REMOVAL OF LEAD AND CADMIUM IONS FROM SYNTHETICALLY CONTAMINATED WATER USING *MORINGA OLEIFER*A AND *M. STENOPETALA* SEED POWDERS

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DECLARATION BY THE CANDIDATE

I hereby declare that no part of this work has been submitted for any degree in any university or institution of learning and either is submitted concurrently. Acknowledgement has been made where other sources of information have been used.

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CERTIFICATE OF APPROVAL

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DEDICATION

I dedicate this thesis to the Late Prof. E.M.T. Henry who was not able to see the completion of this work. I also dedicate this thesis to my wife Loveness who was there to support me even in times of crisis and my two sons Raymond and Desmond. Lastly, but not least I do not forget my parents, Peter and Meriya Mataka who introduced me to both informal and formal education and supported me throughout formal education. I do not take all their contributions for granted.

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ABSTRACT

The discharge of heavy metals into aquatic ecosystems has become a matter of concern in Malawi recently. These pollutants are introduced into the aquatic systems such as rivers and streams as a result of various industrial operations, agricultural and domestic activities. The pollutants of concern include lead and cadmium, which may be derived from sludge disposal, vehicle emissions, paints, batteries, fertilisers, pesticides or preservatives. A recent study of streams and rivers in the city of Blantyre, Malawi revealed lead and cadmium levels exceeding the World Health Organisation acceptable levels for drinking water.

This study was carried out to investigate the possibility of using *Moringa oleifera* and *Moringa stenopetala* seed powders for lead and cadmium ion removal from aqueous solutions by means of batch experiments using jar tests. The following parameters, pH, stirring time, initial metal ion concentration, ionic strength, water hardness and temperature were investigated for lead and cadmium ion removal from aqueous solutions. Equilibrium sorption capacities were evaluated by fitting the sorption data to Langmuir, Freundlich and Dubinin-Radushkevich models. The residual metal ion concentrations were determined by atomic absorption spectroscopy.

M. oleifera and *M. stenopetala* whole seed powders (at a dose of 2.5 g/100 mL) reduced the concentrations of lead ions from aqueous solutions by 78 and 96% respectively. The cadmium ion removals were 53 and 54 % respectively. Generally *M. stenopetala* was more effective than *M. oleifera* in the removal of these cations. Percentage removal increased with an increase in pH and optimum sorption for lead ions was obtained at pH $\geq$ 3 and that for cadmium at pH $\geq$ 5. Lead removal increased with time until equilibrium was reached at 4 h for both powders. However, cadmium removal decreased with stirring time. Results for lead sorption at 30^{o}C were better fitted to Lagergren first order equation and the k value was 2.40 x 10^{-4}/s for both seed powders. For cadmium it was mostly desorption taking place with time and hence the zero order rate constants obtained were negative. Pb^{2+} and Cd^{2+} uptake increased with a raise in initial metal ion concentration and decreased with an increase in ionic strength. Magnesium/calcium water hardness did not show any general trend in percentage

removal for lead ion sorption. A high decrease in percentage removal was observed for cadmium sorption. This was ascribed to the competition between magnesium/calcium ions and cadmium ions for binding sites. Carbonates/bicarbonates hardness enhanced metal ion sorption for both metal ions. This was attributed to the increase in pH and formation of insoluble metal carbonates. Temperature increase enhanced lead ion sorption and reduced cadmium ion sorption for both powders.

Equilibrium studies using whole seed powders showed that lead ion sorption followed the Langmuir sorption isotherm better than Freundlich isotherms and cadmium sorption followed both Langmuir and Freundlich sorption models. Using the Dubinin-Radushkevich sorption model the energies of adsorption for lead ion sorption were 20.41 and 11.95 kJ/mol for *M. oleifera* and *M. stenopetala* respectively. The adsorption energies for cadmium were 3.68 and 4.58 kJ/mol for *M. oleifera* and *M. stenopetala* treatments respectively. Thus, lead ion sorption involved chemisorption, while cadmium ion sorption involved physisorption. Desorption studies showed that metal ions could be desorbed to varying extents from metal loaded powders and the optimum nitric acid concentration for metal desorption was 0.06 mol/L.

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LIST OF ACRONYMS

AAS	ATOMIC ABSORPTION SPECTROSCOPY
D-R	DUBININ-RADUSHKEVICH
CAC	COMERCIAL ACTIVATED CARBONS
ECA	ECONOMIC COMMISSION FOR AFRICA
EDCs	ENDOCRINE DISRUPTING CHEMICALS
SADC	SOUTHERN AFRICA DEVELOPMENT COMMUNITY
UNEP	UNITED NATIONS ENVIRONMENTAL PROGRAMME
US EPA	UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WHO	WORLD HEALTH ORGANISATION

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CHAPTER ONE: INTRODUCTION

1.1 Background

Economic Commission for Africa (ECA, 2001) estimates that up to 16 % of Africa's population will be living in countries facing water scarcity by 2025. Pressure on the water demand in Africa has risen due to uneven distribution of water resources and the region's current state and pace of development and urbanisation. According to the State of Environment in Africa Report (ECA, 2001), the water demand in Southern Africa Development Community region is projected to rise by at least 3 % annually till 2020. This is attributed to rapid population growth, which places high demand on available water resources to meet domestic, agricultural and industrial needs.

The United Nations Environment Programme (UNEP, 2004) accords high priority in its activities to the protection, conservation and more efficient use of freshwater resources, both for human survival and for the maintenance and protection of ecosystems that are of great value to humans. In pursuit of this goal UNEP's Water Policy and Strategy has identified several water focal areas including fresh water scarcity, land based pollution sources, aquatic biological diversity, resource use and management, and knowledge and technology transfer in integrated water management. The Malawi State of the Environment Report has indicated that water degradation is a major environmental problem that threatens the health and well being of humans and ecosystems (Malawi Government, 2002). Improper disposal of various types of waste, deforestation, and poor agricultural practices that encourage soil erosion and deposition of sediments into the water bodies have been identified as the major causes of water degradation in Malawi. The major water pollutants in Malawi include organics and inorganics, which include heavy metals (Malawi Government, 2002).

1.2 Problem Statement

The presence of Cd^{2+} and Pb^{2+} and other heavy metals in the environment has become a major threat to plant, animal and human life due to their bio-accumulating tendency and toxicity and, therefore, must be removed from the water resources (Horsfall and Spiff, 2005a; Waalkes *et al.*, 1992; Elinder, 1992; Ratcliffe and Swanson, 1996). These metals are mainly from

industrial and agricultural sources, and vehicle emissions. Studies in Malawi have revealed high levels of lead and cadmium ions in several streams (Banda *et al.*, 2001; Matope, 2002; Sajidu *et al.*, 2005). The conventional technologies for Cd^{2+} and Pb^{2+} polluted water treatment are not economically feasible in developing economies because they require huge capital investments (Igwe *et al*., 2005a; Horsfall and Spiff, 2004) such that new cost effective technologies are being explored for Cd^{2+} and Pb^{2+} remediation of polluted water (Krishnan and Anirudhan, 2003; Ho *et al.*, 2004; Horsfall and Spiff, 2005a). In this study *M. oleifera* and *M. stenopetala* were chosen as metal coagulants from water because of their well-known water-clarifying properties (Jahn, 1981; Ndabigengesere *et al.*, 1995; Ghebremichael, 2004). Studies have shown that the active agents in *M. oleifera* extracts responsible for water clarification are dimeric cationic proteins with molecular weight of 13,000 Da and isoelectric points between 10 and 13 (Ndabigengesere *et al.*, 1995). The suggested mechanism of the coagulating activity was adsorption and neutralisation of charges, or adsorption and bridging of destabilised particles. The two mechanisms have been assumed to take place simultaneously. A non-protein coagulant has also been identified (Okuda *et al.*, 2001). However, little information is available on the use of *M. oleifera* and *M. stenopetala* for heavy metal removal from contaminated water.

1.3 Objectives and thesis outline

The study was undertaken to investigate the use of *M. oleifera* and *M. stenopetala* seed powders as biosorbents for Pb^{2+} and Cd^{2+} removal from aqueous solutions.

Specific objectives of the study were:

(i) To determine the capacity of *M. oleifera* and *M. stenopetala* seed powders for Cd^{2+} and Pb^{2+} removal from aqueous solutions.

(ii) To determine the effects of conditions, such as dosage, initial pH, contact time, initial metal ion concentration, ionic strength, water hardness and temperature for Cd^{2+} and Pb^{2+} removal using the *M. oleifera* and *M. stenopetala* seed powders.

(iii) To determine the kinetics of Pb^{2+} and Cd^{2+} removal from aqueous solutions

The outline of the thesis is as follows: Chapter 2 provides the literature review and chapter 3 presents the materials and methods used to achieve the goals of this study. The results and discussions of this study are presented in chapter 4. Chapter 5 presents conclusions and recommendations for further study.

CHAPTER TWO: LITERATURE REVIEW

2.1 Sources and toxic effects of Cd^{2+} and Pb^{2+} in water

The United States Environmental Protection Agency has classified lead and cadmium metals as priority pollutants because of their toxicity (US EPA, 1995). Lead is a bluish-white lustrous metal which is very soft, highly malleable, ductile, and a relatively poor conductor of electricity. It is very resistant to corrosion but tarnishes upon exposure to air. Lead pipes used as drains from the baths, are still in service in some countries (Blaylock and Huang, 2000). Lead is present as an alloy in pewter and solder. Tetraethyl lead, $Pb(CH_2CH_3)_4$, is still used in some grades of petrol (gasoline) but is being phased out on environmental grounds. Sources of lead include mined ores, vehicle emissions, canned food from lead soldered containers, paint, ceramics, municipal sludge to land, and industrial manufacturing (Raskin *et al.*, 1994; Cunningham *et al.*, 1997; Blaylock and Huang, 2000). Sources of organic lead such as tetramethyl lead and tetraethyl lead are high-pressure lubricants (lead soaps) and gasoline anti-nock agents (Rahde, 1994).

Cadmium is a relatively rare, soft, bluish-white, transition metal, which usually occurs with zinc ores and is used largely in batteries. Although cadmium has only limited use as a pure metal it forms many binary and more complex alloys, which have useful properties for many commercial applications. Most commercial alloys containing cadmium are used for two major purposes (Cowan, 1982). Firstly, cadmium improves some features of the alloy, such as hardness, wear resistance, castability, mechanical strength and electrochemical properties. Cadmium is added principally to alloys based on copper, tin, lead and zinc although several others benefit from its presence (Cowan, 1982; Elinder, 1985). For example, copper-cadmium alloys which contain between 0.8 to 1.2 per cent cadmium have almost double the mechanical strength and wear resistance of pure copper, yet still retain 90 per cent of its conductivity. Secondly, it lowers melting points for the alloys (Cowan, 1982; Elinder, 1985). These alloys range from the low melting point eutectic (fusible) alloys to high melting point non-eutectic alloys used in metal joining. For example, the tin-lead-bismuth-cadmium alloy which melts at 700^{o}C is more commonly known as Woods Metal and is used in the bonding of metallised

ceramic and glass components to metal frames and chassis where higher soldering temperatures are not possible.

Natural sources of cadmium include volcanic activities and forest fires (WHO 1992; Nriagu, 1980). Anthropogenic sources of cadmium are nickel-cadmium batteries, phosphate fertilisers, cadmium pigmented plastics, cadmium alloys and cadmium stabilised polyvinyl (PVC) products (Nriagu, 1980).

Pb^{2+} and Cd^{2+} have no beneficial biological significance in the human body. However, they are pollutants of global concern with well-known toxic effects (Alloway and Ayres, 1997; Horsfall and Spiff, 2004). Pb^{2+} inhibits the enzymes that catalyse reactions for the biosynthesis of haemoglobin leading to anaemia (Marks, 1985). Furthermore, it is a powerful neurotoxin, which acts by displacing Ca^{2+} in biological systems (Peraza *et al.,* 1998; Bressler *et al.,* 1999). Consequently, a range of pathological conditions are associated with acute Pb^{2+} poisoning, the most characteristic being cerebral oedema (Williams *et al.,* 1999). Organolead compounds such as tetraethyl lead and tetramethyl lead also damage the central nervous system (Rahde, 1994).

Acute cadmium poisoning symptoms are similar to those of food poisoning. It is associated with kidney disease and linked to hypertension. There is also some evidence that cadmium can cause mutations (Carson *et al.,* 1986). The primary adverse health effects which have been observed are lung cancer and kidney damage. Cadmium enters the body by inhalation, ingestion or absorption through the skin, and afterwards it is transported by the blood plasma. The most widely recognized effects are seen in the respiratory system from inhalation (Carson *et al.,* 1986). Acute protenuria also occurs accompanied by symptoms such as fever and chest pain. In extreme exposure cases, pulmonary oedema may develop and cause death. Severe cadmium-induced renal damage may develop into chronic renal failure, and prolonged exposure to fume and dust results in reduced pulmonary function and chronic lung disease, indicative of emphysema (Norberg *et al.,* 1992). Furthermore, cadmium exposure has been associated with skeletal system effects such as osteoporosis and osteomalacia and is known to cause changes in the menstrual cycle (Williams *et al.,* 1999). Also cadmium has the ability to

alter the rate of ovarian and placental steroidogenesis, thereby adversely affecting normal reproduction in both humans and animals. Therefore, cadmium has been added to the list of acknowledged endocrine disrupting chemicals (EDCs) (Chedrese *et al.,* 2006)

2.2 Pb^{2+} and Cd^{2+} pollution in Malawi

Reported studies in the rivers of major cities and towns of Malawi indicate the presence of lead and cadmium ions in these rivers with most sites exceeding WHO recommended limits for drinking water. The Sanitation Master Plan for the City of Blantyre, the industrial capital of Malawi, reported excessive lead and cadmium water pollution in the city (Matope, 2002). The levels of these metals in Mudi and Limbe streams vary from 0.73-0.96 mg/L for lead and a maximum of 0.86 mg/L for cadmium and, therefore, above the WHO (2004) recommended limits of less than 0.05 mg/L for lead and 0.005 mg/L for cadmium. A study in Lunyangwa river basin in Mzuzu city, Malawi, has shown levels of lead metal ions at 0.23 mg/L (Banda *et al.,* 2001). A recent quality inventory compilation of Blantyre streams (Limbe, Nasolo, and Mudi) and wastewater treatment plants (Limbe and Soche) reported levels of lead from 0.027 to 0.118 mg/L and cadmium from 0.002 to 0.015 mg/L with most values exceeding WHO limits (Sajidu *et al.,* 2005).

2.3 Methods for heavy metal removal

A wide range of physical and chemical processes are available for the removal of heavy metals from contaminated water, such as chemical coagulation using aluminium and ferric salts (Fatoki and Ogunfowokan, 2002), and cationic surfactants (Evans, 2003), electro-chemical precipitation, ion exchange and reverse osmosis (Petrell, 2003; Nomanbhay and Palanisamy, 2005). Biological processes have also been investigated using phytoremediation (Prasad and Freitas, 2003; Lyte *et al.,* 1998).

2.3.1 Chemical precipitation

Chemical precipitation of metals is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers (Ahalya *et al.,* 2005). The major advantage in using chemical coagulation is that it provides high efficiency in removing the bulk of metals from solutions at high or moderate concentrations (Yan, 2001). A major drawback with chemical

coagulation is sludge production because its disposal is a problem (Metcalf and Eddy, 1991). When applied to lower metal ion concentrations, especially less than 100 mg/L, the method is also not very efficient and not cost effective (Matheickal *et al.*, 1991; Huang and Huang, 1996). Furthermore, metal salts, such as $Al_2(SO_4)_3$ and $FeCl_3$, if not properly handled may concentrate metal ions in the waters since at low pH, aluminium sulphate is present in a dissolved form and this has been suspected as the cause of death of fish in acidified water (Nomanbhay and Palanisamy, 2005).

Metal ions can also be precipitated as metal hydroxides. In this case the pH of the aqueous solutions is varied and metal ions precipitate out of the solution as metal hydroxides. This process has an advantage in that, apart from the heavy metal removal it also neutralises some acidic pollutants in aqueous solutions. However, it is complex because some metals need pre-treatment to put them into a form that can be easily precipitated (Kumar, 2003). For example, hexavalent chromium wastes are always acidic but the hexavalent chromium must be reduced to trivalent chromium for precipitation to take place. Furthermore, metal ions precipitate at different pH. Therefore, some heavy metals do not form sufficiently insoluble hydroxides to meet the required standards (Kumar, 2003; Kou *et al*., 1999).

2.3.2 Ion exchange

In this process, metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin. This method is considered a better alternative technique than chemical precipitation because it removes metal ions at low concentrations (Herrera *et al.*, 2003), the metal can be recovered as saleable products and it produces consistent performance because it is concentration driven (Kumar, 2003). However, it is not economically appealing because of high operational costs. Furthermore, ion exchange resins are sometimes easily oxidised and hence become ineffective with time (Kuyucak, 1997).

2.3.3 Adsorption using activated carbons

In this method metal ions adsorb onto the activated carbons and hence are removed from aqueous solutions. Adsorption using commercial activated carbon (CAC) can remove heavy

metals from water such as Cd, Ni, Cr and Cu at low concentrations. However, CAC remains an expensive material for heavy metal removal (Nomanbhay and Palanisamy, 2005).

2.3.4 Phytoremediation

Phytoremediation is the use of certain plants to clean up soil, sediments, and water contaminated with metals (Ahalya *et al.,* 2005). The advantages of phytoremediation are that the process occurs in-situ and once the remediation has started there is no need for immediate replenishment (Gosh and Sing, 2005). However, phytoremediation is time consuming hence it is not appropriate for immediate results. Furthermore, the plants accumulate a lot of heavy metals in their system, which hinder their metabolism (Cheng, 2003) and can have a possible effect on the food chain (Todd, 2001). In addition, when the plants die they release heavy metals into the aquatic system and hence there is a need for proper disposal methods to avoid polluting the environment (Todd, 2001).

2.3.5 Reverse osmosis

This is a process in which heavy metals are separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by the dissolved solids in water and wastewater (Ahalya *et al.,* 2005). Reverse osmosis keeps cations concentration at low levels and membranes are chemically resistant to oxidative attack. However, it is not 100 percent efficient and allows some cations and anions across the membrane. Secondly, it has high operational costs because flux rates for contaminant heavy metal cations are low requiring a large membrane surface area and higher equipment costs (Kumar, 2003).

2.4 Biosorption of heavy metals from aqueous solutions

The search for new technologies for the removal of toxic metals from water has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption is the ability of biological materials to accumulate heavy metals from water and wastewater through metabolically mediated or physico-chemical pathways of uptake (Volesky, 1986; Ahalya *et al.,* 2005). 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 (sorbate; metal ions). Due to higher affinity of the sorbent for the sorbate species, sorption process occurs by different mechanisms. The process continues

until equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases. The advantages of biosorption over other conventional water treatment methods are that it has low costs, high efficiency and does not require additional nutrients. Furthermore, the biosorbent can be regenerated for reuse and metal can be recovered (Ahalya *et al.*, 2005).

Various biosorbents have been used to clean up heavy metals in aqueous solutions. These include seaweeds, moulds, yeast, bacteria, crab shells, agricultural products such as wool, rice, straw, coconut husks, peat moss, exhausted coffee (Dakiky *et al.*, 2002), waste tea (Amir *et al.*, 2005), walnut skin, coconut fibre (Espinola *et al.*, 1999), cork biomass (Chubar *et al.*, 2003), seeds of *Ocimum basilicum* (Melo and d'Souza, 2004), defatted rice bran, rice hulls, soybean hulls and cotton seed hulls (Marshall and Champagne, 1995; Teixeria and Zezzi, 2004), wheat bran, hardwood (*Dalbergia sissoo*) sawdust, pea pod, cotton and mustard seed cakes, (Iqbal *et al.*, 2002, Saeed *et al.*, 2002), maize husks and cobs, and millet stalks (Igwe *et al*., 2005a and b).

2.4.1 Mechanisms of metal uptake by biosorption

Various biosorption mechanisms for heavy metals have been reported. These include physical adsorption (Kuyucak and Volesky, 1989), ion exchange (Kuyucak and Volesky, 1989; Muraleedharan and Venkobachar, 1990) and complexation (Aksul, 1992). The various biosorption mechanisms mentioned above can take place simultaneously (Kuyucak and Volesky, 1989; Aksul, 1992)

2.4.1.1 Physical adsorption

Physical adsorption occurs with the help of van der Waals' forces. Kuyucak and Volesky (1989) reported that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomasses of algae, fungi and yeasts took place through electrostatic interactions between the metal ions in solutions and cell walls of microbial cells. Electrostatic interactions were responsible for copper biosorption using bacterium *Zoogloea ramigera* and algae *Chiarella vulgaris,* and also for the biosorption of chromium using fungi *Ganoderma lucidum* and

Aspergillus niger (Aksul, 1992). Horsfall and Spiff (2005b) found that physical adsorption was responsible for Cd^{2+} sorption by *Caladium bicolor* (Wild Cocoyam).

2.4.1.2 Ion exchange

In ion exchange, cell walls of microorganisms contain polysaccharides and the bivalent metal ions exchange with the counter ions of the polysaccharides. For example, the alginates of marine algae occur as salts of K^+, Na^+, Ca^{2+} and Mg^{2+}. These ions can exchange with counter ions such as Co^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+} resulting in the biosorptive uptake of heavy metals (Kuyucak and Volesky, 1989). The biosorption of copper by fungi *Ganoderma lucidium* and *Aspergillus niger* are examples of ion exchange (Muraleedharan and Venkobachr, 1990). Herrera *et al.* (2003) reported the adsorption of silver(I) ions by alfalfa biomass as also ion exchange.

2.4.1.3 Complexation

In complexation, the metal removal from solution takes place by complex formation on the cell surface following the interaction between the metal and the active groups. Aksul (1992) reported that biosorption of copper by C. *vulgaris* and Z. *ramigera* occurred through both adsorption and formation of coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides. Complexation was found to be the only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury adsorption by *Pseudomonas syringae* (Aksul, 1992). Metals may be biosorbed or complexed by carboxyl groups found in polysaccharides and other polymers (Aksul, 1992).

2.4.1.3.1 Complexation via protein functional groups

Proteins are macromolecules of great biological importance made up of α-amino acids of *L* configuration, linked together via peptide bonds, –CONH-. The sequence of amino acids determines the physical and chemical properties of the proteins in terms of the chemical and physical interactions occurring between the side chains. Apart from the carboxamide function group, –CONH-, peptides contain several functional groups in the side chains that are particularly well suited for metal coordination (Lobinski and Potin-Gauter, 1998). These include cysteine ($-CH_2SH$) and methionine ($-CH_2CH_2SCH_3$) which bind metals with sulphur

affinity (Cd, Cu, and Zn) in compounds such as glutathione, and metallothionines; and histidine in which both nitrogen atoms become available for coordination after metal induced deprotonation (e.g. Cu and Zn). The secondary and tertiary structure of the protein is dependent upon the variety of interactions between various groups in the molecule. On heating or change of pH some of these interactions may be affected (denaturation). Metal ions can accept electron pairs and so act as Lewis acids. These electron pairs can be donated by the binding sites on the protein. Binding of metal to protein sites depends on the charges on metal ions, size of metal, statistical factor and pH (Hughes, 1981; Cotton and Wilkinson, 1988)

The size of charge affects the strength of electrostatic interactions between metal ions and the binding sites on proteins. The higher the charge the stronger the complex formed (Hughes, 1981). However, in aqueous solutions there is competition for metal ions between water and the binding sites on proteins (Horsfall and Spiff, 2005a). For metal ions with similar charges, the one with smaller mass has higher charge density (charge/mass) and hence will be more hydrolysed by water (Horsfall and Spiff, 2005a). Consequently, it will be difficult for the binding sites to abstract the metal ion from the water of hydrolysis.

On the effect of size, metal ions with smaller size can easily fit onto the binding sites on proteins; hence they are expected to form stronger complexes with proteins (Hughes, 1981). Similarly, in aqueous solutions, smaller metal ions are strongly hydrolysed by water because of having high charge density and this makes it difficult for them to get sorbed by the protein binding sites from the water of hydrolysis (Horsfall and Spiff, 2005a).

On the other hand, statistical factor relates to the number of different groups available to the metal in addition to reactivity of those groups towards that metal (Hughes, 1981). More groups available for binding provide higher probability for binding between metal ions and binding sites. Nevertheless, for the binding to be sustainable it will depend on the reactivity of the binding sites towards metal ions. In aqueous solutions weak sites face competition for metal ions from water of hydrolysis and this affects the amount of metal ions adsorbed (Horsfall and Spiff, 2005a). Another factor, acidity, affects the competition for donor sites between metal

ions and H^+. This prevents metal coordination, hence metal binding is favoured by a large acid constant for the protonated ligand (Hughes, 1981).

2.5 Factors affecting sorption of heavy metal ions on biomaterial systems

For most metal ion-biomaterial systems, biomass dosage, pH, temperature, stirring time, ionic strength, and the type of metal ions involved affect sorption. Biomass concentration in solution influences metal ion sorption. For lower biomass concentrations there is an increase in the metal uptake as the masses of biomass increase. However, at higher masses there is no significant increase in metal uptake with the increase in biomass dosage (Kuyucak and Volesky, 1989). Gadd (1988) suggested that the increase in biomass concentration leads to interference between the binding sites because a number of binding sites interact with a single metal ion leaving the other metal ions free from binding.

The pH of a solution is another important parameter for adsorption of metal ions from aqueous solutions because it affects the solubility of the metal ions, the concentration of the counter ions on the functional groups of the adsorbent and the degree of ionisation of the adsorbate during reaction (Nomanbhay and Palanisamy, 2005). For most biosorbents an increase in pH generally enhances the sorption capacity of metal cations (Horsfall and Spiff, 2004; Nomanbhay and Palanisamy, 2005). This is attributed to the reduced competition for binding sites between metal ions and the H^+ ion from the acid.

A study of the effects of temperature is useful because it is used to determine whether the adsorption reaction is endothermic or exothermic (Weng, 2002). Furthermore, it also assists in obtaining knowledge on the strength and type of binding between the metal ion and biomaterial-binding sites (Horsfall and Spiff, 2005a). The reported effects of temperature on the biosorption process have indicated different and opposite behaviours. Aksul and Kutsal (1991) reported higher uptake capacity of Pb(II) with an increase in temperature using *C. vurgaris* while Ajmal *et al.* (2003) and Horsfall and Spiff (2005b) reported higher Cd(II) and Pb(II) uptake capacities with the increase in temperature using rice husks and wild cocoyam (*Caladium bicolor*) biomass respectively. This was attributed to the endothermic nature of the metal ion sorption. On the other hand, Ahuja *et al.* (1999), de Rome and Gadd (1987) and Ho

et al. (2004) found temperature independent effects on biosorption of heavy metals using *Oscillatoria anguistissima*, *Rhozopus arrhizus*, and tree fern adsorbent respectively. In contrast, Cruz *et al.* (2004) and Izanloo and Nasseri (2005) reported a decrease in heavy metal uptake capacity with increasing temperature using dead sargassum species biomass and ground pine cone respectively. This was ascribed to weak electrostatic sorption between metal ions and the biomasses.

Ionic strength generally decreased metal uptake from aqueous systems. Schiewer and Wong (2000) reported a decrease in the metal sorption by marine algae with increasing ionic strength. Krishnan and Anirudhan (2003) reported similar trends in cadmium sorption by steam-activated sulphurised carbon prepared from sugar-cane bagasse pith. This was attributed to the suppressing of metal uptake as a result of screening of electrostatic charge and competition with the divalent cations of interest for binding sites on the biomass, affecting biosorption. The type of metal ion will affect such properties as size and charge of the metal ion. Smaller metal ions have higher charge density (charge/mass) than larger metal ions of the same charge. This leads to strong hydrolysis in aqueous solutions. Therefore, in aqueous media there is competition between metal binding sites and water of hydrolysis for the metal ion, which affects metal sorption (Horsfall and Spiff, 2005a).

2.6 Modeling of metal adsorption

Numerous models have been proposed for the adsorption of gases on solid surfaces. In contrast there are few models to describe the adsorption of ions from aqueous solutions. The utilisation of the models rests solely on the adequacy between the experimentally observed tendencies and the shape of the mathematical laws associated with these models (Izanloo and Nasseri, 2005). These models have value in comparing different biosorbents under different working conditions. Furthermore, these models can be used to design and optimise an operating procedure. The most widely used models are Langmiur and Freundlich models. For most metal ion biosorbents both Langmuir and Freundlich isotherms satisfy the metal sorption behaviour comparably (Ahalya *et al.,* 2005; Sharma *et al.,* 2006; Kumari *et al.,* 2006). To determine the characteristic porousity of the adsorbent and mean energy of adsorption the

most widely used model is the Dubinin-Radushkevich model (Horsfall and Spiff, 2004; 2005a).

2.6.1 Langmiur model

The Langmuir adsorption isotherm shows a relationship between the amount of gas adsorbed on a surface and the pressure of that gas (Atkins, 1990). The Langmuir isotherm is based on the following assumptions: metal ions are chemically adsorbed at a fixed number of well-defined sites; each site can hold only one ion; all sites are energetically equivalent and there is no interaction between the ions. The isotherm is often used for adsorption of a solute from a liquid solution and is expressed as (Casey, 1997):

$$q_e = \frac{q_{\max} K C_e}{(1 + K C_e)} \qquad (1)$$

where q_e (g/kg) is the adsorption density at the equilibrium solute concentration; C_e (g/L) is the concentration of adsorbate in solution; q_{max} (g/kg) is the maximum adsorption capacity corresponding to complete monolayer coverage. K (L/g) is the Langmuir constant related to energy of adsorption. The linearised form of equation 1 after rearrangement is given below:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} K_L} + \frac{C_e}{q_{\max}} \qquad (2)$$

A plot of $\frac{C_e}{q_e}$ against C_e is a straight line when the adsorption follows the Langmuir model. The q_{max} is obtained from the slope and K_L from the intercept.

2.6.2 Freundlich model

The Freundlich isotherm is the earliest known relationship describing the adsorption equation. The Freundlich isotherm is based on the following assumptions: adsorption occurs in multilayer coverage on adsorption sites; the energy distribution on adsorption sites is exponential; and the adsorption occurs at constant pH (Adamson, 1990). The Freundlich adsorption isotherm is expressed by equation 3 (Casey, 1997):

$$q_e = K_f C_e^{\frac{1}{n}} \qquad (3)$$

where q_e (g/kg) is the adsorption density; C_e (g/L) is the concentration of adsorbate in solution at equilibrium; K_f is the equilibrium constant indicative of adsorption capacity; and n indicates the affinity of the sorbent towards the powder. This equation is conveniently used in the linear form by taking the logarithms of both sides as:

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \qquad (4)$$

A plot of $\ln q_e$ versus $\ln C_e$ yielding a straight line indicates the confirmation of the Freundlich isotherm for adsorption. The constants n and K_f are determined from the slope and the intercept respectively.

2.6.3 Dubinin-Radushkevich model

The Dubinin-Radushkevich (D-R) equation is not based on a physical adsorption model rather on a model for microporous adsorption (Dubinin and Radushkevich, 1947). The model is based on adsorption potential theory by Polanyi (1916) which had the following assumptions: adsorbed molecules lie in a potential gradient so that, above the adsorbent surface, a number of equipotential surfaces exist each consisting of a finite adsorption volume; adsorption involves the filling of the adsorption volume with potential energy, E, until E is equal to zero; adsorption forces are temperature independent. Dubinin (1966) proposed that the adsorption process is by micropore volume filling as opposed to layer-by-layer adsorption on pore walls. Therefore, the D-R model is used to determine the characteristic porosity and energy of adsorption. The isotherm is given by equation 5:

$$q_e = X_m \exp(-K\varepsilon^2) \qquad (5)$$

where q_e (mol/kg) is the equilibrium concentration, K (mol^2/kJ^2) is related to the energy of sorption and X_m (mol/kg) is the Dubinin-Radushkevich constant related to the degree of sorbate sorption by the biomass surface (Horsfall and Spiff, 2005c). The parameter ε is expressed by the equation (Moaweed, 2004):

$$\varepsilon = RT \ln(1 + \frac{1}{C_e}) \qquad (6)$$

where C_e is the equilibrium concentration of the solute solution, R is the universal gas constant (8.314 J/K.mol) and T (K) is the absolute temperature. The linearised form of the equation is given by equation 7:

$$\ln q_e = \ln X_m - K_{DR}\varepsilon^2 \qquad (7)$$

A plot of lnq_e versus ε^2 is a straight line for the adsorption, which follows Dubinin-Radushkevich model. The mean energy of adsorption is given by

$$E = \frac{1}{\sqrt{2K}} \qquad (8)$$

The energy of adsorption determines whether sorption is physical or chemical (Ceyhan and Baybas, 2001). The energies for chemical ion exchange reactions range within 8-16 kJ/mol and below these energies physisorption is a dominant sorption mechanism (Yan, 2001).

The D-R equation is linear over many orders of magnitude of pressure, but deviations from the predicted model may occur (Marsh and Land, 1970). It was proposed that a completely linear plot is obtained when the micropore volume is expressed mathematically by a Gaussian distribution and any deviation from this linearity is indicative of non Gaussian distribution of micropores (Marsh and Land, 1970).

2.7 Discussion about the coagulants

2.7.1 Characteristics of *M. oleifera* and *M. stenopetala*.

M.oleifera and *M. stenopetala* belong to the family Moringaceae that is represented only by a single genus moringa (Jahn, 1986; Coote *et al.*, 1997; Moges, 2003). The genus is represented by 14 species to which these two species belong. The genus is prevalent and widely distributed in northeast tropical Africa (Mark, 1998). The taxonomic position of the family is not clear (Edwards *et al.*, 2000). It has some features similar to those of Brassicaceae and Capparidaceae but the seed structure does not agree with either of the above families. Pollen studies have not provided any other suggestions and recent molecular studies have pointed to a relationship with the Carricaceae. These indicate that the taxonomic position of the family is not yet settled and is open for further studies (Edwards *et al.*, 2000).

M. oleifera Lam whose synonym is *Moringa pterygosperma* Gaertn is native to sub-Himalayan tracts of northern India but is now grown worldwide in the tropics and sub-tropics (Morton, 1991; Coote *et al*., 1997). The vernacular names include Chamwamba, Kangaluni or sangoa (Malawi), Sangoa or Zalenda (Zimbabwe), Horseradish tree (because of the taste of

roots), Drumstick tree (because of the shape of the pods), West Indian Ben, Idaga Manoye (Yoruba) and Ben Tree (India) and Zakalanda (Zambia) (Williamson, 1975; Morton, 1991; Jahn, 1986). It tolerates a wide range of soil and rainfall conditions. The minimum annual rainfall requirements are estimated at 250 mm with maximum at over 3,000 mm. The presence of a long taproot makes it resistant to periods of drought. Its temperature range of tolerance is 25-35^{o}C, but the tree can tolerate up to 48^{o}C in the shade and can survive a light frost (Coote *et al.*, 1997; Jahn, 1986). A freeze can kill the tree to the ground, but it afterwards sends out new shoots. *M oleifera* tree flowers and fruits annually and in some regions twice annually. During its first year, the *M. oleifera* tree grows up to four meters in height and produces flowers and fruits. Left alone, the tree can eventually reach 12 metres in height with a trunk 30 cm wide. However, the tree can be annually cut back to one metre or less from the ground. Trees can be easily grown from seeds or cuttings (Jahn, 1986).

M. stenopetala, the African moringa, grows naturally in the *Acacia tortilis-Delonix elata-Commiphora spp.* vegetation-complex (Mark, 1998; Moges, 2003). This type of vegetation is often found in well-drained soils at altitudes of 900-1200 m. The species is quite drought resistant. In southern Ethiopia, it is found in areas of mean annual rainfall ranging from 500 to 1400 mm. Cold temperature is a limiting factor for the cultivation of the species because it does not tolerate frost. The tree grows well at an altitude of 400-2100 m, mean annual temperature of 24-30^{o}C and mean annual rainfall of 500-1400 mm.

The species does not have any specific soil requirements, except that it does not grow on waterlogged or swampy soils. The soil pH for the growth of *M. stenopetala* is mostly neutral (Edwards *et al.,* 2000). *M. stenopetala* can be propagated by direct sowing of seeds without pre-treatment, but the standard nursery raised seedlings are also commonly used (Moges, 2003; Edwards *et al.,* 2000). Removing the spongy seed coat improves germination. In a nursery it needs 7-10 days to germinate and use of wide polythene bags is advised as the bulgy root requires large enough space (12 cm diameter flat). In about 3 months the seedlings are ready for planting out. Some farmers occasionally propagate the species by using branch-sized cuttings. Cold temperatures inhibit seeds of *M. stenopetala* and at low temperatures (at and below 15 ^{o}C) an enforced dormancy occurs (Moges, 2003).

2.7.2 Chemistry of the seed kernels of moringa species

The seed kernels of *M. oleifera* are reported to contain 4.08 g H_2O, 34.80 g crude protein, 34.42 g fatty oil, 16.4 g N free extract, 3.5 g fibre, and 3.2 g ash per 100 g (Duke, 1978). The reported composition of *M. oleifera* seeds from Malawi indicates 32.50 g crude protein, 34.34 g lipids, 23.18 g carbohydrates, 5.80 g moisture content and 4.18 g ash content per 100 g (Henry, 2002). Reported analysis of the amino acids of one type of *M. oleifera* (MO 2.1) showed the presence of 60 amino acid residues in the following proportions, arginine (7), histidine (1), alanine (2), tyrosine (1), phenylalanine (1), methionine (1), threonine (2), proline (7), leucine (3), glycine (5), isoleucine (2), cysteine (2), aspartic acid (3), and serine (4) (Gassenschmidt *et al.*, 1995).

Little information is available on the composition of *M. stenopetala* seed kernels, but reports indicate that the fruit pods are rich sources of protein including significant amounts of the sulfur-containing amino acids, methionine and cysteine (Rams, 1994).

2.7.3 Uses of moringa species

2.7.3.1 *M. oleifera*

The plant is described as a multipurpose tree due to its many uses including as food, fodder and medicine (Hunter and Stewart, 1993; Coote *et al.*, 1997). In the Shire valley districts of Malawi, Chikwawa and Nsanje, and in some districts in the central region of Malawi the leaves from the plant are used as leaf vegetables (Coote *et al.*, 1997). Leaves and pods of *M. oleifera* are rich in vitamin A and C (Table 1), and also have high foliar nitrogen content (Hunter and Stewart, 1993; Coote *et al.*, 1997). The leaves also contain calcium, iron, potassium, copper, proteins (Table 1) and all essential amino acids that make them virtually an ideal food supplement (Hunter and Stewart, 1993). The pods are an important source of fibre, potassium, copper, iron, choline, vitamin C and all essential amino acids for growth of children (Hunter and Stewart, 1993). The tree itself is used as live fence for houses, cattle Kraals, bathing enclosures and toilets (Williamson, 1975; Coote *et al.*, 1997).

Table 1: Composition of *M. oleifera* leaves and pods per 100 g of edible portion (Council of scientific and Industrial Research, 1962)

Moringa composition	**Leaves**	**Pods**
Moisture (g)	75.0	86.9
Protein (g)	6.70	2.50
Fat (g)	1.70	0.10
Carbohydrates (g)	13.4	3.70
Fibre (g)	0.90	4.80
Calcium (g)	440	30.0
Phosphorous (mg)	70.0	0.01
Copper (μg/g)	1.10	110
Iodine (μg/kg)	51.0	3.10
Iron (mg)	7.00	18.0
Vitamin A ((μg)	3393	55.0
Nicotinic acid (mg)	0.80	0.20
Vitamin C (mg)	220	120
Vitamin B (μg)	210	-

The seeds of *M. oleifera* contain oil known as Ben oil (Morton, 1991; Coote *et al.,* 1997). The oil has been extracted in Malawi and shown to be easily converted to methyl ester, which is suitable for biodiesel (Henry *et al.,* 2001). The Ben oil has been used in perfumes because it is stable and is able to absorb and retain fragrances. Elsewhere, it has also been used for illumination because it gives a smokeless flame (Morton, 1991).

Almost all parts of *M. oleifera* plants have medicinal properties which are due to the presence of several bioactive components in the moringa family, which include 4-(4-*o*-acetyl-α-*L*-rhamnopyranosyloxy)benzyl isothiocyanate, 4-(α-*L*-rhamnopyranosyloxy)benzyl isothiocyanate**,** niazimicin, pterygospermin**,** benzyl isothiocyanate**,** and 4-(α-*L*-rhamnopyranosyloxy)benzyl glucosinolate (Fahey *et al.,* 2001; Bennet *et al.,* 2003). These

bioactive compounds have shown hypotensive, anticancer, and antibacterial activity. The roots and barks of the tree have antibiotic properties that are due to an alkaloid, pterygospermin and are highly effective in treating cholera and also function as a fungicide (Bailey, 1949). The alkaloid also acts as cardiac stimulant, acts on sympathetic nerve-endings as well as smooth muscles all over the body, and depresses the sympathetic motor fibres of vessels in large doses only. Roots have reportedly been used in curing fevers in children (Duke, 1978). The antibiotic activity is due to one of the chemical component of pterygospermin, isothiocynate, which is produced when it (pterygosperm) breaks down (Figure 1).

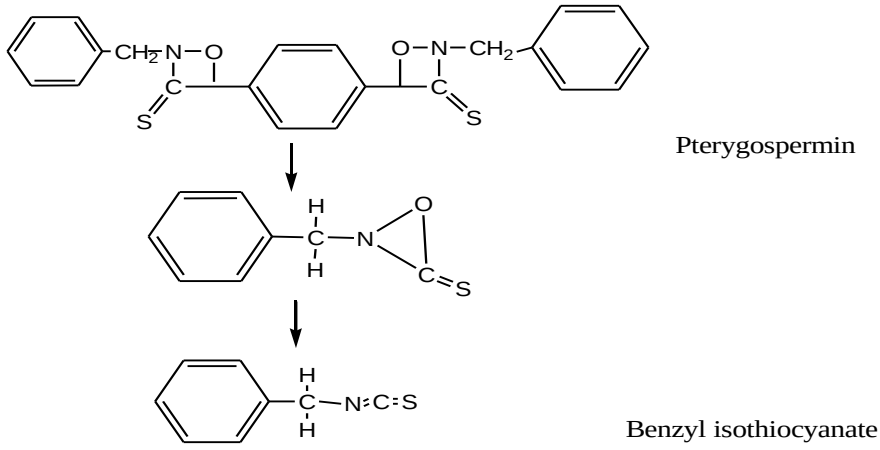

Figure 1: Breakdown of pterygospermin and formation of benzyl isothiocyanate

M. oleifera has also been used as medicine in the treatment of conjunctivitis, scorpion bites, as anti-emetic and in the treatment of diarrhoea. The leaf juice of the plant has a stabilising effect on blood pressure (Maroyi, 2006; Fahey *et al.,* 2001; Bennet *et al.,* 2003). It also controls glucose levels in diabetic patients (Maroyi, 2006); this characteristic is due to 4-(α-L-rhamnopyranosyloxy)benzylglucosinolate and three monoacetyl isomers of this glucosinolate that regulate blood sugar and normalize blood cholesterol (Fahey *et al.*, 2001; Bennet *et al.*, 2003). The moringa seeds are effective against skin-infecting bacteria *Staphylococcus aureus* and *Pseudomanas aeruginosa* (Oliver-Bever, 1986).

Several authors have reported that *M. oleifera* has the capacity to clarify muddy water. Jahn (1984; 1986) reported the use of *M. oleifera* as home based water clarification method by the women of sudan. Sutherland *et al.* (1994) reported effective water clarification on pilot scale at Thyolo treatment plant in southern Malawi. Early work at Chancellor College revealed heavy metal sorption capacity by *M.oleifera* (Sajidu *et al.,* 2005; Mataka *et al.,* 2006).

2.7.3.2 *M. stenopetala*

M. stenopetala has a number of uses, which have been described by several authors. The Turkana tribe of Kenya makes an infusion with the leaves which is used as a remedy against leprosy and given to their livestock to graze on. The Njemps, a people related to the Masai, chew the bark as a treatment against coughs, and use the bark extracts to make fortifying soups (Deleulenaere, 2001). Furthermore, chemical tests of roots and leaf extracts of the plant showed some activity against *Trypanasoma brucei*, a parasite that causes a sleeping sickness like disease of horses, cattle and sheep that is common in tropical Africa (Mekonnen *et al.,* 1999). *M. stenopetala* is also used as herbal medicine against *Visceral leishmaniasis* disease. Studies have shown that crude ethanol extract of leaves and roots have antibiotic activity on *Leishmania donovani*, a parasite that causes the *Visceral leishmaniasis* infection (Mekonnen and Gessesse, 1998). In Somalia, women apparently inhale the smoke released by burning the *M. stenopetala* root during difficult labour. The same smoke is also used as a treatment for epilepsy in the Konso district in Somalia while the leaves are renowned for their effectiveness against diarrhoea (Deleulenaere, 2001). In the same district, *M. stenopetala* (shelaqta in Konso, and also known by the Gamo name of halako) is very widely grown for its edible leaves. The leaves are an ingredient in a daily dish, the dama, which is a major dish in their diet. The leaves are called mida, a term that appears to encompass all plants whose leaves can be eaten in the form of gruel, or pot-herb (Deleulenaere, 2001).

Water clarification ability of *M. stenopetala* has been reported by several authors. Gupta and Chaudhuri (1992) reported the use of the roots of wild *M. stenopetala* to clarify clayey water by nomadic peoples in the Omo valley in Somalia. Further work by Jahn also confirmed that *M. stenopetala* has the capacity to clarify turbid water. It was reported that *M. stenopetala* reduced turbidity levels of muddy water from the Nile. Comparison of effectiveness between

the two species in water clarification showed that 100-150 mg/L dosage of *M. stenopetala* was as effective as 200 mg/L dosage of *M. oleifera* revealing that *M. stenopetala* is more effective than *M. oleifera* (Jahn, 1986). Early work also reported that *M. stenopetala* exhibited some lead ion sorption capacity (Mataka *et al.*, 2006). Therefore, the study was undertaken to investigate the possibility of using *M. oleifera* and *M. stenopetala* seed powders as biosorbents for Pb^{2+} and Cd^{2+} removal from aqueous solutions.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Materials

3.1.1 Coagulants

M. stenopetala seeds were purchased from Kenya (Whizpop Products Ltd., Nairobi) while *M. oleifera* seeds were collected from Chikwawa district in southern Malawi. The seeds of *M. oleifera* and *M. stenopetala* were identified by the Forestry Research Institute of Malawi and were stored at room temperature until used.

3.1.2 Chemicals, reagents and instruments

The following analytical grade chemicals were used: lead nitrate (BDH Chemicals Limited, Poole, UK); cadmium coarse powder, sodium carbonate, sodium bicarbonate and hexane fraction (density = 0.67 g/mL; boiling point range = 65-69^{o}C) (Saarchem (Pty) Krugersdorp Ltd, RSA); sodium chloride, sodium hydroxide and nitric acid (Associated Chemical Enterprises (Pty) Ltd, RSA); hydrochloric acid and diethyl ether (Glassworld, RSA).

Several pieces of equipment were used in the analyses: atomic absorption spectroscopy was done using an AAS (Shimadzu AA-680G V-5); pH was determined using a pH meter (Metrohm 744) calibrated with buffers at pH 4 and 7 (tablets purchased from Saarchem (Pty) Krugersdorp Ltd, RSA); constant temperature treatments were done in a constant temperature water bath (Bath: Haake Type 000-5584, Thermo stirrer: Gallenkamp No. 85); shaking was done using a Griffin shaker; drying was done in a vacuum oven (Gallenkamp OVL 570 010 J) and seeds were ground in a coffee mill (National MX-J210PN).

3.2 Methods

3.2.1 Preparation of seed powders

Seeds were deshelled by hand and the seed kernels were ground in a coffee mill until a consistently fine powder was obtained. The defatted seed powders were prepared by cold solvent extraction of the powdered seeds with hexane fraction. 500 g of seed powders were soaked in 2.0 L hexane fraction and left in the dark for 24 h. The mixture was filtered using Whatman No.1 filter paper, the residue taken up in 2.0 L diethyl ether and left in the dark for

48 h and the mixture was also filtered. To confirm complete defatting, paper test was done by smearing the final residue on a white paper tissue to observe whether the smeared part becomes translucent or not. The residue was dried in a vacuum oven at 40˚C and 600 mbars for 48 h.

3.2.2 Preparation of solutions

3.2.2.1 Reagents

3.2.2.1.1 Nitric acid solutions

The 1+1 nitric acid was prepared by mixing equal amounts of concentrated nitric acid (55 %) and deionised water. A 0.20 mol/L nitric acid was prepared by diluting 17.1 mL of concentrated nitric acid of density 1.34 g/mL to 1 L using deionised water.

3.2.2.1.2 Hydrochloric acid (1.0 mol/L)

This solution was prepared by diluting 98.22 mL of 32 % HCl of density 1.16 g/mL to 1 L.

3.2.2.1.3 Sodium hydroxide (1.0 mol/L)

This solution was prepared by dissolving 40.000 g of sodium hydroxide pellets in deionised water. The solution was poured into a 1 L volumetric flask and diluted to 1 L with deionised water.

3.2.2.1.4 Sodium chloride (2.0 mol/L)

This stock solution was prepared by dissolving 58.442 g of sodium chloride crystals (dried at 110 ^{o}C for 1 h and cooled to room temperature in a desiccator) in deionised water. The solution was poured into a 500 mL volumetric flask diluted to the 500 mL mark with deionised water.

3.2.2.2 Standard metal solutions

3.2.2.2.1 Lead

Stock lead solution (1000 mg/L) was prepared by dissolving 1.598 g of lead nitrate in a minimum amount of 1+1 nitric acid. The solution was poured into a 1000 mL volumetric flask and made up to the mark using deionised water. The intermediate stock solution (100 mg/L)

was prepared by pipetting 10 mL of the stock solution into 100 mL volumetric flask and diluted to the mark with deionised water. The working standards were prepared by pipetting 0, 1, 2, 3, 5, 7 and 10 mL of the intermediate stock solution into 100 mL volumetric flasks and the solutions were made up to the mark using deionised water (APHA, 1989). The concentrations of the solutions were 0, 1, 2, 3, 5, 7 and 10 mg/L respectively.

3.2.2.2.2 Cadmium

Stock cadmium solution (1000 mg/L) was prepared by dissolving 1.000 g of cadmium coarse powder in 4.0 mL and then 8.0 mL concentrated nitric acid. The solution was poured into a 1000 mL volumetric flask and made up to the mark using deionised water. The intermediate stock solution (100 mg/L) was prepared by pipetting 10 mL of the stock solution into 100 mL volumetric flask and the solution was made up to the mark with deionised water. The working standards were prepared by pipetting 0, 1, 2, 3, 5, 7 and 10 mL of the intermediate stock solutions into 100 mL volumetric flasks making up the solutions to the mark using deionised water (APHA, 1989). The concentrations of the solutions were 0, 1, 2, 3, 5, 7 and 10 mg/L respectively.

3.2.3 Determination of metal ion content

The concentration of the lead and cadmium ions was determined using atomic absorption spectroscopy (Shimadzu AA-680G V-5) at 283.3 and 228.9 nm respectively using 7 nm slit with an air-acetylene flame as described by APHA (1989). Known standards (from 0-10 mg/L) were used to calibrate the instruments and to keep good quality control. The analysis of metal ions was done in triplicates and the means and standard deviations were calculated. Biomass-free controls of each of the metal solutions were analysed to detect any possible metal precipitation or contamination. The AAS instrument was cleaned by flashing it with 1 % nitric acid for three minutes before aspirating metal solutions.

3.2.4 Effect of dosage on Pb^{2+} and Cd^{2+} removal from water

A 100 ml of water containing 7 mg/L metal ions was prepared by pipetting 7 mL of 100 mg/L intermediate stock solutions into 100 mL volumetric flasks and the solutions were made up to the mark using deionised water. The triplicates of appropriate masses of moringa powders, 0.0,

0.5, 1.0, 1.5, 2.0 and 2.5 g, were added and the mixture stirred for 1 h. The mixture was filtered by gravity through Whatman No.1 filter paper and the metal ion concentrations of the filtrate were determined using AAS. For the effects of standing time one aliquot was stirred for 1 h, filtered and quantified immediately, and the other aliquot was stirred for 1 h and left for 24 h before filtering. The initial pH for lead ions was pH 2.30 and that for cadmium ions was pH 2.95

3.2.5 Kinetics of Pb^{2+} and Cd^{2+} sorption

A 100 mL of water containing 7 mg/L of metal ions was added to 1.0 g of moringa whole seed powders. The aliquots were stirred and withdrawn at various times (0 - 8 h) in triplicates, and filtered. The residual metal concentration in the filtrate was quantified using AAS immediately after filtration.

3.2.6 Effect of temperature on Pb^{2+} and Cd^{2+} sorption

A 100 mL of water containing 7 mg/L lead or cadmium ion solutions were added to 1.0 g moringa seed powders at various temperatures (0, 25, 40, 60, 80 and 100^{o}C) in triplicates. The moringa suspension was placed in a constant temperature water bath and shaken for 1 h and then filtered immediately. To achieve 100^{o}C heating oil was used. The residual metal ion concentration of the filtrate was determined by AAS (APHA, 1989).

3.2.7 Effect of pH on Pb^{2+} and Cd^{2+} sorption capacity

A 100 mL of water containing 7 mg/L aqueous lead or cadmium solutions of various pH (2 – 10) were prepared in triplicates by adjusting the pH of a solution of 7 mg/L lead or cadmium ions in deionised water using 1 mol/L sodium hydroxide or hydrochloric acid and the resulting metal solutions added to 1.0 g moringa whole seed powders and stirred for 1 h using a magnetic stirrer. Similar solutions were prepared but without adding moringa biomass. The residual metal ion concentration was quantified using AAS (APHA, 1989).

3.2.8 Effect of water hardness on Pb^{2+} and Cd^{2+} sorption capacity

A 100 mL of water containing 7 mg/L lead or cadmium ion solutions in triplicates of different concentrations of Mg^{2+}/Ca^{2+} or HCO_3^-/CO_3^{2-} mixtures were added to 1.5 g of moringa whole

seed powders. The Mg^{2+}/Ca^{2+} solution was prepared as described elsewhere (Anonymous, (1996)). A 1000 mg/L of hard water was prepared by dissolving a mixture of 0.8255 g of $MgSO_4.7H_2O$ and 0.9800 g of $CaCl_2.2H_2O$ in deionised water and the solutions diluted to 1 L. The HCO_3^-/CO_3^{2-} mixture was prepared by adding 1.3768 g $NaHCO_3$ to 1.7662 g Na_2CO_3 and dissolving the mixture to make a 1000 mg/L solution. The aliquots of 0.0, 5.0, 10.0, 18.0, 25.0, and 40.0 mL of this solution were added to 7 mL of 100 mg/L intermediate metal ion solutions and resultant mixture diluted to 100 mL using deionised water. The mixtures were stirred for 1 h, filtered and the residual metal ion concentration of the filtrate determined by AAS (APHA, 1989).

3.2.9 Effect of ionic strength on Pb^{2+} and Cd^{2+} sorption capacity

A set of solutions containing 7 mg/L metal ions and 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mol/L sodium chloride were prepared in triplicates by combining 7 mL of the intermediate stock metal solutions (100 mg/L) with a 2 mol/L stock solution of sodium chloride (0.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mL). (The solutions were diluted to 100 mL with deionised water). The solutions were added to 1.5 g of *M. oleifera* or *M. stenopetala* whole seed powders, stirred for 1 h and resultant mixtures filtered through Whatman No.1 filter paper. The residual metal ion concentration was quantified by AAS (APHA, 1989).

3.2.10 Calculation of efficiencies of metal removal

The percentage sorption by the biosorbent was computed using equation 9:

$$Sorption(\%) = \frac{C_e - C_f}{C_o} x100\% \qquad (9)$$

where C_o (mg/L) and C_f (mg/L) are initial and final concentrations of metal ions in the solution.

3.2.11 Equilibrium sorption studies for Pb^{2+} and Cd^{2+} sorption

Portions of *M. oleifera* or *M. stenopetala* (approximately 0.25 g), in triplicates, were weighed in 50 mL centrifuge tubes using an analytical balance (Metrohm). An aliquot (25 mL) of metal ion solution (Pb^{2+} and Cd^{2+}) with varying initial metal ion concentrations (5 – 15 mg/L) was added and the tubes shaken at a constant speed using the Griffin shaker. After shaking the

tubes for 4 h for lead and 2 h for cadmium, the suspensions were filtered using Whatman No.1 filter paper, filtrates collected in clean sample bottles. The metal contents were determined using AAS (Shimadzu model) (Horsfall and Spiff, 2004).

3.2.11.1 Calculation of metal sorbed at equilibrium

The mean metal ion sorbed by the powder at equilibrium was determined using a mass balance equation as follows:

$$q_e = \frac{(C_o - C_e)v}{m} \qquad (10)$$

where q_e (g/kg) is metal ion adsorption per unit weight of powder at equilibrium, C_o and C_e (g/L) are initial metal ion concentration and the concentration at equilibrium respectively, v (L) is volume of initial metal ion solution used and m (g) mass of powder.

3.2.12 Desorption of metal loaded biomass

Aliquots of metal ions (100 mL, 7 mg/L) were added to 2.0 g of moringa powders in triplicates and stirred for 1 h. The mixture was filtered and the residue was dried in a vacuum oven at 40^{o}C for 24 h. A palm of solid (0.5 g) was transferred into centrifuge tubes, mixed with 25 mL of nitric acid solutions (0.01, 0.02, 0.04, 0.06, 0.08 and 0.10 mol/L) and final mixture shaken for 40 minutes and filtered. The desorbed metal was quantified by AAS. The final pH of the filtrate at each acid concentration was determined.

3.3 Data handling and analysis

Microsoft Excel was used for descriptive statistics and for plotting treatment data. T-test was used to compare individual means. The analyses of variance (ANOVA) were carried out using GenStat Discovery Edition (VSN International, 1999).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Effect of dosage on lead and cadmium removal

The Effects of dosage on Pb(II) and Cd(II) removal from water are shown in Figures 2 and 3. The results show that the effectiveness of removal was enhanced with increasing dosage of both whole seed powders for both metals at 95 % confidence level. This is because higher doses of seed powders resulted in more binding sites being available and hence higher removal of metal ions. Krishnan and Anirudhan (2003) and Izanloo and Nasseri (2005) reported an increase in cadmium ion removal with dosage using steam-activated sulphurised carbon prepared from sugar-cane bagasse pith and ground cone powder respectively and Barros *et al.* (2003) reported enhanced cadmium ion removal with a raise in dosage using *Aspergillus niger*.

In the case of cadmium ion sorption, an increase in metal removal with increasing biomass is observed only up to 2.0 g biomass dosage and the increase was not significantly different above 2.0 g ($p > 0.05$ in Figure 3). This is because of interference between binding sites at higher dose. As the dosage increases the number of binding sites also increases, and this results in different binding sites interacting with a single metal ion which leaves the other metal ions free from binding (Puranik and Paknikar, 1999). Gadd (1988) and Kuyukack and volesky (1989) have reported no further increase in heavy metal sorption at higher dose using yeast biomass and metal adsorption using different adsorbents respectively.

Figures 2 and 3 have clearly revealed that *M. stenopetala* is generally more effective than *M. oleifera* in both lead ion and cadmium ion sorption from aqueous solutions ($p < 0.05$). Further, Jahn (1986) also observed that *M. stenopetala* is a better water clarifier than *M. oleifera*. *M. stenopetala* had higher sorption capacity than *M. oleifera* for lead ion removal. However, for cadmium ion sorption above 2.0 g/100 mL dose, there was no further significant difference. This observation shows that cadmium ion sorption capacities of the two powders are not very different.

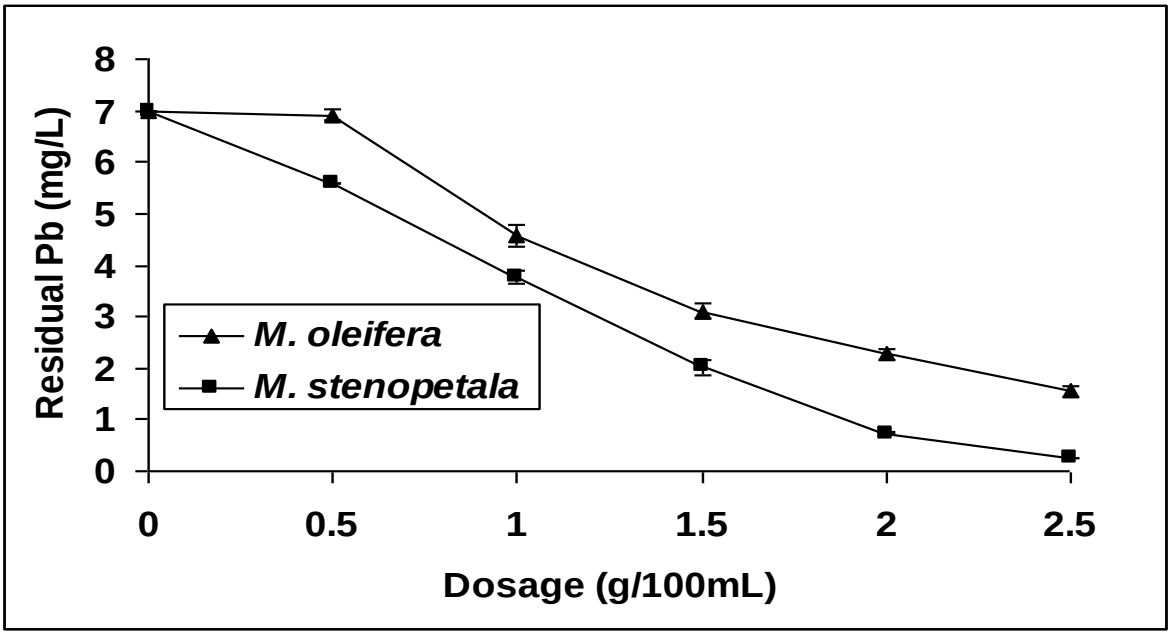

Figure 2: Residual Pb vs dosage using *M. oleifera* and *M. stenopetala* seed powders at initial Pb concentration of 7 mg/L and 30^{o}C.

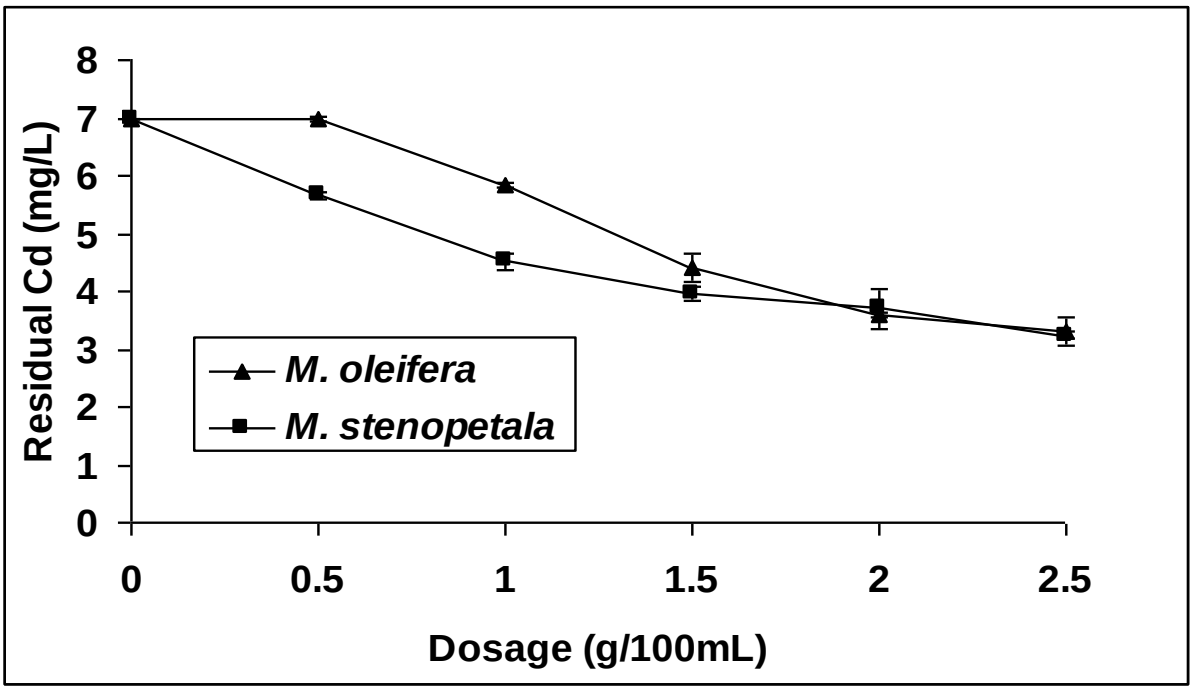

Figure 3: Residual Cd vs dosage using *M. oleifera* and *M. stenopetala* seed powders at initial Cd concentration 7 mg/L and 30^{o}C.

4.2 Effect of type of metal ion on lead and cadmium removal

The results in Figures 4 and 5 clearly indicate that the powders better sorbed lead ions than cadmium ions. The differential sorption of the two ions may be attributed to the difference in their ionic radii; the ionic radius of Pb^{2+} is 1.20 pm while that of Cd^{2+} is 0.97 pm (Horsfall and Spiff, 2005a). The smaller the ionic radius the greater is its tendency to hydrolyse in water leading to reduced sorption (Horsfall and Spiff, 2005a). Further, the greater the atomic weight and ionic size, the greater will be the affinity for sorption (Mattuschka and Straube, 1993; Sag *et al.*, 2002). Although the oxidation number for both metal ions is +2, their charge densities (charge/mass) are different. The charge density of the lead ions is 9.7×10^{-3} C/g and that of cadmium is 1.8×10^{-2} C/g. Cadmium ions, with higher charge density, have consequently been strongly hydrolysed in aqueous solutions, and hence form stronger metal ion-water complexes. Therefore, it is more difficult to displace the water of hydrolysis for a binding site in cadmium ion sorption than in lead ion sorption.

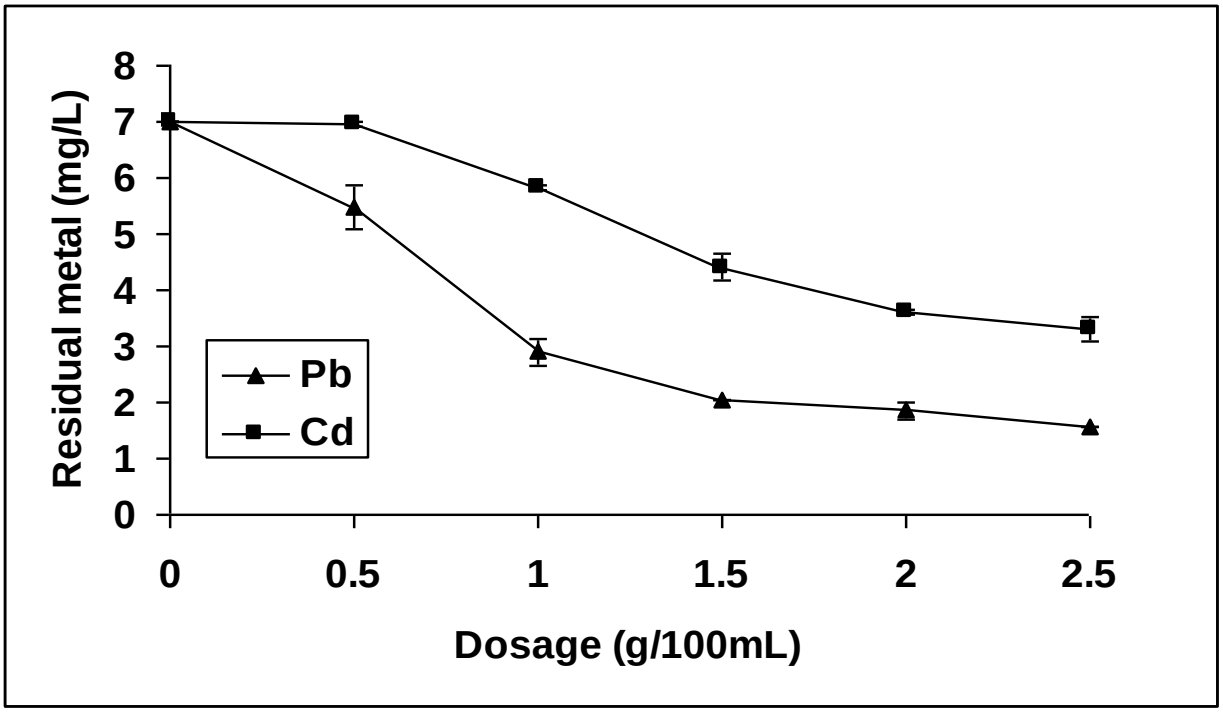

Figure 4: Residual Pb and Cd vs dosage using *M. oleifera* at initial metal concentration of 7 mg/L and 30°C.

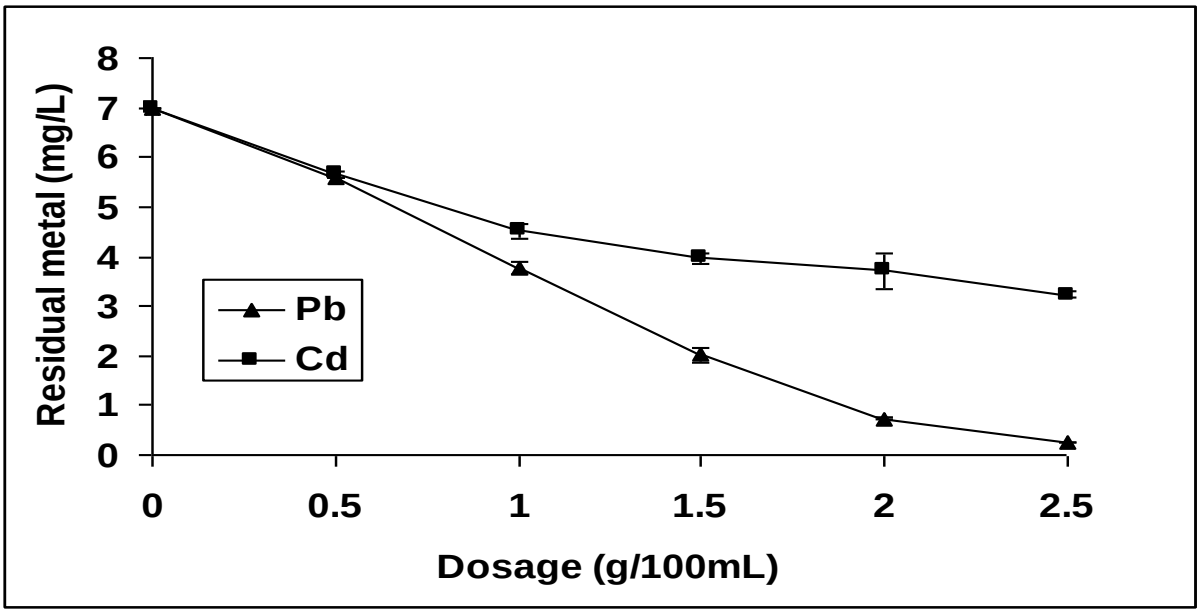

Figure 5: Residual Pb and Cd vs dosage using *M. stenopetala* at initial metal concentration of 7 mg/L and 30^{o}C.

4.3 Effect of standing time on Pb and Cd removal

The results on the effect of standing time are shown in Figures 6 to 9. Standing time did not affect the removal of lead ions by *M. stenopetala* treatment ($p > 0.05$ in Figure 7); doses less than 1.5 g/100 mL showed 24 h higher than 1 h removal and also thereafter 1 h removal was higher than 24 h removal with no significant difference at 1.5 g/100 mL dosage. Lead ions bind to ligands both electrostatically and through formation of inner covalent complexes (Elzinga and Sparks, 2002). At lower biomass concentrations, the ratio of metal ions to biomass is high. Therefore, initially lead ions are expected, largely, to bind through electrostatic interaction. After sometime, inner complexes between lead ions and *M. stenopetala* biomass begin to form. The inner complex formation, presumably, requires more time to get completed. At higher doses more sites become available for binding hence the metal ions bind more quickly to these sites (Nomanbhay and Palanisamy, 2005). However, the sites interfere with each other and hence those sites with weak binding affinity likely lose the metal into aqueous solution through hydrolysis after some time (Gadd, 1988). Therefore, 1 h metal ion sorption is greater than 24 h metal ion sorption.

Use of *M. oleifera* significantly achieved higher lead sorption at 24 h. Results indicated that only two doses, 1.5 and 2.5 g, showed no significant difference with treatment time ($p > 0.05$ in Figure 6). Clearly, more contact time is required for Pb binding to get completed using *M. oleifera* biomass than when using *M. stenopetala*.

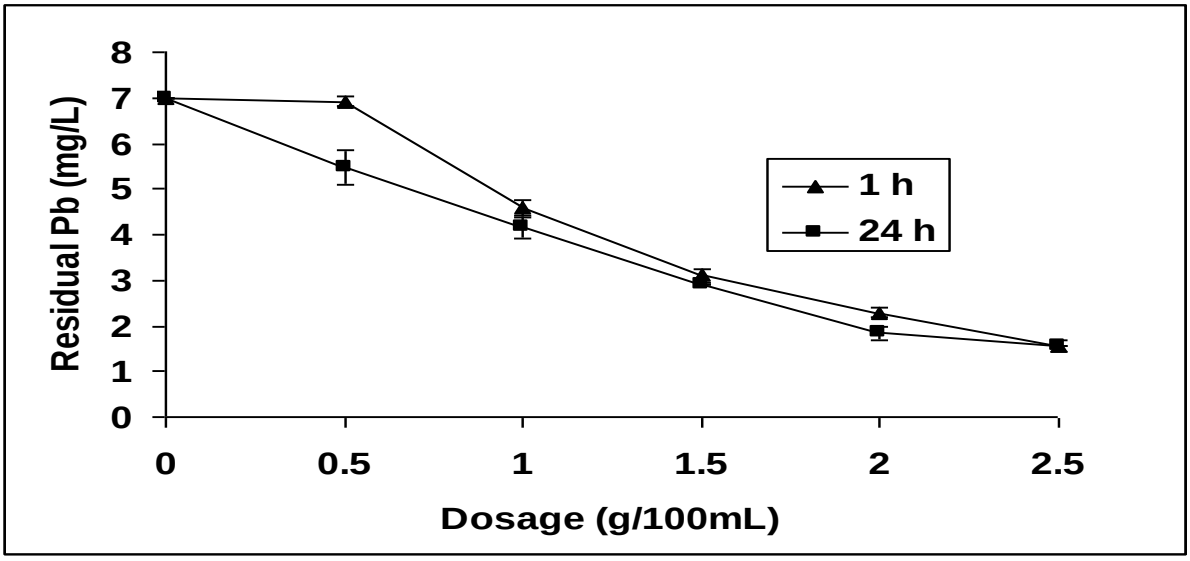

Figure 6: Residual 1 h and 24 h Pb vs dosage of *M. oleifera* at initial metal ion concentration of 7 mg/L and 30 ^{o}C.

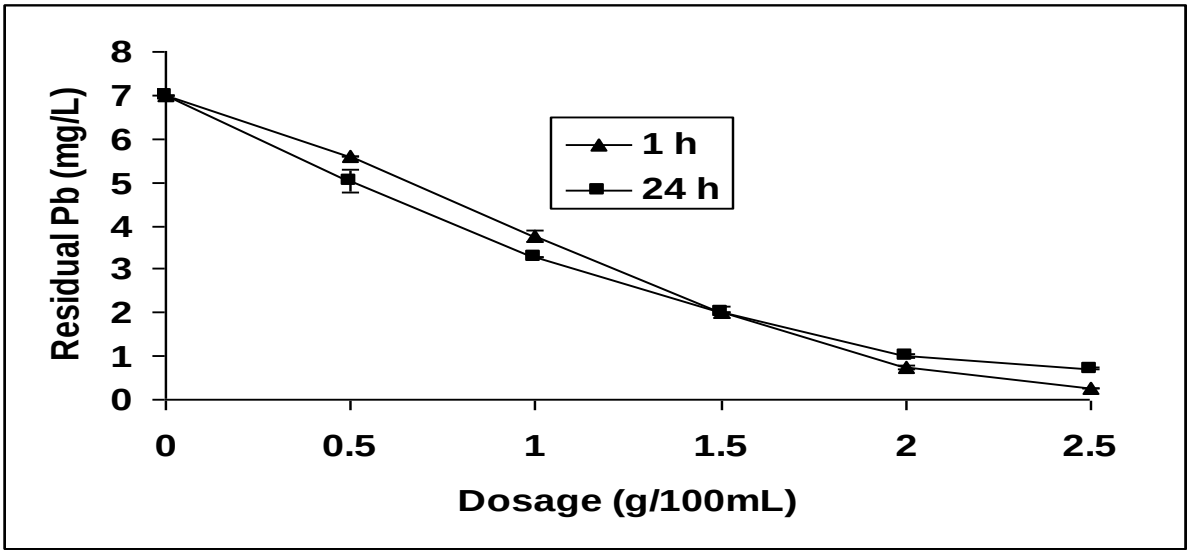

Figure 7: Residual 1 h and 24 h Pb vs dosage of *M. stenopetala* at initial metal ion concentration of 7 mg/L and 30 ^{o}C.

For cadmium ion sorption, a significant difference was observed between 1 h and 24 h (Figures 8 and 9) for both powders ($p < 0.05$). The results showed that for treatment using both powders at lower doses, 0.5 and 1.0 g/100 mL, 1 h sorption gave results higher than 24 h sorption ($p < 0.05$ in Figure 8). At higher dosages, sorption at 24 h standing time was significantly higher than that at 1 h standing time. There was no significant difference on cadmium sorption at a dosage of 1.5 g/100 mL using *M. stenopetala*. Cadmium ions usually bind to low energy sites on the biomasses because cadmium mostly binds through weak electrostatic interaction (Scheiwer and Volesky, 2000). At low biomass dosage there is a higher concentration of metal ions with fewer low energy sites available and hence cadmium metal ions are likely to quickly bind to both low energy and high energy sites. However, the affinity for cadmium ion on the high energy sites is low and, therefore, with time the high energy sites on the biomass likely lose the cadmium metal ions through hydrolysis with the water molecule (Horsfall and Spiff, 2005a). For the higher dosage there are sufficient numbers of low energy binding sites and the cadmium ions presumably bind to these sites. In this case the rate of binding is determined by how fast the metal ions diffuse through to the biomass sites. Since cadmium ions are highly hydrolysed in aqueous medium due to their small size, more time is likely required for the ions to diffuse through to the binding sites (Horsfall and Spiff, 2005a).

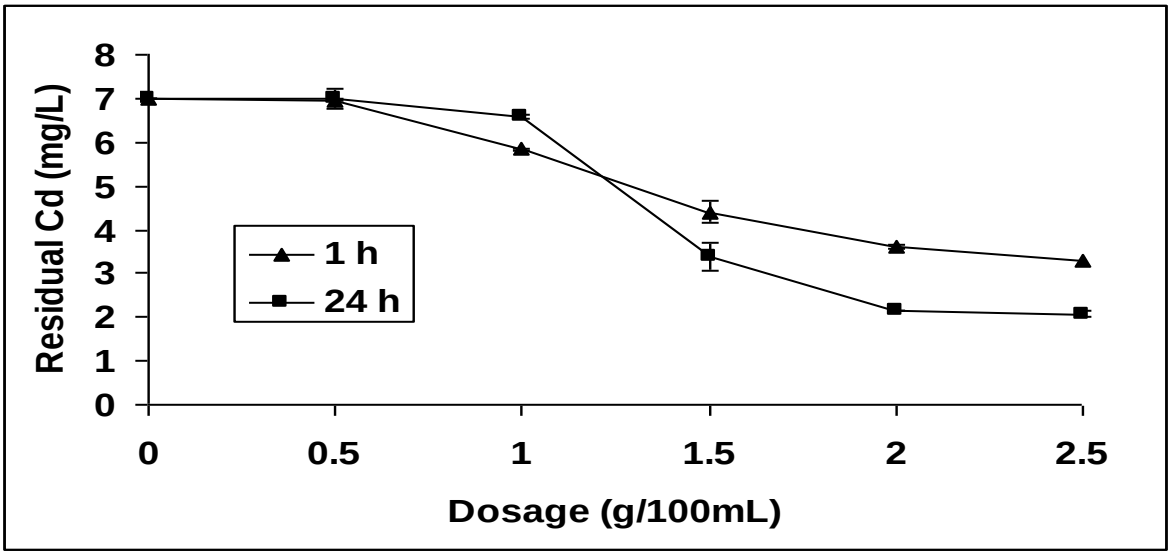

Figure 8: Residual 1 h and 24 h Cd vs dosage of *M. oleifera* at initial metal ion concentration of 7 mg/L and 30 ^{o}C.

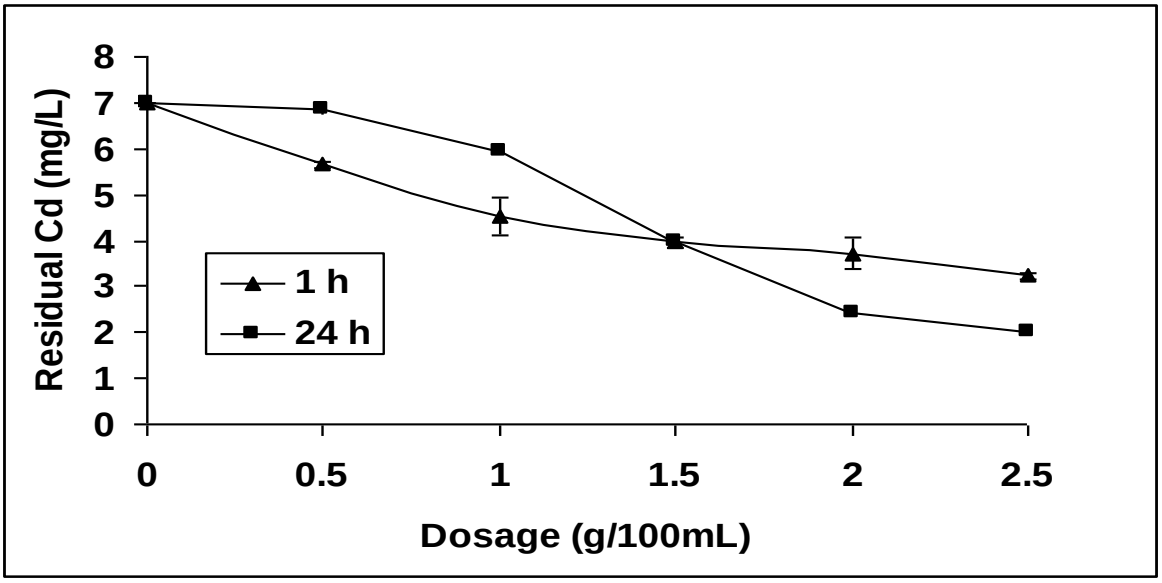

Figure 9: Residual 1 h and 24 h Cd vs dosage of *M. stenopetala* at initial metal ion concentration of 7 mg/L and 30 ^{o}C.

4.4 Comparison between moringa and other biosorbents on lead and cadmium biosorption

Moringa sorption capacity was compared to other biosorbents reported in literature for their metal sorption effectiveness. Moringa powders at the initial concentration of 7 mg/L and pH 3 for lead ion and pH 5 for cadmium ion sorption showed better metal uptake capacities than most biosorbents within similar concentration range (Table 2). This shows that the active agents in the moringa powders have higher effectiveness in metal ion sorption than most other biosorbents. Furthermore, moringa seed powders are easier to prepare than the other biosorbents. This, coupled with their metal sorption effectiveness, makes these more advantageous than other metal biosorbents.

Table 2: Metal ion sorption capacities (mg/L) for different biosorbents

Metal	*Moringa*[1] *oleifera* (7 mg/L)	*Moringa*[1] *stenopetala* (7 mg/L)	Biological[2] activated carbon (10 mg/L)	*Saccharomyces*[3] *cerevisiae* (algae) (50 mg/L)	Waste[4] beer yeast by-product (25 mg/L)	Wild[5] cocoyam biomass (10 mg/L)
Pb	0.612	0.623	-	1.146	0.491	0.255
Cd	0.579	0.495	0.230	0.246	-	0.348

Key: The figures in brackets are initial metal ion concentrations.

[1]This work (treated for 1 h using 1.0 g/100 mL dosage at pH 3 for Pb and pH 5 for Cd); [2]Dianati-Taliki *et al.* (2004); [3]St.Mihova and Godjevargova (2001); [4]Parvathi *et al.* (2007); and [5]Horsfall and Spiff (2004) respectively.

4.5 Effect of using defatted moringa powders on the removal of Pb and Cd from water

The effects of using defatted powders are shown in Figures 10 to 13. Metal ion removal for both lead and cadmium ions also increased with increasing dosage of both defatted seed powders ($p < 0.05$). The results are consistent with those in section 4.1. The increase in the amount of binding sites enhanced metal ion sorption at higher concentrations of biomass.

When compared between 1h and 24 h contact times, differences were observed for lead ion sorption using *M. oleifera* ($p < 0.05$ in Figure 10). Except for 1-1.5 g/100 mL of seed powder, higher doses greater than 2.0 g/100 mL showed that 24 h treatment sorption capacity was higher than 1 h sorption capacity. Figure 11 shows that for lead ion removal using *M. stenopetala* there was no significant difference in metal ion sorption between 1 h and 24 h treatments ($p > 0.05$)

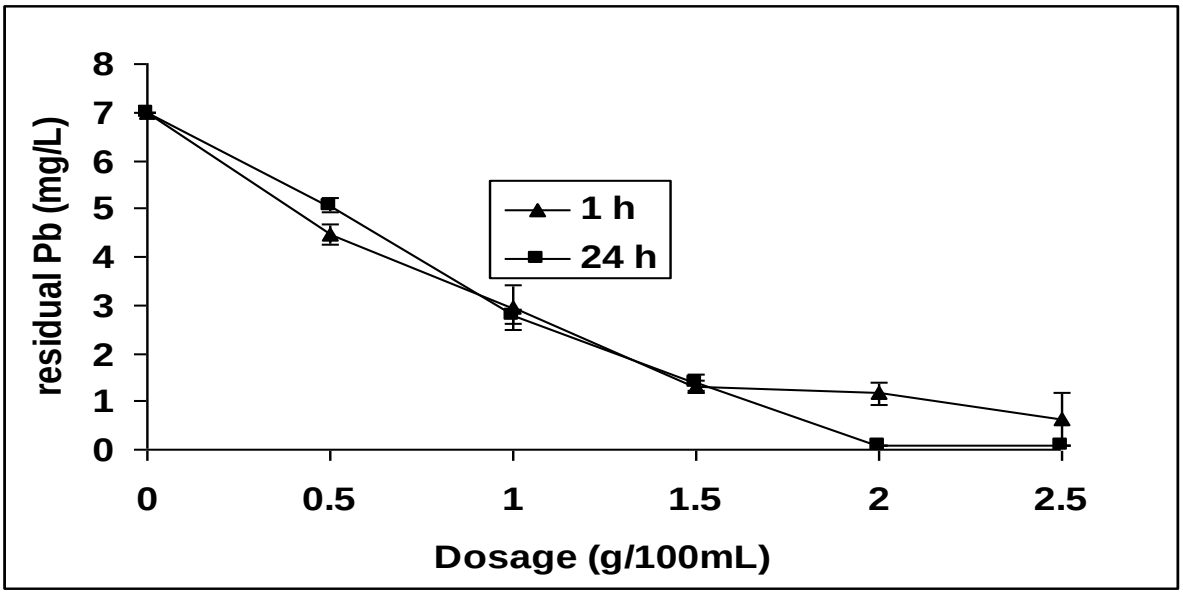

Figure 10: Residual Pb vs defatted *M. oleifera* dosage at initial Pb concentration of 7 mg/L and 30^{o}C.

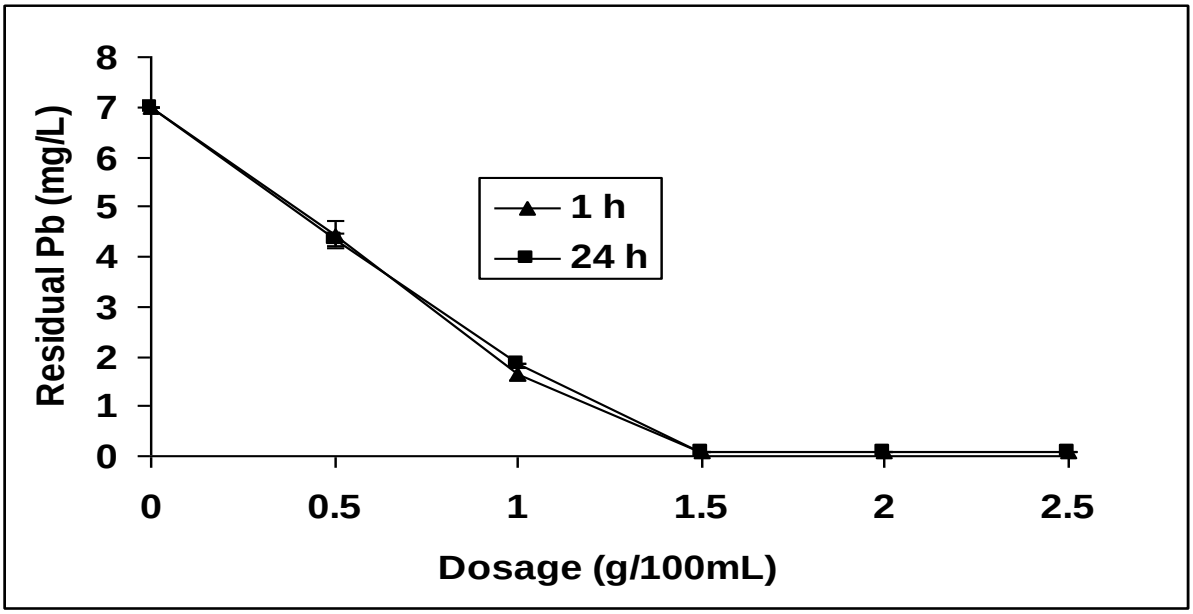

Figure 11: Residual Pb vs defatted *M. stenopetala* dosage at initial Pb concentration of 7 mg/L and 30^{o}C.

A significant difference was observed for cadmium ion removal at 1 h and 24 h treatments using both defatted seed powders (Figure 12). *M. oleifera* treatment results showed that 24 h cadmium ion sorption capacity was higher than 1 h sorption capacity. However, using *M. stenopetala* 1 h sorption capacity was higher than 24 h sorption capacity (Figure 13). Defatting, presumably, exposes more high energy binding sites, but cadmium ions usually bind to low energy sites because they mostly bind through weak electrostatic interaction (Scheiwer and Volesky, 2000). Cadmium ions will still quickly bind to these high energy sites but the sites are likely not stable. Therefore, after sometime, the metal ions are possibly hydrolysed back into solution. This confirms the earlier observation that the binding sites on *M. stenopetala* become less stable with time. Therefore, for cadmium sorption using defatted *M. stenopetala* seed powders, 1 h treatment is sufficient for complete metal treatment

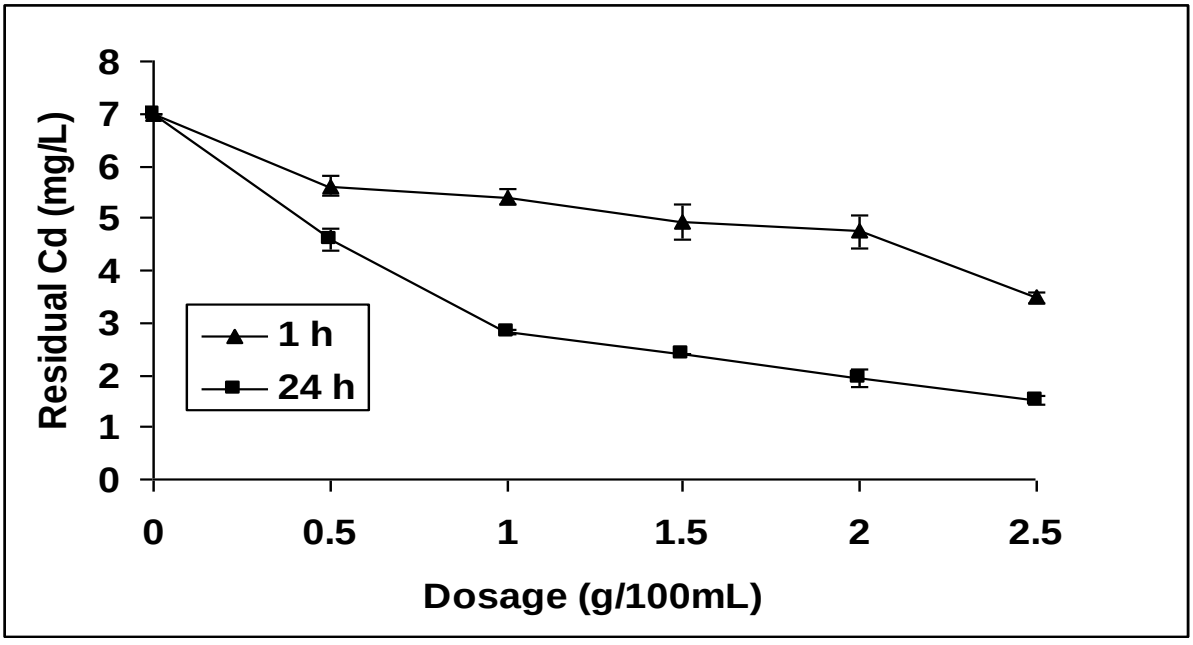

Figure 12: Residual Cd vs defatted *M. oleifera* dosage at initial Cd concentration of 7 mg/L and 30^{o}C.

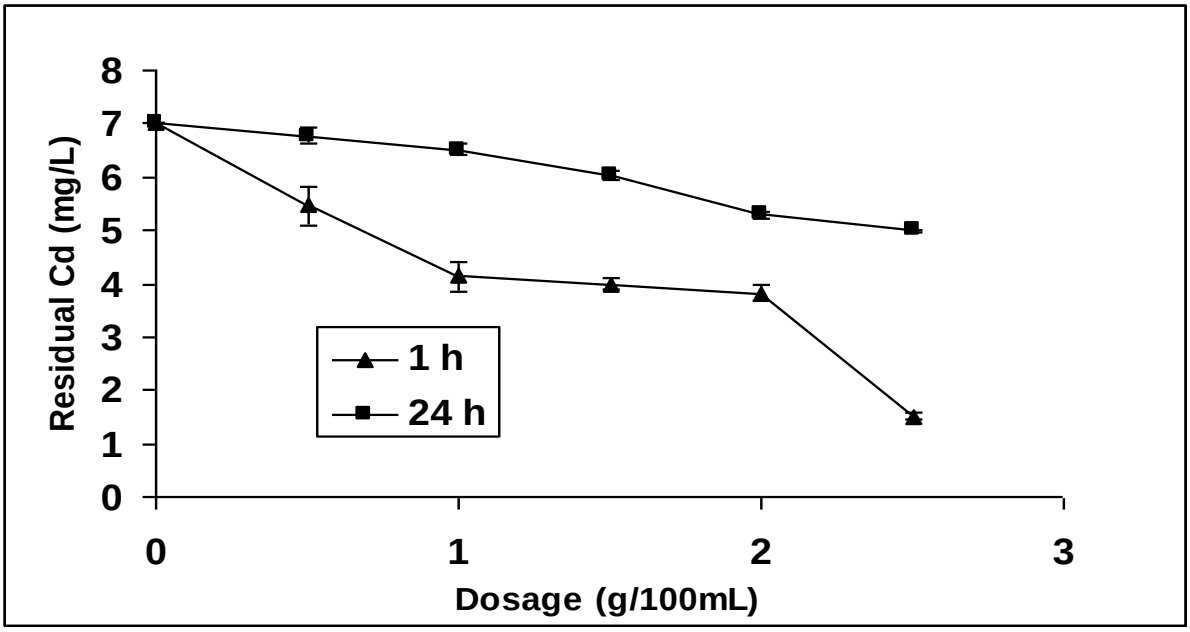

Figure 13: Residual Cd vs defatted *M. stenopetala* dosage at initial Cd concentration of 7 mg/L and 30^{o}C.

4.6 Comparison between whole seed powder and defatted powder on lead and cadmium removal

Figures 14 and 15 show that the defatted powders were more effective in lead ion sorption using both *M. oleifera* and *M. stenopetala* at both 1 h and 24 h. This is likely due to the exposure of more protein binding sites by removing fats.

For cadmium removal Figures 16 and 17 indicate interesting trends at different times. At 1 h using *M. oleifera*, the whole seed powders were more effective than the defatted powders ($p < 0.05$) while at 24 h the defatted powders were more effective than the whole seed powders ($p < 0.05$ in Figure 16). This suggests that cadmium ions need more time to get completely sorbed to *M. oleifera* defatted powders. For *M. stenopetala*, 1 h sorption using defatted powders was more effective than that using the whole seed powders ($p < 0.05$) while 24 h sorption using the whole seed powders was more effective than the defatted powders ($p < 0.05$).

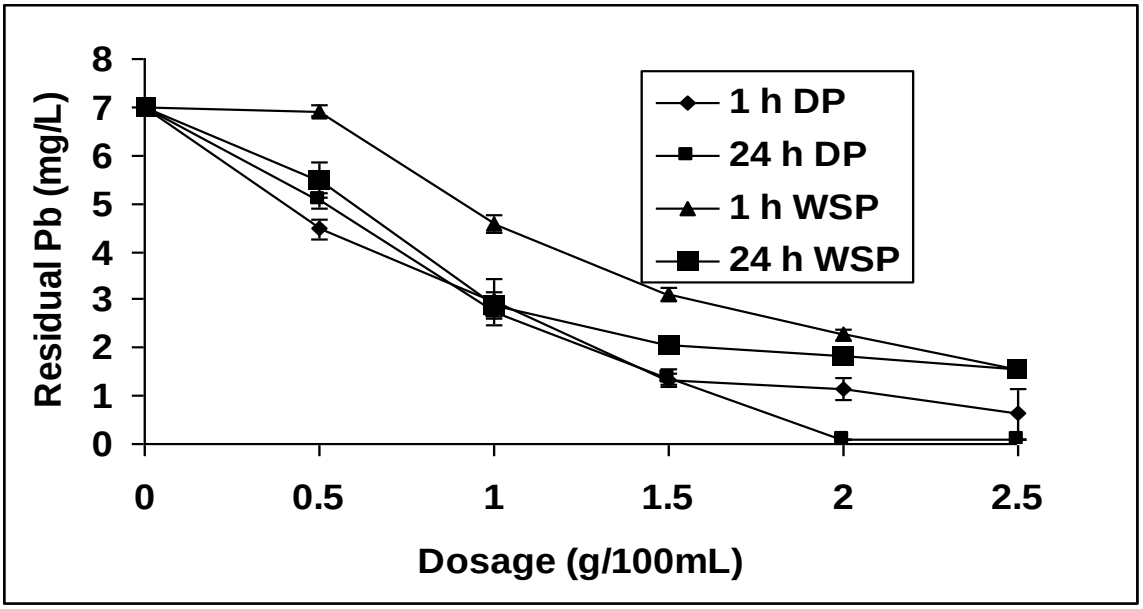

Figure 14: Residual Pb vs dosage using defatted and whole seed powders of *M. oleifera* at initial Pb concentration of 7 mg/L and 30^{o}C.

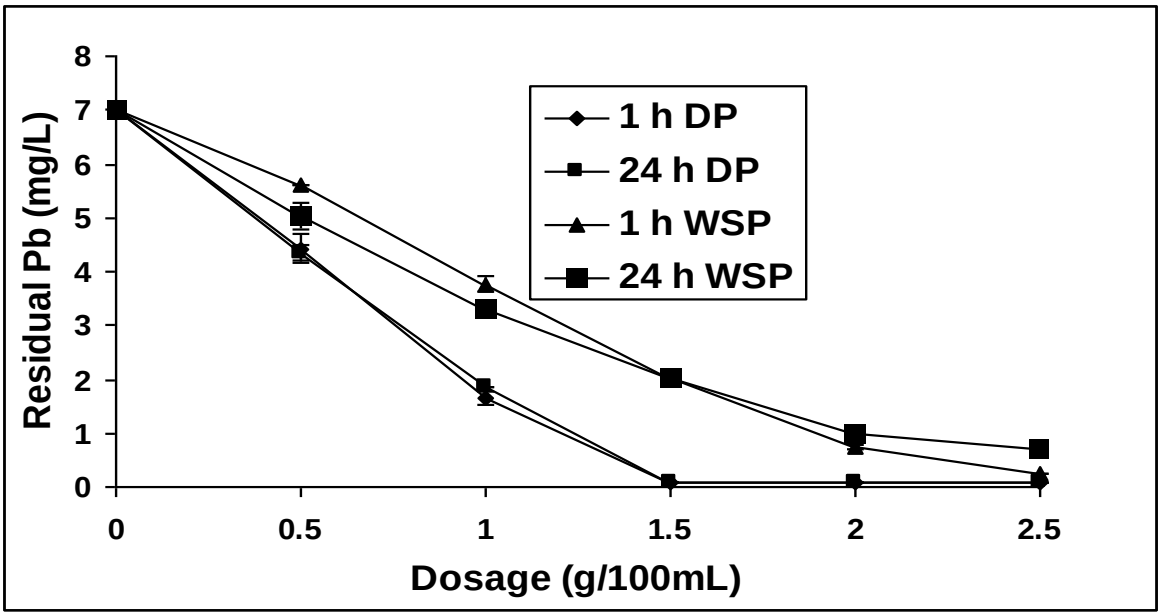

Figure 15: Residual Pb vs dosage using defatted and whole seed powders of *M. stenopetala* at initial Pb concentration of 7 mg/L and 30^{o}C.

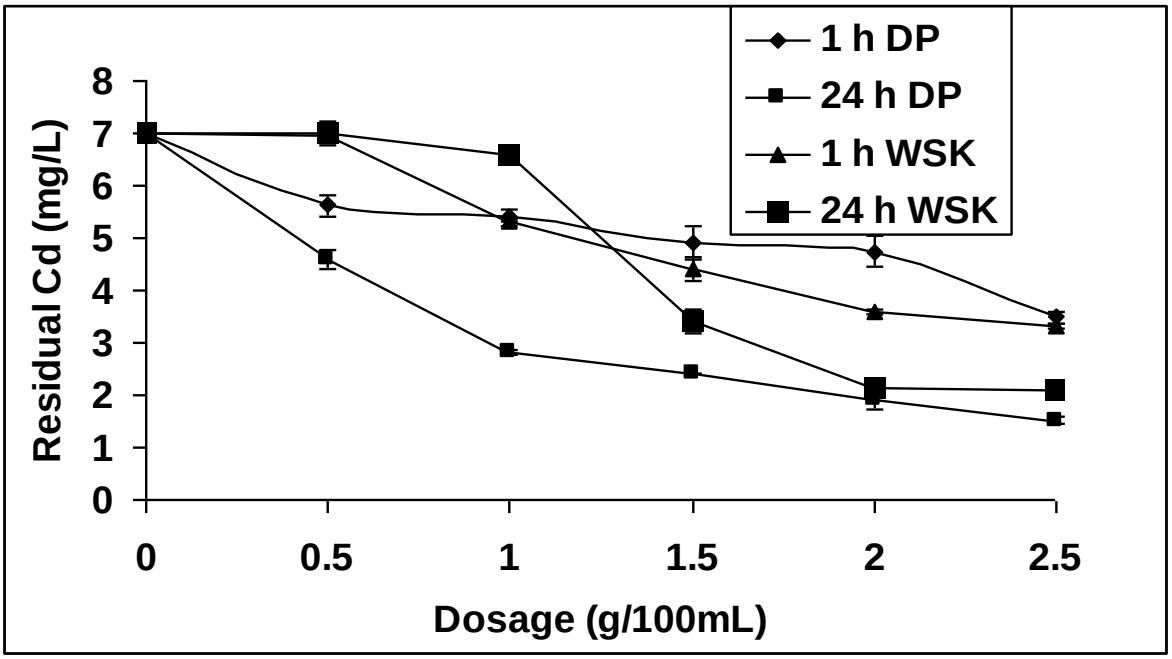

Figure 16: Residual Cd vs dosage using defatted and whole seed powders of *M. oleifera* at initial Cd concentration of 7 mg/L and 30^{o}C.

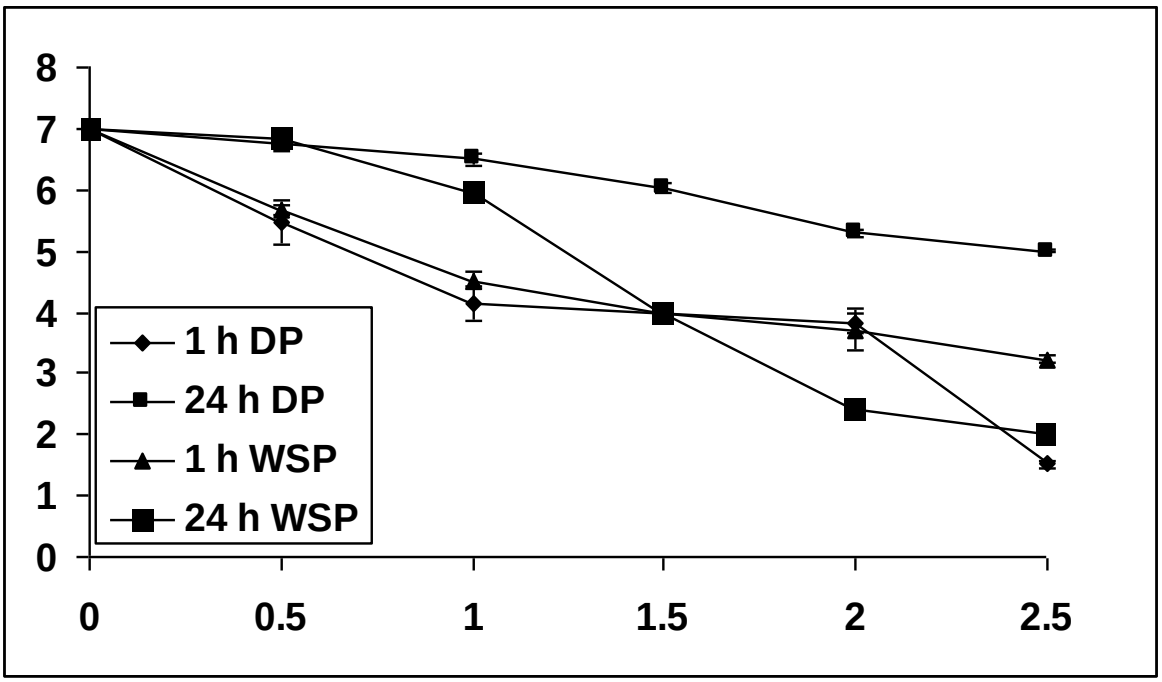

Figure 17: Residual Cd vs dosage using defatted and whole seed powders of *M. stenopetala* at initial Cd concentration of 7 mg/L and 30^{o}C.

4.7 Effect of pH on lead and cadmium removal from water

Figures 18 and 19 show the relationship between pH and total removal percentage. The results showed that the percentage removal was enhanced with an increase in pH. The maximum removals for lead ions, 98.39 % and 93.99 %, and cadmium ions, 93.80% and 88.40%, for *M. oleifera* and *M. stenopetala* respectively were observed at pH 10 indicating that moringa powders are more effective in metal ions removal at higher pH. The same trend has been reported by Herrera *et al.* (2003) on silver metal ion sorption using alfalfa biomass, Raji *et al.* (1997) on heavy metal adsorption using saw dust and Horsfall and Spiff (2005a) on lead and cadmium ion sorption from aqueous solutions using wild cocoyam biomass. The total percentage sorptions obtained at this pH 10 are a result of contributions from the moringa powders and metal precipitation as hydroxide. At pH above 8.0 and 7.7, lead and cadmium ions precipitate as $Pb(OH)_2$ and $Cd(OH)_2$ respectively (Ayres *et al.,* 1994). In this case a significant contribution to metal sorption by the hydroxyl ions was observed above pH 6.

To obtain the optimum pH for metal sorption by the moringa powders, the percentage removal obtained due to metal hydroxide precipitation was subtracted from the combined percentage removal. If the contribution of the hydroxyl ions is considered, the optimum pH for lead ion sorption is pH 3 and that for cadmium ion sorption is pH 5. Above these pH metal ions sorption for both metals might be a result of both precipitation and sorption by moringa seed powders. Before the precipitation pH for both metal ions, significant removal still takes place. Furthermore, the optimum pHs for removal of lead and cadmium ions, pH 3 and 5 respectively, are below the precipitation pH. This shows that metal removal is dominated by sorption by the moringa powders and not precipitation as hydroxides.

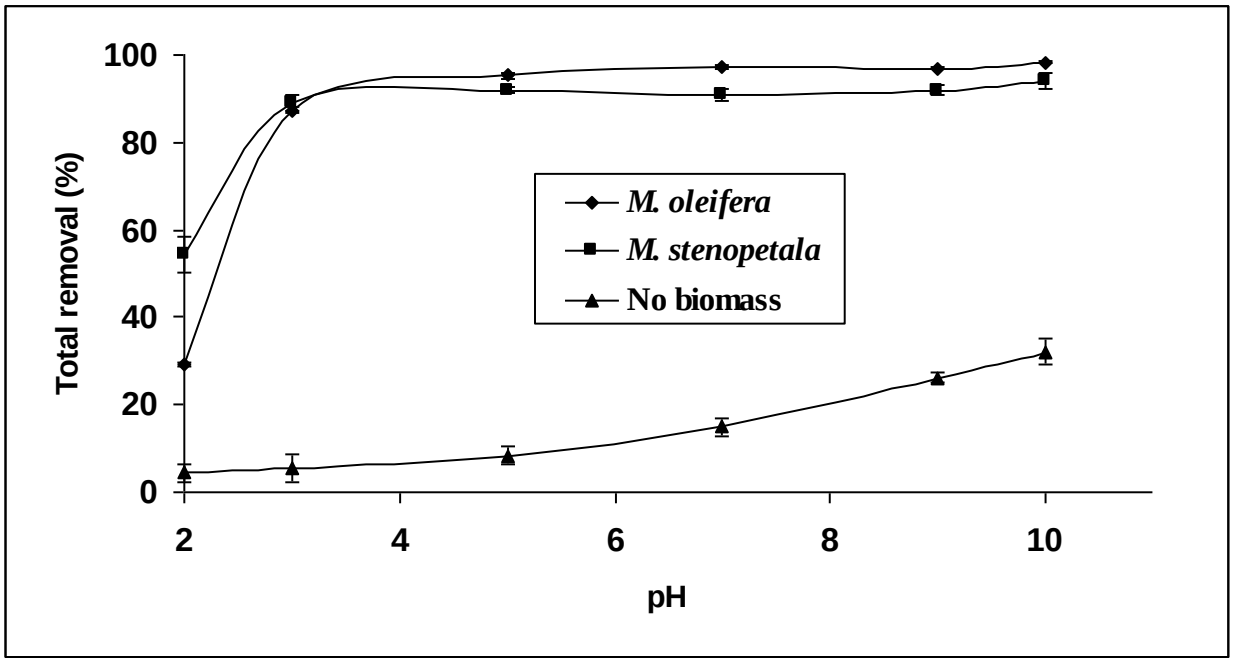

Figure 18: Effect of pH on Pb(II) removal using 1.0 g/100 mL moringa whole seed powders at initial metal ion concentration of 7 mg/L and 30 ^{o}C

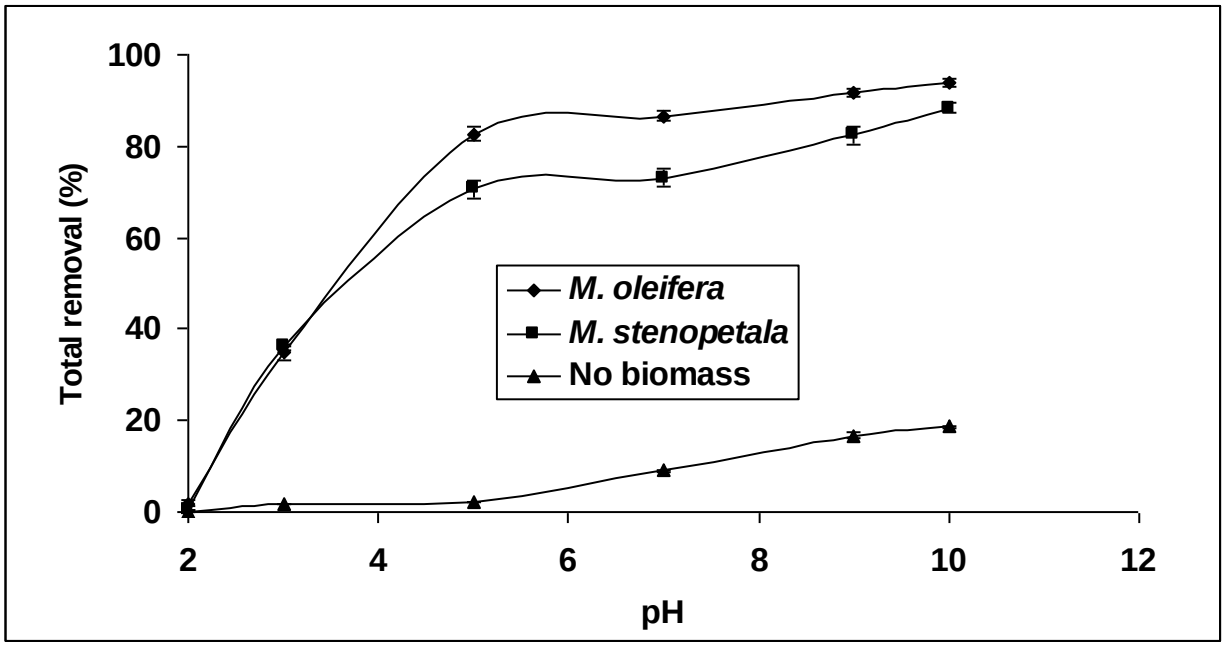

Figure 19: Effect of pH on Cd(II) removal using 1.0 g/100 mL moringa whole seed powders at initial metal ion concentration of 7 mg/L and 30 ^{o}C

The reduced sorption efficiency of lead and cadmium ion removal with the decrease in pH could be attributed to the presence of H^+ ions in the mixture, which compete with Pb^{2+} or Cd^{2+} ions for the binding sites. Furthermore, if the pH is lowered below the isoelectric point, *pI* = 9.6 for *M. oleifera* (Ghebremichael *et al.,* 2005), any basic groups such as nitrogen are protonated, hence the protein will lose its negative charge and contain only positive charges. The positive charges repel the positive ions, and hence make it difficult for the protein to form a complex with the metal ions.

The effects of high pH are analogous to those of low pH. The acid groups of the protein are deprotonated hence it obtains a large negative charge, which can interact more favourably with the positive metal ions (Logan, 1996). At pH 2 the metal uptake is small, especially for cadmium, but not negligible, which can be a result of the presence of very strong acid groups such as sulphur (Fourest and Volesky, 1996). Two sulphur containing amino acids, cysteine and methionine have been identified in *M. oleifera* (Gassenschmdt, 1995).

This study shows that above pH 3, metal ion sorption capacity using *M. oleifera* is higher than that using *M. stenopetala* ($p < 0.05$) and below this pH *M. stenopetala* is better than *M. oleifera*. This agrees with results obtained at the working pH for both lead and cadmium ions removal which is within pH of 2-3 (Figures 2 and 3). On Figures 18 and 19 within pH 5-10, it is observed that percentage metal removal using *M. oleifera* is higher than that using *M. stenopetala* for both lead and cadmium sorption ($p < 0.05$). This suggests that *M. oleifera* metal ion sorption is more pH dependent than *M. stenopetala* sorption especially for cadmium ion sorption. This observation probably indicates that *M. stenopetala* has some binding sites that are less pH dependent than those in *M. oleifera* metal sorption. pH independent biosorption is a result of covalent binding between metal ions and binding sites (Gardea-Torresday *et al.,* 1996). Hence, it can be assumed that *M. stenopetala* metal ion sorption is more covalent than *M. oleifera* sorption. Covalent metal binding has been reported for gold(III) biosorption using alfalfa biomass (Gardea-Torresdey *et al.,* 1998). Furthermore, between the two metal ions, cadmium sorption capacity is more affected by pH changes than lead ion sorption such that at pH 2 there is almost no sorption for Cd^{2+} but significant sorption for Pb^{2+} using both seed powders. This also reflects differences in type and strength of binding

between these two metal ions to the moringa biomass with cadmium ion sorption being more electrostatic than lead ion sorption.

4.7.1 Mechanism of metal ion removal

M. oleifera is a complex material containing organic residues made of several polar functional groups, particularly low molecular weight polypeptides (Jose *et al.,* 1999; Ndabigengesere *et al.,* 1995; Ghebremichael, 2004; Gassenschmidt, 1995). These polypeptides have a variety of structurally related pH-dependent properties of generating either positively or negatively charged sites for attracting anionic and cationic species of metal ions respectively (Costa *et al.,* 1997; Horsfall and Spiff, 2004). The majority of amino acids present in the moringa biomass have isoelectric points in the pH range of 4 – 8 (Devlin, 2002) such that in this pH range, over 90 percent of the amino acid molecules contains cationic sites. With the increase in pH, the carboxylic groups of amino acids would progressively be deprotonated as carboxylate ligands, which become the active sites for metal cations binding. Furthermore, exchange reaction between biomasses and the metal ion reaction may be represented in two ways as shown in the following equations:

$$2B^- + M^{2+} \rightarrow M(B)_2 \qquad (11)$$

$$2HB + M^{2+} \rightarrow M(B)_2 + 2H^+ \qquad (12)$$

where B^- and B are polar sites on the biomass surface, and M^{2+} is either Pb^{2+} or Cd^{2+}. Such reactions are highly pH dependent. According to Gardea-Torresdey *et al.* (1996), pH dependent binding suggests that metal ions are sorbed by biomass through carboxyl, carbonyl or hydroxyl groups.

Since the addition of sodium hydroxide enhanced sorption of metal ions by the seed powders, this means that metal ion binding to some extent was occurring at carboxyl or hydroxyl ligands. Furthermore, Fourier transform infrared analyses have indicated the absence of characteristic peaks of carboxylate ions after Cr(III) removal from aqueous solutions by *M. oleifera* powder (Kumari *et al.,* 2006). Further analysis of proteins from one type of *M.*

oleifera active agents, MO 2.0, showed the presence of high content of side chains, glutamine, proline, serine, cysteine, methionine and asparagines (Gassenschmidt *et al.*, 1995). All of these have either carbonyl, carboxyl or hydroxyl groups which are capable of binding metal ions. Also the presence of other side chains such as arginine, cysteine, histidine and tyrosine was observed (Gassenschmidt *et al.*, 1995). Cysteine and methionine contain sulphur ligands, and both metal ions have higher affinity for these sulphur ligands (Devlin, 2002; Kuyucak and Volesky, 1989). These side chains probably provide binding sites for heavy metal ions (Hay, 1991).

However, there is a need to establish how the metal ions are taken onto the amino acid terminals of the moringa polypeptides. The metal ions hydrolyse in water according to the following equation (Cotton and Wilkinson, 1988; Horsfall and Spiff, 2004):

$$M^{2+} + H_2O \quad \leftrightarrow \quad M(OH)^{+} + H^{+} \qquad (13)$$

where M^{2+} is either Pb^{2+} or Cd^{2+}. Usually the resulting metal ion solution becomes more acidic. The equilibrium is set up where when the removal involves M^{2+} the equilibrium position shifts to the left indicating that the resulting solution will become more neutral. When the removal involves $M(OH)^{+}$ the equilibrium position shifts to the right indicating that the resulting solution becomes more acidic. Removal of M^{2+} is attributed to ion exchange and that for $M(OH)^{+}$ to complexation. At pH 7 there is a 1:1 ratio of Pb^{2+} and $Pb(OH)^{+}$, and Cd^{2+} and $Cd(OH)^{+}$ in lead and cadmium ion solutions respectively (Horsfall and Spiff, 2004). This means that there is a possibility of having simultaneous ion exchange (due to M^{2+}) and complexation (due to $M(OH)^{+}$).

These results show that at relatively low initial pH (pH 3 and 5 for lead and cadmium ions respectively), neutral pH and higher pH, significant metal sorption still takes place (Figures 18 and 19). Thus, removal is not only due to ion exchange (lower pH) or complexation (higher pH), but is a combination of both ion exchange and complexation between metal ions and the amino acid terminals of moringa polypeptides. Hence the proposed mechanism for metal ion removal by the moringa is ion exchange and complexation, both taking place simultaneously.

4.8 Effect of stirring time on lead and cadmium removal

The effects of stirring time are shown in Figures 20 and 21. The results showed that the rate of lead ion sorption was fast during the first 1.5 h ($p < 0.05$), and slowed before becoming constant at 4 h for both *M. oleifera* and *M. stenopetala* treatments (Figure 20). At equilibrium 51 % of lead was removed by *M. oleifera* and 55 % of lead was sorbed by *M. stenopetala*. The increase in stirring time enhanced the time for diffusion of lead ions towards the surface of the adsorbents and hence raised sorption. Nomanbhay and Palanisamy (2005) and Ahalya *et al*. (2005) reported a raise in various metal removal with stirring time using chitoson coated oil palm shell charcoal and chromium(IV) removal using the husks of bengal gram (*Cicer arientinum*) respectively.

It is interesting that cadmium removal quickly increased to the maximum quantity in the first 15 minutes and decreased subsequently with stirring time (Figure 21). The decrease was significant between 0.25 to 2 h ($p < 0.05$). Subsequently, there was little decrease in metal desorption with additional stirring time. Herrera *et al.* (2003) reported reduced biosorption of silver ions to alfalfa biomass with time. This is probably due to the formation of unstable complexes, which easily break as the time of stirring increases. The weakness of cadmium ions-moringa complexes is enhanced by the fact that cadmium, due to its high charge density and small size, is more hydrolysed by water than lead. This results in the competition for cadmium between water and binding sites on the moringa. Hence with increase in stirring time cadmium ions go back into the aqueous solution. Clearly, lead ions form stronger complexes with moringa seed powders than cadmium ions.

The comparison between effects of stirring time for cadmium and the effects of standing time as given in Figures 8 and 9 shows that there are contrasting results. Effects of standing time at a dose of 1.0 g/100 mL show that for both seed powders stirring for 1 h only and filtering after 24 h resulted in higher cadmium ion sorption than stirring for 1 h and filtering immediately. This is the case since without a further increase in stirring time the complexes formed between moringa binding sites and cadmium metal ions do not further disintegrate. Hence there is more time for metal ions to diffuse towards the binding sites when the aliquot is filtered after 24 h than when it is filtered after 1 h.

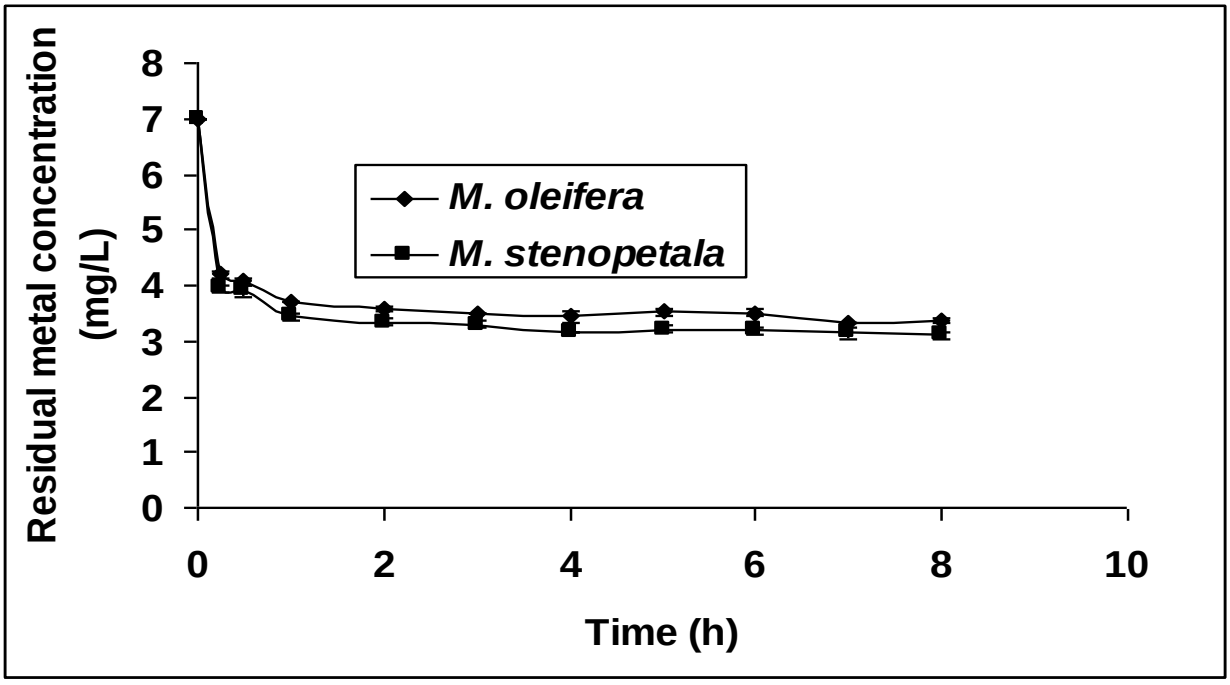

Figure 20: Residual Pb vs time using 1.0 g/100 mL moringa seed powders at initial Pb(II) concentration of 7 mg/L and 30^{o}C.

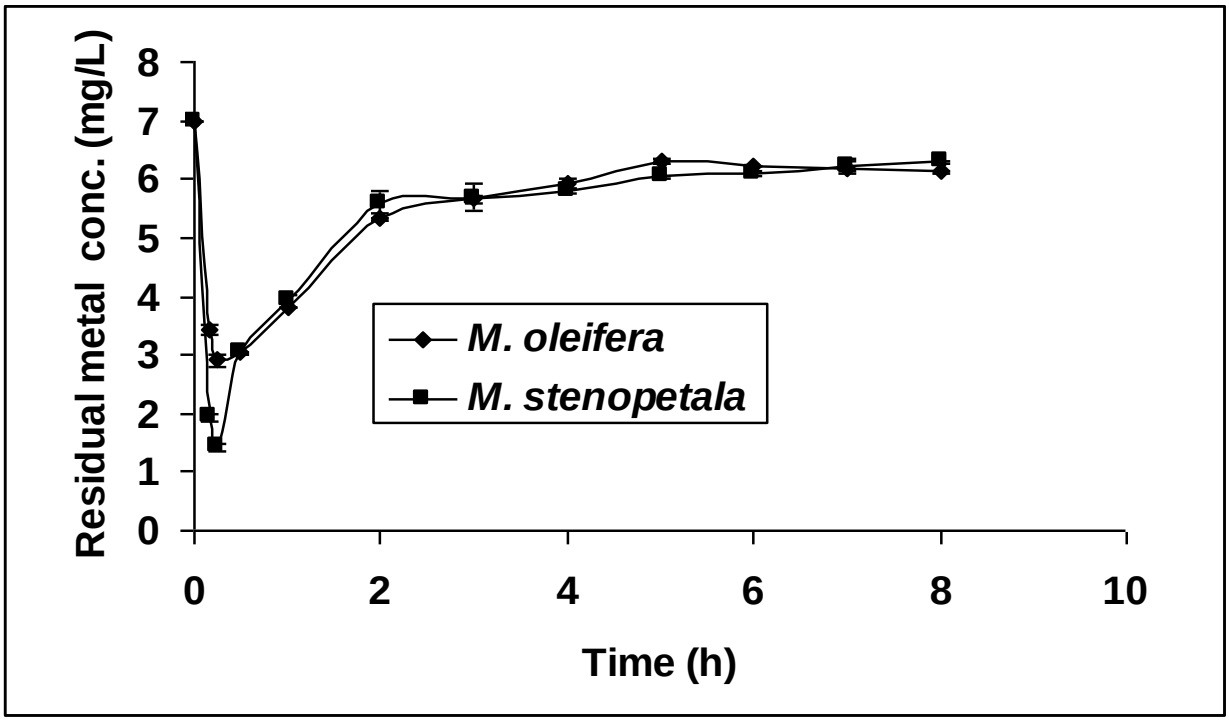

Figure 21: Residual Cd vs time using 1.0 g/100 mL moringa seed powders at initial Cd(II) concentration of 7 mg/L and 30^{o}C.

4.9 Effect of initial metal ion concentration on lead and cadmium removal

The relationship between metal uptake and initial metal concentration is shown in Figures 22 and 23. The effect of initial metal ion concentration is an important parameter, which determines the maximum uptake capacity of the metal ion-biosorbent systems (Reddy and Reddy, 2003). The increase in initial Cd(II) and Pb(II) concentrations enhanced Cd(II) uptake per unit mass of the moringa powders (mg/g) and Pb(II) uptake per unit mass for *M. stenopetala* treatment ($p < 0.05$). However, the metal uptake for lead *M. oleifera* treatment did not change significantly ($p > 0.05$ in Figure 22). The lead uptake ranged from 0.349 to 0.354 mg/g and 0.375 to 0.448 mg/g for *M. oleifera* and *M. stenopetal*a respectively and that for Cd(II) ions ranged from 0.065 to 0.092 mg/g and 0.065 to 0.113 mg/g for *M. oleifera* and *M. stenopetala* respectively. The enhanced metal uptake with a raise in initial metal ion concentration might be due to an increase in the ratio of initial number of moles of Pb(II) or Cd(II) to the available surface area of the powders. Horsfall and Spiff (2004) and Krishnan and Anirudhan (2003) reported increase in metal uptake with initial metal concentration using wild cocoyam and steam activated sulphurised carbon prepared from sugar cane bagasse pith respectively. However, when saturation of binding sites is achieved no significant increase in metal ion removal is observed hence *M. oleifera* treatment for lead ions suggests that the binding sites were already saturated even at lower initial concentrations of lead ions since there was no significant increase in metal uptake. Figures 22 and 23 clearly show that *M. stenopetala* is more effective than *M. oleifera* for both lead and cadmium ion sorption. This is consistent with results described elsewhere (section 4.1) which show that *M. stenopetala* is more effective than *M. oleifera*. Furthermore, lead ions are better sorbed than cadmium ions ($p < 0.05$).

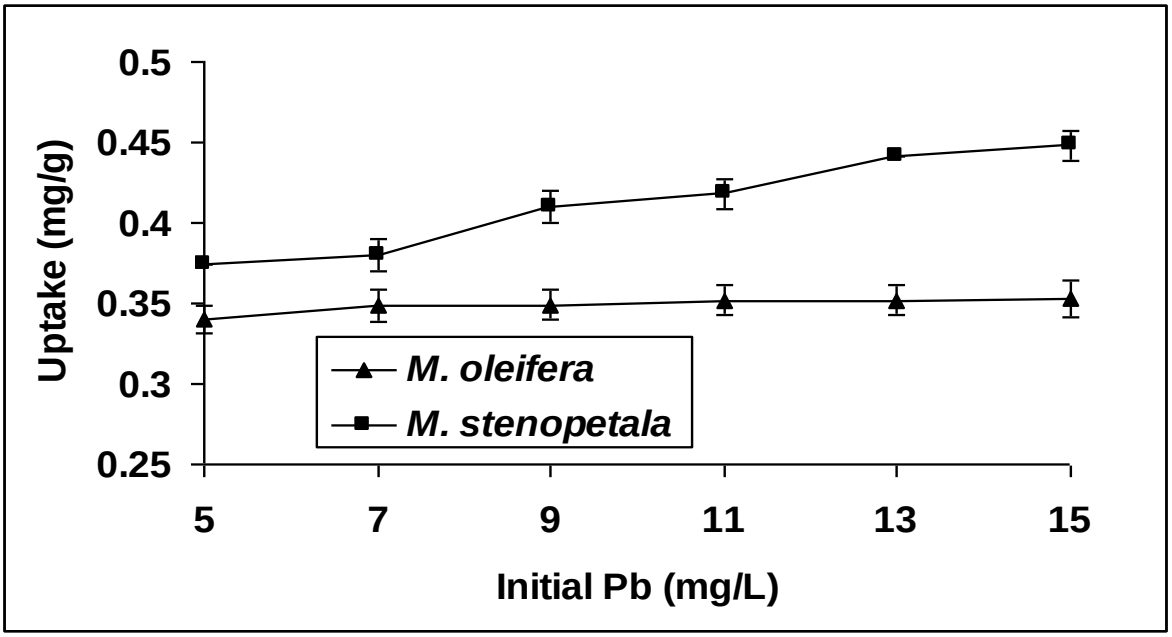

Figure 22: Pb uptake vs initial Pb concentration using 1.0 g/100 mL moringa powders at 30ºC.

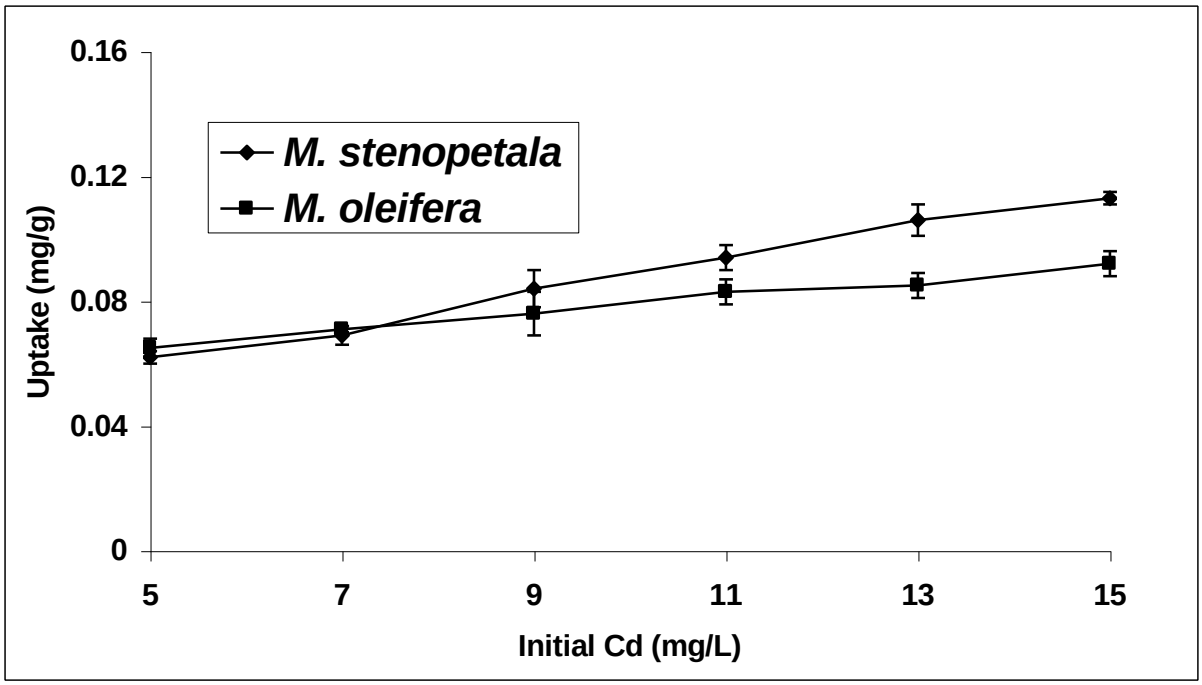

Figure 23: Cd uptake vs initial Cd concentration using 1.0 g/100 mL moringa powders at 30ºC.

4.10 Effect of ionic strength (mol/L sodium chloride) on lead and cadmium removal

The effects of ionic strength on lead and cadmium removal are shown in Figures 24 and 25. The results, generally, showed that both Pb(II) and Cd(II) uptake decreased with an increase in the concentration of sodium chloride from 0.0 to 1.0 mol/L. An increase in ionic strength suppresses metal uptake as a result of screening of electrostatic charge (Scheiwer and Volesky, 1997). Alternatively, other sorbable ions can compete with the divalent cations of interest for binding to the biomass, affecting biosorption (Scheiwer and Wong, 2000; Krishnan and Anirudhan, 2003). The competition depends on two major factors, namely, the competition of cations for the same binding sites and the strength of these binding sites. In general, ions like sodium, which are weakly bound through mostly electrostatic attraction, are effective in competing only with other weakly bound ions. However, even heavy metals, which tend to form inner complexes are partially bound through electrostatic attraction as a consequence of the higher concentration of all metals near binding sites, than in the bulk solution (Scheiwer and Volesky, 2000). Therefore, their covalent binding is consequently also reduced when background salts are added.

The decrease in lead ion sorption with the increase in ionic strength for both moringa seed powders is less than that for cadmium sorption. This is the case since cadmium ions are bound more weakly to the binding sites of the powders than lead ions. This observation is consistent with the trend observed on the effects of contact time and hence confirms that cadmium adsorption involves weak physisorption.

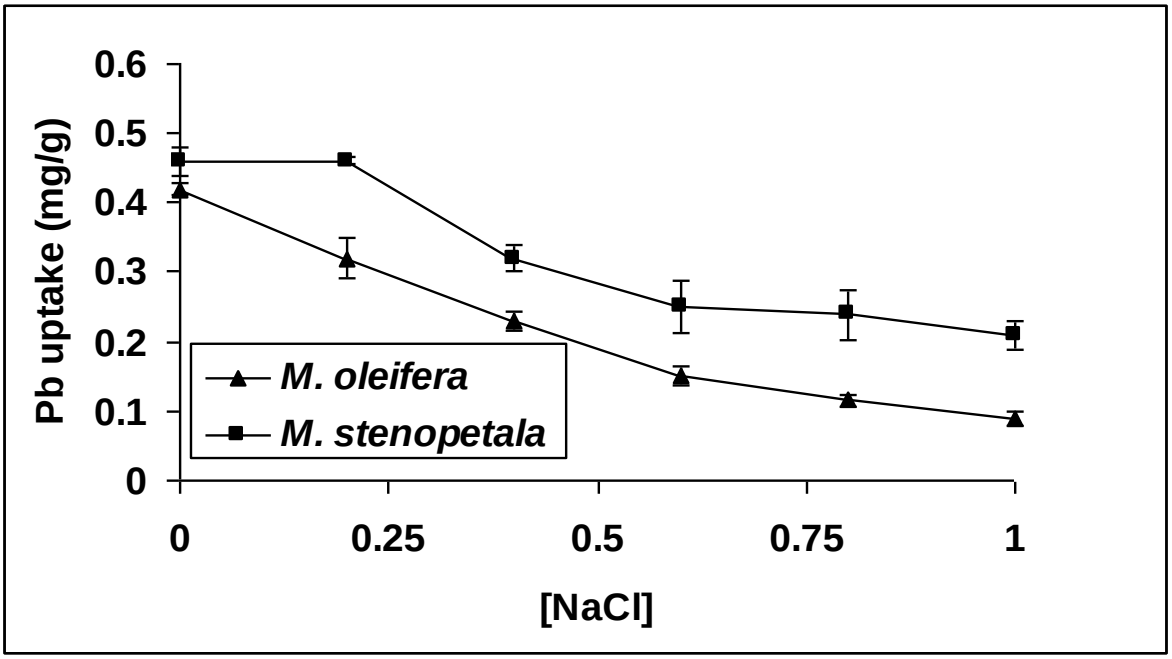

Figure 24: Pb uptake vs ionic strength using 1.5 g/100 mL moringa dosage at initial Pb concentration of 7 mg/L and 30ºC.

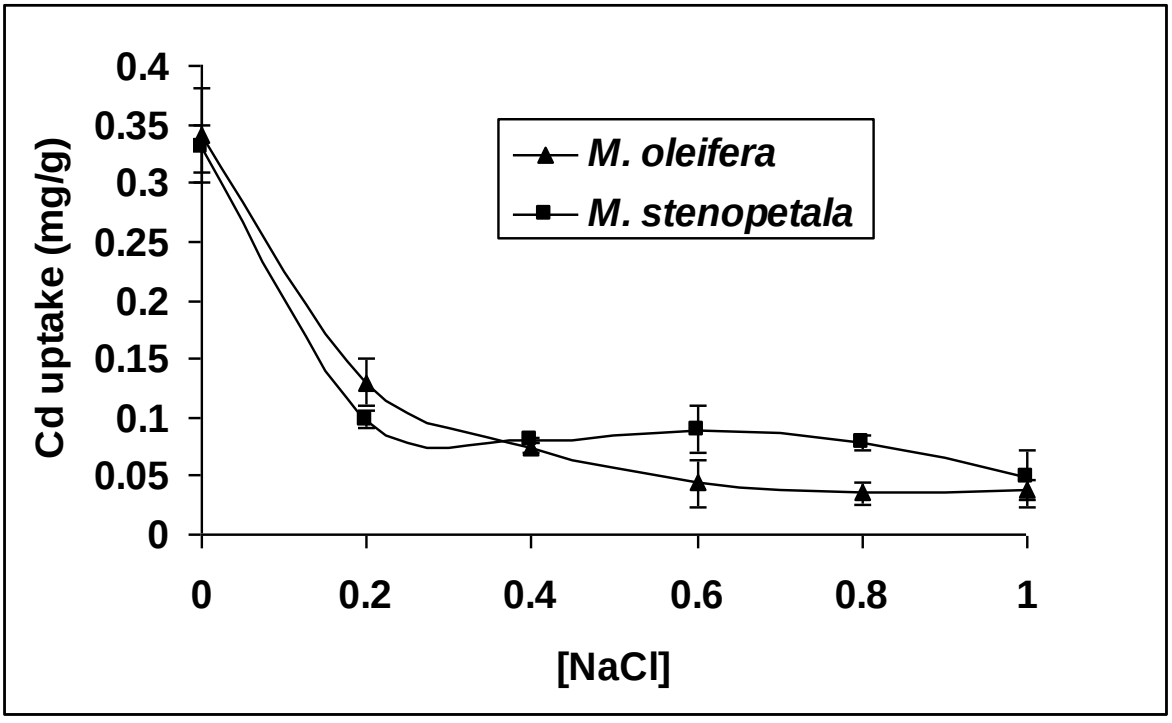

Figure 25: Cd uptake vs ionic strength using 1.5 g/100 mL moringa dosage at initial Cd concentration of 7 mg/L and 30ºC.

4.11 Effect of water hardness ions on metal removal

The effects of different concentrations of carbonates/bicarbonates and magnesium/calcium mixtures are shown in Figures 26 and 27. The effect of water hardness varied depending on the ions involved. Generally, the results showed that as the concentration of carbonates/bicarbonates was raised the total metal ion removal increased. The optimum carbonates/bicarbonates hardness was 100 mg/L for lead ion sorption (Figure 26) and 50 mg/L for cadmium ion sorption (Figure 27). This observed trend is likely due to the formation of low solubility lead carbonates and cadmium carbonates (K_{sp} =7.4 x 10^{-14} and 1.0 x 10^{-12} respectively), which precipitate out of the solution (McGinnes, 2002). Furthermore, as the concentration of carbonate/bicarbonates mixture increased the pH of the resulting solution also increased. Hence, the competition for binding sites between metal ions and H^+ ion is likely reduced. The final pH at optimum carbonates/bicarbonates for lead ion sorption was 3.03 using *M. oleifera* and 3.05 using *M. stenopetala*. This compares well to the optimum pH results obtained in section 4.7. On the other hand the final pH at the optimum carbonates/bicarbonates for cadmium ion sorption concentration was 6.34 using *M. oleifera* and 3.30 using *M. stenopetala*. Generally, the pHs obtained showed that metal ions are predominantly sorbed by the moringa powders as compared to precipitation as hydroxides or carbonates. More information on water hardness and pH is in appendix 1

For the Mg/Ca concentration there is no general trend for lead ion sorption presumably because lead ions form stronger complexes with binding sites on the moringa powders hence it is able to compete well with the other metal ions for binding sites. A slight decrease was observed for cadmium removal. Herrera *et al.* (2003) also reported a decrease in silver ion sorption capacity from aqueous solutions in the presence of Mg^{2+} and Ca^{2+} independently. Magnesium and calcium ions are hard acids. Therefore, they are likely to replace cadmium ions (soft acid) from their binding sites when binding involves oxygen ligands, which are hard bases (Herrera *et al.*, 2003). This suggests that oxygen ligands are involved in metal ion sorption from water.

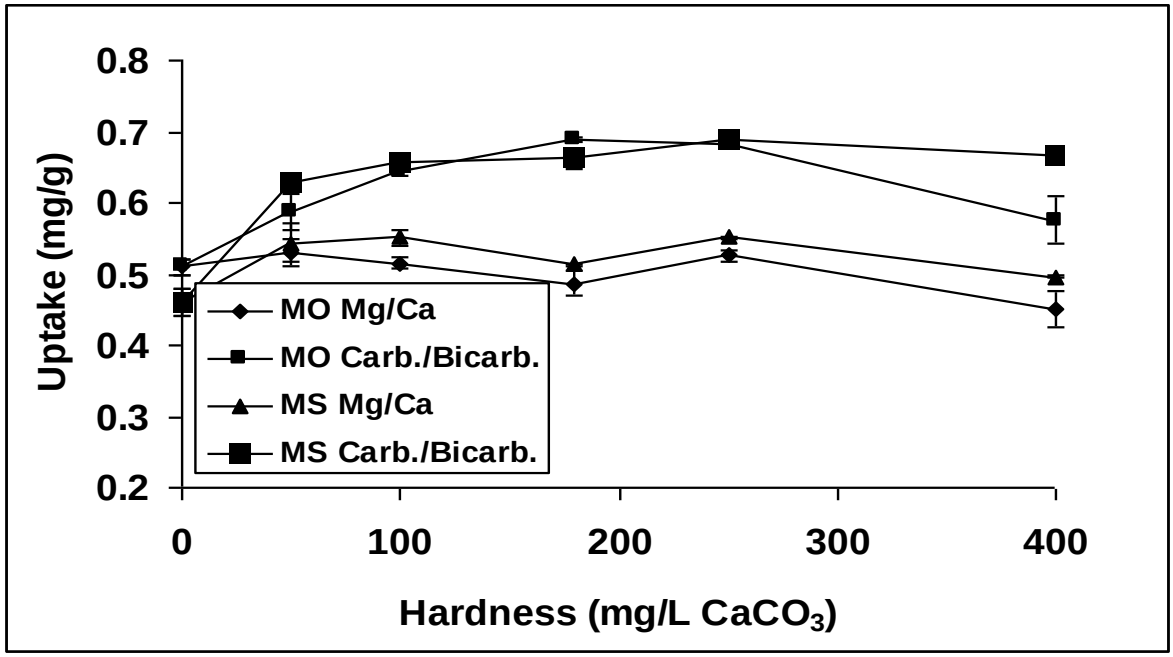

Figure 26: Pb uptake vs water hardness using 1.5 g/100 mL of moringa whole seed powders at 7 mg/L Pb concentration and 30^{o}C.

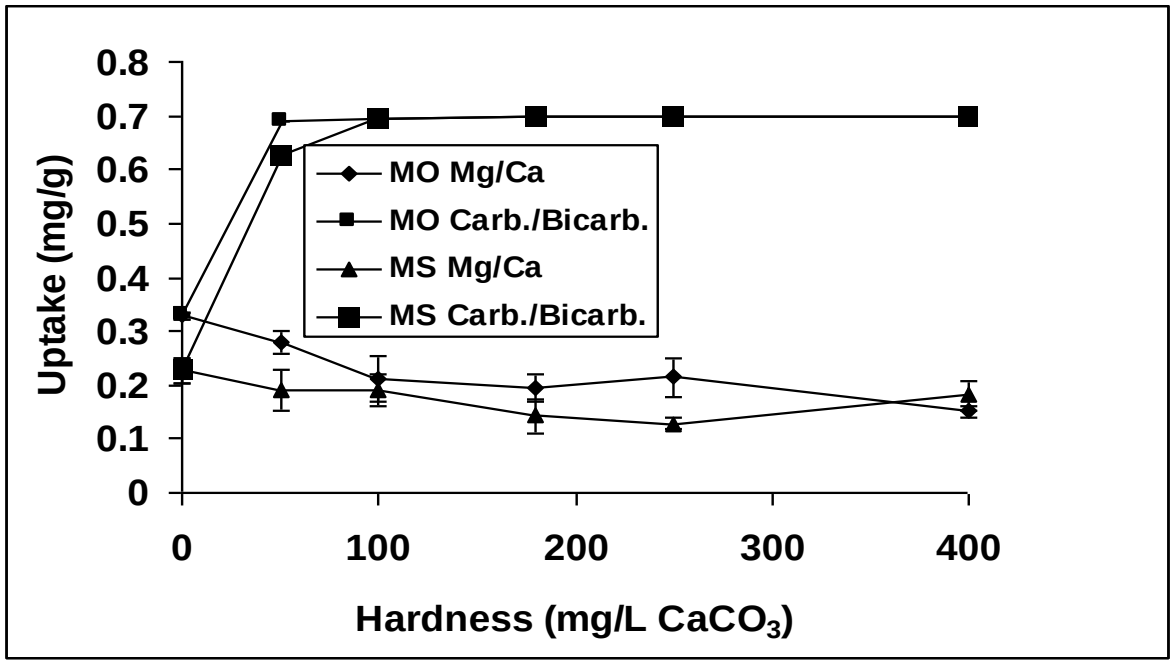

Figure 27: Cd uptake vs water hardness using 1.5 g/100 mL moringa whole seed powders at 7 mg/L Cd concentration and 30^{o}C.

4.12 Adsorption isotherms for metal removal

4.12.1 Langmuir isotherms

Lead ion sorption data fitted well to the Langmuir isotherms for both *M. oleifera* and *M. stenopetala* (R^2 = 0.9996 and 0.9957 respectively) (Figure 28). They seem to show that monolayer sorption is more favoured than multilayer (Casey, 1997). When compared, no significant difference exists in lead ion sorption by *M. oleifera* and *M. stenopetala* ($p > 0.05$). However, q_{max} for *M. stenopetala* treatment (0.416 g/kg) is 16 % higher than that for *M. oleifera* treatment (0.351 g/kg). This shows that *M. stenopetala* has better sorption capacity than *M. oleifera*; consistent with results described elsewhere (Section 4.1).

The cadmium data also fitted well to the Langmuir sorption model for both moringa seed powders, $R^2 > 0.93$, (Figure 29). This suggests that monolayer sorption is also present in the removal of cadmium ions from water using both moringa powders. No significant difference exists in cadmium sorption between the two seed powders ($p > 0.05$). From Table 3, however, the Langmuir data shows that the maximum adsorption, q_{max}, for *M. stenopetala* is 115 % higher than that for *M. oleifera*.

Comparing K_L values for Pb^{2+} and Cd^{2+} clearly shows that lead which has a smaller K_L value is better sorbed by moringa than cadmium because the higher the K_L value the larger the hydrolysis energy. Metals with higher hydrolysis energy form strong metal aquo complexes hence it is difficult to displace the water molecule for a binding site and therefore lower sorption capacity (Horsfall and Spiff, 2005a). This is consistent with results described in section 4.2.

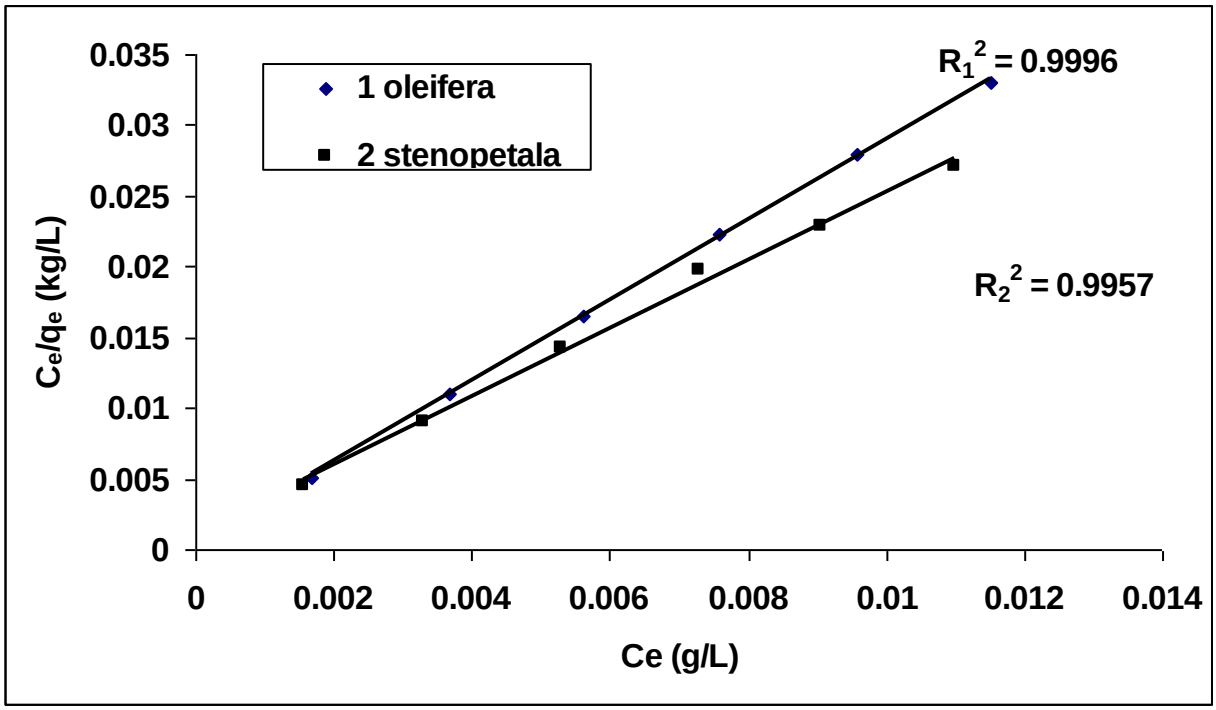

Figure 28: Langmuir isotherms for Pb sorption using 1.0 g/100 mL moringa powders at 30^{o}C.

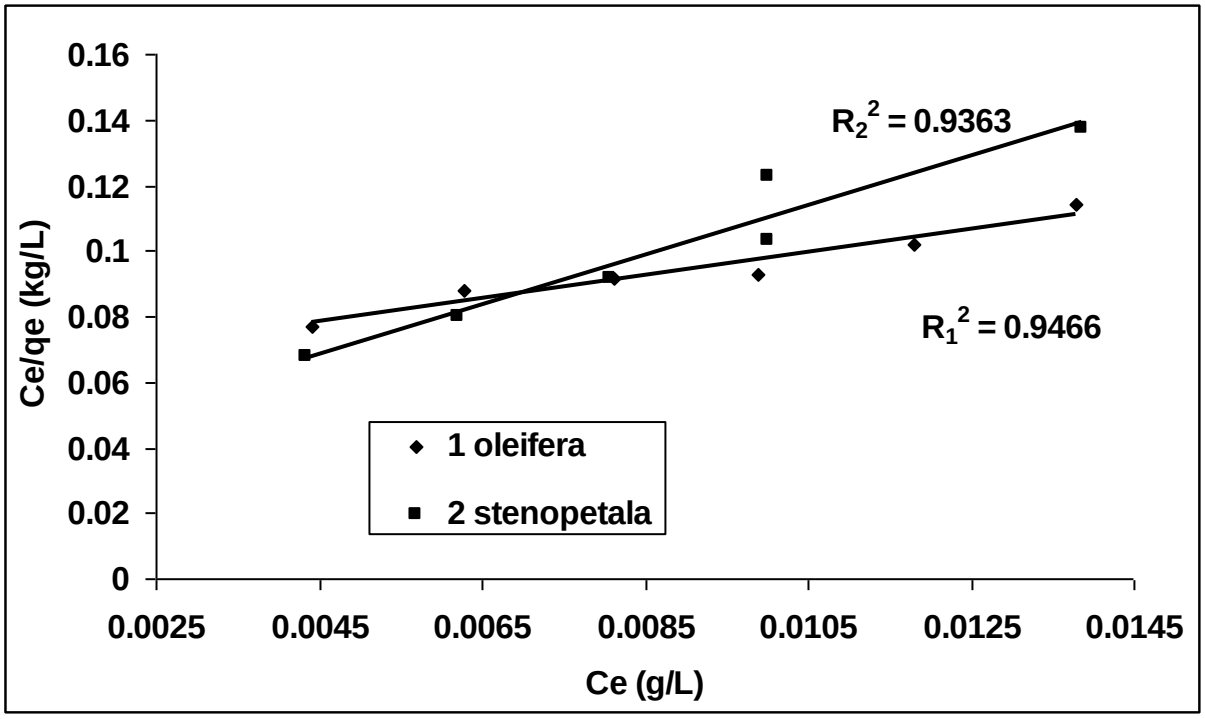

Figure 29: Langmuir isotherms for Cd sorption using 1.0 g/100 mL moringa powders at 30^{o}C

Table 3: Key parameters from Langmuir adsorption isotherms

Langmuir constants						
Metal coagulant	Cadmium			Lead		
	q_{max} (g/kg)	K_L	R^2	q_{max} (g/kg)	K_L	R^2
M. oleifera	0.132	0.0045	0.9466	0.351	0.0018	0.9996

4.12.2 The Freundlich isotherms

The lead ion sorption data fitted as well to the Freundlich isotherms for both *M. oleifera* and *M. stenopetala* treatments (R^2 = 0.8715 and 0.8821 respectively in Figure 30). This suggests that multilayer sorption mechanism is also involved in lead ion sorption (Casey, 1997). The values of n and K_f for *M. stenopetala* treatment are greater than those for *M. oleifera*. This agrees with the observation that *M. stenopetala* is more effective than *M. oleifera* in metal ion sorption from aqueous solutions.

The cadmium data fitted much better to the Freundlich sorption model, $R^2 > 0.93$, (Figure 31) than lead data. There was also no significant difference on cadmium ion sorption by the two powders ($p > 0.05$). From Table 4, the values of $1/n$ for both powders, from Freundlich plots, were slightly less than unity. This indicates that although adsorption is favoured there is also significant desorption taking place as the reaction progresses (Horsfall and Spiff, 2005a). This observation confirms the trends observed in the effects of stirring time on cadmium sorption and that the interaction between moringa seed powders and cadmium ions is weak.

The dependence of cadmium adsorption on two different isotherms (Langmuir and Freundlich) suggests that there are both strong binding sites and weak binding sites on the moringa biomass surface (Duta *et al.,* 2004). If adsorption occurs until all the strong binding sites are occupied, adsorption process follows Langmuir isotherm. When most of the strong binding sites have bonded, weaker sites will likely dominate the metal sorption. However, there are still both strong binding sites and weak binding sites involved in the metal sorption that result

in heterogeneity of the adsorption surface. This heterogeneous adsorption follows the Freundlich model. The adsorption of arsenate and arsenite on titanium dioxide suspension has been explained in similar way (Duta *et al.*, 2004). From the Freundlich parameters, *n* and K_f for lead ion sorption are greater than those for cadmium ion sorption. These support the observation that moringa powders are more effective in lead ion removal than cadmium ion.

Table 4: Key parameters from Freundlich adsorption isotherms

Freundlich constants						
Metal coagulant	Cadmium			Lead		
	n	K_f	R^2	*n*	K_f	R^2
M. oleifera	1.42	2.66	0.9708	11.31	385	0.8715
M. stenopetala	1.37	2.08	0.9458	35.19	1.74×10^{14}	0.8821

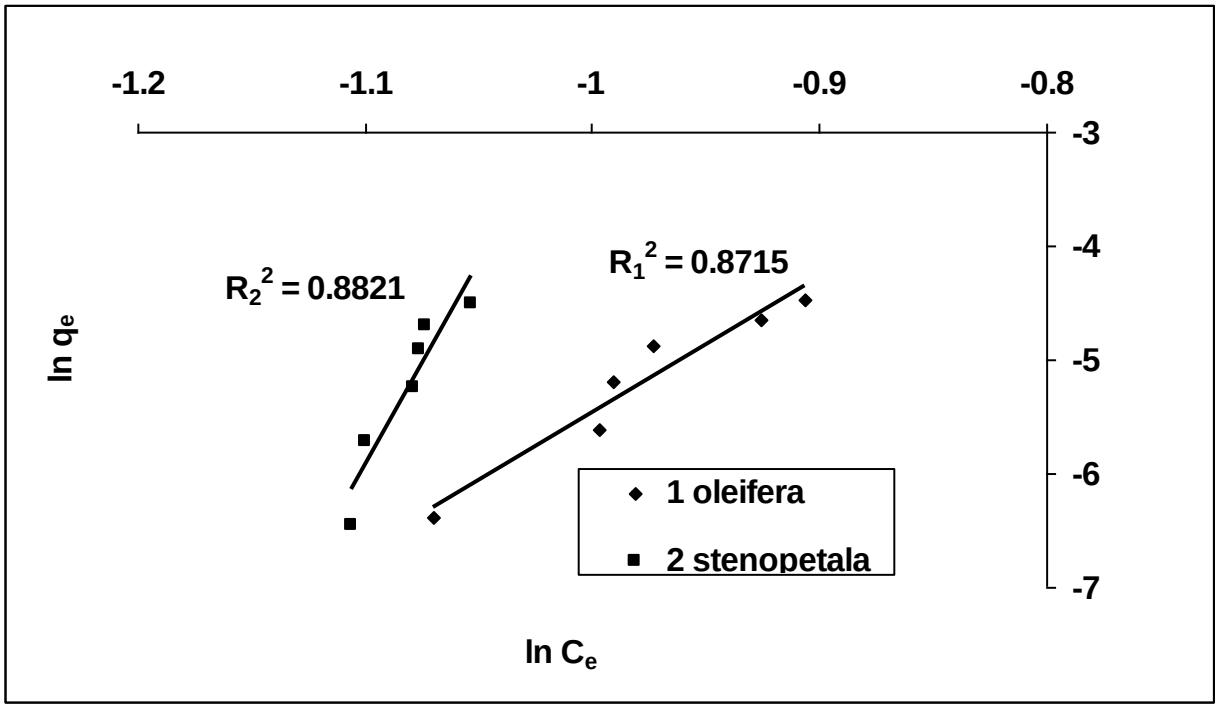

Figure 30: Freundlich isotherms for Pb sorption using 1.0 g/100 mL moringa powders at 30ºC

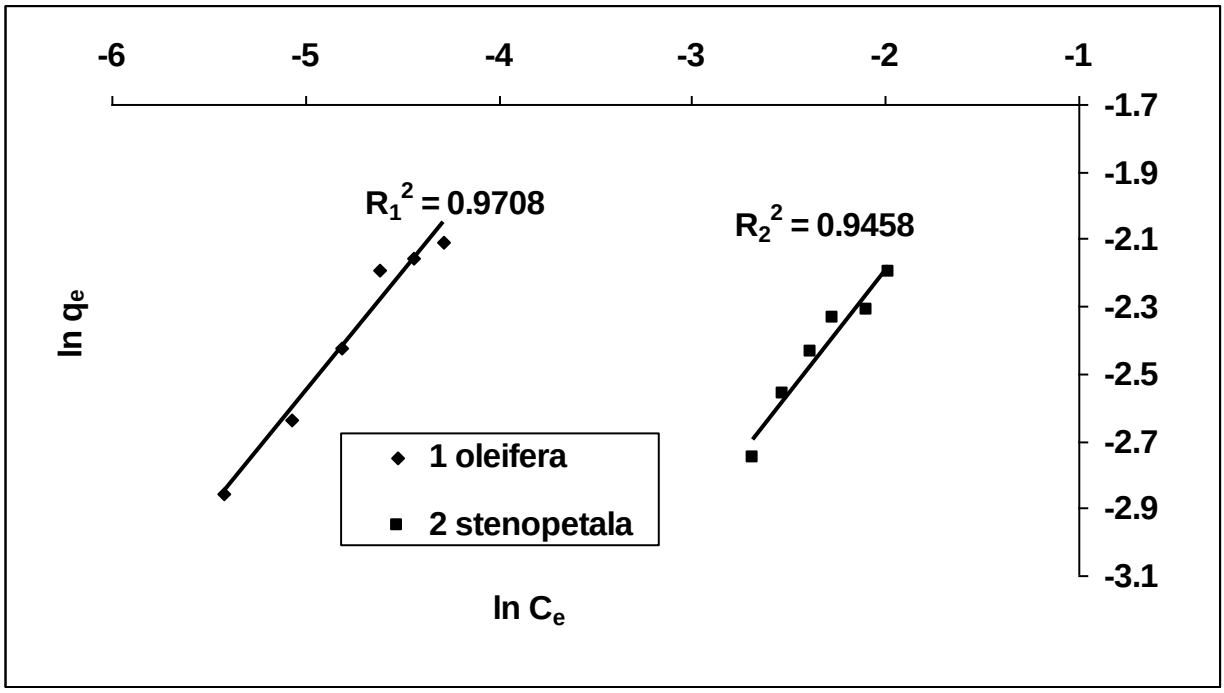

Figure 31: Freundlich isotherms for Cd sorption using 1.0 g/100 mL moringa powders at 30^{o}C

4.12.3 Dubinin-Radushkevich Isotherms

The sorption data was plotted according to the linearised form of the Dubinin-Radushkevich isotherm (Figures 32 and 33). Lead ion sorption fitted well to the D-R model ($r > 0.90$). This suggests that (i) lead ion sorption on both moringa powders can be perfectly explained by Gaussian distribution curve (Marsh and Land, 1970), and (ii) lead ion adsorption process is dominated by micropore filling as assumed by the D-R adsorption model (Marsh and Land, 1970). Cadmium ion sorption fitted better to the D-R plot ($R^2>0.97$) than lead sorption. This suggests that cadmium sorption more dominated by micropore filling than lead sorption and can be explained by the Gaussian distribution curve. The values of maximum adsorption capacities (X_m), correlation coefficient (R^2), a constant related to energy of adsorption (K) and mean energy of adsorption (E_a) are given in Table 5. For both metal ions, X_m for metal sorption using *M. stenopetala* is higher than that for *M. oleifera*. Energies of adsorption for lead ions using *M. oleifera* and *M. stenopetala* treatments, 20.41 kJ/mol and 11.95 kJ/mol respectively, are greater than 8 kJ/mol suggesting that lead ions bind to higher energy sites and hence their removal from aqueous solutions involve a chemical change, possibly strong ion exchange. Energy of adsorption less than 8.0 kJ/mol means the dominant mechanism of metal

sorption is physisorption (Yan, 2001). The values of energy of adsorption for cadmium ion sorption for *M. oleifera* and *M. stenopetala* are 3.68 kJ/mol and 4.58 kJ/mol respectively. This indicates that Cd(II) binds to low energy sites and hence involves physisorption through weak electrostatic interaction. The weak binding is also responsible for the decrease in metal uptake with an increase in time of stirring as reported in section 4.7 because the complexes formed are easily broken.

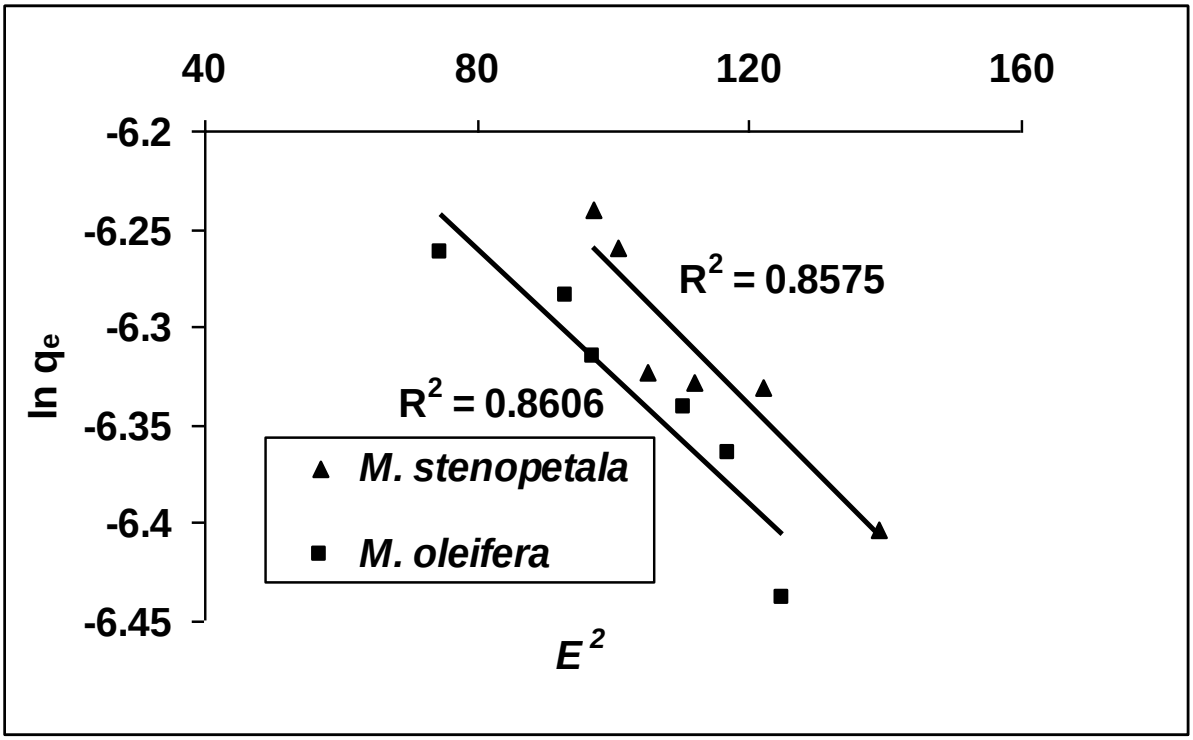

Figure 32: Dubinin-Radushkevich isotherms for Pb sorption using 1.0 g/100 mL moringa powders at 30 ^{o}C

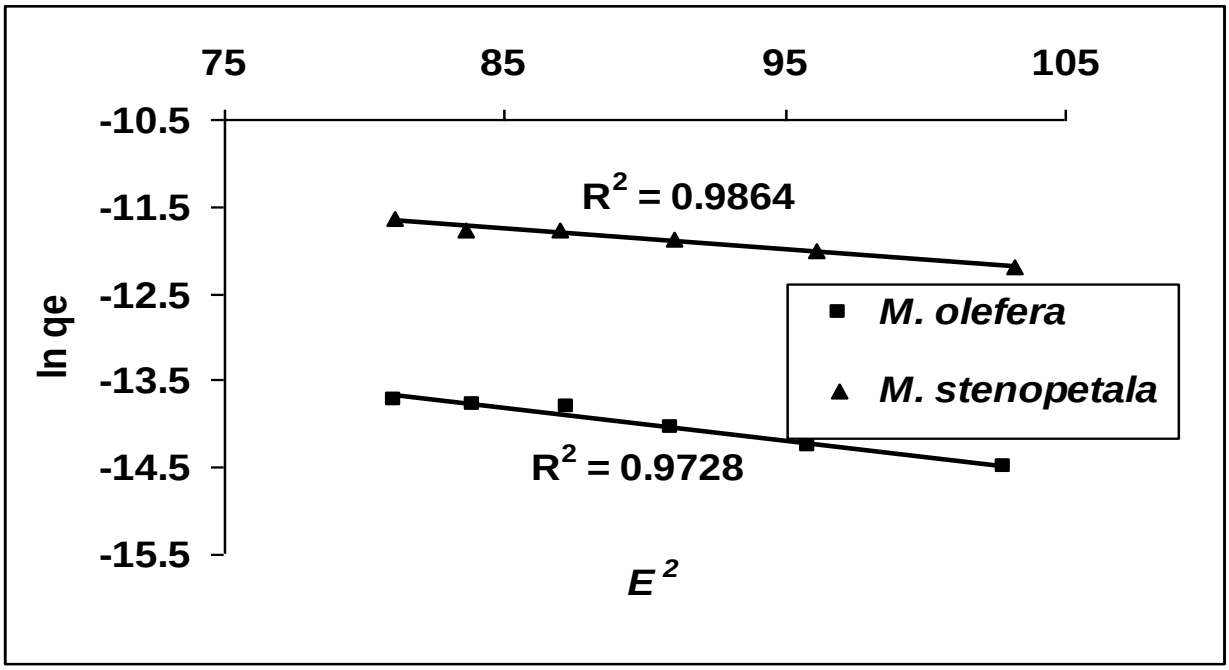

Figure 33: Dubinin-Radushkevich isotherms for Cd sorption using 1.0 g/100 mL moringa powders at 30 ^{o}C

Table 5: Key parameters from Dubinin-Radushkevich isotherms.

Metal ions	Powder	X_m (molkg^{-1})	K (mol^{-2}kJ^{-2})	E (kJmol^{-1})	R^2
Pb^{2+}	*M. oleifera*	0.00185	0.0012	20.41	0.8606
	M. stenopetala	0.0200	0.0035	11.95	0.8575
Cd^{2+}	*M. oleifera*	0.0229	0.0370	3.68	0.9728
	M. stenopetala	0.0598	0.0238	4.58	0.9864

4.13 Kinetics of lead and cadmium ion sorption from water

The kinetics of lead ion sorption over 0 - 3 h period for both seed powders are shown in Figures 34 and 35 respectively. The data was tested against the Lagergren pseudo first order kinetics equation:

$$\ln(q_e - q_t) = kt \qquad (14)$$

where q_e and q_t, (g/kg) are the amount of lead ions adsorbed per unit mass at equilibrium and at time *t* respectively and *k* is the rate constant. The results show that lead ion sorption data fitted well to Lagergren first order kinetics for both *M. oleifera* and *M. stenopetala* treatments of lead water (R^2 = 0.9748 and 0.8620 respectively). The rate constants were 2.410 x $10^{-4}s^{-1}$ and 2.394 x $10^{-4}s^{-1}$ for *M. oleifera* and *M. stenopetala* treatments respectively at 30^{o}C, suggesting that the rates of lead ion sorption using both moringa powders are not significantly different ($p > 0.05$). Izanloo and Nasseri (2005) and Jianlong *et al.* (2001) have reported first order kinetics on cadmium and lead ion sorption from aqueous solutions using pine cone powders and fungal biomass of *Aspergillus niger* respectively. Reported first order rate constants for other biomasses, the two strains of yeast *Saccharomyces cerevisiae* and *Phaenerochete chrysosporium* on copper sorption from aqueous solutions are 9.440 x $10^{-6}s^{-1}$ and 6.340 x $10^{-5}s^{-1}$ respectively at biomass concentration of 1.0 g/100 mL (St. Mihova and Godjevargova, 2001). Also first order rate constant for the biosorption of lead ions from aqueous solutions at a concentration of 5 mmol/L was 1.690 x $10^{-5}s^{-1}$ (Nabizadeh *et al.*, 2005). This shows that metal biosorption using the studied moringa biomasses is faster than that of *Saccharomyces cerevisiae* and *Phaenerochete chrysosporium* on copper sorption from aqueous solutions.

The kinetics of cadmium ion removal using *M. oleifera* and *M. stenopetala* are shown in Figures 36 and 37 respectively. Zero order kinetics was integrated using the equation:

$$C_e = -kt + C_o \qquad (15)$$

where C_e (mg/L) is the concentration at time *t* and C_o (mg/L) is the concentration at time t_o and *k* is the rate constant. The negative rate constants obtained suggest that desorption was more favoured than adsorption.

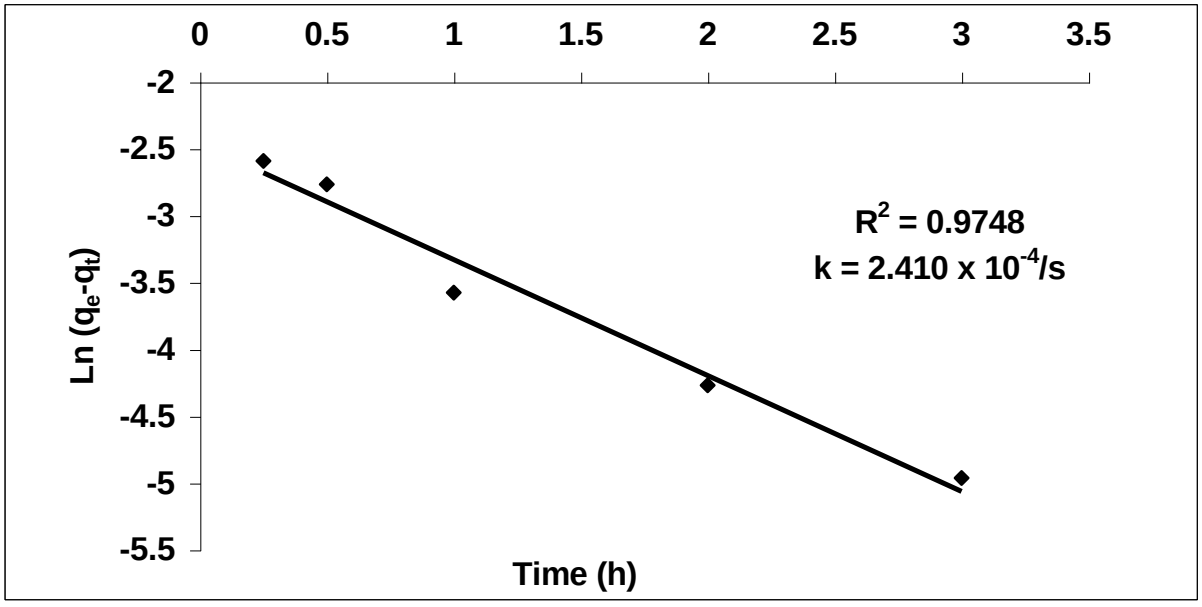

Figure 34: Lagergren plot for lead sorption using 1.0 g/100 mL *M. oleifera* whole seed powder at initial metal concentration of 7 mg/L and 30°C.

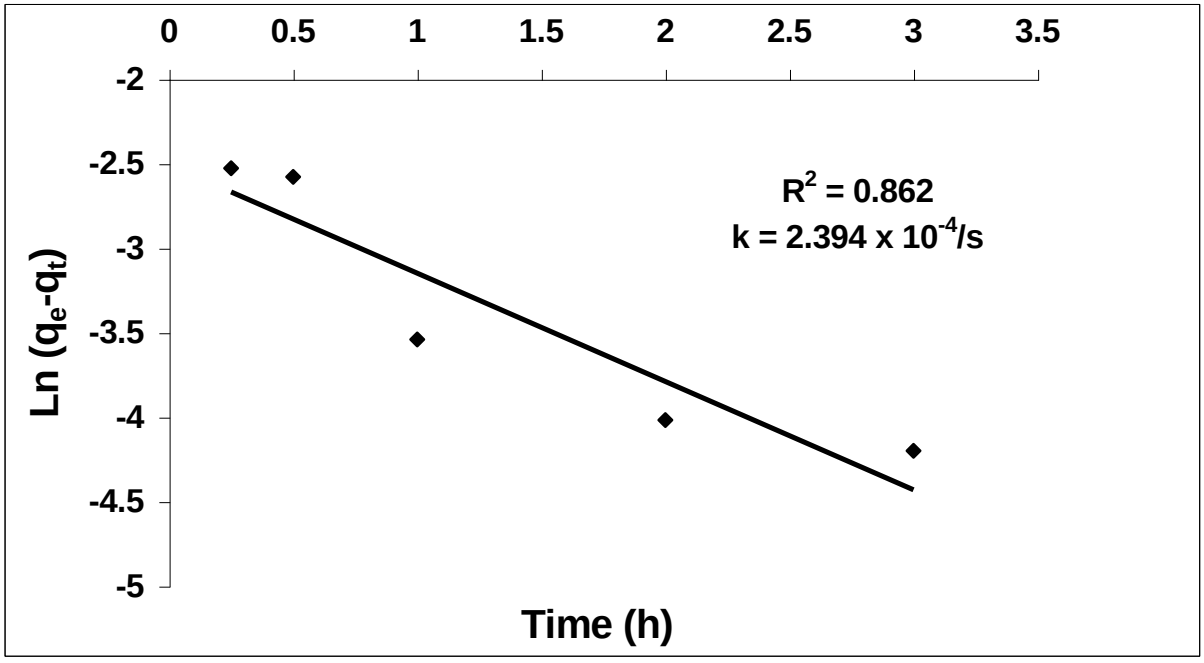

Figure 35: Lagergren plot for lead sorption using 1.0 g/100 mL *M. stenopetala* whole seed powders at initial metal concentration of 7 mg/L and 30°C.

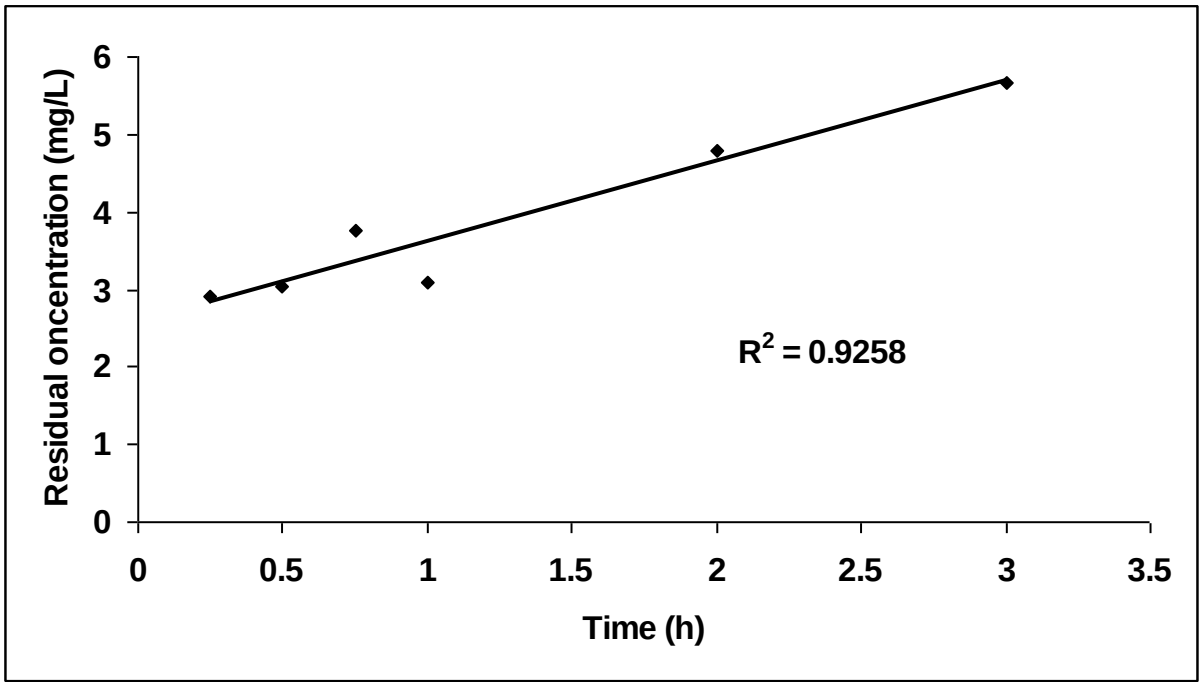

Figure 36: Kinetics of cadmium sorption using 1.0 g/100 mL *M. oleifera* whole seed powder at initial metal concentration of 7 mg/L and 30^{o}C.

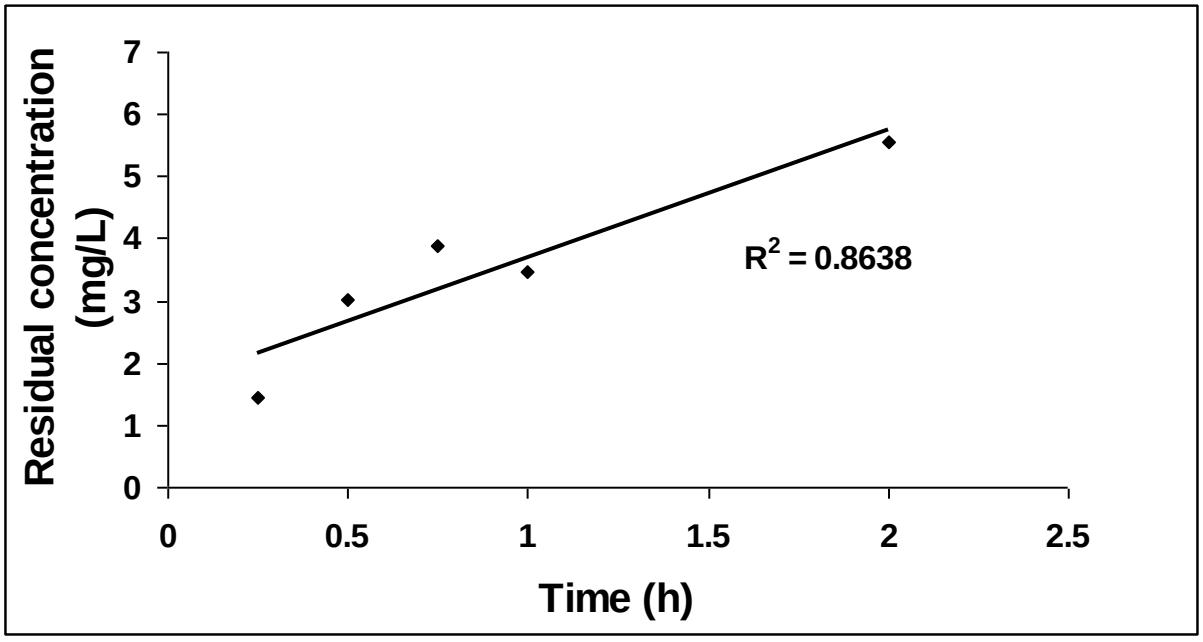

Figure 37: Kinetics of cadmium sorption using 1.0 g/100 mL *M. stenopetala* whole seed powders at initial metal concentration of 7 mg/L and 30^{o}C**.**

4.14 Effect of temperature on lead and cadmium removal from water

The effects of temperature on metal uptake are shown in Figures 38 and 39. The lead uptake increased with temperature (Figure 38). This indicates strong binding between lead ions and moringa powders binding sites. Furthermore, the reaction is likely to be endothermic (Weng, 2002). Manju *et al.* (1998) and Ho (2003) reported similar results on metal biosorption using coconut husk carbon and tree fern respectively.

However, the cadmium uptake decreased as the temperature increased in the range of 0 - 40^{o}C (0.46 – 0.34 mg/g) for *M. oleifera* and 0 - 60^{o}C (0.43 – 0.19 mg/g) for *M. stenopetala* treatment before increasing to 0.67 and 0.69 mg/g for *M. oleifera* and *M. stenopetala* respectively at 100 ^{o}C (Figure 39). Results of temperature dependence were similar to those found in stirring time dependence. This observed initial decrease in cadmium uptake with increasing temperature also suggests weak binding interaction between the binding sites and the cadmium ions, which supports physisorption. This conclusion supports the values of adsorption energies obtained from D-R isotherms. Furthermore, physical adsorption reactions are normally exothermic, hence the extent of adsorption generally increases with a decrease in temperature (Salinas *et al.,* 2000). Izanloo and Nasseri (2005) and Herrera *et al.* (2003) have reported a decrease in cadmium removal using ground cone powders and silver(I) ions adsorption by alfalfa biomass respectively with temperature. The sorption of cadmium starts to increase at 40^{o}C for *M. oleifera* and at 60^{0}C for *M. stenopetala*. As the temperature is increased, a number of bonds in the polypeptides, which are the likely sorption agents, are weakened. The first to be affected are the long range interactions that are necessary for the presence of tertiary structure. As these bonds are first weakened and are broken, the polypeptide obtains a more flexible structure and the groups are exposed to solvent (Hay, 1991). This exposure of more groups to the solvent presumably increased the number of binding sites for enhanced cadmium ion sorption.

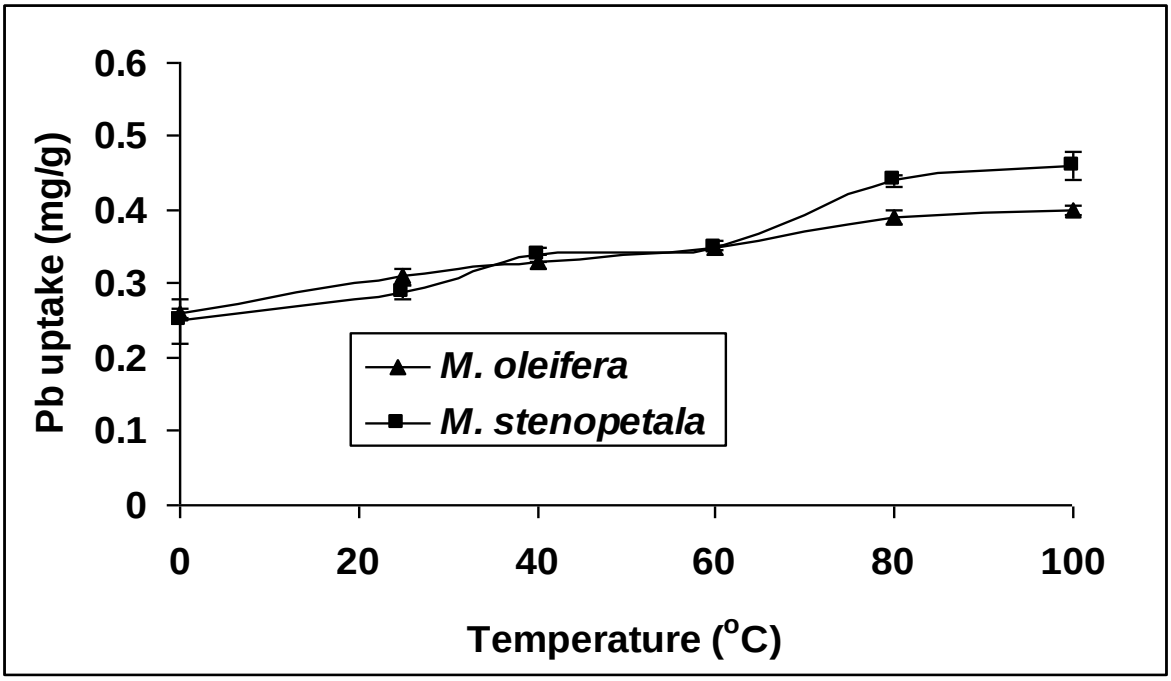

Figure 38: Pb uptake vs temperature using 1.0 g/100 mL moringa powders at initial Pb concentration of 7 mg/L

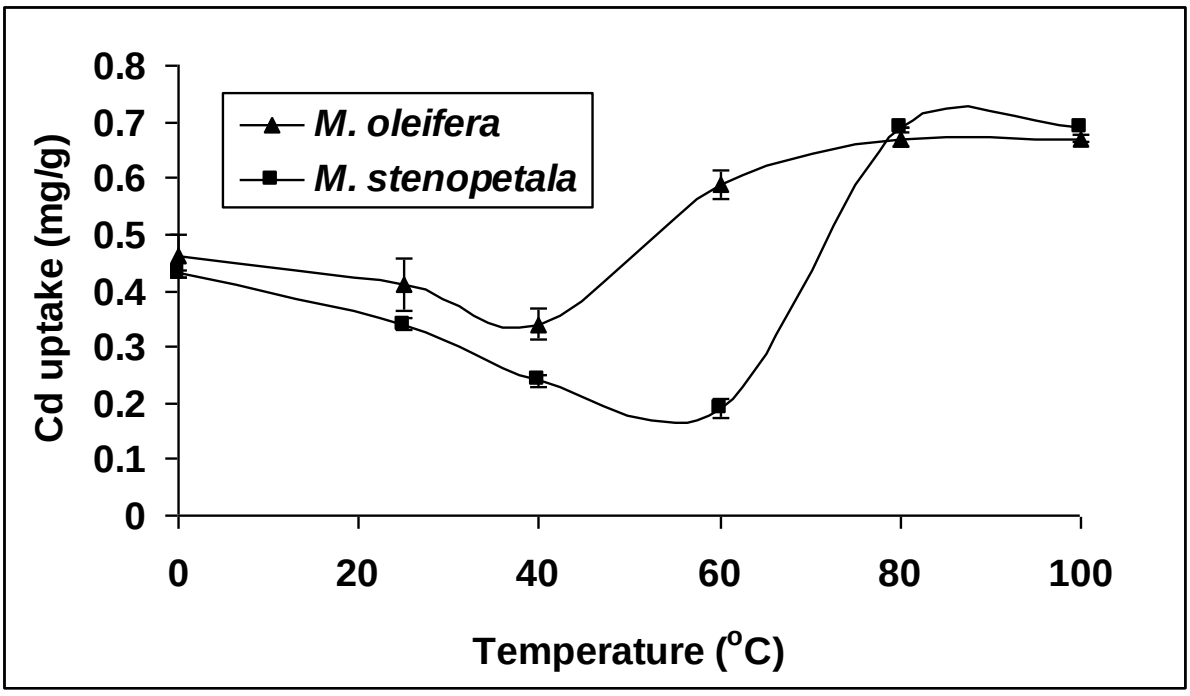

Figure 39: Cd uptake vs temperature using 1.0 g/100 mL moringa powders at initial Cd concentration of 7mg/L

4.15 Desorption of metal loaded biomass

From the observations on the effects of pH on metal ion sorption, it was hypothesised that a decrease in pH could lead to desorption of metal ions from the biomass. It was found that in the presence of different concentrations of nitric acid metal ions adsorbed to the moringa biomass were being desorbed (Figures 40 and 41). The increase in acid concentration enhanced desorption of metal ions from the moringa biomass. The optimum desorption for both metals using both moringa powders was observed at 0.06 mol/L of nitric acid. Metal ion desorption was also reported by Baig *et al.* (1999) on metal biosorption using the biomass of *Solanum elaeagnifolium* (silverleaf nightshade) and Krishnan and Anirudhan (2003) using steam activated sulphurised carbon prepared from sugarcane bagasse. Sharma *et al.'s* (2006) concurrent desorption studies found that the optimum metal ion desorption using *M. oleifera* seed powder was obtained at 0.05 mol/L nitric acid. This compares well with results from this study.

The optimum amounts of metal ions are desorbed at pH 1.64 and 1.59 for lead ions desorption and 1.90 and 1.70 for cadmium ions desorption using *M. oleifera* and *M. stenopetala* respectively. The desorption pH values are indicated in appendices 4 and 5. The desorption capacity for lead ions is less than that for cadmium ions ($p < 0.05$). This observation agrees with that of effects of stirring time and temperature. Since cadmium ions bind mostly through weak electrostatic interactions towards the binding sites on the moringa biomass, they are likely to be more easily desorbed with a decrease in pH than lead ions that bind strongly to the biomass sites.

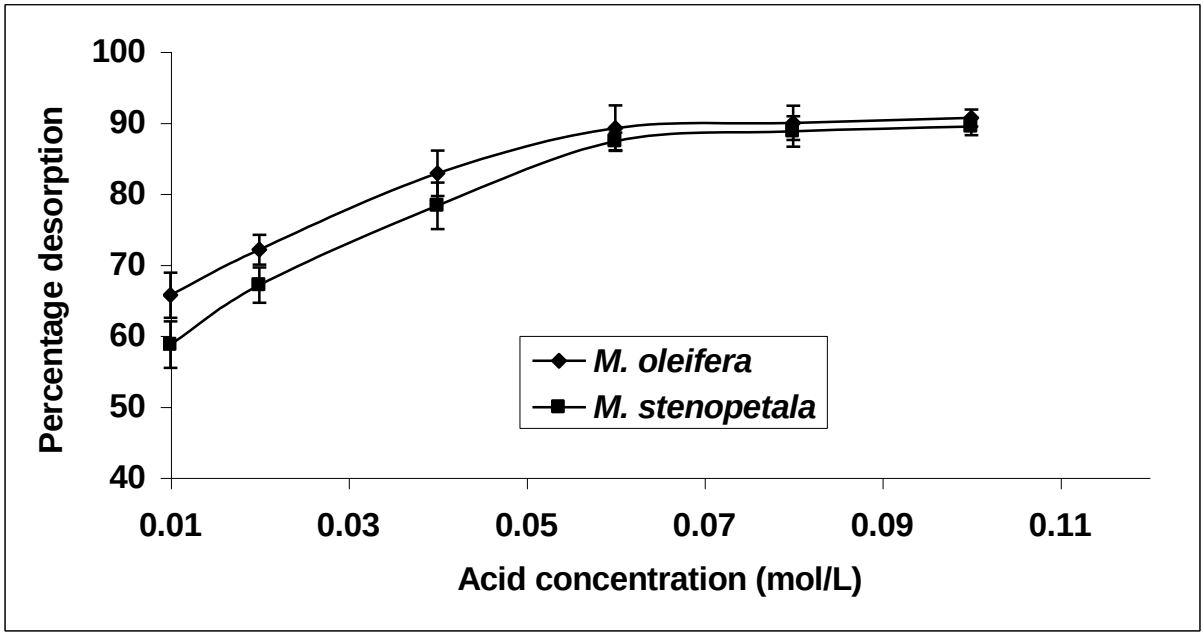

Figure 40: Lead ion desorption from 0.50 g/25 mL lead loaded moringa powders in various concentrations of nitric acid at 30 ^{o}C.

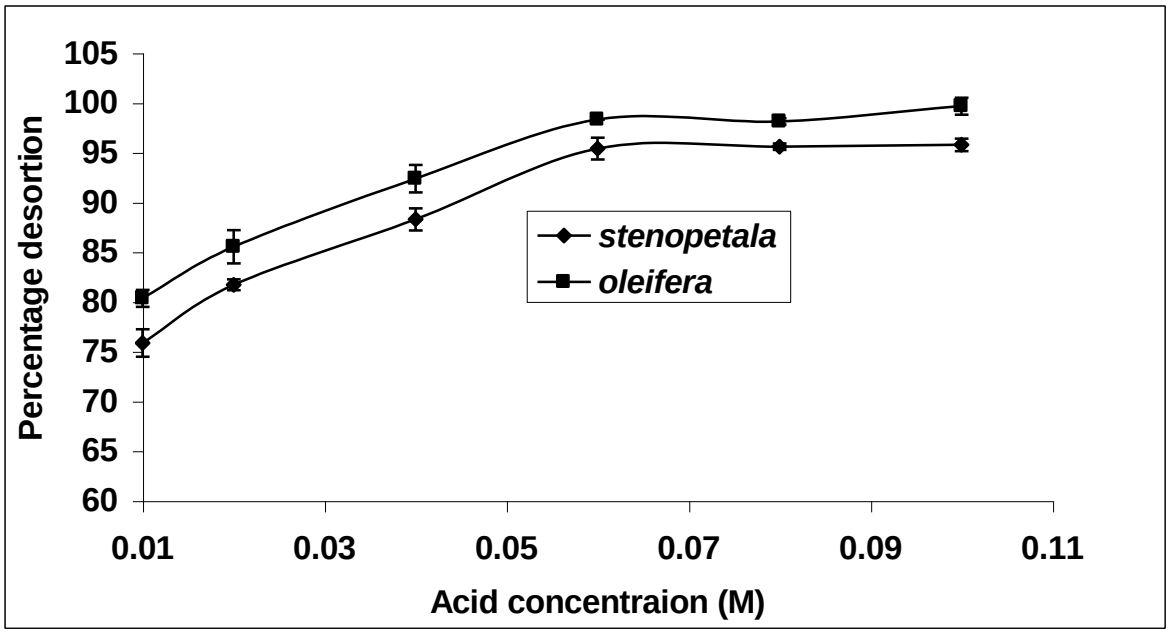

Figure 41: Cadmium ion desorption from 0.50 g/25 ml cadmium loaded moringa powders in various concentrations of nitric acid at 30 ^{o}C.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The sorption studies have shown that *M. oleifera* and *M. stenopetala* have the capacity to remove Cd^{2+} and Pb^{2+} from aqueous solutions, and the sorption for both metal ions increase with the raise in biomass dosage for both moringa powders. At 2.5 g/100 mL dose lead had a sorption efficiency of 78 % using *M. oleifera* and 96 % using *M. stenopetala* while cadmium had 53 % using *M. oleifera* and 54 % using *M. stenopetala*. Further, *M. stenopetala* is more effective in both lead and cadmium ion sorption from aqueous solutions than *M. oleifera* at the operating pH of 2-3. Furthermore, lead ions were better sorbed from aqueous solution than cadmium ions.

Generally, metal ion removal increased with rising pH, suggesting that sorption may be largely due to the presence of hydroxyl, carboxyl or carbonyl functional groups. The optimum pH for metal ion removal was pH 3 for lead ion and pH 5 for cadmium. A significant metal sorption was observed at low pH (pH = 2) using both moringa powders. This indicates the presence of strong acid groups one of which is sulphur. The metal ions are sorbed both as $M(OH)^{+}$ and M^{2+}, and the mechanism of metal removal is complexation and ion exchange.

Metal ion sorption was also affected by initial metal ion concentration, ionic strength, water hardness and temperature. The metal uptake increased with rising initial metal ion concentration but decreased with an increase in ionic strength. However, the effect of water hardness varied depending on the source of hardness ions. The presence of carbonates/bicarbonate mixture in the metal ions solution enhanced metal uptake, which was likely due to the formation of low solubility lead and cadmium carbonates. Generally, the presence of Mg^{2+}/Ca^{2+} did not show any trends in metal uptake apart from slightly decreasing metal ion sorption, which was attributed to competition for binding sites between Ca^{2+} and Mg^{2+} on one hand and Pb^{2+} or Cd^{2+} on the other hand. Temperature also affected the metal ion being sorbed. While lead sorption increased with temperature cadmium sorption decreased. This indicated weak binding between cadmium ions and the moringa binding sites. This also showed that the reaction involved was likely physisorption for cadmium and chemisorption for

lead. Therefore, to effectively use moringa species, there is a need to optimise these parameters for metal ion removal from aqueous solutions.

The equilibrium sorption studies have shown that lead and cadmium ion removal follow both Langmuir and Freundlich isotherms. Langmuir model describes lead ion sorption better than the Freundlich model for both seed powders suggesting that monolayer sorption is the major sorption mechanism. Cadmium ion sorption Langmuir and Freundlich models described both monolayer and multiplayer coverage taking place simultaneously.

The energies of adsorption for Pb^{2+} and Cd^{2+} show that Pb^{2+} adsorption is chemisorption and Cd^{2+} adsorption is physisorption. This difference is due to differences in charge density between Pb^{2+} and Cd^{2+} with Cd^{2+} having higher charge density. The kinetic studies indicated that lead ion sorption followed Lagergren first order kinetics and cadmium desorption followed zero order kinetics. Both lead and cadmium ions are easily desorbed from the moringa biomass; 91 and 89 % desorptions are achieved for lead using *M. oleifera* and *M. stenopetala* respectively while 96 and 99 % desorptions are achieved for cadmium using *M. oleifera* and *M. stenopetala* respectively at 1.0 mol/L HNO_3 and 30^{o}C after 40 minutes of shaking. This indicated that both the biomass and the metal ions could be reclaimed for further use.

5.2 Recommendations

Conventional heavy metal sorption techniques from water are not cost effective and may have side effects on the consumer. Therefore, it is imperative to develop alternative methods for heavy metal remediation of contaminated water. The use of moringa species offers greater opportunities since the cake is effective in reducing turbidity, and decreasing sludge production. Further studies should be directed at (i) determining the effectiveness of moringa species in removing other pollutants such as anions from water, (ii) exploring the extraction and purification of protein from moringa biomass and testing the effectiveness of extracted protein on metal removal, (iii) identification of actual agents for metal ion sorption from moringa, the mechanism of metal sorption and (iv) determination of energy changes involved during metal sorption by moringa.

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APPENDICES

Appendix 1: Relationship between final pH and water hardness.

		Pb^{2+}		Cd^{2+}	
Type of hardness	Hardness (Mg/L)	Final pH (MOWSK)	Final pH (MSWSK)	Final pH (MOWSK)	Final pH (MSWSK)
HCO_3^- /CO_3^{2-}	0	2.27	2.30	2.79	2.81
	50	2.83	2.82	6.34	3.30
	100	3.03	3.05	7.40	7.64
	180	4.25	4.24	8.69	8.14
	250	8.10	8.07	9.08	8.90
	400	8.25	8.29	9.45	9.08
Mg/Ca	0	2.27	2.30	2.79	2.81
	50	2.64	2.64	2.83	2.85
	100	2.64	2.61	2.90	2.94
	180	2.65	2.65	2.84	2.84
	250	2.63	2.64	2.87	2.84
	400	2.65	2.64	3.19	2.99

Appendix 2: Final pH after pH effects treatment of metal ion water using moringa whole seed powders.

	Pb^{2+}		Cd^{2+}	
Initial pH	Final pH (MOWSK)	Final pH (MSWSK)	Final pH (MOWSK)	Final pH (MSWSK)
2	2.20	2.23	2.27	2.29
3	3.02	3.09	3.25	2.99
5	3.43	3.42	3.47	3.21
7	4.01	4.04	3.49	3.21
9	4.06	4.10	4.19	3.21
10	4.14	4.21	5.94	3.20

Appendix 3: Percentage metal removal at different pH using moringa whole seed powders.

	Percentage removal			
pH	*M. oleifera*	*M. stenopetala*	*M. oleifera*	*M.stenopetala*
2	29.21 ± 0.60	54.31 ± 4.25	1.59 ±1.15	0.47 ± 0.63
3	87.42 ± 0.70	89.06 ±1.85	34.80 ±1.4	36.09±0.52
5	95.38 ± 0.70	91.97 ± 0.77	82.70 ±1.66	70.7 ±1.93
7	97.14 ± 0.43	90.89 ±1.47	86.53 ±1.07	73.05 ± 2.04
9	96.98 ± 0.13	91.95 ±1.22	91.73 ± 0.90	82.37 ±1.92
10	98.39 ± 0.43	93.99 ±1.98	93.80 ± 0.87	88.40 ± 0.31

Appendix 4: Desorption of lead ions from lead loaded moringa biomass.

Seed powder	Acid Conc. (M)	Metal adsorbed (mg/L)	Desorbed metal conc. (mg/L)	Residual metal in solid (mg/L)	Desorption (%)	Equilibrium pH
M. oleifera	0.01	5.14	3.37± 0.16	1.76±0.17	65.67±3.27	2.03
	0.02	5.14	3.70±0.16	1.43±0.13	72.09±2.47	1.85
	0.04	5.14	4.21±0.23	0.92±0.17	82.84±3.27	1.76
	0.06	5.14	4.58±0.08	0.55±0.06	89.22±1.24	1.64
	0.08	5.14	4.62±0.08	0.52±0.11	89.94±2.14	1.58
	0.10	5.14	4.65±0.00	0.48±0.06	90.64±1.24	1.57
M. stenopetala	0.01	5.24	3.04±0.16	2.20±0.17	58.00±3.20	1.94
	0.02	5.24	3.52±0.16	1.73±0.11	67.09±2.10	1.79
	0.04	5.24	4.10±0.23	1.14±0.17	78.26±3.20	1.66
	0.06	5.24	4.58±0.16	0.66±0.17	87.35±3.20	1.59
	0.08	5.24	4.65±0.16	0.59±0.13	88.75±2.42	1.53
	0.10	5.24	4.69±0.08	0.55±0.06	89.45±1.21	1.48

Appendix 5: Desorption of cadmium ion from cadmium loaded moringa biomass.

Seed powder	Acid Conc. (M)	Metal adsorbed (mg/L)	Desorbed metal conc. (mg/L)	Residual metal in solid (mg/L)	Desorption (%)	Equilibrium pH
M. oleifera	0.01	4.035	3.06±0.05	0.97± 0.06	75.84± 1.38	2.50
	0.02	4.035	3.30± 0.01	0.74± 0.02	81.69± 0.55	2.22
	0.04	4.035	3.56± 0.03	0.47± 0.04	88.27±1.10	1.90
	0.06	4.035	3.85±0.03	0.19± 0.04	95.39±1.10	1.90
	0.08	4.035	3.86± 0.02	0.18± 0.01	95.58±0.32	1.70
	1.00	4.035	3.86± 0.03	0.17± 0.03	95.76±0.63	1.61
M. stenopetala	0.01	3.984	3.20±0.03	0.97±0.06	80.34±0.84	2.35
	0.02	3.984	3.41±0.07	0.74±0.02	85.52±1.67	2.18
	0.04	3.984	3.68±0.06	0.47±0.04	92.37±1.40	1.85
	0.06	3.984	3.92±0.00	0.19±0.04	98.30±0.00	1.70
	0.08	3.984	3.92±0.02	0.18±0.01	98.30±0.56	1.58
	1.00	3.984	3.93±0.03	0.17±0.03	98.67±0.84	1.56