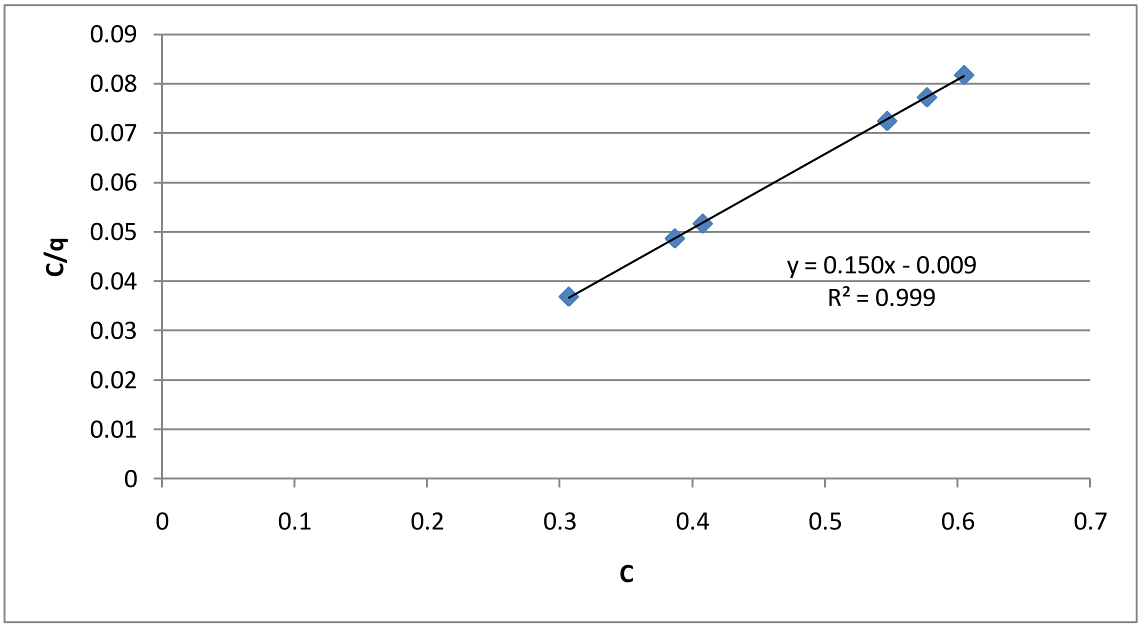


Figure 4: Langmuir isotherm for Mn^{2+}

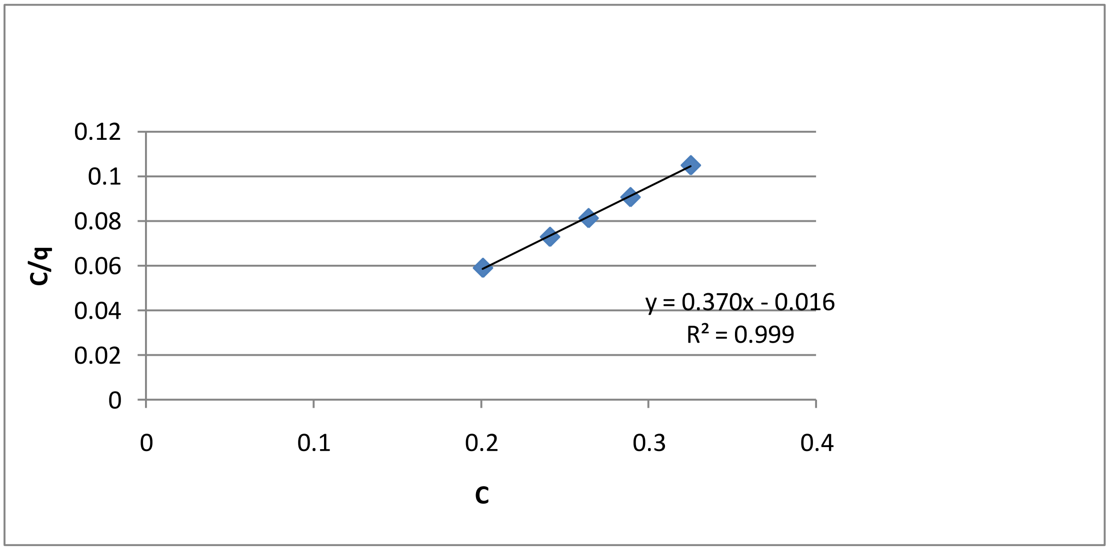


Figure 5: Langmuir isotherm for Cd^{2+}

Appendix III: Freundlich Isotherms

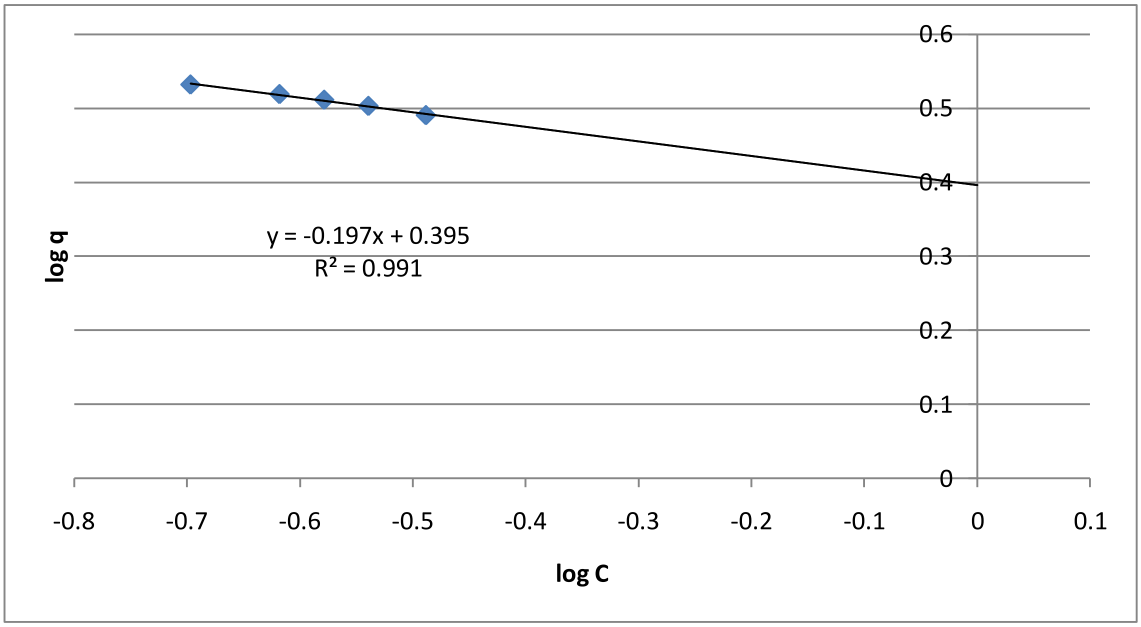


Figure 1: Freundlich isotherm for Cd^{2+}

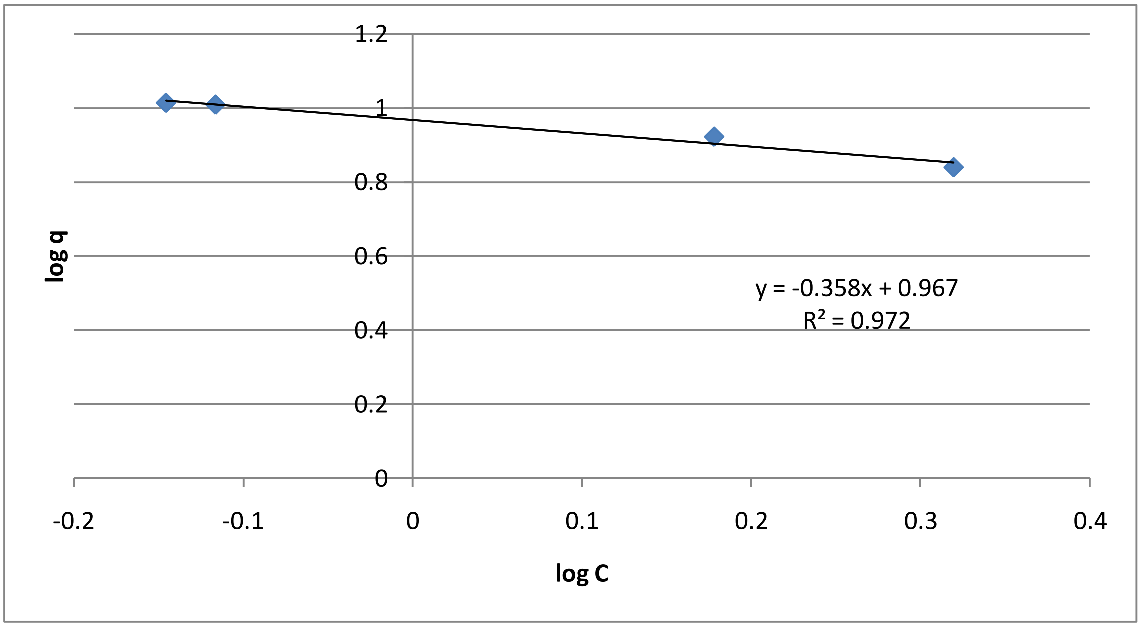


Figure 2: Freundlich isotherm for Cr^{2+}

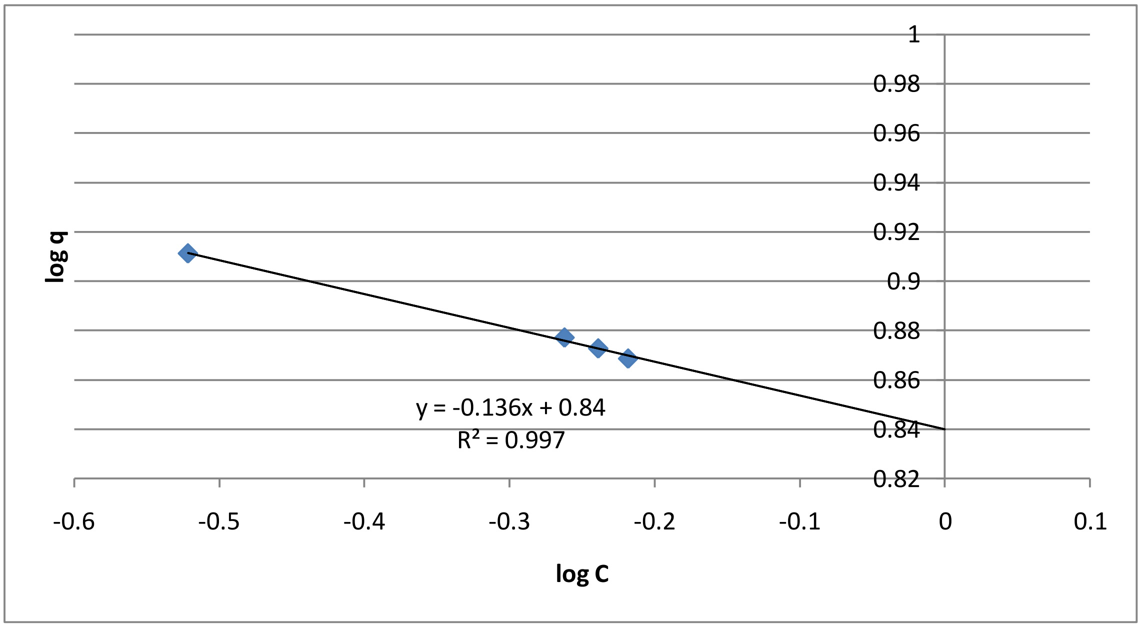


Figure 3: Freundlich isotherm for Mn^{2+}

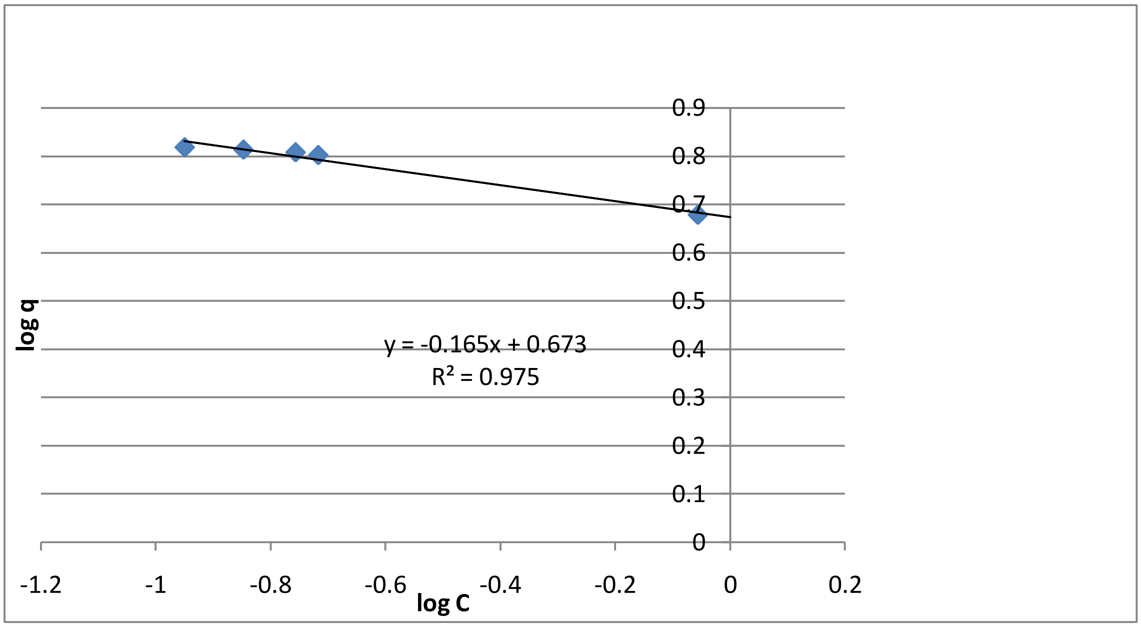


Figure 4: Freundlich isotherm for Zn^{2+}

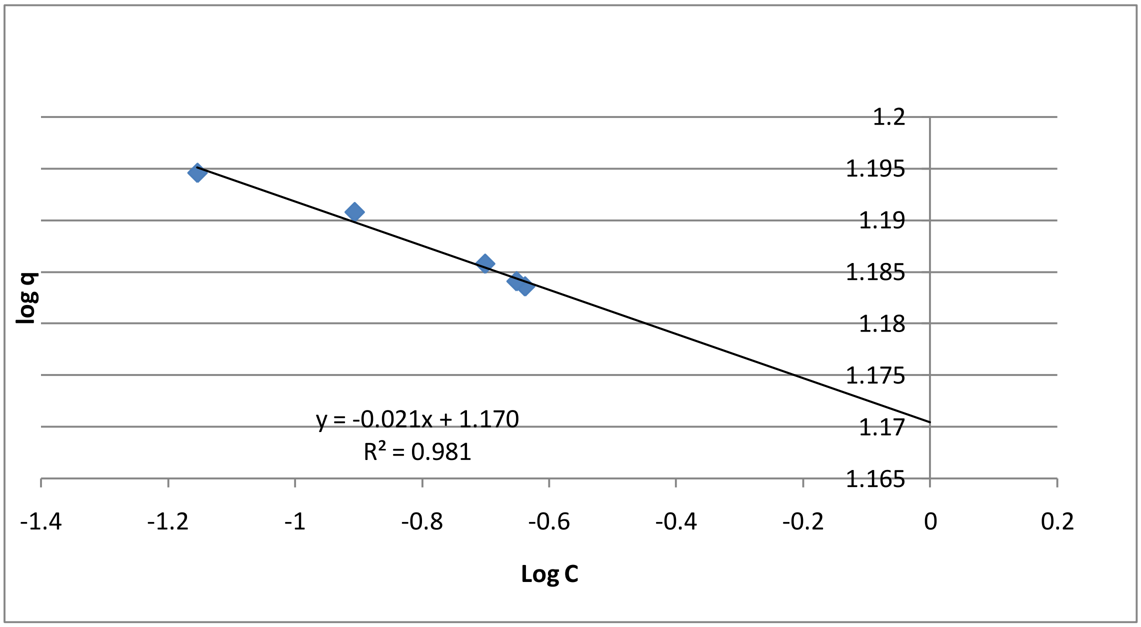


Figure 5: Freundlich isotherm for Zn^{2+}

Appendix IV: Pseudo-First Order Kinetics

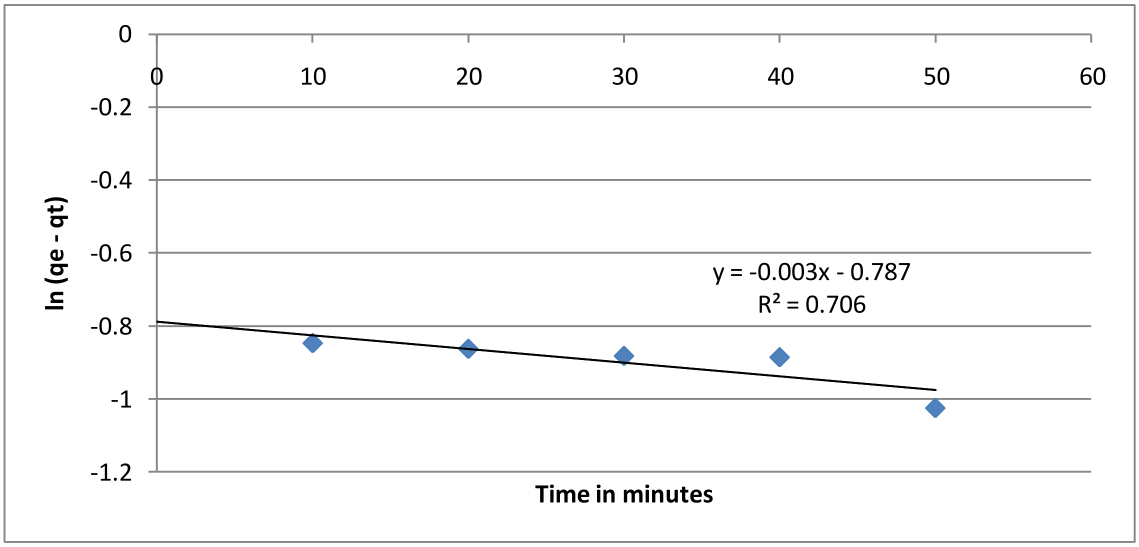


Figure 1: Pseudo-first order kinetic for Cu^{2+}

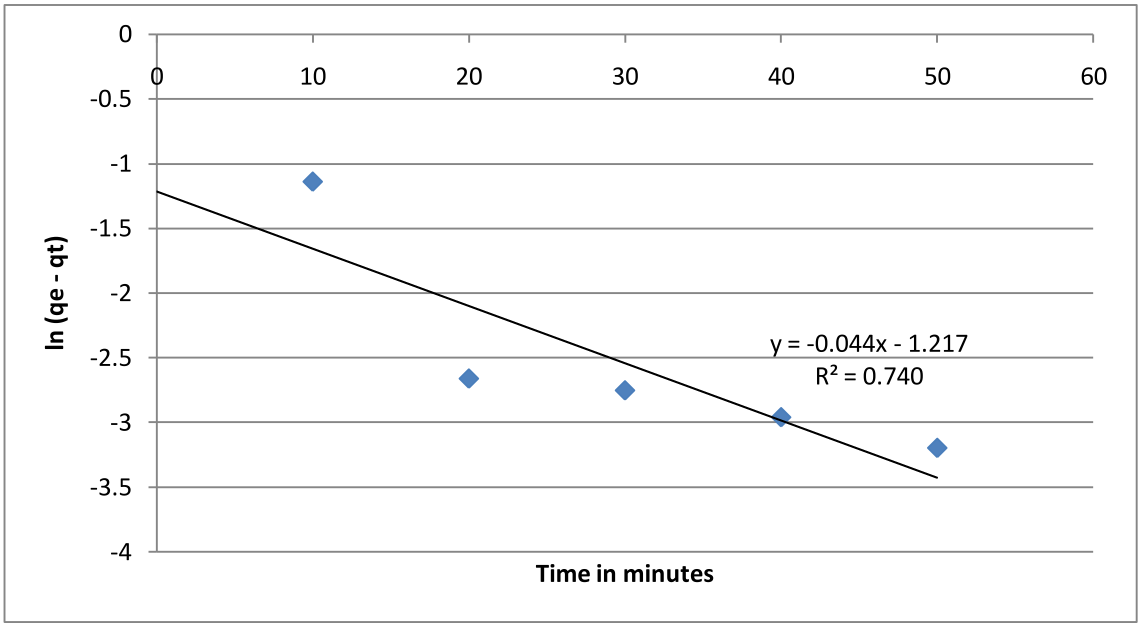


Figure 2: Pseudo-first order kinetic for Zn^{2+}

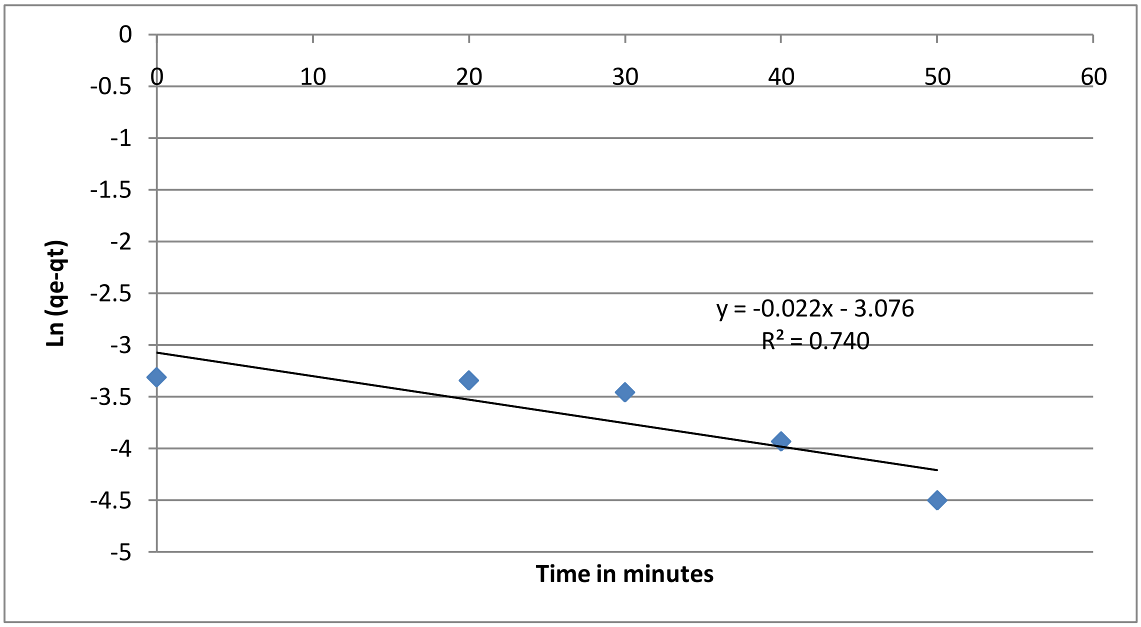


Figure 3: Pseudo-first order kinetic for Pb^{2+}

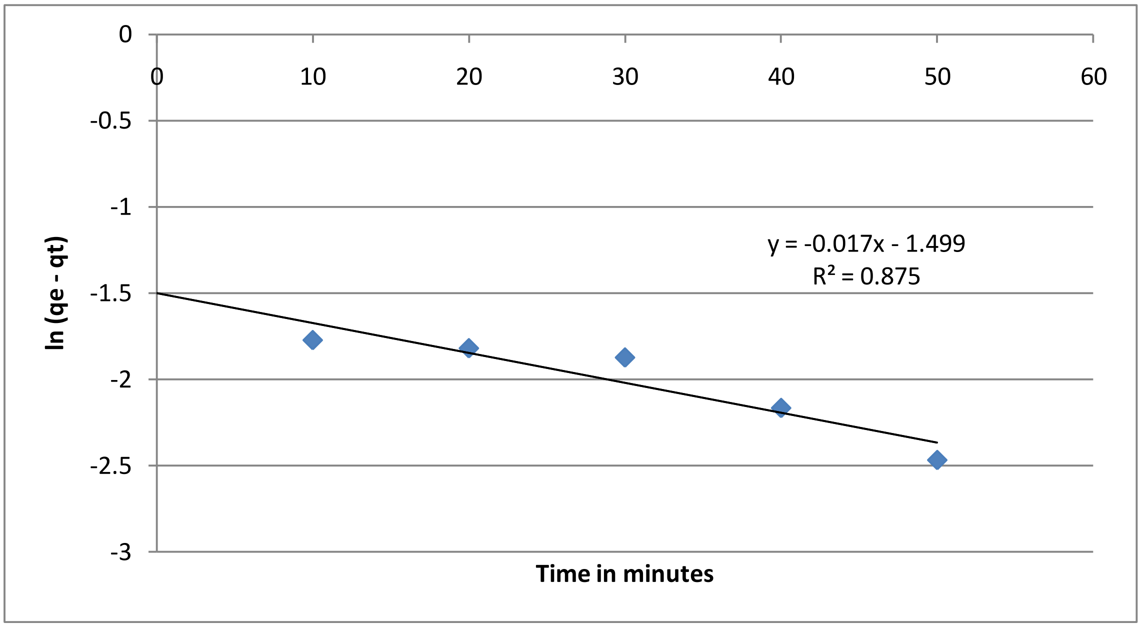


Figure 4: Pseudo-first order kinetic for Mn^{2+}

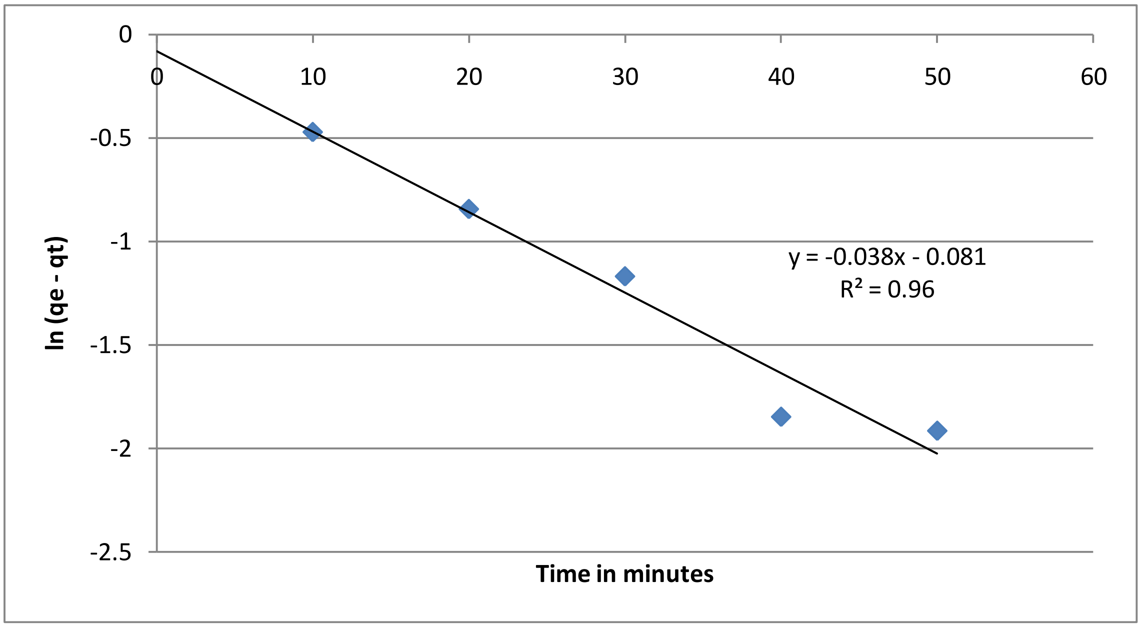


Figure 5: Pseudo-first order kinetic for Cr^{2+}

Appendix V: Pseudo Second Order Kinetics

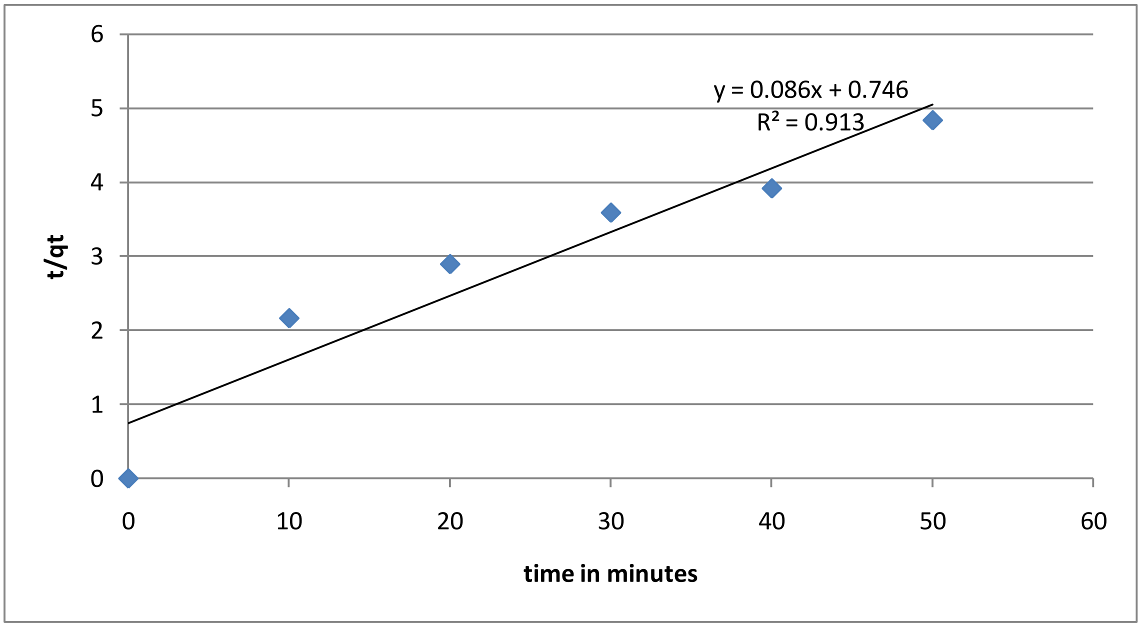


Figure 1: Pseudo-second Order kinetic for Cr^{2+}

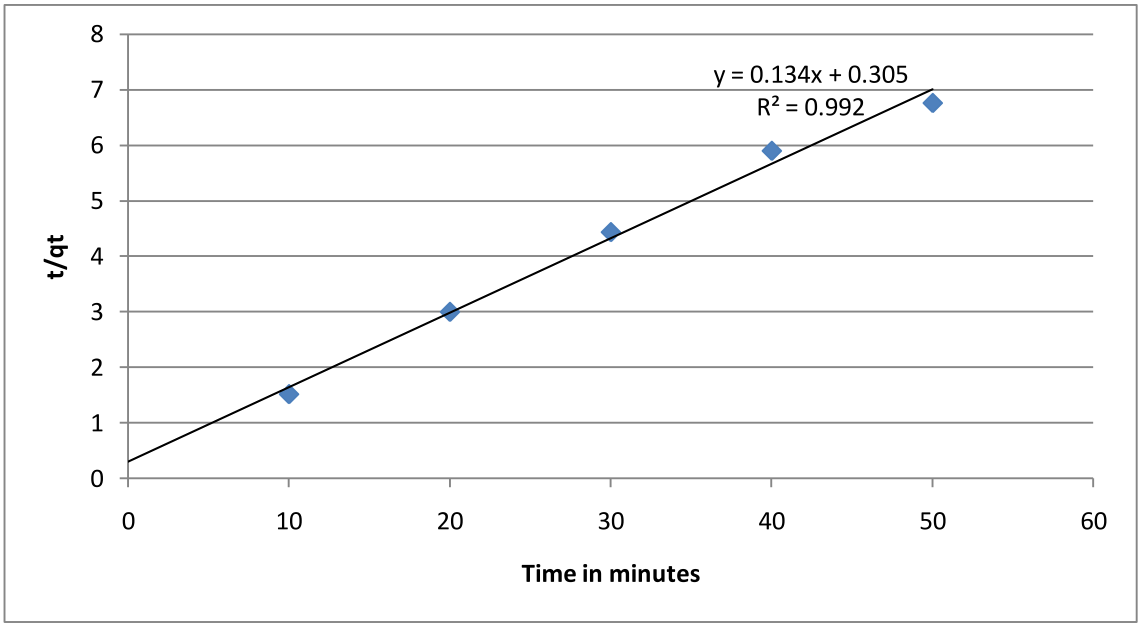


Figure 2: Pseudo-second Order kinetic for Cu^{2+}

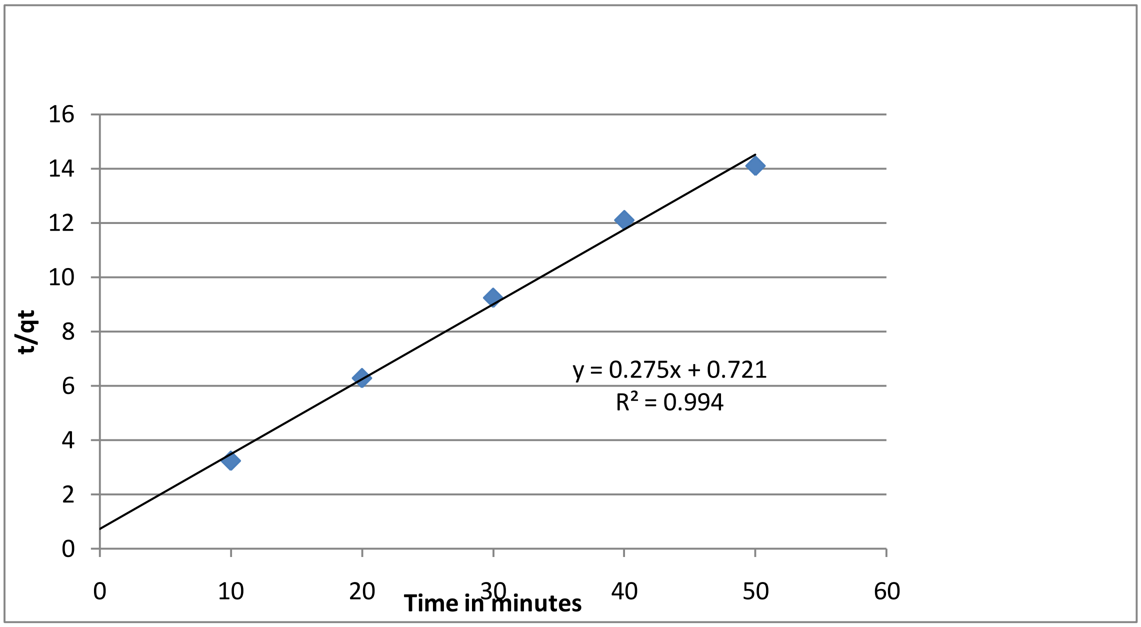


Figure 3: Pseudo-second Order kinetic for Cd^{2+}

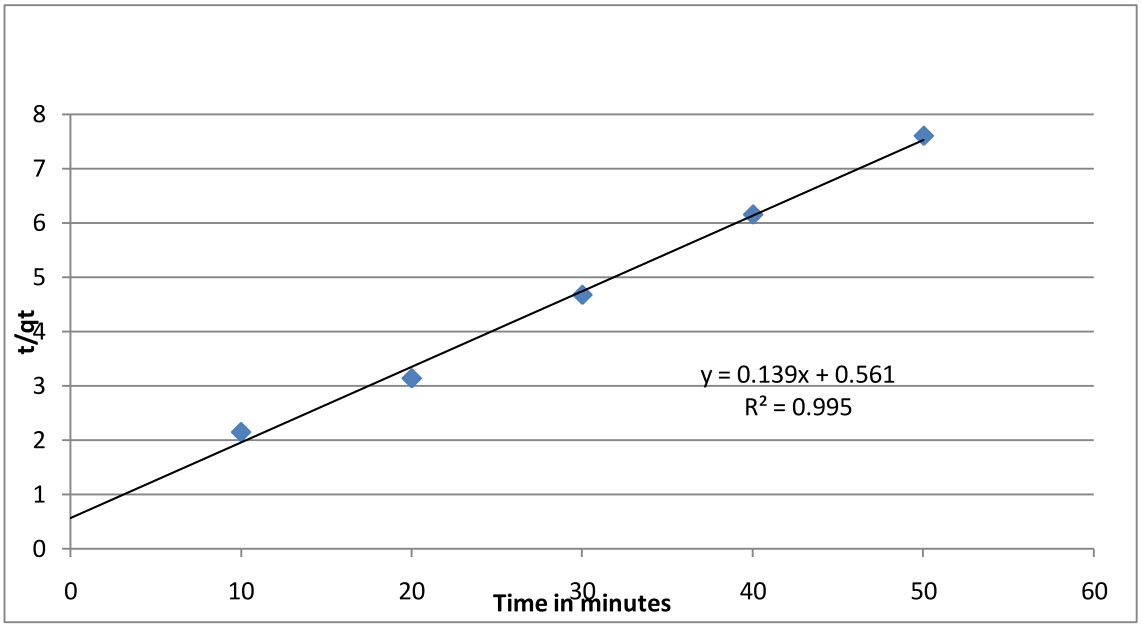


Figure 4: Pseudo-second Order kinetic for Zn

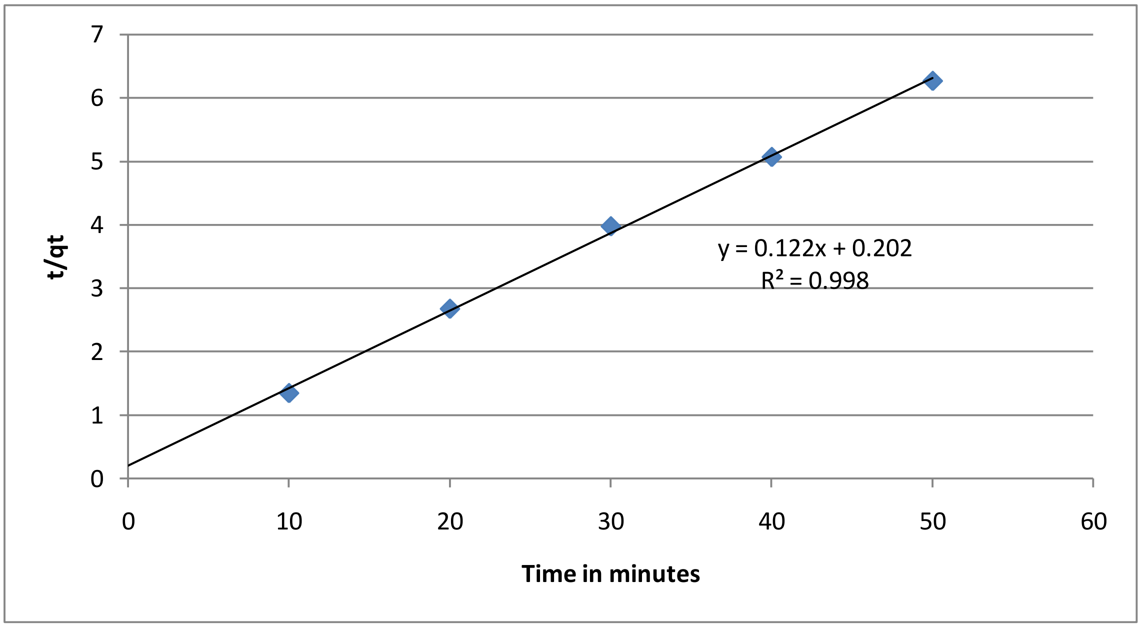


Figure 5: Pseudo-second Order kinetic for Mn

Appendix VI: Concentration of Heavy metals (ppm) in Winam Gulf

Site Metal	Lwangni	Kibos	Nyamasaria	Dunga	Yatch	Kisumu beach	Kissat	Tilapia Beach
Cu	ND	ND	ND	1.543±0.030	ND	ND	ND	1.03±0.011
Cd	0.032 ±0.002	0.013±0.001	0.028±0.002	0.030±0.003	0.036±0.001	0.032±0.021	0.035±0.001	0.037±0.007
Pb	1.13±0.011	0.92±0.008	1.04±0.011	ND	ND	ND	0.99±0.01	1.12±0.031
Zn	3.06±0.015	0.301±0.01	2.00±0.05	3.47±0.032	3.002±0.041	3.00±0.031	3.03±0.051	2.94±0.025
Cr	ND	0.459±0.021	ND	0.315±0.013	0.212±0.011	0.342±0.02	0.401±0.021	0.033±0.005
Mn	4.967±0.04	1.365±0.021	1.102±0.04	1.21±0.061	0.153±0.001	1.302±0.006	1.302±0.044	1.243±0.017

ND = Not detected