BIOSORPTION OF LEAD FROM AQUEOUS SOLUTION BY *Ziziphus mauritiana* SEED SHELLS

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By

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Declaration

I declare that the work in this dissertation entitled “Biosorption of lead from aqueous solution by *Ziziphus mauritiana* seed shells” has been carried out by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and a list of references is provided. No part of this dissertation was previously presented for the award of another degree or diploma at this or any other Institution.

Name	Signature	Date

Certification

This dissertation entitled “Biosorption of lead from aqueous solution by *Ziziphus mauritiana* seed shells” by Hope Babvoshia BITIYONG (P13SCBC8029) meets the regulations governing the award of the degree of Master of Science Biochemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dedication

I dedicate this wok to my family.

ABSTRACT

Presence of heavy metals in the aquatic systems has become a serious problem. Lead is a metal which has been associated with various human activities However, it is also toxic to humans, with the most deleterious effects on the hemopoietic, nervous, reproductive systems and the urinary tract. Biosorption is potentially an attractive technology which utilizes biomass to sequester heavy metals from wastewater. Studies carried through the environmental biotechnology have shown that many biosorbents present in the nature have great capacity for removal of heavy metals. The aim of the present study was to utilize *Ziziphus mauritiana* Lam. seed shells for biosorption of Pb (II) ions from aqueous solutions. These seed shells were used for the biosorption Pb (II) ions from aqueous solution under batch mode. The concentration of Pb(II) ions sorbed was determined using atomic absorption spectrophotometry. The influence of biosorbent dose, initial lead (II) concentration, contact time, temperature, solution pH and agitation rate on the removal process, were investigated. Equilibrium studies, kinetics and thermodynamics of Pb (II) biosorption were also investigated. About 4 g of the adsorbent was obtained as the optimum biosorbent dose which showed a metal uptake capacity (qe) of 2112.03 mg g^{-1} for an initial metal ion concentration of 50 mg g^{-1}. Increase in initial metal ion concentration led to a decrease in percentage removal efficiency of the *Z. mauritania* seed shells with 10 mg g^{-1} having the highest percentage Pb removal of 82.05%. The equilibrium contact time for the metal was found to be 60mins with 80.69 % uptake. At temperature of 70^{o}C, the maximum percentage removal (89.48%) was observed. The optimum uptake of Pb (II) occurred at pH 7 with the highest percentage removal of 88.39%. Results of agitation rate showed a maximum uptake (85.18%) at 300 rpm. The equilibrium data was analyzed by the Freundlich and Langmuir adsorption isotherm models and was found to conform to the Freundlich isotherm with R^2 value 0.952. The maximum adsorption capacity of the

Z. mauritania seed shells for Pb (II) was 500 mg g^{-1}. The kinetics of Pb (II) ion was modeled using pseudo-first-order and second-order models as well as the intraparticle diffusion model. It was shown that the adsorption could be described by the pseudo-second order kinetic model with R^2 value 0.997. Thermodynamic parameters such as Gibbs free energy (ΔG^o), enthalpy (ΔH^o) as 15807.88 kJ/mol and entropy change of the biosorption (ΔS^o) as 50.07 kJ/K/mol indicated that the biosorption process was endothermic in nature, spontaneous and feasible. FT-IR analysis of *Z, mauritiana* seed shells showed that O-H, C-O, C=O, C=N, NO_2 are functional groups involved in the biosorption process. It was concluded that *Z. mauritania* seed shells can be used as an effective, low-cost and environmentally friendly biosorbent to remove Pb (II) ions from aqueous solutions.

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List of Abbreviations

AAS	Atomic Absorption Spectrophotometer
Al	Aluminium
ATSDR	Agency for Toxic Substances and Disease Registry
C_0	Initial lead (II) ion concentration/ liquid-phase concentration of lead at initial time
$CaCI_2$	Calcium chloride
C_{Ae}	Equilibrium concentration on the sorbent in mg/L
C_e	Equilibrium concentration in solution in mg/L
cm^{-1}	per centimeter
Co	Cobalt
COOH	carboxyl
Cr (VI)	Chromium (VI) ions
Cu	Copper
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid.
Fe	Iron
Fe (II)	Iron (II) ions
FTIR	Fourier Transform Infrared
GI	Gastrointestinal tract.
H_2SO_4	Sulphuric acid
H_3PO_4	Phosphoric acid

NH_4OH	Ammonium hydroxide
HCI	Hydrochloric Acid
HNO_3	Nitric acid
I.Q	Intelligence quotient
K	Kelvin
K_c	Equilibrium constant
K_F	constant of Freundlich isotherm
Kg	Kilo gram
$KHCO_3$	Potassium hydrogen carbonate
kJ/mol	kilojoules per mole
K_L	Langmuir constant
KSCN	Potassium thiocyanate
LGA	local government area
M	Molar
mg l^{-1}	milligram per litre
mg/g	milligram per gram
mg/l	Milligram per litre
mL	millilitres
Mn	Manganese
MNR	Monitored natural recovery
Mo	Molybdenum
mol g^{-1}	mole per gram
n	adsorption intensity
Na_2CO_3	Sodium carbonate

NaOH	Sodium Hydroxide
NH_2	amino group
Ni	Nickel
OH	hydroxyl
Pb (II)	Lead (II) ions
Pb $[NO_3]_2$	Lead Nitrate
pH	Potential of Hydrogen Ions
PO_3O_2	Phosphoryl group
Ppb	parts per billion
Se	Selenium
q_e	Amount of biosorption at equilibrium
q_t	Amount of biosorption at time t.
R	molar gas constant (8.314 $Jmol^{-1}K^{-1}$)
R^2	correlation coefficient
rpm	Revolutions per minute
SEM	Scanning Electron Microscopy
S_F	separation factor
SH	Sulphydryl
W.H.O	World Health Organization
Zn	Zinc
Zn (II)	Zinc (II) ions
ΔG^0	Change in free energy
ΔH^0	Change in enthalpy
ΔS^0	Change in entropy

µm	micrometer
µm²	micrometer square

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Heavy metals are natural constituents of the earth's crust, though indiscriminate human activities have drastically altered their geochemical cycles and biochemical balance resulting in the accumulation of these metals in plant parts. In recent years, pollution by heavy metals has become one of the most serious environmental problems (Nilanjana *et al*., 2008). Prolonged exposure to heavy metals such as cadmium, copper, lead, nickel, and zinc can cause deleterious health effects in humans (Singh and Arora, 2011).

The major source of heavy metals pollution in the environment is mainly the industries (Wierzba, 2015). The release of such toxic substances in the wastewater from industrial activities such as metallurgy, electroplating, metal processing and finishing, tanneries, chemical manufacturing, mining, pharmaceuticals, pesticides, organic chemicals, rubber and plastics, among others may cause serious effects on the environment and human health (Lairini and Espinola, 2012; Rodriguez-Tirado *et al*., 2012). It is evident that the release of these toxic metals and their dispersal in the environment may cause tragic effects on the exposed populations. For some decades, there has been an increased flux of metallic substances in aquatic environment which is due to a huge increase in the use of heavy metals in industries (Kailas, 2010). The most important characteristics of these heavy metals are that they are non-degradable and are therefore persistent. More so, most of them are toxic to living organisms. This is because the toxic substances have a tendency to accumulate in the soil, sea water, fresh water and sediment due to their high dispersion from where they enter into the food chain (Rathnakumar *et al.,* 2009). Heavy metals are toxic to aquatic flora

and fauna even in relatively low concentrations (Monika *et al*., 2014). Some of these metals can be assimilated, stored and concentrated by organisms (Mohan and Singh, 2002). Therefore, there is need for these toxic heavy metals to be removed from wastewater before its disposal, in order to have a pollution-free environment. (Esmaili *et al.*, 2003).

Despite the toxic effects of heavy metals, it is important to note that some of the heavy metals such as Cu, Fe, Mn, Co, Mo, Ni and Zn, are essentially required for various physiological functions of both plants and animals though they are required in minimal quantities. In general, they play important roles in functioning of living **organisms**. In addition, these elements could also participate in other activities: (i) forming the structure of proteins and pigment (ii) redox processes (iii) regulation of the osmotic pressure (iv) maintaining the ionic balance and (v) acting as enzyme component of the cells (Kosolapov *et al.,* 2004; Zaidi *et al*., 2012). Among these elements, Al, Co, and Se, play a role in promoting plant growth and may be essential for particular taxa (Pilon-Smits *et al.,* 2009). Similarly, Zn plays a significant role in cellular division and amplification, protein synthesis, and contributes in carbohydrate, lipid, and nucleic acid metabolism (Collins, 1981). On the other hand, structure and composition of microbiota (Wani and Khan, 2010) and plant growth (Stoyanova *et al*., 2008; Ahmad *et al*., 2012; Reddy *et al*., 2013; Oves *et al,* 2013) are reported to be significantly affected when the concentrations of such trace elements exceed the normal level.

According to WHO (2003), the maximum discharge limits for lead in wastewater is 0.5 mg l^{-1}. Maximum limit in drinking water is 0.01 mg l^{-1}. There is no safe level for lead metal in drinking water because at very dilute concentration it can cause adverse health effects. Lead is known to cause mental retardation, reduction in haemoglobin production which is necessary for oxygen transport and it interferes with normal cellular metabolism (Monika *et al*., 2014). Lead has

damaging effects on the nervous system and it reduces intelligence quotient (I.Q) level (Mohanty *et al.,* 2005). Some of the commonly used methods for the removal of metals include; electrochemical methods, reverse osmosis, chemical precipitation, ion exchange, biological processes, floatation and membrane process (Park *et al*., 2010).

Biosorption can be seen as the property of certain bio-molecules (biomass) to bind and concentrate selected ions or other molecules from aqueous solutions (Volesky, 2007). Biosorption as a process involves a solid phase (sorbent or biosorbent) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ion). The sorbate species is usually attracted and bound with different mechanisms to the sorbent. This interaction results from the high affinity which the sorbent has for the sorbate species. This process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution (Hussain *et al.,* 2010). Biosorption is a promising technique for the removal of heavy metals from aqueous environments especially when biosorbents are derived from lignocellulosic materials (Coelho, *et al.,* 2007). The biosorbent materials have metal-sequestering property and can be used to decrease the concentration of heavy metal ions in solution from ppm to ppb level. Park *et al.* (2010) reported that biosorbents that have been used for the removal of metal ions can be categorized into bacteria, fungi, algae, industrial wastes, agricultural wastes, natural residues and other biomaterials, with the former two giving broader choices.

Agricultural by-products are generated in high amounts; some of these products are not used economically although they can be used as biosorbent to remove toxic heavy metals which cause environmental issues. One of such example is the cassava peels derived from cassava (*Manihot esculents cranz*) a root crop cultivated and consumed as a staple food in many regions of the developing world (Ashaye *et al.,* 2010). The use agricultural residues for wastewater treatment has

a lot of advantages which includes simplicity of the technique, little processing requirement, good adsorption capacity, selective adsorption of heavy metal ions, low cost, availability, and easy regeneration (Osman *et al.*, 2010).

This research focuses on the investigation of the potential of *Ziziphus mauritiana* Lam. seed shells for the removal of Lead (II) ions from aqueous solution. *Z. mauritiana,* an agricultural product belongs to the family *Rhamnaceae*. It is commonly called jujube, Chinese date, Indian plump, Chinee apple. In northern Nigeria it is called *Magarya* (Hausa). It is widely distributed in the Northern part of Nigeria.

1.2 Statement of Research Problem

High concentrations of metals above the tolerance limits are found in aquatic environment due to rapid industrialization and increase in population. (Tewaria *et al*., 2005).The presence of heavy metals in the water environment is a major concern due to their toxic effects since they cause severe health problems to animals and human beings. According to the World Health Organization (WHO), the metals of most immediate concern are Lead, Chromium, Manganese, Iron, Cobalt, Nickel, Copper, Zinc, Cadmium, Mercury and Aluminum (El-Sayed *et al*., 2010).

In the northern part of Nigeria where gold mines are found, series of lead poisonings occur which led to deaths of at least 163 people between March and June 2010 in Zamfara state including Children. More recently, about 28 Children were reported to have died due to lead poisoning in Rafi LGA of Niger state (BBC, 2010, June 4; The guardian, 2015, May 14).

Several conventional physical and chemical treatment techniques may be used for the removal of lead ions from aqueous solutions. However, such processes are not only expensive and highly

energy intensive, but also lead to production of harmful by-products and end-products, the ultimate disposal of which again causes secondary pollution (Sen and Ghosh, 2010).

1 Heavy metal ions such as lead do not degrade into harmless end products, unlike organic pollutants of which majority are susceptible to biological degradation (Yu, 2005).

Exposure to lead is associated with multiple health effects, including systemic effects such as gastrointestinal effects, anemia, hypertension and hearing loss; effects on the nervous system (e.g. on behavior and cognition), on development, and on the reproductive system, as well as genotoxicity, carcinogenicity and social effects (Sati *et al.*, 2015). These effects can be found in both children and adults.

The hard stone/shell of *Z. mauritiana* seeds restricts its germination. Cracking of the shell or extraction of seeds hastens germination. These shells once cracked are considered as agricultural wastes materials and are commonly found in our environment having potentials to serve as biosorbents.

1.3 Justification

Although, removal of toxic heavy metals from industrial wastewater has been practiced for several decades by the conventional physico-chemical removal methods, such as chemical precipitation, electroplating, membrane separation, evaporation and resin ionic exchange, these methods are usually found to be inefficient and expensive, especially when treating wastewater with low concentration of heavy metals (Volesky and Leusch, 1995; Dabrowski *et al.*, 2004; El-Ashtoukhy, 2008).

Alternative methods such as those involving the use of low cost materials like agro residues to remove heavy metals by a simple biosorption process will not produce chemical sludge, hence no risk of secondary pollution and it is more efficient and easy to operate compared to other methods (Rathnakumar *et al.*, 2009).

Use of cheap agricultural residues such as rice straw (Farooq *et al.*, 2010), sawdust (Bulut *and* Tez, 2007), carrot residues (Nasernejad *et al.*, 2005), tobacco dust (Qi and Aldrich, 2008), orange residue (Khormaei *et al.*,2007), apple residue (Lee *et al.*,1998), coconut shell powder (Pino *et al.*, 2006) and powdered leaves of castor tree (Al Rmalli *et al.*, 2008) as adsorbents have been reported for heavy metals removal from wastewater. *Z. mauritiana* which is also an agro residue may also have the potentials for biosorption.

Z. mauritiana is a common plant in the northern part of Nigeria; it is produced in large quantity which leads to the availability of *Zizipus mauritiana* seed shells in large quantity that could always be put into use.

Z. mauritiana seed shells just like other seed shells may contain cellulose and hemicelluloses, which have biosorption properties hence can bind to lead II ions.

1.4 Aim

The aim of this study is to investigate the ability and effectiveness of *Z. mauritiana* seed shells to be used as biosorbent for the removal of lead ions from aqueous solution.

1.5 Specific Objectives

i. To examine the effect of process parameters (initial concentration of adsorbate, dosage of biosorbent, agitation rate, pH, Temperature, and contact time) on the biosorption efficiency of Pb (II) ions from aqueous solution by *Z. mauritiana* seed shells.

ii. To evaluate the mechanism of the biosorption process using Langmuir and Freundlich isotherms

iii. To determine the kinetics of Pb (II) ions biosorption by *Z. mauritiana* seed shells.

iv. To determine the thermodynamics of Pb (II) ions biosorption by *Z. mauritiana* seed shells.

v. To study the degree of interaction between *Z. mauritiana* seed shells and the adsorbate through change in morphology of the biosorbent using scanning electron microscopy and; to investigate the functional groups implicated in the biosorption by seed process using FTIR.

CHAPTER TWO

LITERATURE REVIEW

2.1 Heavy Metals Pollution

In recent years, heavy metal pollution has become one of the most serious environmental problems (Aktan *et al.,* 2013). Rapid industrialization and urbanization have resulted in the generation of large amounts of aqueous effluents, which contain high level of toxic pollutants such as heavy metals (Krishnani and Ayyappan, 2006; Hashem, 2007; Monika *et al.,* 2014). Despite the higher technological level and increased awareness regarding to industrial safety, the number of industrial accidents continues to rise at an alarming rate. Heavy metal Pollution refers to the introduction of heavy metals into the biosphere. As xenobiotics, these heavy metals sometimes find their way into the body system through the food chain (Bayo, 2012). They resist chemical and biological transformation and accumulate in the tissues, including the nerves, to cause toxicity. Some heavy metals are neurotoxic for example lead, mercury, nickel, zinc, cadmium, chromium and manganese (Munoz *et al*., 2012; Rodriguez-Tirado *et al*., 2012).

Industrial activities such as metal plating, fertilizer production, mining operations, tanneries, paper, batteries and pesticides production among others have been found to contaminate the environment directly or indirectly by discharging wastewater containing heavy metals into the environment (Fu and Wang, 2011). These activities consequently result in contamination of natural waters (Monika *et. al.,* 2014). Hence pollution problem of industrial wastewater is becoming more and more serious; thereby making the treatment of these polluted industrial wastewaters a global concern especially since wastewater from municipalities, communities, and industries ultimately return to receiving waters or to the land (Monika *et. al*., 2014). Heavy metals released by a number

of industrial processes are major pollutants in marine, ground, industrial and even treated wastewaters (Dixit *et al*., 2015). Since heavy metals are hazardous to the environment, it is therefore necessary that they are appropriately removed from waste stream before being discharged into the environment (Dixit *et al*., 2015).

2.2 Bioremediation

Bioremediation involves the use of biological methods to remove pollutants from the environment. Bioremediation can occur as a natural process or as a facilitated process whereby microorganisms or other enhancers are introduced into the environment in order to enhance the breakdown of the contaminants by resident microorganisms (Shilpi, 2012). Bioremediation can be classified as *in-situ* or *ex-situ* depending on whether it is used at the site of contamination or outside the original site respectively (Perelo, 2010). Bioremediation has advantages over the conventional dredging and capping methods. These include cost effectiveness and complete mineralization of pollutants. Its non invasiveness also leaves the ecosystem intact (Shilpi, 2012). Bioremediation is also a more suitable technique because it is effective even at lower concentration of contaminants. Its major disadvantage however is that it is less predictable than other conventional methods and it takes longer time (Perelo, 2010). Common bioremediation techniques include bioaugumentaion, monitored natural recovery (MNR), biostimulation, rhizoremediation and phytoremediation.

2.2.1 Bioaugumentaion

Bioaugumentaion involves the introduction of microorganisms into the contaminated environment with the aim of supplementing the indigenous population and speed up degradation of pollutants (Perelo, 2010). The major challenges with this process is that the process may be inhibited by factors such as pH, redox reactions, concentration and bioavailability of contaminants, presence

of toxic contaminants and the absence of key co substrates as this will affect the survival and activities of the introduced organisms (Thompson *et al.,* 2005). It is however important to select strains of microorganisms similar to the microbial communities present in the habitat (Thompson *et al.,* 2005).

2.2.2 Monitored natural recovery (MNR)

Monitored natural recovery (MNR) is the use of natural capacities of the native microbial population in combination with naturally occurring physical and chemical processes (Perelo, 2010). MNR involves leaving the contaminated sediments in place and allowing natural ongoing processes such as aquatic sedimentation, biological and chemical transformation to immobilize or degrade the contaminants *in-situ* hence reducing its bioavailability (Magar, 2001). The uniqueness of this method is that it requires no action to initiate or continue the process. Particle bound sediments may leave the site by erosion, transportation and dispersion. This may however lead to an increase in the contaminant concentration in the downstream areas. Another disadvantage of this technique is that it requires extensive long term monitoring and takes time. Its cost effectiveness is however a major advantage (Perelo, 2010).

2.2.3 Biostimulation

Biostimulation is a technique which involves influencing the availability of certain factors such as cations, sulfates, humic substances and nitrates required for microbial growth or even injecting electron donors such as methanol and acetate into the microbial population in order to increase indigenous microbial population (Lovely, 2000; Couto *et al.,* 2010). Biostimulation can also be seen as a process which enhances the bioavailability of contaminants to the degrading organisms by the use of surfactants like bile salts, sodium taurochlorate, Triton X-100; among others

(Kanissery and Sims, 2011). These surfactants act to lower the surface tension between the aqueous medium and the solid (adsorbent) medium. A major disadvantage is that the surfactants may become substrates or toxic to the degrading microorganisms. The surfactants may also inhibit the degradation by detaching the microbial cells from the contaminant-water surface (Kanissery and Sims, 2011).

2.2.4 Phytoremediation

Phytoremediation involves the use of plants or algal species to extract the contaminants such as heavy metals from the environment. Although this technique is not very effective on organic pollutants, it has the potential to completely transform the pollutant into a less or completely non toxic component (Perelo, 2010). Adsorption, translocation, transformation and mineralization by plant enzymes are some of the chemical and biophysical processes embedded in the mechanisms of phytoremediation (Meagher, 2000). The accumulation of toxic compounds in plant tissues due to incomplete decomposition of the organic pollutants is a major disadvantage of this technique. The partially decomposed pollutants may be released into the environment or enter the food chain and thus cause health hazards (Aken, 2008).

2.2.5 Rhizoremediation

Rhizoremediation combines both phytoremediation and biostimulation techniques to remove contaminants from the environment. The applicability of this technique is based on the fact that the use of plants as bioremediators could enhance the activity of degrading microorganisms in their root system (Perelo, 2010).

2.3 Heavy Metal Toxicity

Heavy metals are elements having atomic number greater than 20, and a specific gravity greater than 5.0 (Srivastava and Majumder, 2008). Heavy metals are ubiquitous in the environment (Mahajan and Sud, 2013). Most of the heavy metals are dangerous to health or to the environment. Their free radicals are highly reactive attacking other cellular structures (Fu and Wang, 2011). The ability of metals to disrupt the function of essential biological molecules, such as protein, enzyme and DNA is the major cause of their toxicity. Displacement of certain metals essential for cellular functions by a similar metal is another cause of toxicity. Heavy metals in industrial wastewater include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold, and nickel (Fu and Wang, 2011). International bodies such as the WHO have extensively studied and reviewed the effects of these heavy metals on human health and have established that the main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (Rodriguez-Tirado *et al*., 2012). Chronic heavy metal exposure has been implicated in several degenerative diseases of the central nervous system, the cardiovascular and gastrointestinal (GI) systems, lungs, kidneys, liver, endocrine glands, and bones and may increase the risk of some cancers (Bahadir *et al*., 2007; Rodriguez-Tirado *et al*., 2012; Zabochnicka-Aswia and Krzywonos, 2014). The acute heavy metal intoxications may damage these same systems.

2.4 Lead and Lead Poisoning

Lead is one of the most prevalent metals detectable in the waste streams from mining operations, electroplating and petrochemical industries, (Kalyani *et al*., 2009). Lead is widely used in many industrial applications such as storage battery manufacturing, printing, pigments, fuels and explosive manufacturing (Jalali *et al*., 2002). Lead has been cited as one of the most toxic heavy metals that have long-term negative impacts on health, causing anemia, encephalopathy, hepatitis and nephritic syndrome (Sati *et al*., 2015). Lead is a systemic poison retained by mammals in liver,

kidney, muscles and other organs and its presence in drinking water, even in low concentrations may cause anemia, kidney malfunction/ nephritic syndrome, hepatitis, tissue damage of brain and even death in extreme poisoning (Zulkali *et al*., 2006; Ozer, 2007). Severe lead poisoning has also been associated with abortion, stillbirth and neonatal deaths while moderate lead poisoning can cause neurobehavioral and intelligence deficit (Bahadir *et al*., 2007; Rodriguez-Tirado *et al*., 2012). It is therefore, essential to remove Pb (II) and other heavy metals from wastewater before disposal (Dixit *et al*., 2015).

In their review, Abdi and Kazemi (2015) discussed various methods for the removal of heavy metals such as chemical precipitation, ion exchange, adsorption, reverse osmosis, electrodialysis, ultrafiltration, nanofiltration, coagulation, flocculation, floatation, etc stating their merits and demerits. Among these methods, adsorption is the simplest and relatively cost-effective, thus has been widely used. These processes are generally efficient in removing the bulk of metals from solution at high or moderate concentrations. However, they produce a large amount of metallic sludge, making metal recovery difficult (Fu and Wang, 2011).

2.4.1 Chemistry of lead

Lead is a chemical element with the symbol **Pb** with atomic number 82 it has 82 electrons, arranged in an electronic configuration of $[Xe]4f^{14}5d^{10}6s^{2}6p^{2}$. It is a soft, odourless, malleable and heavy post-transition metal. Lead in its solid state has a bluish-white color that soon tarnishes to a dull grayish color when exposed to air; in its liquid state, the metal has shiny chrome-silver luster. Lead's characteristic properties include high density, malleability, softness, ductility, poor electrical conductivity compared to other metals, high resistance to corrosion, and ability to react with organic chemicals (Wani *et al*., 2015). Lead exists in the valence states of +2 and +4 and has

four naturally occurring stable isotopes: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. Inorganic lead compounds usually consist of lead in the divalent state (+2), and the chemistry of divalent lead is similar to that of group 2 metals (HSDB 2009).

Lead has a high density of 11.3g cm-3. Its high atomic number and hence high relative atomic mass of 207, along with its face centered cubic structure (the metal atoms or, more precisely, ions) are arranged in a dense, close packed structure greatly accounts for its high density. The high density of lead has important bearings on other properties, particularly attenuation of X-rays, gamma rays and sound waves (Akron 2010).

Lead form compounds that are relatively soluble in water and those that are relatively insoluble in water. The soluble compounds of lead include, lead acetate, lead acetate trihydrate, lead chloride, lead nitrate, and lead acetate; all are soluble in water, and lead acetate trihydrate is miscible with water (HSDB, 2009). Lead also forms insoluble compounds such as Lead carbonate, lead chromate, lead iodide, lead oxide, and lead phosphate among others (HSDB 2009; Akron 2010).

2.4.2 Applications of lead

Lead is a metal which has been associated with human activities for the last 6000 years. It has its largest use is in lead-acid storage batteries for motor vehicles and general industry. Lead metal also is used particularly in products such as construction materials, ammunition, cable covering, paint, piping, brass and bronze, bearing metals for machinery, and sheet lead (ATSDR 1999). Lead is known to have radiation resistance; it is therefore, used by hospital personnel and people working in high radiation environments to protect them from radiation. In addition, it can also be used in the post and telecommunications industries, metallurgy, chemical industry, railways, transportation, construction, weapons, aerospace, aviation, oil and other industries. All of the major soluble and insoluble compounds of lead have a variety of industrial uses. Lead nitrate is

used in the manufacture of matches and explosives, as a heat stabilizer in nylon, and as a coating on paper for photothermography, Lead acetate is used as a water repellent, for mildew protection, and as a mordant for cotton dyes. Lead carbonate, lead fluoride, lead fluoborate, and lead naphthenate are used as catalysts, with additional uses in various industries (HSDB 2009; Guberman 2010).

2.4.3 Toxicity of lead

Lead is a widely used metal, but it is at the same time a subtle, persistent and versatile poison. The characteristic features of lead toxicity, such as neuropathy, anemia, nephropathy, colic, sterility, and coma, were noted both by Hippocrates and Nikander from time past (Landrigan *et al*., 1990). Lead may gain entrance into the body by direct swallowing and ingestion through the intestines, through the skin or through the lungs by inhalation (Markowitz, 2000; Moyo and Chikazaza, 2013). For adults with occupational exposure, the most significant route for absorption is through the respiratory tract.

Lead affects almost every system in the body. Although, it has deleterious effects on children and adults, children are more susceptible to lead exposure because of their higher rate of intestinal absorption and retention, the frequency of pica and hand-to-mouth activity. The most serious effects of lead are seen on erythropoiesis, renal function, and the central nervous system (Papanikolaou *et al*., 2005; Sati *et al*., 2015).

2.4.3.1 *Toxicity on hemopoietic system*

Lead causes aneamia by affecting the process of heme synthesis, it inhibits three important enzymes delta aminolevulinic acid dehydratase, deltaaminolevulinic acid synthase, and ferrochelatase known to participate in the the synthesis of heme. The adverse effects of lead appear even with blood concentrations as low as 10 μg/dl. (Ahamed *et al*., 2005). It is reported that the

inhibition of delta aminolevulinic acid dehydrates starts at values as low as 5 μg/dl resulting in the accumulation of delta aminolevulinic acid in the plasma and its excretion in urine (Lyn, 2006; Vij, 2009). The enzyme ferrochelatase which catalyzes the incorporation of iron into the porphyrin ring can also be inhibited and its pathway is interrupted as a result of lead toxicity. The major consequences of this effect, which have been evaluated in both adults and children, are reduction of circulating levels of hemoglobin and the inhibition of cytochrome P 450-dependent phase I metabolism (Mugahi *et al*., 2007). Lead clearly inhibits normal hemoprotein function in both respects.

2.4.3.2 ***Effect on nervous system and neurodevelopment***

The nervous system is the most vulnerable organ system to lead-induced toxicity (Cory-Slechta 1996). Although, both the central and peripheral nervous systems can be affected, the peripheral nervous system is mainly affected in adults, whereas in children, the central nervous system is primarily affected (Bellinger, 2004). The developing nervous system of the child makes it more sensitive to lead-induced impairment (Kim *et al*., 2015). When the impact of lead poisoning on the central nervous system is severe, it results in acute encephalopathy. The symptoms of which include persistent vomiting, headaches, poor attention span, ataxia, seizures, pappiledema, irritability, impaired consciousness, loss of memory, dullness and coma (Flora *et al.,* 2006).

Peripheral neuropathy, however, is the most common manifestation among adults with occupational lead exposure. It is rarely seen in children except for those with sickle cell disease (Bellinger, 2004; Kim *et al*., 2015). The effect of childhood lead exposure associated with deficits in central nervous system functioning may persist into adulthood.

2.4.3.3 ***Effect on kidney***

Renal toxicity in sub-clinical lead poisoning involves inhibition in the proximal tubular lining cells and renal insufficiency (Suradkar *et al.*, 2009). Early or acute nephropathy is characterized by dysfunction of the proximal tubules (Fanconi's syndrome), which manifests as glycosuria, aminoaciduria, increased sodium and decreased uric acid excretion, and phosphaturia with hypophosphatemia. These effects are reversible. Chronic lead nephropathy is however, characterized by irreversible effects such as progressive interstitial fibrosis, azothemia and a reduction in the glomerular filtration rate. The acute form of nephropathy is most frequently reported in children, while the chronic form is mainly reported in adult (Rastogi, 2008).

2.4.3.4 *Effect on other systems*

Low doses lead in is often associated with increased blood pressure in adults which increases the risk from cardiovascular disease (Navas-Acien *et al*., 2007; Alwaleedi, 2015). The effects are noted mainly in systolic pressure and are associated with blood concentrations starting at approximately 30 μg/dl (Bergeson, 2008). Lead toxicity typically causes constipation and colic (Kosnett, 2005). Reduced sperm counts and motility have also been associated with chronic lead exposure (Navas-Acien *et al.*, 2007; Mohsen *et al.*, 2011).

2.4.4 Modes of bioremediation of lead

Lead can be removed from the environment through bioremediation using biological means where microorganisms, plants or other enhancers are employed to remove such contaminants (Shilpi, 2012). Examples of methods of bioremediation include biosorption, monitored natural recovery (MNR), bioaugumentaion, biostimulation, rhizoremediation and phytoremediation

2.5 Conventional Methods of Heavy Metal Removal

There are various chemical and physicochemical methods for treatment of wastewaters containing heavy metals; these methods include chemical precipitation, electrochemical reduction, ion

exchange, electrodialysis, coagulation/ flocculation, ultrafiltration, and adsorption (Fu and Wang, 2011). The choice of treatment depends on effluent characteristics such as concentration of the heavy metal, pH, temperature, flow volume, biological oxygen demand, the economics involved and the social factor like standard set by government agencies (Fu and Wang, 2011; Abdi and Kazemi, 2015).

2.5.1 Electrodialysis

Electro Dialysis (ED) is a membrane process, which involves the transportation of ions through a semi permeable membrane, under the influence of an electric field (Fu and Wang, 2011). The membranes used are cation-or anion - selective, basically implying that either positive ions or negative ions will flow through. Cation-selective membranes are polyelectrolytes with negatively charged matter, which rejects negatively charged ions and allows positively charged ions to flow through (Ahluwali and Goyal, 2007).

2.5.2 Coagulation/ Flocculation

Coagulation is a chemical reaction which occurs when a chemical called coagulant is added to the water. This coagulant causes the colloidal material in the water to form aggregates called flocs. Suspended matter is then attracted to these flocs. Flocculation is a slow gentle mixing of the water to encourage the flocs to form and grow to a size which will easily settle out. Both coagulation and flocculation are essential processes of drinking water treatment as well as wastewater treatment (Park *et al.*, 2010; Fu and Wang, 2011).

2.5.3 Ultrafiltration

Ultrafiltration is a pressure – driven purification process it involves the use of membranes with pore sizes in a range of 0.1 to 0.001 microns (Ramachandra *et al.,* 2008). Typically, ultrafiltration will remove high molecular-weight substances, colloidal materials, and organic and inorganic polymeric molecules (Park *et al.,* 2010). In this separation process, water and low molecular weight substances permeate a membrane while particles, colloids, and macromolecules are retained. The major removal mechanism is size exclusion, although the electrical charge and surface chemistry of the particles and/or membrane may affect the purification efficiency (Fu and Wang, 2011). The coefficient of contaminant separation increases with increase in solvent evaporation time and decrease in temperature of casting solution (Ramachandra *et al.,* 2008).

2.5.4 Reverse osmosis

Reverse osmosis involves the transport of a solvent across a semi permeable membrane as a result of pressure difference between the interior and the exterior portions of the membrane. The process is suitable for removal of ions and larger particles from contaminated effluents (Ramachandra *et al.,* 2008) and have been reported to find application in processes which includes the treatment of wastewater containing organic substances, wastewater from electroplating and metal finishing, pulp and paper, mining and petrochemicals, textile, food processing industries, radioactive wastewater, municipal wastewater, and contaminated groundwater. The major disadvantage of reverse osmosis is the high power consumption due to the pumping pressures and the restoration of the membranes (Fu and Wang, 2011).

However, some of these conventional treatment technologies are not able to effect complete removal of the pollutants, as they even lead to production of toxic chemical sludge, and its treatment becomes a costly affair and is not eco-friendly (Fu and Wang, 2011). Therefore, removal of toxic heavy metals to an environmentally safe level in a cost effective and environment

friendly manner assumes great importance (Gakwisiri *et al*., 2012). More so, these methods require a high concentration of the heavy metals to be effective (Vieira and Volesky, 2000; Volesky, 2007). Biosorption of heavy metals from aqueous solution can be considered as an alternative technology in industrial wastewaters treatment (Zabochnicka-Aswia and Krzywonos, 2014).

2.5.5 Adsorption

The various physicochemical and biological processes such as precipitation, coagulation, flocculation, membrane process, sedimentation, filtration, flotation, electrochemical techniques, biological process, ion exchange and adsorption among others are all employed to remove pollutants from industrial wastewater before discharging into the environment (Hai *et al.,* 2007). The use and invention of this wide range of treatment techniques with varying levels of success has accelerated progress in scientific community (Marti *et al.,* 2008). Adsorption process is of major interest. It is a surface phenomenon in which a multi-component fluid (gas or liquid) mixture is attracted to the surface of a solid (adsorbent) forming attachment through physical or chemical bonds. Adsorption is seen as the most promising, efficient and widely used fundamental technique in wastewater treatment processes (Liang *et al.,* 2009). This is because of its economical viability, simplicity, social acceptability and technical feasibility (Nouri *et al.,* 2007). Another advantage of this technique is that it leads to minimization of chemical or biological sludge; there is also a possibility of metal recovery and adsorbent regeneration after adsorption (Volesky, 2007). The disposal of the adsorbed materials is however, a major challenge for this technique.

2.6 Biosorption

Biosorption is defined as a process in which solids of biological origin, such as microorganisms or their derivatives and plant materials are employed for sequestration of heavy metals from an

aqueous environment (Wang and Chen, 2009). Biosorption is a rapid phenomenon of passive metal uptake sequestration by non-growing biomass. It is a property of certain types of inactive, non-living microbial biomass to bind and concentrate selected heavy metals or other molecules from even very dilute aqueous solution through metabolically mediated or physico-chemical pathways (Volesky, 2007; Shaaban *et al*., 2015). Biomass exhibits this property by acting as a chemical substance or as ion exchangers of biological origin. Particularly, it is the cell wall structure of certain algae, fungi and bacteria that was found to be responsible for this phenomenon. The transfer of metal ions from aqueous to solid biosorbent phase can be due to passive, facilitated or active transport (Volesky, 2007).

The process of biosorption usually involves two phases, a solid phase (sorbent or biosorbent) and a liquid phase (solvent, normally water) which contains a dissolved species to be sorbed (sorbate, metal ion). Due to higher affinity of the sorbent for the sorbate species, the sorbate is attracted and bound with different mechanisms. This process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution (Hussain *et al*., 2010).

According to Coelho *et al*. (2007), biosorption is a promising technique for the removal of heavy metals from aqueous solutions especially if the biosorbents are of lignocellulosic origin. The adsorption of toxic waste from industrial wastewater using agricultural waste and industrial by-products has been massively investigated (Xining *et al*., 2014). Various agricultural by-products both in their raw and modified forms have been studied for their ability to be used as biosorbents; these includes melon seed husk (Giwa *et al*., 2013), sawdust (Kaczala, 2009), Potato peels (Aman *et al*.,2008), tobacco dust (Qi and Aldrich, 2008), coconut shell powder, orange residue (Khormaei *et al*., 2007), (Pino *et al*., 2006), powdered leaves of castor tree (Al Rmalli *et al*., 2008), seed

shells (Amudaa *et al*., 2009), rice straw (Farooq *et al*., 2010) and carrot residues (Nasernejad *et al*., 2005), among others. However, it is still necessary to carry out more work and investigations on some other locally available agricultural residues.

2.6.1 Factors affecting biosorption

In order to understand the quality of the biosorption process and to design the system for the removal of contaminants, there is need to evaluate the effect of factors which influences the performance of biosorption. There are three major factors affecting metal biosorption. These include:

i. The characteristics of biosorbents - surface area, porosity, presence and number of functional groups such as carboxyl, hydroxyl and amino groups.
ii. The characteristics of metals that are being sorbed; which deals with the metal speciation in solution.
iii. Operational parameters such as pH, temperature, contact time, agitation rate, initial concentration of the metal ions and interference of other ions (Aksu *et al.,* 1992; Ahmady – Asbchin *et al.,* 2008).

The major challenging factor for the biosorption process is pH. It seems to be the most important parameter in the biosorption process since, it influences not only the chemistry of the metals in solution but also the activity of the functional groups in the biomass and the competition of metallic ions (Dursun, 2006). These functional groups create a negatively charged surface at above the isoelectric point of the biomass and electrostatic attractions between the metals and the surface of the biomass bring about the biosorption process. At low pH values, the functional groups are protonated thus causing a lower metal uptake when the metal is in the cationic form such as Pb (II) this is due to the blocking of the functional groups responsible for the metal uptake. However,

in the case of Cr (VI) which is anionic in nature, it can bind at lower pH values, that is, the surface charge of the biomass is positive in nature (Friis and Myers-Keith, 1986; Galun *et al*., 1987) .

The concentration of biomass in the solution can also influence the metal removal. If the amount of biomass is increased it influences the solution chemistry of the metals, the activity of the functional groups in the biomass, and also the competition between metallic ions by making available more binding sites for the metal ions in the solution. This phenomenon can be attributed to the interference between the binding sites (Gadd *et al.*, 1988; Fourest and Roux, 1992).

In most cases, biosorption is utilized to treat wastewater which could contain more than one type of metal ions. Hence, the presence of other interfering ions can decline the uptake of the metal of interest. Tsezos and Volesky (1982) reported that the presence of Fe (II) and Zn (II) influenced the uptake of uranium by *Rhizopus arrhizus.* More so, cobalt uptake by different microorganisms also seemed to be completely inhibited by the presence of uranium, lead, mercury and copper (Sakaguchi and Nakajima, 1991; Volesky, 2007).

In 1887, Arrhenius suggested that, when the temperature of a reaction is increased, the rate constant and the reaction rate also increases. Uslu and Tanyol (2006) studied the equilibrium and thermodynamic parameters of biosorption of lead (II) and copper (II) ions onto *Pseudomonas putida*; lead (II) and copper (II) adsorption rate increased when the temperature was increased from 20 to 25^{o}C and 20 to 30^{o}C, respectively. A subsequent increase in temperature reduced the adsorption rate. Zhou *et al*., (2007) reported that the rate of Chromium (VI) biosorption by dead *Bacillus licheniformis* biomass was accelerated when the temperature was elevated, and the metal uptake also increased by the effect of the rise in temperature. All these observations indicate the significance of temperature on biosorption. Biosorption process may be accelerated or decelerated with the increase in temperature, depending on the particular metal and the specific biomass.

Biomass size, structure, and morphology also affect the amounts of metal biosorbed by different biomass. The greater the biomass surface area to dry weight ratio, the greater the quantity of metal biosorbed by a biomass surface per unit weight (Giwa *et al.,* 2013).

2.6.2 Advantages of biosorption

Biosorption has several superiorities over the traditional metal removal methods which make it to be more preferential. The main advantage of biosorption is that it is a cheap process; the cost of biosorbents is low as they are often abundant locally or are made from residual byproducts of industrial plants, mainly agricultural wastes. It yields good metal recovery as the metals can be recovered after being sorbed from the aqueous media using a suitable eluting solution. Biosorption is a regenerative process as the biosorbent can be reused after the metal is desorbed and recycled. No secondary pollution with sludge occurs with biosorption as is the case with many other metal removal technologies hence biosorption can be said to be environmentally friendly (Abdi and Kazemi, 2015).

2.7 Biosorbents

One of the major challenges for biosorption is the selection of the most promising type of biomass from a large option of readily available and inexpensive biomaterials. Ion exchange resins and activated carbon have long been identified as effective commercial adsorbents for treating industrial wastewater but their high cost and low efficiency have limited their use. A good number of natural inorganic materials like clay, synthetic materials such as zeolite, industrial organic wastes substances such as ash and living or non-living materials have found application as good sources of cheap adsorbents capable of replacing the popularly used but more expensive ones (Zouboulis *et al.,* 2010). The biomass based biosorbents provide excellent alternatives to ion

exchange resins and activated carbons due to their low cost and high efficiency (Vijarayaghavan and Yun, 2008a). At the moment, a good number of biomass types have been examined for metal binding capabilities under various conditions. These biosorbents can be sourced from waste biomaterials generated from agriculture (Zouboulis *et al*., 2010), food and pharmaceutical industries; making biosorption an economical alternative treatment method. Different inexpensive, locally and abundantly available biosorbents such as sugarcane bagasse (Ihsan *et al*., 2013), mustard oil cake (Khan *et al.,* 2012), olive-stone (Blázquez *et al.,* 2011), among others have been successfully employed for removal of various heavy metals. Biomass rich in acidic groups such as carboxyl, hydroxyl, and weak basic groups such as amides/amines are efficient in adsorbing metal ions (Joo *et al*., 2010). A large number of microorganisms such as fungi, bacteria, yeasts and algae, they have also been reported to bind a number of heavy metals to varying extents (Wang and Chen, 2006).

2.7.1 Fungi and yeast as biosorbents

The use of fungi as biosorbent material has been shown to be efficient. It is also one of the cost-effective and eco-friendly methods which serve as an alternative to chemically bound treatment process. Fungal and yeast biomass have been demonstrated to have excellent metal-binding properties when compared to other groups of microorganisms. The binding of metals on their cell wall usually involves rapid uptake of large amounts of metals and is independent of metabolism. This adsorption efficiency can be attributed to their high percentage of cell wall material. In fungi and yeast, the biosorption capacity of dead biomass may be greater, equal to or less than that of the living cells (Zouboulis *et al*., 2010). Examples of fungal biosorbents include *Aspergillus sp*

(Yakup and Bayramoglu, 2005; Zeng *et al.*, 2015), *Penicillium sp* (Tan and Cheng, 2003; Fan *et al*., 2008), *Rhizopus sp* (Park *et al*., 2005), etc. Fungi have the benefit of being relatively uncomplicated to grow in fermenters, therefore ,being appropriate for large scale production. Another benefit is the easy separation of fungal biomass by filtration because of its filamentous structure. In comparison to yeasts, filamentous fungi are less sensitive to variations in nutrients, aeration, pH, temperature and have a lower nucleic content in the biomass (Li *et al*., 2015).

2.7.2 Algae biosorbents

Algae are efficient and cheap biosorbents as their nutrient requirement is little. Based on statistical analysis on the potentials of algae in biosorption, it has been reported that algae absorb about 15.3% - 84.6% which is higher as compared to other microbial biosorbents. In all the types of algae, brown alga is known to have high absorption capacity (Mustapha and Halimoon, 2015). Living or dead algal cells are being increasingly used as biosorbents to remove heavy metals from aqueous solutions due to their high sorption uptake and their availability in practically unlimited quantities in the seas and oceans and their reasonable regular quality (Apiratikul and Pavasant, 2008). The marine algae is a major source of potential metal-sorbing biomass and has been a candidate of interest due to its availability from water bodies and the presence of alginate usually in gel form in their cell walls (Vijayaraghavan and Yun, 2008b). In algae, accumulation of metals is rapid and independent of metabolism. It involves adsorption onto the cell surface (wall, membrane or external polysaccharides) and binding to cytoplasmic ligands, phytochelatins and metallothioneins, and other intracellular molecules (Gonzalez *et al*., 2012; Bulgariu and Bulgariu, 2014**)**. The cellulose, proteins polysaccharides and uronic acid present in algal cell wall are potential binding sites (Zouboulis *et al*., 2010). The algal cell wall has many functional groups, such as hydroxyl (OH), phosphoryl (PO_3O_2), amino (NH_2), carboxyl (COOH), sulphydryl (SH),

etc., which confer negative charge to the cell surface (Gonzalez *et al.*, 2012; Bulgariu and Bulgariu, 2014**.)**

2.7.3 Bacteria

Bacterial cell walls are powerful metal chelators. Metal binding occur as a two stage process involving interaction between metal ions and reactive groups followed by inorganic depositions of increased amounts of metals. The carboxyl group of the glutamic acid of the peptidoglycan is the major site for metal deposition (Zouboulis *et al.*, 2010). Although biosorption is generally independent of metabolism, but a metabolism dependent microbial environment may enhance metal deposition on the bacterial biosorbent. Example of bacteria that have found application include the genre *Pseudomonas* (Hasan and Srivastava, 2009), *Escherichia coli* (Souiri *et al*., 2009; Quintelas *et al*., 2009), *Bacillus* (Green-Ruiz *et al.*, 2008). Bacteria have evolved a number of efficient systems for detoxifying metals ions as they develop these resistance mechanisms mostly for their survival. Their isolation, screening and harvesting on a larger scale may be complicated but still remain one of the efficient way of remediating pollutants (Zouboulis *et al.*, 2010).

2.7.4 Agricultural materials/ residues

Recently, attention has been channeled towards the biomaterials which come out as byproducts or wastes from large scale industrial operations and agricultural waste materials. The application of such waste derived, low cost biological material as biosorbents for the removal of heavy metals can be said to be “killing two birds with one stone”. For it uses waste to dispose waste. This waste biomass can be sold to earn some money, at the same time; the cost associated with disposing the waste biomass produced can be saved (Osman *et al.*, 2010; Xining *et al.*, 2014).

Agricultural materials mainly those that are rich in cellulose and lignin show great potential metal binding capacity. The basic components of the agricultural waste biomass include hemicelluloses, lignin, lipids, starch, proteins, extractives, simple sugars, water hydrocarbons among others. These components contain a variety of functional groups which facilitates metal complexation or precipitation and hence assists the heavy metals sequestration process (Bailey et al., 1999). Environmentally friendly and economical agricultural waste materials that are of renewable nature, unique **chemical composition**, abundance and more efficient seem to be viable option for heavy metal remediation. Several Studies reveal that various agricultural waste materials have been tried and were found to have significant metal adsorption capacity. These promising agricultural waste materials can be used in the removal of metal ions either in their natural form or after they undergo some physical or chemical modification (Sud et al., 2008).

There are certain inherent demerits associated with the use of microorganisms for the biosorption of heavy metals. Firstly, algal and fungal biomass which are rich in protein have limitations as proteinaceous materials are likely to putrefy under moist conditions. More so, most metal sorption reported in literature is based on algal and fungal biomass which must be cultured, collected from their natural habitats and pre-processed and transported under special conditions, hence introducing the factor of additional costs (Kumar and Bandyopadhyay, 2006).

2.8 *Ziziphus mauritiana*

Z. mauritiana Lam belongs to the family *Rhamnaceae*. It is widely grown in mild-temperate, rather dry areas, of both hemispheres and is adapted to warm climates. *Z. mauritiana* grows either as shrublets, shrubs or trees with thorny branches and are used as a hedge to form defensive fences for animals (Cherry, 1985). It is often called jujube, Chinese date, Indian plump (Morton, 1987). In northern Nigeria it is called "*Magarya*" (Hausa). It is widely distributed in the Northern part of

Nigeria and is produced in large quantity. The horticultural varieties of *Z. mauritiana* are mainly grown for fruit and can produce large quantities, up to 600 kg/tree per year. The fruit is eaten fresh, dried, pickled or made into conserves. The fruit contains a fleshy edible portion and a stony shell covering the seeds. Although the seed shells are readily available at low-cost but their characteristics on lead adsorption are yet to be systematically studied and just like other seed shells, the stony seed shells of *Z. mauritiana* may also contain cellulose and hemicelluloses, which have adsorption properties and hence can bind to lead II ions (Bailey et al., 1999).

2.9 Recovery of Metals and Regeneration of Biosorbents

A major and important industrial application of biosorption is recovery of loaded pollutants especially valuable metals from the biosorbent and the regeneration of the biosorbents for reuse (Donghee *et al.,* 2010). The biosorption capacity of a biomass and the relative ease with which it can be regenerated and reused determines the worthiness for its use as a biosorbent. (Bishnoi and Garima, 2005).

Generally adsorbates are removed from solution either through metabolism-independent biosorption and metabolism dependent biosorption. The adsorbates that bind onto the surface of a biosorbent through metabolism-independent biosorption could be easily desorbed by simple non-destructive physical methods such as micro-waving and heating. Non-destructive chemical methods can also be used to desorb the adsorbate on the biomass using chemicals such as organic solvents (methanol, ethanol and acetone), alkalis (NaOH and NH_4OH), acids (HCI, H_2SO_4, HNO_3, H_3PO_4 and acetic acid) and other chemicals like $CaCI_2$, KSCN, Na_2CO_3, $KHCO_3$ and EDTA). However, adsorbate bound intracellularly through metabolism-dependent bioaccumulation can only be released using strong acids or alkalis (Donghee *et. al*., 2010). The choice between the uses

of living or dead biomass system is therefore very important because of the implication of the later metal recovery (Donghee *et. al*., 2010). Dilute mineral acids or alkalis are commonly used because they allow efficient desorption from the biosorbent. A major disadvantage is the fact that they also cause serious structural damage to the biosorbent and this may cause a drop in the biosorption capacity of the biomass (Monika *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Lead Nitrate (Pb $[NO_3]_2$) (Merck Germany)

Sodium Hydroxide (NaOH) (The Science company, Assay: 97)

Hydrochloric acid (HCl) (The Science company, Assay: 37%)

All the chemicals used were of analytical grade.

3.1.2 Equipment

Atomic absorption spectrophotometer (FS 240 AA Spectrophotomerter)

FTIR spectrometer (Nicolet IS10, Thermoscientific (UK))

Scanning Electron Microscope (Phenom ProX Desktop)

Magnetic stirrer (IKA*C-MAG HS7 digital)

pH meter (Thermoscientific)

Weighing balance (Mettler Toledo, JS703 C/A, capacity: 700ct)

Grinding mill (Retsch RM 100)

3.1.3 Biosorbent

The fruits and leaves of *Z. mauritiana* were collected from the wild in Zangon kataf LGA, Kaduna state. It was authenticated at Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria, with a voucher number 0295.

3.2 Methodology

3.2.1 Preparation of the biosorbent

The *Z. mauritiana* seeds were broken using pestle and mortar to separate the seeds from the shells, after which the seed shells were washed several times using distilled water to remove the dirt particles. The seed shells were then dried for 24 hours in an oven at 40 °C. The dried seed shells were ground into small particles using a grinding mill (Retsch RM 100) and stored in an airtight container for future use.

1.1.1.1 *3.2.2 Preparation of stock Pb (II) solution*

A stock solution of lead nitrate (1000 mg/l) was used as the adsorbate and solutions of varying concentrations were obtained by diluting the stock solution with distilled deionized water. All pH adjustments were made with 0.1 M hydrochloric acid and 0.1 M sodium hydroxide depending on the pH value required. Lead concentrations were determined using atomic absorption spectrophotometer.

1.1.1.2 *3.2.3 Batch adsorption studies*

The Batch experiment was carried out according to the method described by Das *et al*., (2013) with slight modifications.

3.2.3.1 *Effect of Biosorbent Dose (Mass of Z. mauritiana) on Pb (II) biosorption*

Varying amounts of the biosorbent ranging between 1 – 40 g at an interval of 4 g were equilibrated for 30mins with 200 ml of lead solution at an initial concentration of 50 mg/L. The mixture was filtered and the residual concentration of lead in the filtrate was determined using atomic absorption spectrophotometer. For subsequent analysis, 4 g of the biosorbent was used based on the percentage biosorption obtained for the varying adsorbent doses evaluated.

3.2.3.2 *Effect of Initial Lead Nitrate Concentration on Pb (II) biosorption*

Z. mauritiana powder (4.0 g) was added to 200 ml of Pb (II) solutions within the concentration of 10–50 mg/L at an interval of 10 mg/L. The mixture was stirred for 30 mins, and filtered through Whatman number 1 filter paper, and the residual lead (II) ion concentration was quantified using atomic absorption spectrophotometer.

3.2.3.3 *Effect of contact time on Pb (II) biosorption*

The biosorbent (4.0 g) was mixed with 200 ml of lead nitrate solution of 10 mg/L concentration. The mixture was stirred at 100 rpm and withdrawn at different time intervals; it was filtered using Whatman No 1 filter paper and the residual Pb (II) after adsorption was determined using atomic absorption spectrophotometer. The amount of biosorption at equilibrium, q_e (mg/g) was calculated thus:

$$q_e = \frac{(C_0 - C_e)\ V}{W} \quad \text{----------3.1}$$

Where C_0 and C_e (mg/L) are the liquid-phase concentration of lead at initial and equilibrium time, respectively, V (L) is the volume of the solution and W (g) is the mass of dry biosorbent used. The biosorption efficiency of Pb (II) was calculated as:

$$\text{Percentage biosorption} = \frac{(C_0 - C_e)\ x\ 100}{C_0} \quad \text{----------3.2}$$

3.2.3.4 *Effect of temperature on Pb (II) biosorption*

The biosorbent (4.0 g) was added to 200 ml of lead solution of 10 mg/L concentration within a temperature range of 0 ^{0}C – 100 ^{0}C at an interval of 10 ^{0}C. The mixture was shaken at 100 rpm for 1 hr. The mixture was then filtered and the residual lead ion concentration of the filtrate was determined using atomic absorption spectrophotometer.

3.2.3.5 *Effect of pH on Pb (II) biosorption*

The biosorbent (4.0 g) was mixed with 200 ml of lead solution of 10 mg/L concentration at different pH values 3 – 9 at an interval of 1. The pH was adjusted with 0.1 M NaOH or 0.1 M HNO_3 solutions and measured with a pH meter. Agitation was at a constant stirring speed of 100 rpm for 1hr. The solution was filtered and the residual lead concentration in the filtrate was determined using atomic absorption spectrophotometer.

3.2.3.6 *Effect of agitation rate on Pb (II) biosorption*

The effect of agitation rate on Pb (II) biosorption was investigated by adding 4.0 g of biosorbent into 200 ml of lead solution of 10 mg/L concentration at pH 7 and at a constant temperature of 70°C. The solution was agitated at different stirring rates ranging from 100 rpm – 500 rpm at an interval of 100 rpm. The solution was filtered and the residual lead concentration in the filtrate was determined using atomic absorption spectrophotometer.

3.2.4 Determination of the Mechanism of Pb (II) Biosorption

Batch adsorption studies was carried out at the optimum conditions previously determined and the modelling of the biosorption process on *Z. mauritiana* seed shells was obtained by using different adsorption isotherms. In order to determine the adsorption isotherms, Langmuir and Freundlich equations were used. The Langmuir Equation is expressed as:

$$\frac{C_e}{q_e} = \frac{1}{q_m K_1} + \frac{C_e}{q_m} \qquad \text{----------}$$

3.4 where q_e is the amount of metal ion biosorbed on a biosorbent (mol g^{-1}) at equilibrium time, q_m is monolayer capacity of the biosorbent (mol g^{-1}), K_L is the biosorption constant (L mol^{-1}), and C_e is equilibrium concentration of metal ion in solution (mol L^{-1}).

The shape and favourability of the biosorption process was identified by means of a dimensionless separation factor (S_f). S_f is calculated using Equation 3.5

$$S_f = \frac{1}{1+K_L C_0} \quad \text{----------3.5}$$

Where K_L is the Langmuir constant and *Co* is the initial concentration of lead (II) ions. The value of separation parameter (S_f) provides important information about the nature of adsorption. The value of S_f indicates the type of Langmuir isotherm to be irreversible where ($S_f = 0$), favorable ($0 < S_f < 1$), linear ($S_f = 1$) or unfavorable ($S_f > 1$).

The Freundlich equation in logarithmic form can be given as

$$\text{Log } q_e = \log k_f + \frac{1}{n} \log c_e \quad \text{---------- 3.6}$$

where K_F and *n* are empirical Freundlich constants, and indicative of adsorption capacity and adsorption intensity, respectively; q_e and C_e are the amount of metal ion biosorbed on a biosorbent (mol g^{-1}) at equilibrium time and the equilibrium concentration of metal ion in solution (mol L^{-1}), respectively.

3.2.5 Kinetics Studies of Pb (II) Adsorption

The kinetics study of Pb (II) biosorption was carried out by batch adsorption at optimum conditions evaluated previously. The samples were taken at preset time intervals of 15minutes up to 60 minutes. The residual Pb (II) concentration after adsorption was determined using AAS and the amount of biosorption at time *t*, q_t (mg/g) was calculated using Equation 3.7:

$$q_t = \frac{(C_o - C_t)\, V}{W} \quad \text{----------3.7}$$

1.1.1.3 *Where C_t (mg/L) is the liquid-phase concentrations of Pb (II) solutions at any time, t.*

1.1.1.4 *The biosorption kinetics of the removal of Pb (II) was analysed using Lagergren Pseudo-first-order, second-order kinetic equations and the intraparticle diffusion model.*

3.2.5.1 *Pseudo-first order kinetic model*:

The pseudo-first order rate expression given by Lagergren can be represented as follows:

$$\mathrm{Log}(q_e - q_t) = \mathrm{Log}q_e - \frac{k_1 t}{2.303} \quad \text{----------3.8}$$

where k_1 (s^{-1}) is the rate constant of biosorption, q_e and q_t are amounts of metal ion biosorbed (mol g^{-1}) on biosorbent at equilibrium, and at time t (s), respectively. The rate constants, k_1, will be calculated from the plots of log (q_e - q_t) versus t for different concentrations of Pb (II).

3.2.5.2 *Pseudo-second order kinetic model*:

The pseudo-second order kinetic model based on adsorption equilibrium capacity can be expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad \text{---------- 3.9}$$

where k_2 is the pseudo-second order rate constant (g mol^{-1} s^{-1}).

3.2.5.3 *Intra-particle diffusion model:*

The Weber and Morris intra-particle diffusion model was to elucidate the diffusion mechanism. The model was expressed using Equation 3.10:

$q_t = K_d\ t^{1/2} + I$ ----------3.10

Where I is the intercept and K_d is the intra-particle diffusion rate constant.

3.2.6 Biosorption Thermodynamics

Batch adsorption study was carried out at the optimum conditions determined previously but at varying temperatures ranging between 0 ^oC – 70 ^oC at an interval of 10 ^oC. The thermodynamic parameters for the biosorption process such as changes in free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0) were evaluated using the following Equations:

$$K_c = \frac{C_{Ae}}{C_e} \quad \text{----------3.11}$$

$\Delta G^0 = -RT \ln K_c$ ---------- 3.12

$$\text{Log } K_c = \frac{\Delta S^0}{2.303R} - \frac{\Delta H^0}{2.303RT} \quad \text{----------3.13}$$

Where C_e is the equilibrium concentration in solution in mg/L and C_{Ae} is the equilibrium concentration on the sorbent in mg/L and K_c is the equilibrium constant. *R* is the gas constant (8.314 J mol^{-1} K^{-1}), and *T* is the absolute temperature (K). The values of ΔH^0 and ΔS^0 will be calculated, respectively, from the slope and intercept of the van't Hoff plot of logK_c versus 1/*T*.

3.2.7 Scan Electron Microscopy

Scan electron microscopy was used to study the surface morphology of the biosorbent to provide information on the degree of interaction between the biosorbent particles and the metal ions. This analysis was carried out by placing a double adhesive on a sample stub after which the biosorbent was sprinkled on the double adhesive. It was placed on a sample cutter and 5nm of gold deposited on the sample to make it conductive, it was then put on a sample holder and inserted into the scan electron microscope column, after which the sample was viewed under the navigation camera in order to adjust the contrasts, clarity, magnification and was sent to electron mode where the morphology was studied.

3.2.8 Fourier Transform Infrared (FTIR) Analysis

FTIR spectroscopy was used to identify the chemical groups present in seed shells which are possibly implicated in the biosorption process. The analysis was carried out using KBr as the background material within a spectral range of 4000 to 500 cm^{-1}. 40 mg of the ground biomass was encapsulated in 400 mg.

CHAPTER FOUR

RESULTS

4.1 Effect of Batch Parameters on Pb (II) Biosorption

4.1.1 Effect of Adsorbent Dose

Different biosorbent doses were selected ranging from 1 g to 40 g while the concentration of Pb (II) was fixed at 50 mg/L. Figure 4.1 shows that the percentage Pb (II) ion removal increased with increase in biosorbent dose. The equilibrium adsorption capacity Figure 4.2 however showed an opposite trend; the adsorption capacity reduced from 7810 to 226.63 mg/g for an increase in adsorbent dosage from 1 g to 40 g, respectively. More so, maximum Pb (II) ion removal (90.65 %) was recorded at 40 g of *Z. maritaiana* seed shells.

4.1.2 Effect of initial Pb (II) ion concentration

Figure 4.3 shows the effect of different initial Pb (II) ion concentration on biosorption of Pb (II) ion onto *Z. mauritiana* seed shells. The percentage removal of Pb (II) ion decreased with increasing initial Pb (II) ions concentration. The maximum Pb (II) ion removal (82.05%) occurred at 10 mg/L with 50 mg/L having the minimum (71.94%). On the other hand, the adsorption capacity at equilibrium increased with increase in initial Pb (II) ion concentration as seen in Figure 4.4 where it increased from 410.25 to 1798.45 mg/g as the initial concentration was changed from 10 to 50 mg/L, respectively.

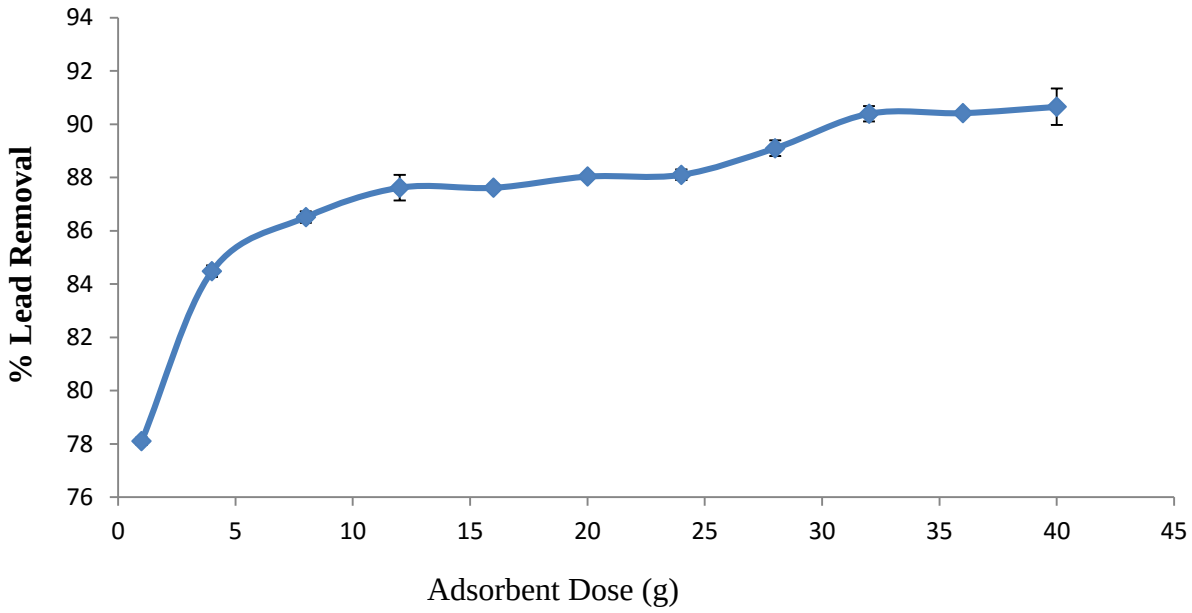


Figure 4.1: Effect of Adsorbent Dose on Biosorption of Lead (II) by *Z. mauritiana* Seed shells. Biosorption Conditions: 1-40 g sorbent, 200 ml of sorbate, pH 5.0, incubation time: 30 mins, temperature 25±2 ^{o}C.

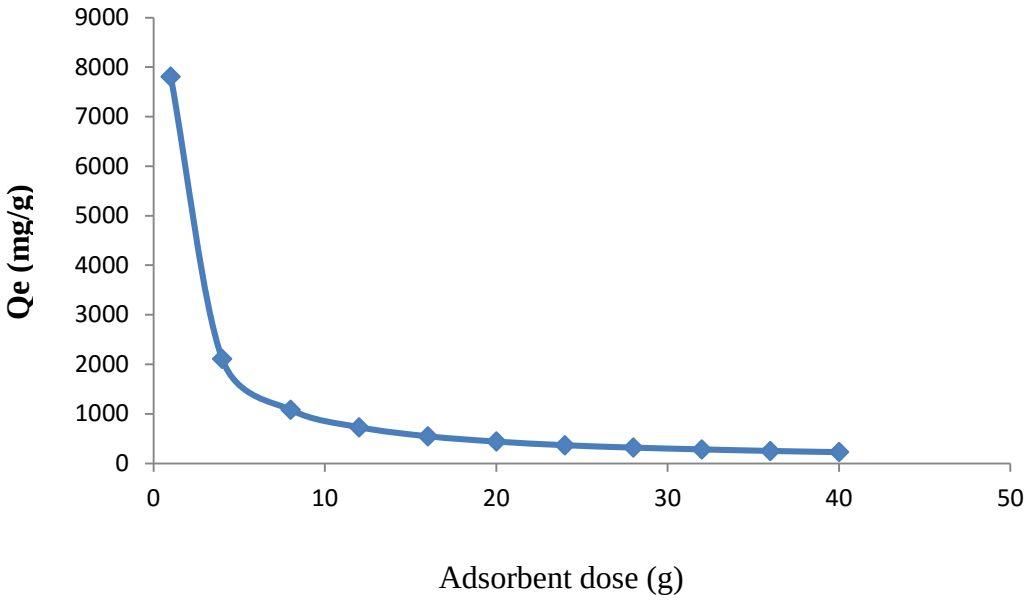


Figure 4.2: Effect of Adsorbent Dose on Adsorption Capacity for Lead (II) by *Z. mauritiana* Seed Shells. Biosorption Conditions: 1-40 g sorbent, 200 ml of sorbate, pH 5.0, incubation time: 30 mins., temperature 25±2 ^{o}C.

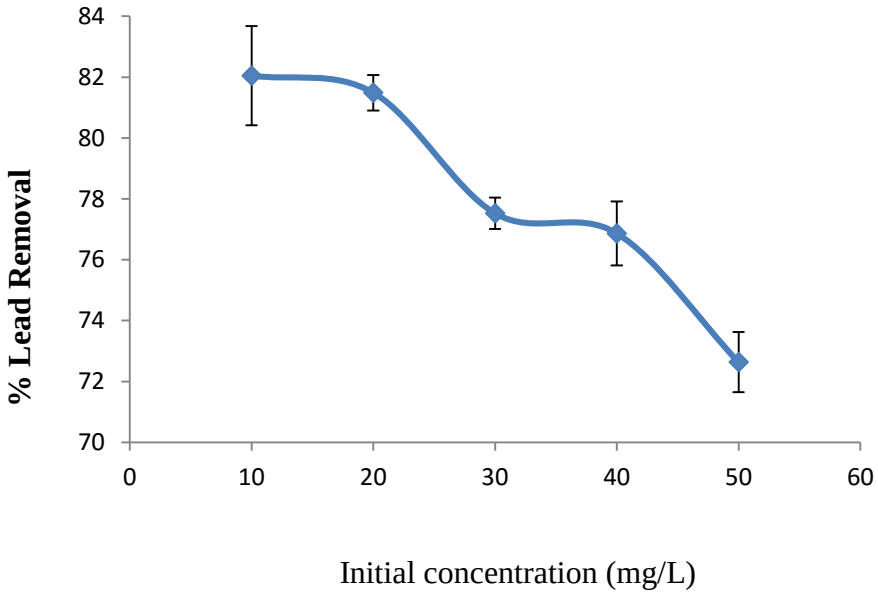


Figure 4.3: Effect of Initial Pb (II) Ions Concentration on Biosorption of Lead (II) by *Z. mauritiana* Seed Shells. Biosorption Conditions: 4 g sorbent, 200 ml of sorbate, pH 5.0, incubation time: 30 mins

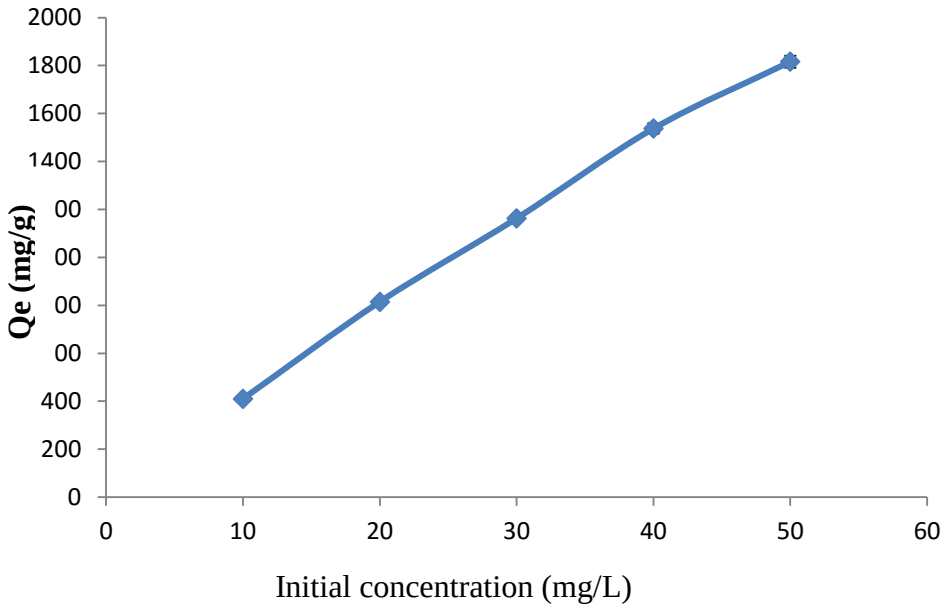


Figure 4.4: Effect of Initial Pb (II) Ions Concentration on Adsorption Capacity for Lead (II) by *Z. mauritiana* Seed Shells. Biosorption Conditions: 4 g sorbent, 200 ml of sorbate, pH 5.0, incubation time: 30 mins.

4.1.3 Effect of contact time

The biosorption of Pb (II) ion as a function of contact time was examined by varying the contact time within a range of 15 -120 minutes as shown in Figure 4.5. The result shows that, biosorption of Pb (II) ion markedly increased with an increase in contact time up to 60 minutes (80.69%). Although there was a further increase in adsorption with increase in contact time but the equilibrium was nearly reached after 60 minutes as the rapidity of percentage Pb removal was seen to reduce after the first 60 minutes.

4.1.4 Effect of temperature

The effect of temperature was examined by varying the incubation temperature within a range of 0 ^{o}C -100 ^{o}C with the initial concentration of Pb (II) fixed at 10 mg/L. The results obtained shown in Figure 4.6 shows that biosorption of Pb (II) ion by *Z. mauritiana* seed shells initially increased with increasing temperature with a maximum percentage Pb (II) removal (89.48%) observed at 70 ^{o}C with further increase in temperature leading to a decrease in the Pb (II) ion biosorption.

4.1.5 Effect of pH

The effect of pH on the removal efficiency of Pb (II) ion was studied at different pH values ranging from 3.0 to 9.0, and at an initial lead ion concentration of 10 mg/L. From Figure 4.7, it was observed that there was an increase in Pb (II) ions removal as the pH increased from 3.0-7.0 with a maximum percentage Pb (II) ion (88.39%) removal at pH 7.0. A decrease in the Pb (II) ion removal occurred when the pH value of the solutions was further increased from 8.0 to 9.0.

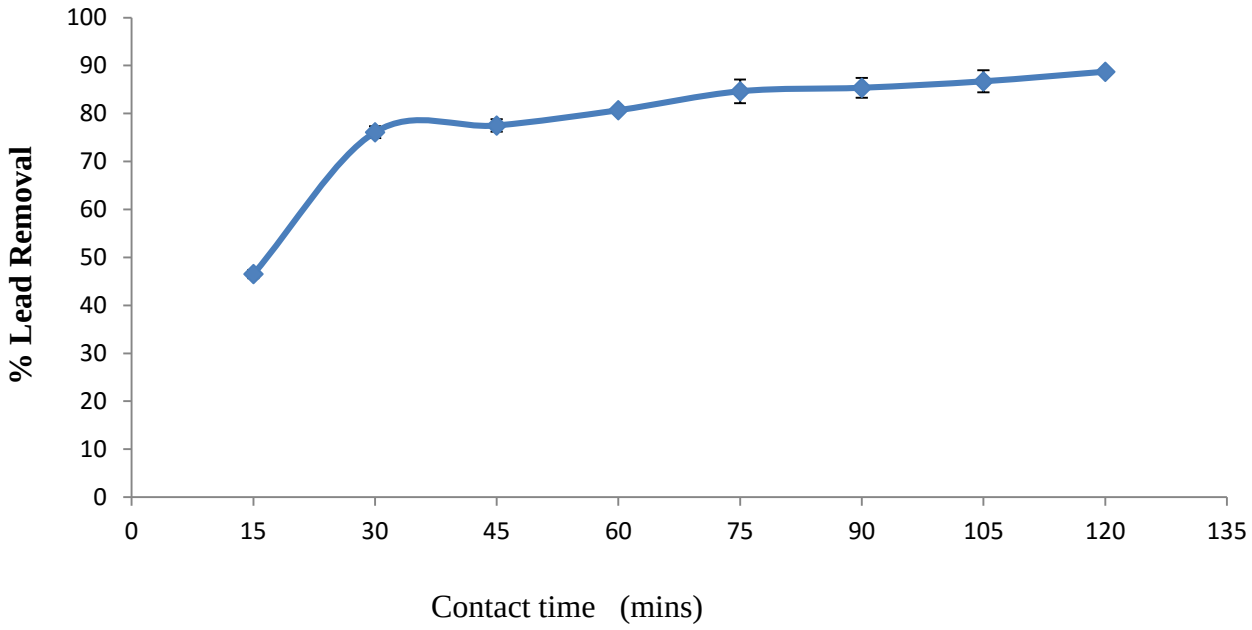


Figure 4.5: Effect of Contact time on Biosorption of Lead (II) by *Z. mauritiana* Seed Shells for Lead (II). Biosorption Conditions: 4 g sorbent, 200 ml of sorbate (10 mg/L), pH 5.0, Temperature 25±2 ^{o}C.

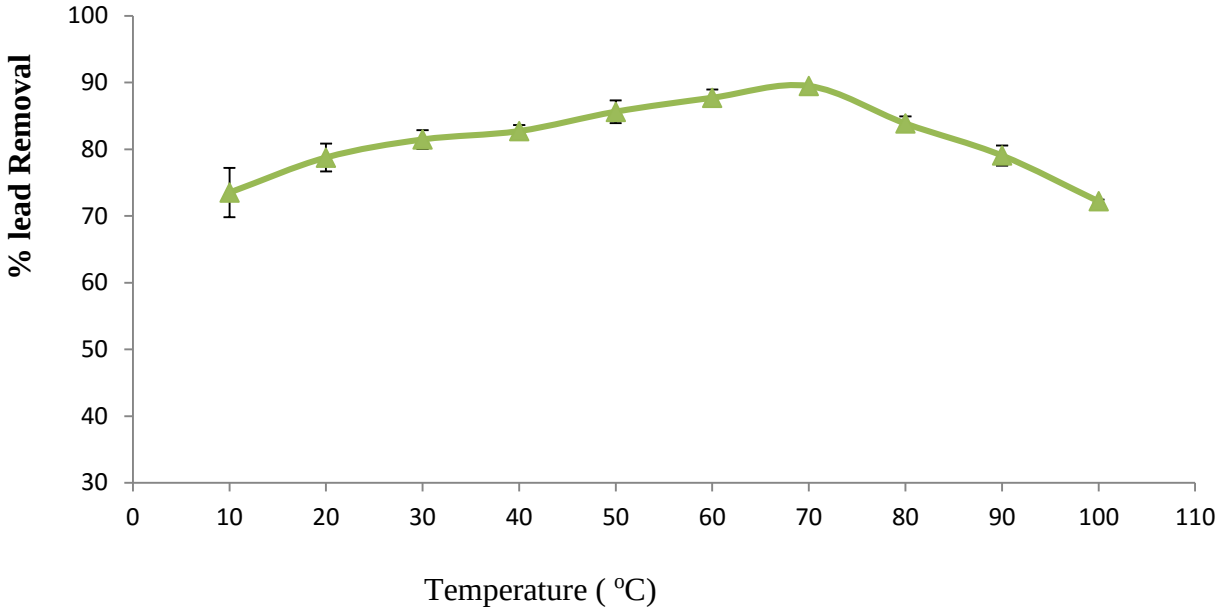


Figure 4.6: Effect of Temperature on Biosorption of Lead (II) by *Z. mauritiana* Seed Shells. Biosorption Conditions: 4 g sorbent, 200 ml of sorbate (10 mg/L), pH 5.0, incubation time, 60 mins, speed, 100 rpm

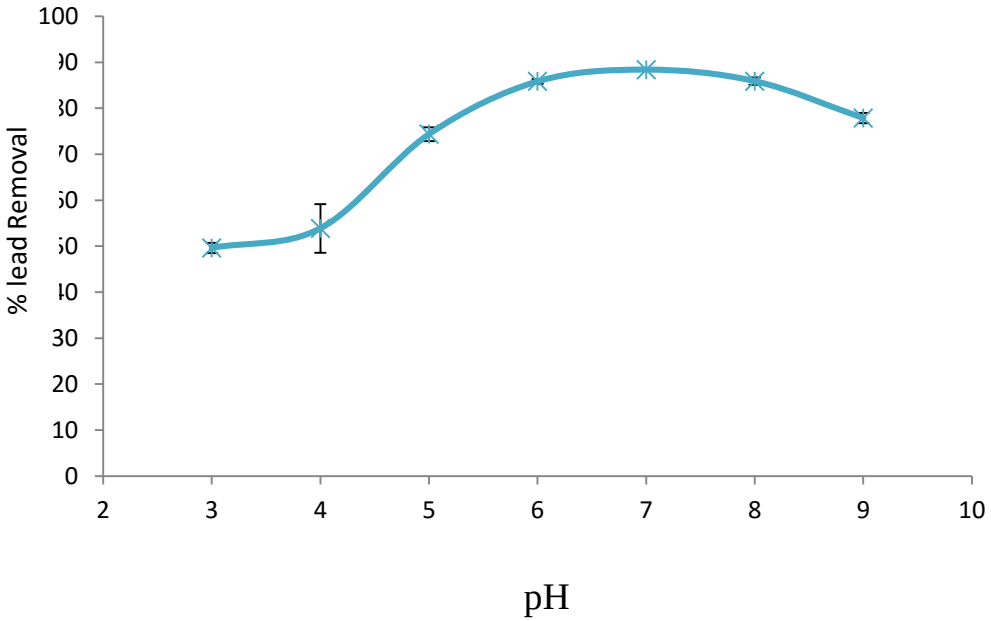


Figure 4.7: Effect of pH on Lead (II) Biosorption by *Z. mauritiana* Seed Shells. (Experimental conditions: Initial Pb (II) concentration: 10 mg/L, Adsorbent dose: 4 g, 200 mL of sorbate (10mg/L), Agitation speed: 100 rpm, Temperature: 70^{o}C, Contact time: 60 mins)

4.1.6 Effect of agitation rate

Figure 4.8 shows the effect of agitation rate on Pb (II) biosorption. The result shows that shaking rate has pronounced effect on the amount of Pb (II) biosorbed. The result shows an increase in percentage removal of Pb (II) from 56.87% to 85.18% as the agitation rate increased from 100 to 300 rpm respectively. Beyond 300 rpm, the percentage Pb (II) removal remained relatively constant.

4.2 Mechanism of the Biosorption

The plots of Langmuir and Freundlich equations representing Pb (II) biosorption by *Z. mauritiana* seed shells are illustrated in Figures 4.9 and 4.10, respectively. The adsorption constants of Langmuir and Freundlich equations and their correlation coefficients (R^2) were presented in Table 4.1. The R^2 values for the Langmuir and Freundlich plots were 0.721 and 0.952, respectively; with Langmuir constant (K_L) of 0.0181 and the monolayer capacity of the biosorbent (q_{max}) of 500mg/g. From Table 4.1, the K_F constant of Freundlich isotherm, was 82.79 L/g, whereas the 1/*n* value was found to be 0.648.

4.3 Thermodynamics of the Biosorption.

The Van't Hoff plot of log K_c versus 1/T is presented in Figure 4.11 with a correlation coefficient of 0.874. The values of ΔS^0 and ΔH^0 determined from the intercept and slope of the plot were found to be 50.07kJ/mol and 15807.88kJ/mol, respectively as presented in Table 4.2. The values of ΔG^0 at different temperatures are also presented in Table 4.2. As the temperature increased, ΔG^0 values also decreased.

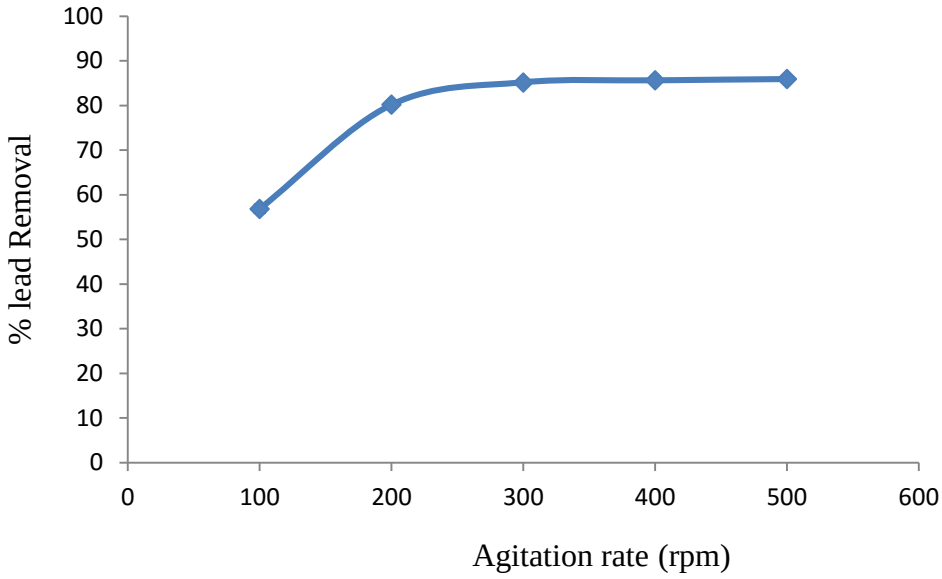


Figure 4.8: Effect of Agitation Rate on Biosorption of Lead (II) *Z. mauritiana* Seed Shells. (Experimental Conditions: Initial Pb (II) concentration: 10 mg/L, Adsorbent dose: 4 g, 200 mL of sorbate (10 mg/L), Contact time: 60 min, pH 7.0).

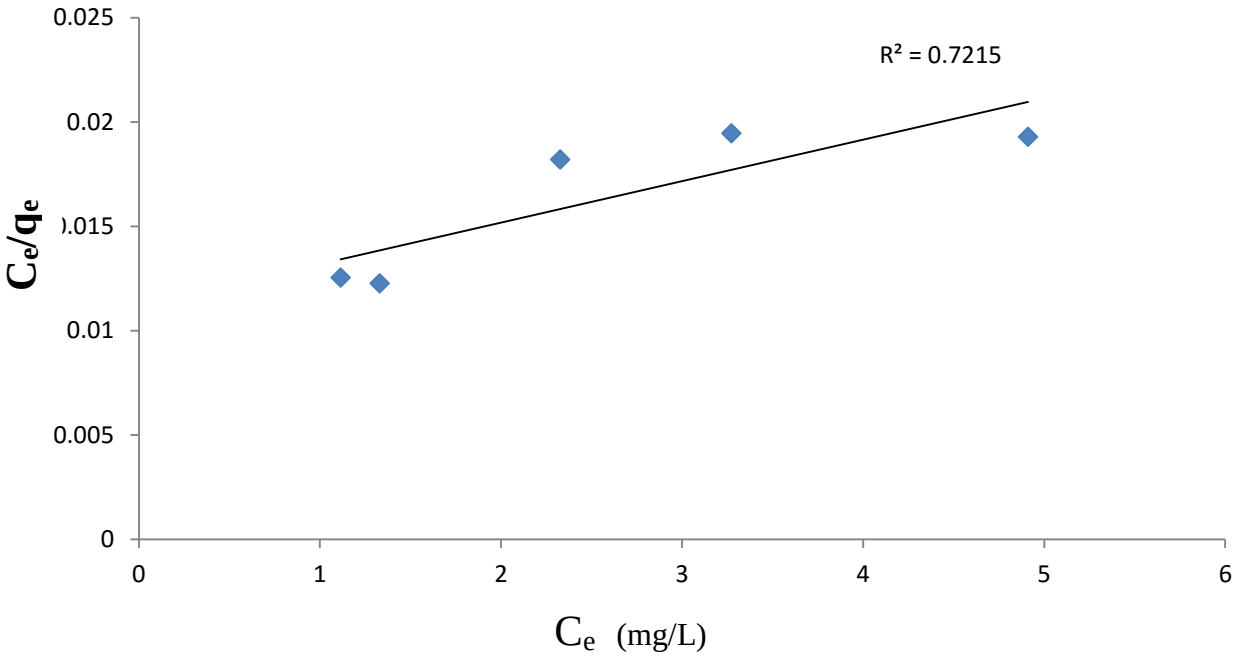


Figure 4.9: Langmiur Isotherm for Lead Biosorption onto *Z. mauritiana* Seed Shells. At 10 mg/L Pb (II) concentration, 70 °C, 300 rpm, pH 7 and for 60 mins.

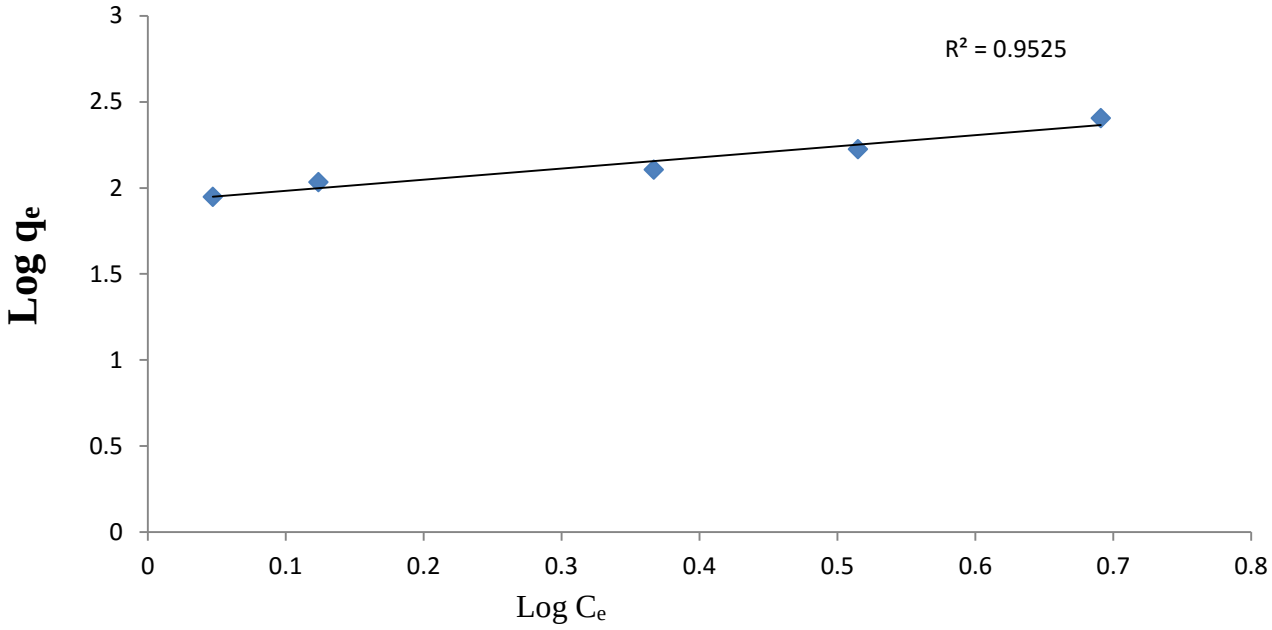


Figure 4.10: Freundlich Isotherm for Lead (II) Biosorption onto *Z. mauritiana* Seed Shells. At 10 mg/L Pb (II) concentration, 70 °C, 300 rpm, pH 7 and for 60 mins.

Table 4.1: Adsorption Isotherm Constants for Biosorption of Pb (II) onto *Z. mauritiana* seed shells

Adsorption Isotherms	Parameters		R^2	S_F
Langmuir Isotherm	q_{max}, mg/g	500	0.721	0.3549
	K_L, L/mg	0.1818		
Freundlich Isotherm	K_F, mg/g	82.79	0.952	0.0012
	1/n	0.648		
	n	1.543		

(q_{max} is the maximum adsorption capacity (mg/g); K_L is the Langmuir constant related to the energy of adsorption (L/mg),; K_F is the Freundlich Constant measuring adsorption capacity; n is the adsorption intensity)

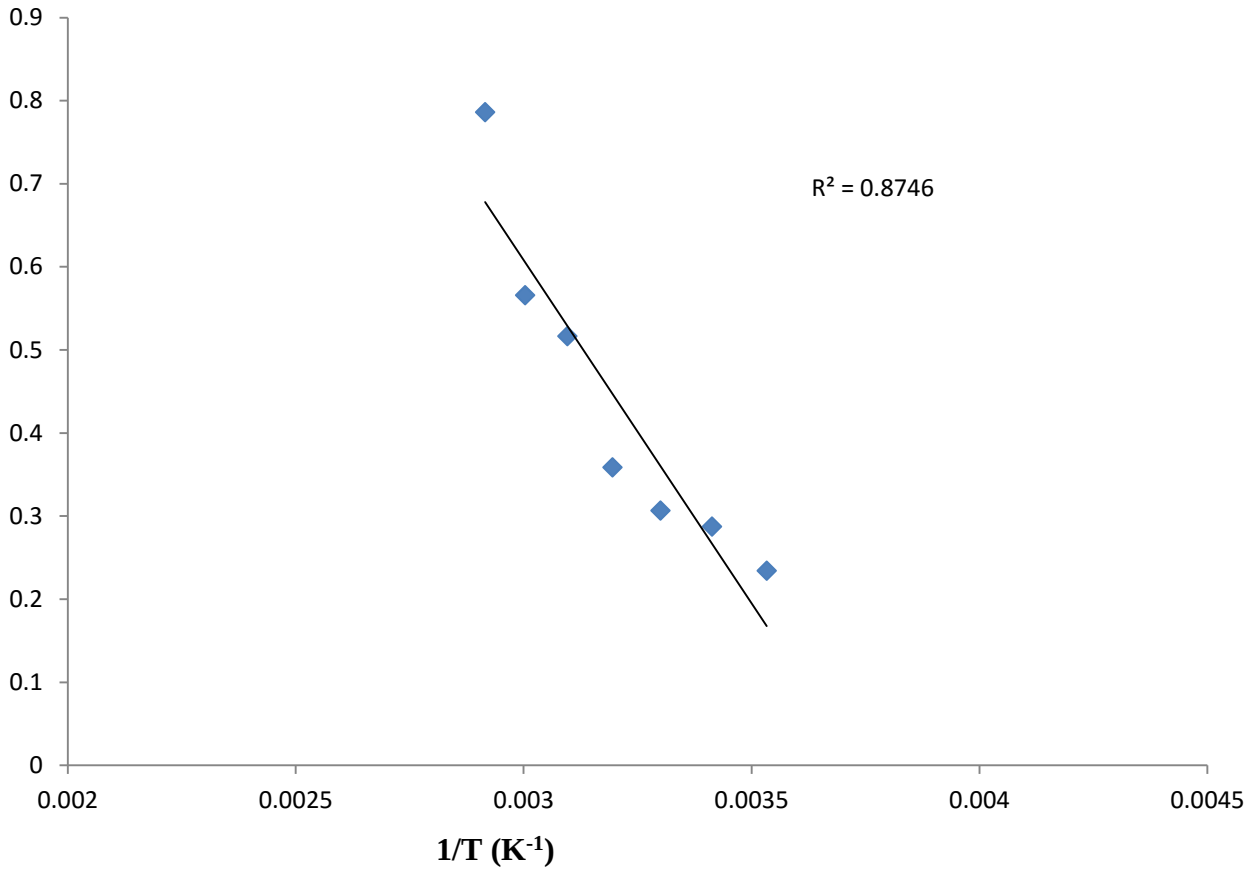


Figure 4.11: Van't Hoff Plot of log K_c versus 1/T

Table 4.2: Thermodynamic Parameters for Biosorption of Pb (II) onto *Z. mauritiana* seed shells

Temperature (**K**)	ΔG^0 (kJ/mol)	ΔS^0 (kJ/K/mol)	ΔH^0 (kJ/mol)
283	-551.52	50.07	15807.88
293	-700.31		
303	-772.56		
313	-933.73		
323	-1387.26		
333	-1566.41		
343	-2242.04		

4.4 Kinetics of the Biosorption.

Figures 4.12, 4.13 and 4.14 represent the Lagergren Pseudo first, second order and the intra-particle diffusion model plots for lead biosorption onto *Z. mauritiana* seed shells, respectively. Their respective R^2 values were found to be 0.882, 0.997 and 0.908. The value of the theoretical equilibrium uptake, ($q_{e, cal}$) obtained for second-order equation and the experimental value ($q_{e, exp}$) are in good agreement as represented in Table 4.3. The other kinetic parameters K_1 and K_2 were found to be 0.0299 and 4 x 10^{-4} g/mg/min, respectively (Table 4.3). K_d and I were found to be 28.29 mg/g.$min^{1/2}$ and 260.4, respectively.

4.5 Scan Electron Microscopy (SEM)

The SEM micrographs of biomass surface before and after Pb (II) ions biosorption are shown in Figures 4.15a and 4.15b, respectively. The porous and irregular surface structure of the adsorbent can be clearly observed in the SEM image shown in Figures 4.15a and 4.15b. There is a clear demarcation in the surface morphology of biomass as observed when the total number of pores on the biomass surface reduced from 60 to 47 after biosorption. The average pore area ratio also reduced from 9.35% to 5.95%. Other morphological changes are represented in Appendix 1.

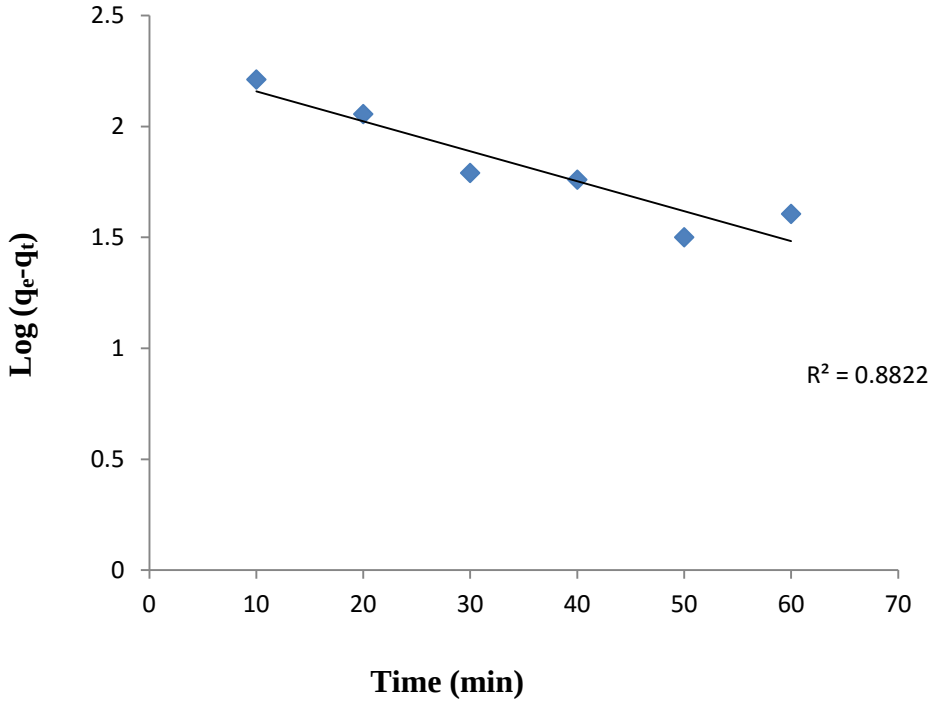


Figure 4.12: Pseudo-first-order Kinetic Plot for Lead Biosorption onto *Z. mauritiana* Seed Shells. At 10 mg/L Pb (II) concentration, 70 °C, 300 rpm, pH 7 and for 60 mins.

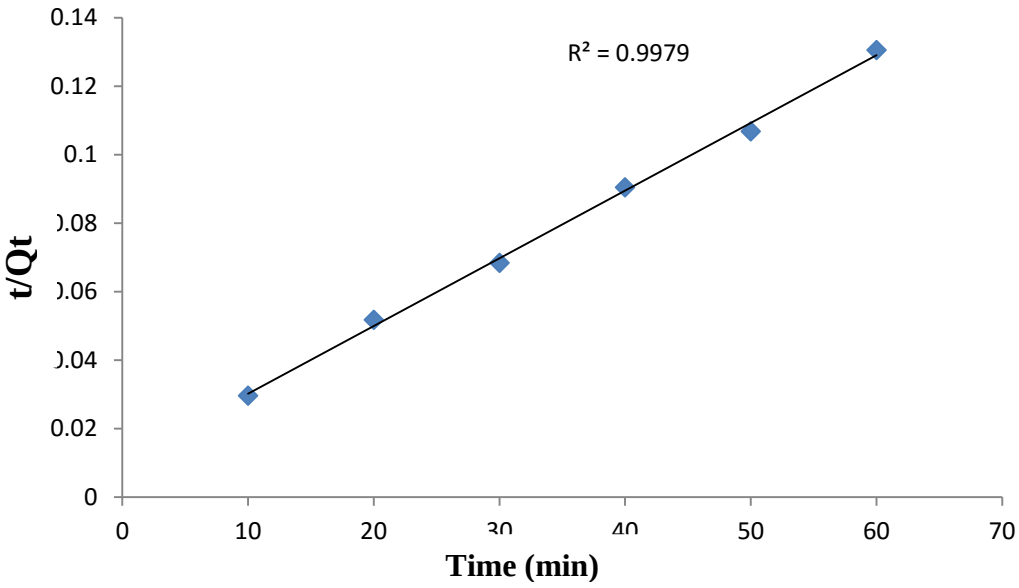


Figure 4.13: Pseudo-second-order Kinetic Plot for Lead (II) Biosorption onto *Z. mauritiana* Seed Shells. At 10 mg/L Pb (II) concentration, 70 °C, 300 rpm, pH 7 and for 60 mins.

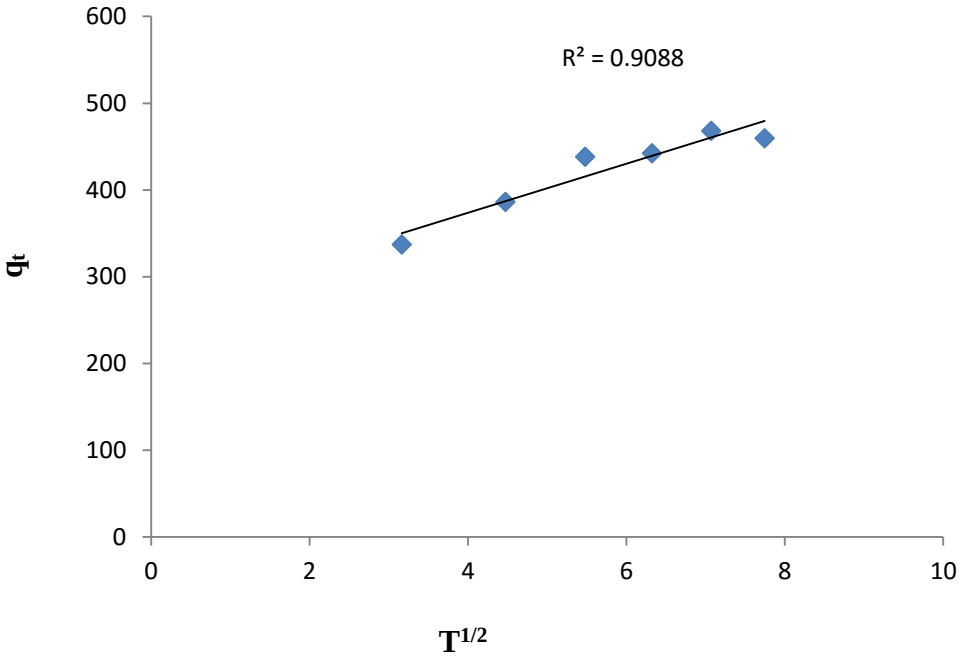
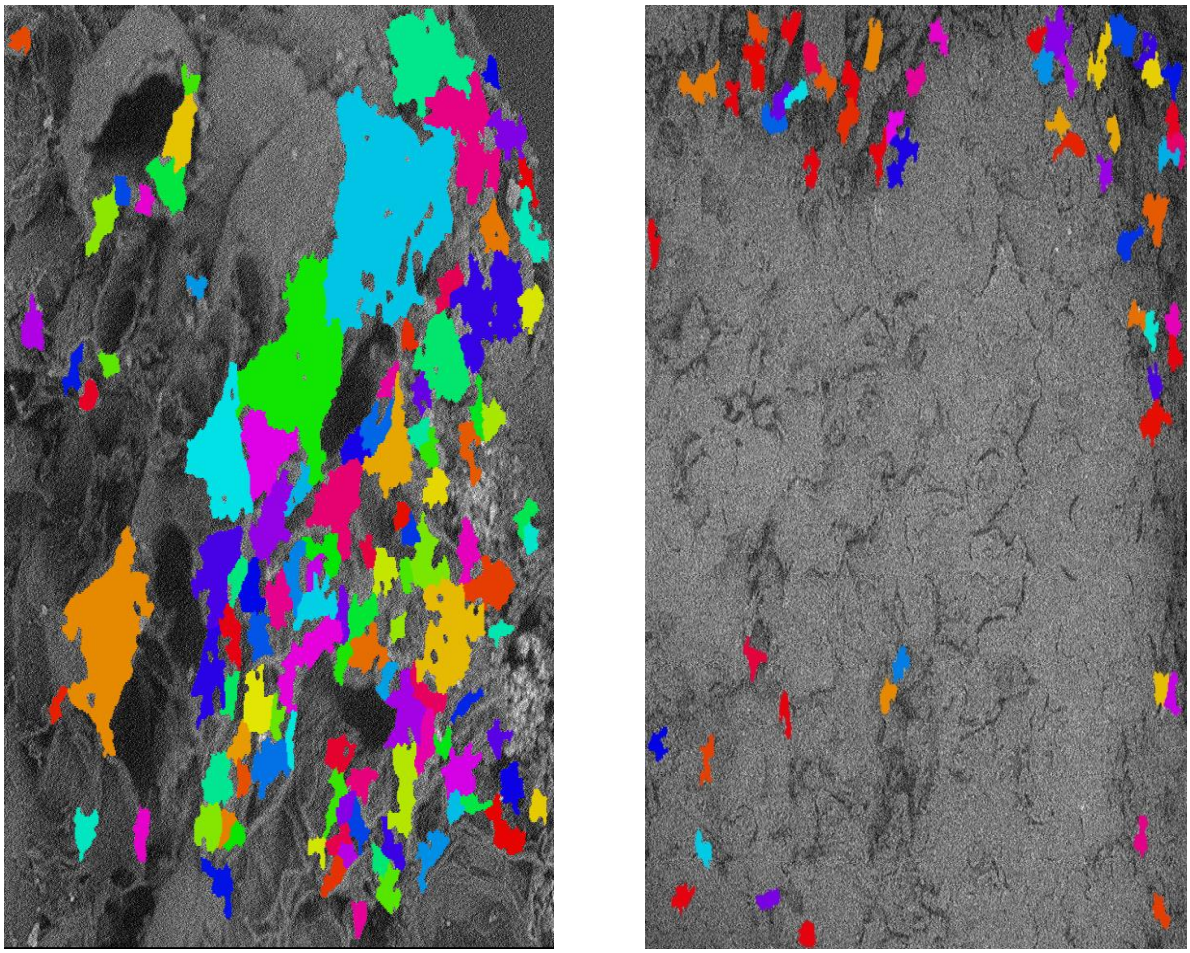


Figure 4.14: Intra-particle Diffusion Model for Lead (II) Biosorption onto *Z. mauritiana* Seed Shells. At 10 mg/L Pb (II) concentration, 70 °C, 300 rpm, pH 7 and for 60 mins.

Table 4.3: Kinetic Parameters for Biosorption of Pb (II) onto *Z. mauritiana* seed shells

Kinetic model	Parameters	
Pseudo-first-order	$q_{e\ exp.}$ (mg/g)	500
	K_1(g/mg/min)	0.0299
	$q_{e,\ cal.}$ (mg/g)	196.34
	R^2	0.882
Pseudo-second-order	K_2 (g/mg/min)	4×10^{-4}
	$q_{e,\ cal}$ (mg/g)	500
	R^2	0.997
Intra particle diffusion model	K_d (mg/g.min$^{1/2}$)	28.29
	I	260.4
	R^2	0.908



(a) (b)

Figure 4.15: SEM Image of Biomass (a) before Biosorption and (b) after Biosorption. Magnification: 500x

4.6 Fourier Transform Infrared (FTIR)

The FTIR spectra of the *Z. mauritiana* seed shells before and after Pb (II) biosorption were shown in Figure 4.16a and b, respectively. The spectra in Figure 4.16a showed peaks at 3295, 1636, 1505, 1461, 1371, 1319, 1241 and 1032cm^{-1}.whose intensity were either minimized or shifted slightly into 3423, 1637,1541, 1458, 1378, 1320, 1251 and 1046cm^{-1}, respectively in Figure 4.16b. Some of the basic frequencies of *Z. mauritiana* seed shells and the corresponding functional groups are displayed in Appendix 2.

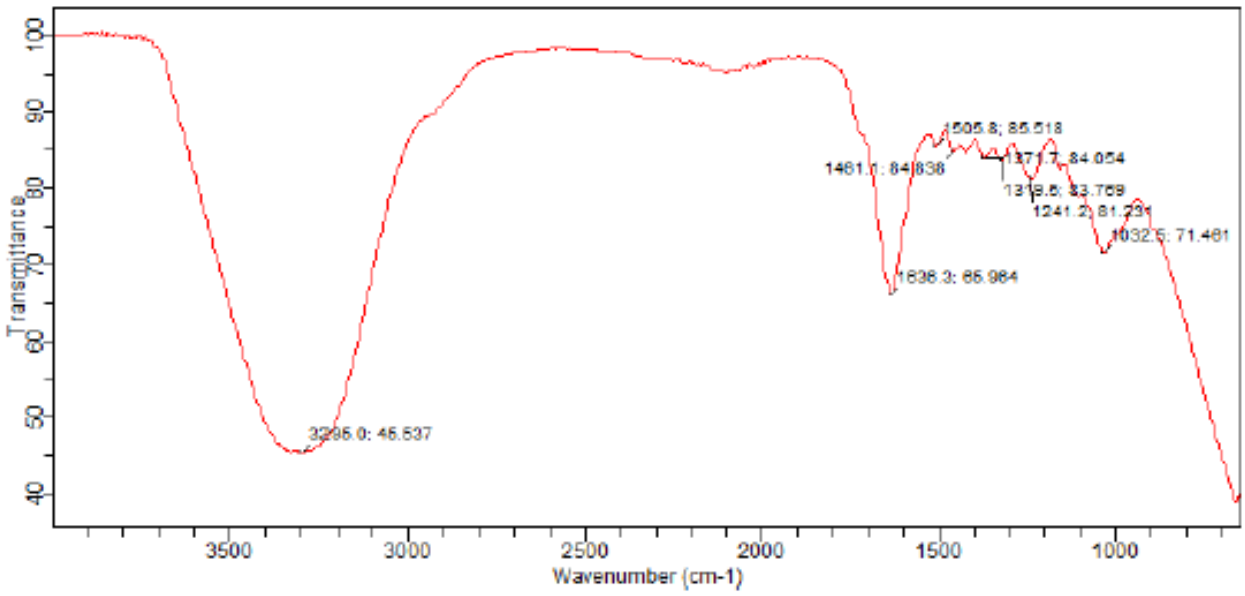


Figure 4.16a: FTIR of biomass before biosorption

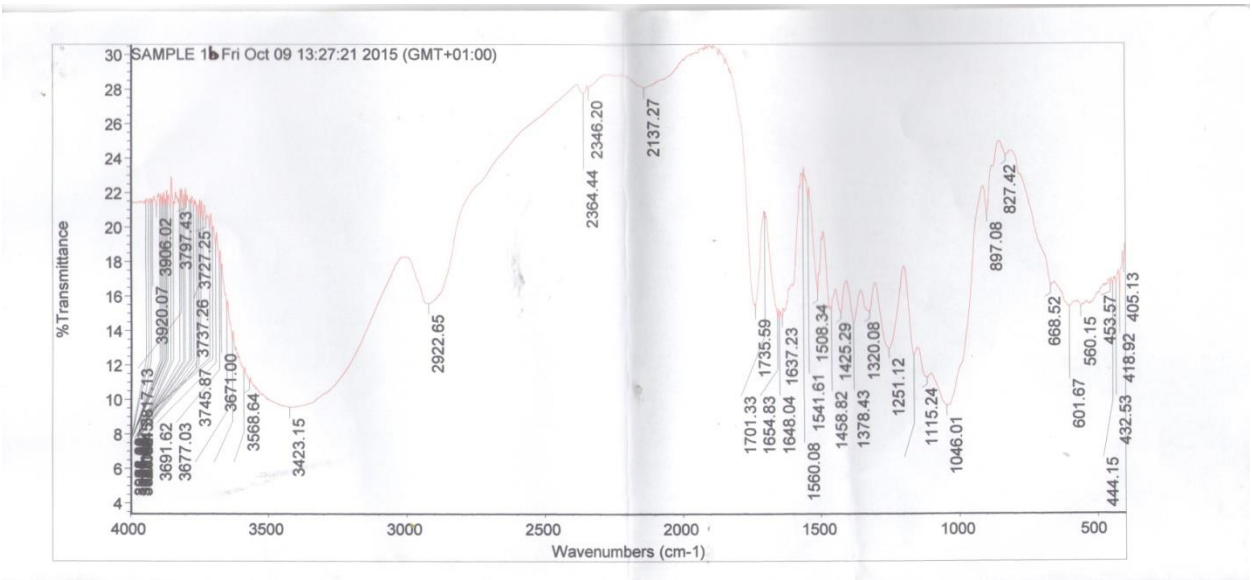


Figure 4.16b: FTIR of biomass after biosorption

CHAPTER FIVE

5.0 DISCUSSION

The increase in percentage Pb (II) removal by *Z. mauritiana* seed shells with increasing adsorbent dosage could be attributed to increase in the adsorbent surface area consequently leading to an increase in the number of binding sites on the adsorbent surface. Hence, allowing the binding of more metal ions (Esposito *et al*., 2001). The marked reduction in adsorption capacity as the adsorbent dosage was increased from 1g to 40 g indicated in Figure 4.2 may be due to the decrease in total adsorption surface area available to Pb (II) ions resulting from overlapping or aggregation of adsorption sites (Sarin and Pant, 2006; Akar *et al*., 2009). Maximum Pb (II) ion removal (91.61%) was recorded at 40 g of *Ziziphus maritaiana* seed shells. This result agrees with those earlier reported by Awwad and Salem, (2012) on biosorption of copper (II) and lead (II) ions by modified loquat (*Eriobotrya japonica*) leaves. Mataka *et al.* (2010) also had similar findings on cadmium sorption using *Moringa stenopetala* and *Moringa oleifera* seed powder.

Considering that the rate of adsorption is a function of the initial concentration of the adsorbate, initial sorbate concentration becomes an important factor to be considered for effective adsorption. The percentage removal of Pb (II) by *Z. mauritiana* seed shells decreased with increasing initial Pb (II) ion concentrations as presented in Figure 4.3. This may be because of greater numbers of ions in solution at high metal concentration collide more frequently with each other and with those bound to the adsorbent surface thereby leading to desorption of the metal ions from the binding sites of adsorbent particles (Ekpete *et al*., 2010). Moreover, all adsorbents have a limited number of binding sites and at a certain concentration the binding sites become saturated (Tsai and Chen, 2010). The enhanced removal of Pb (II) ions at low concentration can hence be accounted for by the high availability of free reactive/ binding sites. However, in higher concentration, the reduced

availability of reactive sites may slow down the biosorption process (Ihsan *et al*., 2013). The adsorption capacity at equilibrium however, increased with increase in initial Pb (II) ion concentration (Figure 4.4). It is possible that the initial concentration of the metal ions provides the necessary driving force to overcome the mass transfer resistance of Pb (II) ion between the aqueous and the solid phase (Chowdhury and Saha, 2010). This trend was earlier reported by Ekpete, *et al*. (2010) for the adsorption of chromium (VI) and zinc (II) ions onto the skin of orange peels.

As observed in Figure 4.5, there was a quick uptake of the metal ions in solution by the biomaterials within the first 60 minutes after which further increase in contact time did not increase the Pb (II) adsorption process markedly. This observed phenomenon, is a sign that binding may have occurred on the cell walls of the biomass (Vijarayaghavan and Yun, 2008b). The fast adsorption rate at the initial stage may be explained by an increased availability in the number of active binding sites on the adsorbent surface (Asrari *et al.,* 2010). The sorption rapidly occurs and is normally controlled by the diffusion process from the bulk to the surface. In the later stage, the sorption is likely an attachment-controlled process due to less available sorption sites. Similar findings have been reported by other investigators for Pb (II) adsorption onto other adsorbents (Asrari *et al.,* 2010; Awwad and Salem, 2012; Mousa *et al.,* 2013). Ekpete *et al*., (2010) also reported same in the adsorption of chromium (VI) and zinc (II) ions on the skin of orange peel.

The observed increase in percentage lead removal as temperature was increased as presented in Figure 4.6 may be due to the fact that at higher temperatures, entropy of a system tends to increase, which leads to increase in randomness and consequent reduction in the stability of metal ions. Hence, the attraction potential between the metal and the binding sites on the adsorbent surface is increased (Daneshvar *et al*., 2002). The result agrees with those earlier reported by Sulaymon *et*

al. (2013) on column biosorption of lead, cadmium, copper, and arsenic ions on Algae (Mixture of green algae; Chlorophyta and blue-green algae; Cyanobacteria). The decrease in biosorption efficiency as temperature was increased above 70^0C may be attributed to an increase in the relative desorption of the heavy metals from the solid phase to the liquid phase. It may also be as a result of deactivation of the biosorbent surface and/or destruction of active sites on the biosorbent surface due to bond disruption (Meena *et al*., 2008). Moreover, it could be due to the weakness of the sorbent active site binding forces and the sorbate species and also between the adjacent molecules of the sorbed phase (Sari and Tuzen, 2008).

The pH of a solution affects metal ion solubility and biosorbent total charge. Metal biosorption is said to be directly dependent on pH value (Romera *et al*., 2007). At low pH, occupation of the negatively charged sites of the biosorbent by hydrogen and hydroxonium ions leads to the reduction of available sites for metal ion uptake (Mataka *et al*., 2010). As the pH increases, the ability of metal ions to effectively compete with hydroxonium ions for the sorbent binding sites also increase there by facilitating the uptake of more metal ions. Further biosorption after pH 7 was hampered due to precipitation of metals as hydroxides during the biosorption process. It may also be due to occupation of the adsorption sites by anionic species which repelled the further binding of such ions on to the biosorption surface (Donmez and Aksu, 2002). The decrease in adsorption at high pH values may be due to the competitiveness of the oxyanion of Pb and OH ions in the bulk solution (Agarwal *et al.*, 2006). Sati *et al*. (2015) reported similar results for biosorption of Pb (II) by *Spirogyra communis*.

The agitation rate had a pronounced effect on Pb (II) adsorption as seen in Figure 4.8, where the percentage lead removal increased with increase in agitation speed. This may be due to turbulence (Evans *et al*., 2002) and reduction of the diffusion boundary layer thickness around the adsorbent

particles (Hubbe *et al*., 2012). This usually results in increased diffusion rate of the solute from the bulk liquid to the liquid boundary layer (Vijayaraghavan and Yun, 2008b), thereby facilitating rapid uptake of the metals. The uptake capacity decreased at higher speeds beyond 300rpm probably because of the dispersion of the adsorbent particles which hindered the binding of metal ions to the binding sites on the biomass surface. Similar trend was reported by Mane *et al*. (2010) on the effect of pretreatment of algal biomass on bioadsorption of manganese. Das *et al*., (2013) also reported same for Chromium (VI) removal from aqueous solution using *Pistia stratiotes* biomass.

From the correlation coefficient (R^2) values obtained from the two adsorption isotherm used, it can be said that the Pb (II) biosorption fits in well with Freundlich when compared with Langmuir which implies that the biosorption took place on heterogeneous surfaces not only a monolayer adsorption. The *n* value indicates the degree of non-linearity between the solution concentration and biosorption. The *n* value in Freundlich equation was found to be 1.543. Since *n* lie between 1 and 10, this indicates the biosorption of Pb (II) onto *Z. mauritiana* seed shells is a physical process (Awwad and Salem, 2012). The essential properties of Langmuir isotherm model expressed in terms of a dimensionless constant called separation factor (S_F) is less than unity. The small S_F value indicates a highly favorable adsorption implying that *Z. mauritiana* seed shell is suitable adsorbent for Pb (II) ions (Sampranpiboon and Charnkeitkong, 2010).

The negative value of ΔG^0 at the different temperatures used is an indication that the biosorption process was feasible and spontaneous while the enthalpy value confirms the biosorption process as endothermic. The positive value of ΔS^0 shows that there is an increase in the randomness in the system solid/solution interface during the biosorption process. This is in agreement with the work of Farhan and Khadom, (2015) on the biosorption of heavy metals from aqueous solution by

Saccharomyces cerevisiae. This further indicates that *Z. mauritiana* seed shells have a great affinity for Pb (II) ions and there are some structural changes at Pb and *Z. mauritiana* seed shells interface (Ho, 2003).

It is evident from the Table 4.3 that the pseudo-second-order kinetic model showed excellent linearity with high correlation coefficient (R^2=0.997) under the studied experimental conditions in comparison to the pseudo-first-order kinetic model (R^2=0.882) and the intra-particle diffusion model (R^2=0.908) suggesting that chemisorption was a rate determining step. The intra-particle diffusion model plot did not pass through the origin. This deviation from the origin is due to the difference in the rate of mass transfer in the initial and final stage of the sorption indicating the existence of some boundary layer effect and further showing that intra-particle diffusion was not the only rate-limiting step (Das *et al*., 2013). More so, the calculated q_e value in the case of pseudo-second-order kinetic model agrees with the experimental q_e obtained. This agrees with results of Osasona *et al.*, (2013) on biosorption of Zn (II) from aqueous solution using powdered cow hooves. Studies on the surface topography of an adsorbent could provide important information on the degree of interaction between the adsorbent and the adsorbate (Giwa *et al.*, 2013). The textural structure of *Z. mauritiana* seed shells as shown in the SEM image before biosorption showed the irregular texture and porous nature of the surface of the biosorbent. This seemingly rough surface of the native biosorbent (Figure 4.15a) is an indication of high surface area (Demirbas *et al.*, 2004). Figure4.15b displayed evidently that over the biosorption period, the surface morphology of the native biosorbent had undergone remarkable physical disintegration resulting in emergence of a smoother and more even surface area.

The FT-IR spectra of *Z. mauritiana* seed shells before adsorption displays a number of absorption peaks, reflecting the complex nature of the seed shells. A strong and broad peak at 3295 cm^{-1} results due to the stretching of the O–H bond and is indicative of bonded hydroxyl group of alcohols and phenols (Garg *et al*., 2007). A change in peak position to 3423 cm^{-1} in the spectrum of the lead loaded Z. *mauritiana* seed shells, indicates the binding of lead to the hydroxyl groups. C=O stretching is confirmed by the peak at 1636 cm^{-1} in the biomass before biosorption. The shift in position of 1636 to 1637 cm^{-1} in the lead loaded biomass indicates binding of lead to the C=O group. The peak in the biomass before biosorption at 1319, 1241and 1032 cm^{-1} were shifted to frequency of 1320, 1251, and 1046 cm^{-1} respectively; this may be due to the involvement of carbonyl –CO group from carboxylic acids, it can also be attributed to the presence of OCH_3 of ether, a linkage component of carbohydrates and lignin. The change in peaks at 1371 and 1319 cm^{-1} to 1378 and 1320 cm^{-1}, respectively indicates the involvement of NO_2 in the binding of lead. C=N stretching is also indicated by the peaks at 1636 and 1505 cm^{-1}; the change in their position to 1637 and 1541 cm^{-1} shows the involvement of C=N functional group in the biosorption process. Also, the peaks around 432 and 418 cm^{-1} (Figure 4.16b) indicates the presence of metal complex such as Pb $(OH)_2$ which may have participated in metal binding (Saroj *et al*., 2006).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- The potential of *Z. mauritiana* seed shells for biosorption of Pb (II) is dependent on numerous factors such as the adsorbent dosage, initial metal ions concentration, contact time, temperature, solution pH and agitation rate.
- The study on equilibrium sorption revealed that Freundlich isotherm model gave the best fit to experimental data. The monolayer adsorption capacity of biomass was found to be 500 mg g^{-1} at 10 mg L^{-1} initial Pb (II) concentration. The n value also indicates that the process is a physical process.
- The thermodynamics of the biosorption is such that the process is endothermic with enthalpy change (ΔH^o) as 15807.88 kJ/mol, spontaneous and feasible with negative free energy (ΔG^o) change under the experimental conditions and an entropy change (ΔS^o) of 50.07 kJ/K/mol.
- In the study of kinetics of the sorption, the pseudo-second-order kinetic model provides better correlation of the sorption data hence chemisorption is the rate limiting step. Intra-particle diffusion was not the sole rate controlling factor.
- The biosorption involves both physical and chemical process.
- Fourier transform infrared (FT-IR) analysis showed that functional groups such as O-H, C-O, C=O, C=N, NO_2 are involved in the binding of Pb (II) to the *Z. mauritiana* seed shells based on the changes in the peak positions observed from the spectra of the biomass before and after biosorption.

- *Z. mauritiana* seed shells proved to be suitable for the removal of Pb (II) from aqueous solution. Since the *Z. mauritiana* seed shells is an easily, locally available, low-cost adsorbent and has a considerable high biosorption capacity, it may be treated as an alternative adsorbent for treatment of wastewater containing Pb (II) ions.

6.2 Recommendations

- Based on this study, *Z. mauritiana* seed shells can be recommended for use in biosorption of Pb (II) ions from aqueous solutions at an adsorbent dose ranging from 1-4 g
- *Z. mauritiana* seed shells could be used in the production of microfilters for trapping of Pb (II) ions for bioremediation of lead in lead contaminated effluents.
- Other heavy metals like cadmium (Cd^{2+}), mercury (Hg^{2+}), chromium, etc. should also be tested in order to establish which of the heavy metals is more adsorbed by *Z. mauritiana* seed shells.
- Further research can be attempted to enhance the existing results by chemical modification of *Z. mauritiana* seed shells using acids or bases. As modification may affect the number of active binding sites or functional groups on the adsorbents hence, improve the adsorption capacity of adsorbent.
- Particle size of *Z. mauritiana* seed shells should be varied in future research so as to ascertain the effect of various particle sizes on the adsorption efficiency of the biosorbent.
- Future research should focus on desorption of Pb (II) ions from *Z. mauritiana* seed shells to determine if the biosorbent can be regenerated and the metals recovered.
- Future research should focus on the continuous mode of biosorption in order to evaluate the prospects of the industrial application of the process

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APPENDICES

Appendix 1: Pore properties of *Z. mauritiana* seed shells before and after Biosorption

Property	Median Before biosorption	Average	Median after biosorption	Average
Circle equivalent diameter	32.9 µm	37.5 µm	16.7 µm	17.7 µm
Major axis	24.9 µm	28.3 µm	21.8 µm	23.1 µm
Minor axis	14.6 µm	16.3 µm	13.1 µm	13.7 µm
Circumference	195 µm	196 µm	73 µm	77.8 µm
Convex hull	132 µm	146 µm	64.2 µm	68.1 µm
Circumscribed circle diameter	51.2 µm	56 µm	25.7 µm	27.2 µm
Area	859 µm²	1.35E+03 µm²	219 µm²	250 µm²
Volume by area	1.93E+04 µm³	4.62E+04 µm³	2.43E+03 µm³	3.09E+03 µm³
Pixel count	1344	2731	796	912
Aspect ratio	0.787	0.719	0.611	0.61
Circularity	0.364	0.39	0.528	0.537
Convexity	0.74	0.768	0.901	0.886
Elongation	0.42	0.411	0.389	0.39
Grayscale	102	102	163	165
Inscribed circle diameter	195 µm	196 µm	10.7 µm	10.9 µm
Total Pores calculated for 1 image	60		47	
Average Pore Area Ratio for 1 image	9.35%		5.95%	

Appendix 2. FTIR spectral characteristics of *Z. mauritiana* before and after Pb (II) biosorption

IR Peak	Frequency (cm^{-1}) before adsorption	Frequency (cm^{-1}) after adsorption	difference	Functional group	Family of compounds
1	3295	3423	128	O-H stretching	Alcohols
2	1636	1637	1	C=O stretching	Amides,
				C=C stretching	Alkenes, Aromatics
				C=N	Imines.
3	1505	1541	36	C=C stretching,	Aromatic,
				NO_2 stretching,	Nitro
				C=N stretching	Imines
4	1461	1458	- 3	C=C stretching,	Aromatic
5	1371	1378	7	C-H Bend	Alkanes,
				NO_2 Stretching	Nitro.
6	1319	1320	1	NO_2 Stretching C-O	Nitro. Ethers, Esters, Alcohols, Carboxylic acids
7	1241	1251	10	C-O stretching	Ethers, Esters, Alcohols, Carboxylic acids
8	1032	1036	14	C-O stretching	Ethers, Esters, Alcohols, Carboxylic acids