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DEPARTMENT OF CHEMISTRY

USE OF *MORINGA OLEIFERA* (MORINGA) SEED PODS AND *SCLEROCARYA BIRREA* (MORULA) NUTSHELLS FOR METAL REMOVAL FROM WASTEWATER AND BOREHOLE WATER

BY

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CERTIFICATION

The undersigned certifies that they have read and hereby recommend, for the acceptance by the Department of Chemistry, the University of Botswana, a thesis entitled **“USE OF MORINGA SEED PODS AND MORULA NUTSHELLS FOR METAL REMOVAL FROM WASTEWATER AND BOREHOLE WATER”** as part of the work recommended in fulfilment of the requirements for a Master’s degree in chemistry at the University of Botswana.

Supervisors

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Internal examiner

Date: ------------------------------ Signature---------------------------

External examiner

Date: -------------------------------- Signature --------------------------

DECLARATION

I hereby declare that this dissertation submitted for the degree of Master of Science in Analytical Chemistry to the University of Botswana, is original ecxept where due to reference is made. This work has not been submitted wholly or in part for the award of a degree or diploma to this or any other insitution of higher learning.

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Maina Irene Wangari

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Date

DEDICATION

In memory of my late dad Peter Maina Muchai,

And

I dedicate this work to my beloved family.

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ABSTRACT

A study to investigate the possible use of non-edible seed pods of *Moringa Oleifera* (moringa) and *Sclerocarya birrea* (morula) nutshells for removal of metal ions from wastewater and borehole water samples was carried out. One variable at a time method was used to optimize parameters that affect sorption capacity of both sorbents. Contact time, pH, temperature, particle size, sorbent dose and initial metal concentration were the parameters investigated. Removal of seven selected metal ions were studied including lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), iron (Fe), zinc (Zn) and magnesium (Mg). Determination of residual metal ions after employing sorbent was done using flame atomic absorption spectroscopy (FAAS). Using 200 ng metal ion mixture in 50 mL of water sample, the optimized parameters for Moringa seed pods were 60 min contact time, 1 g of sorbent dose, pH 8, 100 µm sorbent particle size and extraction temp 35°C. While using Morula nutshells, the optimized conditions were 120 min contact time, 2 g sorbent dose, pH 8, 100 µm sorbent particle size and extraction temp 35°C. The percentage removal of Fe, Zn, Cu, Cd, Mg, Mn and Pb from water samples using treated MNS was 79.6, 52.9, 78.2, 58.6, 90.3, 100.0 and 93.4 while using treated MSP was 80.9, 57.6, 89.0, 65.5, 88.2, 100.0 and 94.7 respectively. Moringa seed pods was found to be a better sorbent in comparison to morula nut shells since the percentage removal of metals was higher while using Moringa seed pods. The developed methods were validated and the % removal was found to range between 86.49 ± 4.33 to 99.63 ± 3.36%. The method indicated good linearity ($R^2 > 0.99$) for all selected metals and also proved to be sensitive as low LODs were achieved ranging from 0.010 ± 0.003 to 0.067 ± 0.02 mgL^{-1}. Determination of the functional groups in the sorbents was done using Fourier Transform Infrared spectroscopy (FTIR). The experiments were done for sorbents before and after metal removal by sorbents. Shift in transmittance (%) and intensity of the peaks of the different functional groups in the sorbents was attributed to the metal binding to these functional groups and hence responsible for removal of metal from water samples. SEM-EDX was used to determine the morphology and the elemental analysis of the sorbents. Presence of irregular surface and pores showed that the sorbent has different sites that can act as adsorbing sites. The removal efficiency of acid treated sorbents was compared to that of untreated sorbents and it was found to be higher for acid treated sorbents for both Morula nutshells and Moringa Oleifera seed pods. These non-edible plant parts for Morula and Moringa Oleifera plants are proposed as a cheap, simple and an effective alternative for purification of water contaminated with heavy metals.

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1.0 INTRODUCTION

Demands on water resources for households, commercial, industrial and agricultural activities are increasing on daily basis. Agriculture and domestic sector account for more than 70% of global freshwater withdrawals (FAO, 2012). For example, in Botswana 80% of the population depends on groundwater due to poor rainfall and water scarcity (Central Statistics office 2009, unpublished data).

Wastewater resulting from homes and industries is often discharged into rivers after treatment. Recycling and reuse of wastewater can be a supplementary source to already existing water sources, especially in arid and semi-arid regions. The major source of wastewater in sewage treatment plants is municipal wastewater which may contain pathogens and potentially toxic elements and organic compounds. For example, diarrhea is responsible for the deaths of 1.8 million people every year and it mostly affects children in developing countries, because of unsafe water supplies and poor sanitation facilities. It is also of critical concern that the additional trace inorganic compounds such as heavy metals (Toze 2007, Lintelmann *et al.,* 2003) could be present in treated water due to treatment failure or inability to remove them. Wastewater from municipal, agricultural and industrial activities is normally discharged into designated oxidation ponds, treated and discharged to rivers. It has been demonstrated, that before being discharged to rivers, removal of most pollutants can be achieved by treatment technologies employed in wastewater treatment plants such as screening, primary treatment, secondary and tertiary treatment (Ternes *et al.,* 2002; Buser *et al.,* 1999; Ternes 1998). For example, in Gaborone, Botswana, Gaborone sewage treatment plant; (GWTP) treats all sewage that is generated in Gaborone and surrounding areas. This wastewater contains sewage from homes, waste from brewery, chemical industries and pharmaceutical waste. Below are some of the typical treatment processes that are involved.

Screening and pretreatment

This is the first stage of the wastewater treatment process. It involves filtering debris; removal of gross, suspended and floating solids from raw sewage. It includes screening to trap any solid objects that escaped the first screening and sedimentation by gravity to remove suspended organic and inorganic solids. This level is sometimes referred to as “mechanical treatment”, Primary treatment can reduce the BOD of the incoming wastewater by 20-30% and the total suspended solids by some 50-60%.

Tertiary treatment

The water, at this stage is put into large rectangular tanks called aeration lanes. Air is pumped into the water to encourage bacteria to breakdown the tiny bits of sludge that escaped the sludge scrapping process. Aeration tanks comprise of aerobic zone for COD removal and anoxic zone.

Secondary treatment

This stage biological treatment is used to remove the dissolved organic matter that escapes primary treatment. This is achieved by microbes consuming the organic matter as food, and converting it to carbon dioxide, water, and energy for their own growth and reproduction. The biological process is then followed by additional settling tanks (“secondary sedimentation") to remove more of the suspended solids. About 85% of the suspended solids and BOD can be removed by a well running plant with secondary treatment. Secondary treatment technologies include the basic activated sludge process, the variants of pond and constructed wetland systems, trickling filters and other forms of treatment which use biological activity to break down organic matter.

The treated wastewater may be used for irrigation, watering parks and possibly recycled for drinking purposes (Van Haute 1978; Ratanatamskul *et al.*, 1996; Kung 1998; Lazarova *et al.*, 2003). The nutrients such as nitrogen, phosphorus and potassium, that can be in wastewater are very essential such that they can assist plant growth (Zhang *and* Cornel 2005; Lone *et al.*, 2012; He *et al.*, 2008; Al Salem 1996). Despite the usage of wastewater in several parts of the world, the safety and quality of wastewater reuse still remains a problem. There is a high probability that the water discharged to the rivers after treatment in oxidation ponds, potable and ground water as well could contain heavy metals not benign to the environment.

1.1 Heavy metals in wastewater

Heavy metals occur in aquatic systems from natural sources and anthropogenic activities. Such activities include metal plating, mining activities, agricultural activities, solid waste from animals, smelting, battery manufacture, tanneries, petroleum refining, paint manufacture, pesticides, pigment manufacture, printing and other industrial effluents (Zheng *et al.*, 2007; Kumari *et al.*, 2005). The human body needs some of these metals at trace levels for correct nutrition, but further accumulation in the body results in various health effects (Singh *et al.*, 2010; Wang *et al.*, 2005; Khan *et al.*, 2008). For example, disasters due to pollution by methyl mercury in aquatic streams have been recorded such as Minimata tragedy in Japan and Cd contamination in in Jintsy river of Japan (Friberg and Elinder, 1985). In Owino Uhuru Slums, Mombasa Kenya the water, soils and dust samples from the houses around a Pb acid battery recycling company were found to have high concentration of Pb which was posing a great health risk to the people living in the slums (Benards, 2012).

Bioaccumulation of heavy metals vary between species, ages, sex and organs. The target tissues of heavy metals are metabolic active ones which accumulate high level of metal such as liver, kidney and fish gills, whereas in muscles where the metabolic activity is relatively low accumulates less level of heavy metals (Sasowsky *et al.*, 2004). Some metals are known to be toxic even at low concentrations. These include Cr, Pb, Cd, As and Hg (Nguyen *et al.*, 2005; Yetilmezsoy *et al.*, 2008). Pb causes various severe health problems in vital organs of human beings even at low concentrations, such as the kidney, liver, blood composition, nervous system and also retards the reproductive system. It also reduces chlorophyll production and can inhibit plant cell growth at very high concentrations (Yetilmezsoy *et al.*, 2009; Ronteltap *et al.*, 2007). Cd is very toxic to both human beings and animals. The International Agency for Research on cancer classified Cd as a human carcinogen (IARC, 1993). Sources of this metal include cadmium batteries, metal coating and alloys. However, the concentration in environmental samples is low. Cd may induce kidney dysfunctions, osteomalacia and reproductive deficiencies. It can also cause damage to the central nervous system and produce psychological disorder (Strömgren, 1998). It was found to decrease seed germination, lipid content and growth in plants (Mathew, 2005).

While a number of other metals such as Cu, Fe, Zn, Mn and Co, are known to be essential elements and play important roles in biological metabolism at very low concentrations and either an excess or deficit can disturb biochemical functions in both humans and animals (Yilden, 2003). Maximum allowed limits set by EPA 2004 for contaminants in treated wastewater enforced in developed and developing countries are 0.01, 0.2, 5.0, 0.2, 5.0 and 2.0 mgL^{-1} for Cd, Cu, Pb, Mn, Fe and Zn, respectively. While the Botswana bureau of standards (BOBS) and water utilities company (WUC) drinking water quality standards for Pb, Cu, Cd, Ni, Mn and Zn are 0.1, 1.5, 0.05, 0.4, 0.05 and 1.5 mg L^{-1} respectively. Intake of higher concentrations more than the allowed of these metals could cause severe effects.

Fe can contribute to soil acidification and loss of essential elements such as phosphorus and molybdenum. It can also cause pathological events such as iron oxides deposition in Parkinson's disease (Matusch *et al.*, 2010). Mn is toxic to a number of crops at a few-tenths to a few mgL^{-1} in acidic soils (USEPA, 2004). Zn on the other hand causes reduction in immune function and the levels of high density lipoproteins (spears, 2000). Cu has been associated with liver damage and when it interacts with Zn it produces adverse nutrient interactions (Altamura and Muckenthaler, 2009), disrupts photosynthesis, plant growth and reproductive processes (Mathew 2005).

Heavy metals such as copper Cu, Zn, Ni and Cr, are discharged in wastes (Sun and Shi, 1998; Fernandez *et al.*, 2005); Cd, Cr, Zn and Pb can be found in agricultural fertilizers (Nicholson *et al.*, 2003; Otero *et al.*,2005), and also in manure sewage sludge (Cheung and Wong 1983;Walter *et al.*, 2006). Pb, Cd, Hg, Pt, Au, Cr, As, Ni and Mn are commonly in electronics (Veglio *et al.*, 2003), effluent from industries such as alloy and steel industries, electroplating, paints and pigments industries (Rule *et al.*, 2006; Cheng 2003; Zhao *et al.*, 1999; Alvarez-Ayuso *et al.*, 2003; Davis and Burns 1999). These numerous application of the heavy metals in industries contributes a challenge on how to completely eradicate their presence in the environment. Therefore the biggest predicament is to optimize the benefits of wastewater as water sources and the nutrients it contains while minimizing negative impacts on human health.

Fu and Wang (2011) reported that the trend in heavy metals pollution in the ecosystem was increasing consistently throughout the world especially among developing countries. Hence, a need arises for developing means of purifying water, which is effective and also cost effective even at very low concentrations. Removal of heavy metals from wastewaters has been achieved through chemical precipitation (Tuenay and Kabdasli, 1994), ion exchange (Baltpurvins *et al.,* 1999), ion flotation (Garcia-Sanchez and Alvarez-Ayuso, 2003), adsorption (Doyle and Liu, 2003), reverse osmosis (Ozaki *et al.,* 2001) and membrane filtration (Dabrowski *et al.,* 2004), some of which have been discussed in the following Section 1.2 below.

1.2 Conventional methods for removal of heavy metal

1.2.1 Precipitation Methods

Most soluble metals precipitate when pH is alkaline (8 to 14). Precipitation of metals as insoluble hydroxides, carbonates or sulphides is used in about 75% of facilities for treating wastewater (Barakat 2011; Kurniawan *et al.,* 2006). The resultant precipitate is then separated from the water by sedimentation and/or filtration or flotation (Matis *et al.,* 2004; Zamboulis, 2001). Examples of precipitating agents include fly ash (Wang and Wu, 2006), lime (Tadesse *et al.,* 2006) and carbon dioxide (Lee *et al.,* 2005). Lime precipitation was employed for the removal of heavy metals such as Zn(II), Cd(II) and Mn(II) cations (Charerntanyarak 1999). Lime was successfully used to remove Cr, Cu, Pb and Zn from industrial wastewater, in the order Cur > Pb > Cr and Zn was least removed (Tadesse *et al.,* 2006; Kurniawan *et al.,* 2006). The efficiency of metal precipitation was optimum in the alkaline pH (8 to 10). Lime (CaO) is a preferred precipitant for the removal of heavy metals from industrial wastewater, however, high amounts are required and heavy metals may not be reduced to an acceptable level for discharge of water into rivers and ponds; due to poor inadequate settling and the dissolution of precipitates (Tadesse *et al.,*2006). These chemicals are expensive to buy and are toxic to human beings (Barakat 2011).

Fly ash gives rise to an alkaline pH of 10 to13 when mixed with water at a solid/liquid ratio of around 10. Hence, it can be reasonably expected that metal ions can be removed from aqueous solutions by precipitation and adsorption onto fly ash particles. The optimum pH range for chromium precipitation using fly ash and lime was 7 to10. The efficiency of fly ash in metal removal has been found to be affected by presence of CO_2 (Alinnor 2007; Cho *et al.,* 2005; Wang *and* Wu 2006; Ngah and Hanafiah, 2008). Carbon dioxide was found to neutralize lime and reduce the solubility of some heavy metal compounds and in the process reducing the leaching of the heavy metals (Hills *et al.,* 1999; Lee *et al.,* 2005). Chemical precipitation of heavy metals from acid mine drainage samples using 1, 3-benzenediamidoethanethiol dianion (BDET) has also been developed to selectively and irreversibly bind heavy metals from aqueous solution. BDET was found to remove approximately >90% of several toxic or problematic metals from acid waste waters (Wilmoth and Kennedy, 1979). BDET was found to reduce the concentrations of a wide variety of divalent metals in water and sediments to very low percentages. Furthermore, it has been demonstrated that the metal-BDET precipitates are insoluble in aqueous solution and in common organic solvents and are stable over a wide pH ranges (Sellmann, and Heinemann 2000; Matlock *et al.,* 2002). However, use of BDET has some limitations. For example, high costs are involved in the synthesis of the compound, involve use of chemicals that causes secondary pollution to the environment and high cost on disposal of sludge (Fu and Wang 2011).

1.2.2 Electrocoagulation/flocculation

This is an electrochemical method of treating polluted water whereby sacrificial anodes dissolve to produce active coagulant precursors into solution (Linares-Hernández *et al.,* 2009). This process normally include adjusting of pH and addition of ferric/alum salts as coagulants in order to overcome repulsive forces between particles (Licsko 1997). A direct current voltage is applied, and Al^{3+} or Fe^{2+} ions are produced, hydrolyzed and form a series of metal hydroxides at the anode that are able to destabilize dispersed particles such as heavy metals present in the wastewater. At the same time, hydrogen bubbles produced at the cathode can float most of the flocks formed from the destabilized particles (Shammas 2004; Chou 2010). To increase particle size, flocculation of unstable particles is done, these forms aggregates that can be removed by decantation or flotation from the wastewater (Semerjian and Ayoub 2003). Plattes *et al.*, (2007) employed precipitation, coagulation and flocculation processes using ferric chloride to remove tungsten from industrial wastewater. Tungsten removal was found to be most efficient (98–99%) in acidic conditions (pH < 6). Bojic *et al.*, (2009) explored spontaneous reduction–coagulation process using micro-alloyed aluminium composite in a laboratory semi-flow system to treat model heavy metal wastewater. The residual concentrations of metals were at admissible levels after only 20 minutes of treatment.

This technique can treat inorganic effluents with metal concentration less than 100 mgL^{-1} or higher concentration greater than 1000 mgL^{-1}. To treat wastewater contaminated with copper, Li *et al.*, (2003) modified a coagulation-flocculation process by using sodium diethyl-dithiocarbamate (DDTC) as a trapping agent and both poly-ferric sulphate and poly-acrylamide as the flocculants. DDTC is a used as metal precipitant and forms insoluble metal-sithio salts (Andrus 2000). Using a 25 mgL^{-1} coagulant dose at optimum pH of 10 to 11.5, a percentage removal of 99.6% and 95% of 20 ppm Cu was possible using Poly-ferric sulfate and Poly-acrylamide respectively. 100 mgL^{-1} of Na_2S as a coagulant was also used for removal of 450 ppm Zn (II), 1085 ppm Mn (II) and 150 ppm Cd (II). At pH of 11.0, the removal efficiency was 99.91%, 99.73 % and 99.95% for Zinc, cadmium and Mn respectively (Charerntanyarak, 1999). For removal of 50 ppm of Cu, Zn and Ni, the iron/iron electrode combination produced a higher percentage of copper and zinc removal efficiency >95% and > 80%, respectively, while for nickel the aluminum/iron and iron/aluminum electrode combinations >95 and >85%, respectively (Prica *et al.,* 2015). Besides its good efficiency this methods has shortcomings that hinder its adoption as a global strategy for wastewater treatment. Such as large consumption of expensive chemicals, generations of large amounts of sludge and high cost of disposal of contaminated sludge (Ayoub and Semerjian, 2001; Barakat 2011; Farooq *et al.,* 2010). Generally, coagulation–flocculation cannot remove heavy metals effectively (Chang and Wang, 2007). Therefore, additional treatment processes are needed.

1.2.3 Membrane techniques

Membrane technologies show great promise for heavy metal removal for their high efficiency, easy operation and space saving. The membrane processes used to remove metals from the wastewater are reverse osmosis, electrodialysis, filtration and filtration.

Reverse Osmosis

It is a process where heavy metals are separated by a semi-permeable membrane at a pressure greater than osmotic pressure which is caused by the dissolved solids in waste waters (Bohdziewicz *et al.,* 1999). Environmental legislation requires that the membrane used in reverse osmosis to have small pore sizes (down to 10^{-4} µm) (Benito and Ruiz 2002). Reverse osmosis works effectively at a pH range of 3 to 11 and at 4.5 to 15 bar of pressure; however that depends on porosity, material, thickness, roughness, hydrophobicity and charge of the membrane. Pressure is the most significant factor, the higher the pressure, the higher the metal removal efficiency. Reverse osmosis membranes has been employed for metal removal from industrial wastewater.

The initial concentration of 500 ppm of Cu^{2+} and Cd^{2+} were reduced to 3 ppm using a 2.5 m^2 polyamide membrane, at 45°C (Qdais *and* Moussa 2004). The removal efficiency was 98% and 99% for copper and cadmium, respectively effectively. In a comparative study, reverse osmosis was found to be better than resin ion exchange and membrane filtration processes. Over 90% of As (VI) and 99% of As (V) was removed using reverse osmosis membranes compared to 55% from the other two methods. However, low efficiencies (25%) were recorded for As (III). This is due to its neutral molecules that readily pass through the membranes. Therefore a prior oxidation pretreatment of As (III) to As (V) is needed (Chan *et al.,* 2008).

Reverse osmosis is robust, effective and capable of removing a wide range of dissolved species from water (Fu and Wang 2011). However, this method has some limitations. Such as membrane are prone to fouling due to suspended solids, or oxidized compounds for example chlorine oxides, the small pores of the (Potts *et al.*, 1981). The performance of the membranes decreases over time, thereby decreasing permeate flow rate (Ning, 2002). Replacing of the membranes, high energy consumption, and need for experienced personnel to run the process makes this method to be expensive (Fu and Wang 2011; Kurniawan, *et al.,* 2006)

Electro-dialysis

This is a process where the ions of the heavy metals are separated through the use of semi-permeable ion selective membranes by applying an electric potential (Bruggen *et al.,* 2002). When a solution containing ions passes through the cell compartment, the anions migrate towards the anode and the cations towards the cathode (Itoi *et al.,* 1980; Chen 2004; Ebbers *et al.,* 2015). Electro-dialysis has been used for removal of metal cations from wastewaters (Gering *and* Scamehorn 1988; Gnusin *et al.,* 1984; Nichols *and* Allen 1982; Oka *and* Takatama 1978; Uosaki

1974; Anon 1984) including production of drinking water from black water and seawater (Sadrzadeha *et al.,* 2009). Cation exchange membranes (Perfluorosulfonic Nafion 117) removed 90% of Co (II) and 69% of Ni (II) from plating wastewater (Tzanetakis *et al.,* 2003) and Nafion 450 removed 13% of Cd(II) (Marder *et al.,* 2003). Increasing voltage and temperature has been reported to increase the efficiency of an electrodialysis cell (Mohammadi *et al.,* 2004; Lambert *et al.,* 2006)

Due to the spacing of cation and anion permeable membranes, cells of concentrated and dilutes salts are formed. This technique is also prone to clogging of the membranes due to formation of metal hydroxides. It requires careful operation, clean feed and periodic maintenance to prevent damages of the membranes (Pedersen *et al.,* 2015).

Membrane filtration

They are pressure driven membrane operations that use porous membranes for the removal of heavy metals. Ultrafiltration uses permeable membranes to separate heavy metals, macromolecules and suspended solids from inorganic solution on the basis of pore size (5 to 20 nm) and molecular weights of separating compounds. Metal removal through use of membrane impregnated with chelators has also been documented. For example, wastewaters containing the ions of Co (II) and those of Ni (II) (a representative of the heavy metals) were treated by a combination of chelation and ultrafiltration (Geckeler *et al.*, 1996, Geckeler et al., 1999, Baticle *et al.*, 2000, Vonk *et al.*, 1997 and Marty *et al.*, 2000), the percentage removal was in the range of 63-86% for both metal ions. Pb and As ions were also removed effectively from industrial wastewaters by surfactant-enhanced ultrafiltration (Fillipi *et al.*, 1999, Purkait *et al.*, 2004, Purkait *et al.,* 2005).The surface active agents used were either anionic (dodecylbenzenesulfonic acid) or cationic (dodecylamine) surfactants. Nanofiltration has been used for removal of metal ion such as nickel (Murthy and Chaudhari, 2008; Murthy and Chaudhari, 2009), chromium (Muthukrishnan and Guha, 2008), copper (Cséfalvay *et al.*, 2009 and Ahmad and Ooi, 2010) and arsenic (Nguyen *et al.,* 2009 and Figoli *et al.,* 2010) from wastewater. An increase of pH and a decrease of operating temperature and metal ions feed concentration led to higher removal for of Arsenic using NF90 and N30F membranes (Figoli *et al.,* 2010). Use of membrane filtration process has benefits from ease of operation, reliability and comparatively low energy consumption as well as high efficiency of pollutant removal (Erikson, 1988).

The main disadvantages of this process are the resultant sludge, which also poses a problem of its disposal, fouling of membranes, biodegradation of membranes and high operational costs for the membrane systems (Kurniawan, *et al.,* 2006).

1.2.4 Ion-exchange

In ion exchange, a reversible interchange of ions between the solid and liquid phases occur. A resin (insoluble) removes ions of like charge in a chemically equivalent amount without any structural change of the resin (Rengaraj *et al.,* 2001 and Vigneswaran *et al.,* 2004). Synthetic ion exchange resins such as Amberlite IR-120 and Dowex 2-X4 are common matrices used in ion exchange system (Chan, *et al.,* 2006 ; Sapari *et al.,* 1996). These resins were employed to remove Zn(II), Cr(III) and Cr(VI) and the ion exchange system was found to remove all heavy metal (100%) from plaiting wastewater. Amberlite IR-120 is a strongly acidic resin with a sulfonic acid functionality and metal removal in ion exchanger works effectively in acidic conditions with pH between 2 and 6 (Lin and Kiang 2003; Kabay *et al.,* 2003). Similarly, Rengaraj *et al.,* 2001 reported 100% removal of Cr (III) when using IRN77 and SKN1 from a solution containing 100ppm of the Cr. Comparative study to recover Chromic acid from synthetic plating solution reported that while using of Ambersep 132 resin, metal exchange capacity in the column operation (100 mg g^{-1}) was higher than that of batch studies (92.1 mg g^{-1}) at the same concentration of 750 mgL^{-1} of the contaminated solution (Lin and Kiang 2003). This implied that more cations were exchanged in the column than in batch studies. This could be attributed to the fact that in batch studies, concentration gradient decreased with an increasing contact time, while in the column operation, the resin had continuous physic-chemical contact with fresh feeding solution (Ko *et al*., 2001; Kabay *et al*., 2003; Gode and Pehlivan 2003; Dries *et al*., 2005). Clinoptilolite, Amberjet 1200h, Ambersep 132, lewatit TP 207 resins have also been used in an ion exchanger to remove zinc, nickel, and cadmium (Papadopoulos *et al.*, 2004; Alvarez-Ayuso 2003). They were found to have a removal efficiency of >90% from solution with 100 ppm of the metal ions (Juang *et al*., 2003; Kabay *et al.*, 2003; Gode and Pehlivan 2003). The shortcoming of this method is that ion exchange is nonselective, highly sensitive to the pH of solution, some metal ions are removed partially and it is an expensive method since the synthetic resins used are expensive to purchase (Barakat 2011).

Although these conventional methods have higher capacity for the removal of toxic heavy metals, their utilization may require several pre-treatments as well as additional treatments, thereby incurring high installation and operating cost (Kam *et al.*, 2002; Kim, 2002; Volesky, 1990). Some of these processes also produce large volumes of highly concentrated metalliferous sludge that may be difficult to dewater and dispose of (Sandau *et al.*, 1996; Babel and Kurniawan, 1999). Due to these challenges, there is need for safer, economical and effective ways for elimination of heavy metals from waters. Use of low cost sorbents has focused attention on use of biological materials as a considerable potential solution for removal and recovery of pollutants from industrial effluents (Wang, 2002; Vieira *et al.,* 2010; Bhatti *et al.,* 2007; Kazemipour *et al.,* 2008; Garg *et al.,* 2007; Mishra and Patel 2009; Vaghetti *et al.,* 2009; Garg *et al.,* 2008; Reddy *et al.,* 2010; Wan Ngah and Hanafiah 2008; Helen Kalavathy and Miranda 2010; Amuda *et al.,* 2007; Saka *et al.,* 2012; First *et al.,* 2007; Annadural *et al.,* 2003). Biosorbents are prepared from naturally abundant materials such as plant-derived materials, algae, bacteria and from by-products or waste biomass from other industries.

1.3 Biosorption methods for removal of metals

Biosorption has gained important credibility during recent years as a low-cost, readily available and efficient treatment technology for effective removal of heavy metals from wastewaters (Chandrasekhar *et al.,* 2001; Basu *et al.,* 2003; Kumar *et al.,* 2006; Fourest and Roux, 1992). Plant materials normally contain components such as hemicelluloses, lignin, lipids, proteins, simple sugars, water hydrocarbons and starch (Cafer Saka *et al.,* 2012). This components contains functional groups like amines, sulphyl, carboxyl, carbonyl, hydroxyl that may be involved in forming metal complexes and hence helping in removal of metal from the samples. Use of these materials has the following advantages (Ngah and Hanafiah, 2008): they (i) are readily available, (ii)require little or no processing, (iii) have got good adsorption capacity for low-level metal concentrations, (iv) have got selective adsorption for heavy metal ions, and (v) can be easily regenerated (Gupta and Babu 2009; Nasiruddin Khan *and* Farooq Wahab 2007; Shukla *et al.,* 2002; Annadural *et al.,* 2003).

1.3.1 Utilization of nonedible parts from food and multipurpose plants as biosorbents

Some parts of food and indigenous plants are considered to be nonedible and therefore in most cases they are considered as waste and of less importance. These nonedible parts can be used as sorbents as will be discussed in the Section 1.3.2 below.

1.3. 2 Moringa Oleifera and Morula trees as multipurpose plants

Botswana is endowed with a variety of indigenous tree including *Sclerocarya birrea* (morula) and of recent, many people plant *Moringa oleifera (*MO*)* tree. The plant parts of these trees are widely used locally mostly for medicinal purposes. These plants are essential for food security, health and nutrition, and economic welfare of rural communities in the developing world (Saka *et al.,* 2007). For example morula tree is a multipurpose tree with highly nutritive fruits which can be consumed fresh or commercially processed. Its fruits are used in preparation of juices, jams, jellies and alcoholic beverages as they contain high amounts of vitamin C. The kernel contains proteins and oils believed to preserve meat (Mosase and Aganga, 2001; Venter and Venter, 1996; Van-Wyk *et al.,* 1997), the oil extracted from the seeds is highly beneficial for human health and is used for cooking, skin and hair (Mojeremane and Tshwenyane 2004; Bhattacharya, 2012). The oils sources from the seeds of these plants can be used for cosmetics, (Kleiman *et al.,* 2008). However they have a marked difference in fatty acid and oxidative properties. For example, MO seed oil had less than 1% polyunsaturated and morula had 6.7% of the unstable materials. Fatty acids in morula nuts has oils that include palmetic acid (12 g/100 g), stearic acid (9.2 g/100 g), oleic acid (69.9 g/100 g) and linoleic acid (7.8 g/100 g) (Wynberg, 2002)

Morula tree is one of plant species used widely in traditional medicine in different parts of the world including most African countries. It is used for many diseases and affections such as hypertension, dysentery, stomach aches or gastro-entries (Dimo *et al.,* 2007). The stem bark extract of *Sclerocarya birrea* exhibited a significant reduction in blood glucose and increased plasma insulin levels in diabetic rats (Moatshe *et al.,* 2011). Therefore it was concluded that the stem bark extract could treat diabetics due to stimulation of insulin secretion. Some of the mineral contents it contains include Cu, Mn and Zn (Balemtougri *et al.,* 2001; Glew *et al.,* 2004). The nuts of these trees have high levels of protein (28-31% or 30.9 g/100 g) and energy. They are rich in minerals like iron (31 mg/g), magnesium (467 mg/100 g), phosphorus (836 mg/100 g), Potassium (677 mg/100 g) and copper (54.8 mg/100 g) which contributes to their importance in diets of some rural communities (Wynberg *et al.,* 2002). Some of the products of morula have been commercialized and marketed in Southern Africa over the last 20 years (Hall, 2002). This includes popular morula liquer-Amarula from South Africa, ‘Marulaan’ wine in Zambia (Leakey, 1999) and pasteurized juice in Botswana (Taylor and Kwerepe, 1995; Shackleton *et al.,* 2002).The leaves are cooked as relish (Fox and Young 1982) and also used for hypertension and other infections (Belemtougri *et al.,*2001). A study of the fruit pulp and skin volatiles using SPME (Solid phase micro-extraction) and GC-MS has been reported. The study revealed that the major compounds in the fruit pulp were β-caryophyllene and α-humulene. (Z)-3-decen-1-ol was the major alcohol detected in the head-space of the whole fruit (Viljoen *et al.,* 2008).

Moringa Oleifera (MO) is another interesting plant of the developing world, as it can easily be cultivated and adaptable semi-arid climates. It is a multipurpose plant as it is commonly used plant worldwide in traditional medicine, and a rich source of nutrients and antioxidants (Coppin *et al.,* 2015; Katayon, *et al.,* 2006; Stevens *et al.,* 2013). The leaves, the stem bark, flowers and seeds have been used for medical purposes. The leaves from MO contain elements (micro and macro) such as Na, Mn, Fe, Zn, Ca and Cu (Szymczycha-Madeja *et al.,* 2014).

Moringa Oleifera (MO) seed biomass as biosorbent

MO seed biomass has been promoted for water purification. The flowers are known to be rich in minerals such as $Ca^{2+,}$ Mg^{2+} and K^{+}, that were found to improve the breast milk production and possess in women (Michel *et al.*, 2012). The seeds have been used for water treatment for generations in countries like China, India, Egypt and Sudan by local communities (Foidl *et al.*, 2001). The seed are ground, then mixed with water and agitated for few minutes then left for about an hour. The water is then filtered out using a woven fabric. The purified water is decanted leaving the coagulated particles on the bottom (Gassenschmidt *et al.*, 1995) . This treatment was found to reduce turbidity and water hardness (Ghebremichael *et al.*, 2005; Yin 2010). Some studies (Okuda *et al.*, 2001; Ghebremichael *et al.*, 2005) indicated that the presence of bivalent cations such as Ca^{2+} and Mg^{2+} significantly enhanced the coagulating properties of MO seeds, where the cations may have adsorbed the active components to form an insoluble net-like structure to capture suspended particles of kaolin clay that was in water samples. Tablets made from MO seeds were able to remove oil from water in petroleum extraction with efficiency ranging from 76 % (coagulant extracted from aqueous medium) to 96% (coagulant extracted from saline) (Michel *et al.*, 2010)

MO seed biomass has not only been studied for its coagulating properties but also its removal of heavy metals from aqueous samples. ((Mataka *et al.*, 2010; Obuseng *et al.*, 2012; Sharma *et al.*, 2005) . The research indicated that perfomance of this sorbent could work at near neutral to pH 8 and that it was possible to adsorb a mixture of metal ions without affecting sortpion capacity. At a high pH, the surface charge of the sorbent is negatively charged and therefore the sorption of metal ions on the sorbent is high, at low pH the metals ions compete with the H^+ ions for the active sites therefore decreasing the sorption (Adelaja *et al.*, 2011; Jimoh, T. O. 2012). Arsenic was removed from aqeous solution. The removal efficiency was 60.21% for As(III) and 85.60% for As (V) at optimum conditons of 2.0 g of sorbent, 25 mgL^{-1} metal concentration, pH 7.5 and 2.5 respectively (Kumnari *et al.*, 2005). Protein/amino acid-metal ion interactions were reported to be responsible for sorption phenomenon. In a relatively similar study, the bark of MO was used to study the biosorption of Pb^{2+} in aqueous solution. Likewise, parameters that influence the biosorption such as pH, biosorbent dose, contact time and concentration of metal ion were investigated. MO bark was considered a viable alternative to activated carbon, ion-exchange-resin and other synthetic adsorbents used for this purpose (Reddy *et al.*, 2010).

In the study of Bhatti *et al.*, (2007), biomass from MO pods was also used to investigate the removal of Zn(II). It was compared to the biomass treated with NaOH and it was noted that removal efficiency increased of 10% when treated biomass was used. The experimental results showed that the maximum pH for efficient sorption of Zn(II) was 7 ± 0.1 at which evaluated biosorbent dosage and biosorbent particle size, were 0.5 gL^{-1}, <0.255 mm, respectively. Increase in sorbent dose increases the number of particles and hence increasing the binding sites for sorption hence increasing removal efficiency and small size of particle increases offers large surface area for the adsorption of the metal ions on the sorbent (Arunlertaree *et al.*, 2007; Garg *et al.*, 2007; Garg *et al.*, 2008). The seed pods have also been used to remove organics (benzerne, toluene, cumene) from aqeous solution (Akhtar *et al.*, 2007). It was noted that the removal of the organic contaminants onto the pods was in order of cumen > toluene> benzene.

Some parts of these plants that are not edible and are considered as waste, for example MO seed pods and Morula nut shells (see figure 1). Recycling and use of these plant waste material could offer effective, economic and readily available materials that can be used to remove metal ions from water samples. This can be beneficial to rural areas where resources to acquire the expensive and complicated methods are limited. There is limited research on the application of these nonedible parts as biosorbents for the removal of toxic metal from water samples. However, literature is available where similar nonedible materials from plants and other materials have been used in water treatment (Hawrhorne-Costa, Winkler Hechnleitner and Gomez-Pineda 1995; Maranon and Sastre 1991; Dakiky *et al.,* 2002). Such materials include egg shells from poultry (Arunlertaree *et al.*, 2007), fly ash (Mishra and Patel 2009) nutshells (Kazemipour, *et al.*, 2008; Vaghetti *et al.,* 2009) and fruit husks (Marshall *et al.,* 1999; Ajmal *et al.,* 2006; Agbozu and Emoruwa 2014 ; Meena *et al.,* 2008). Some of these materials have been effective for removing of heavy metals. For example, untreated Soybean hulls removed >65% of metals (Nagar and Singh 2003; Quadri *et al.,* 2004; Kurniawan, Gilbert Y S Chan, W. Lo, *et al.,* 2006; Rizzuti and Nguyen 2007; Yadav *et al.,* 2008; Ye *et al.,* 2012) and acid treated soybean hulls were used to effectively remove metal ions (Cd, Ni, Cu, Pb and Zn) from industrial and municipal wastewater, and the efficiency was increased by 26% (Marshall *et al.,* 1998). Orange peels have also been used for removal and recovery of Ni^{2+} ions from electroplating water. The effectiveness of metal removal was dependent on operational conditions such as temperature, pH, initial concentration and sorbent dose. The sorbents could be regenerated for reuse with no reduced efficiency (Ajmal *et al.,* 2000).

The current work therefore, seeks to explore the unexploited property of Moringa seed pods (Figure 1.1 a) and Morula nutshells (Figure 1.1 b) as a bio remedial approach for removal of toxic metals from aqueous samples.

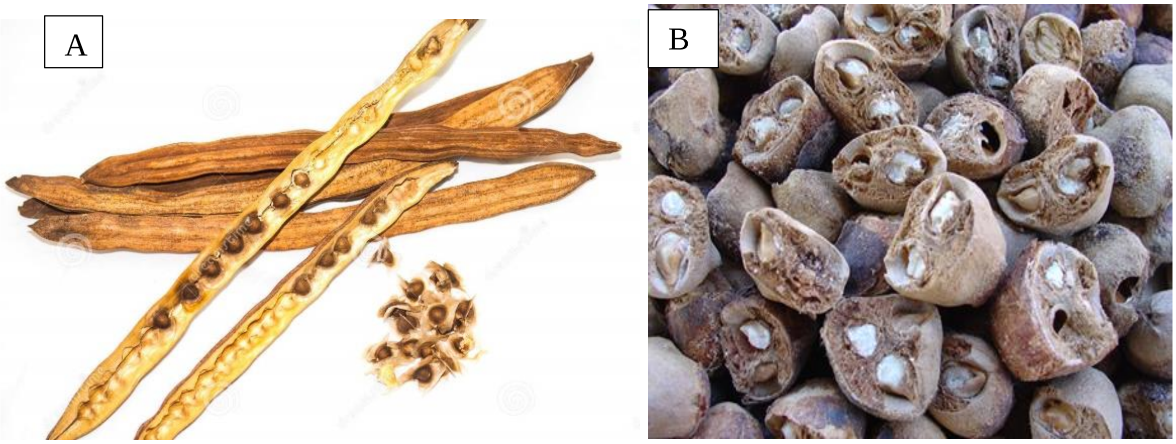

Figure 1.1: *Moringa Oleifera* seed pods (A) and morula nutshells (B).

Use of non-edible plant parts as biomass to remove metals is gaining interest in many studies. However, it is very important and economic to develop a method for the regeneration and reuse of sorbent since the saturated sorbents that contain the metal ions are not safe for disposal as they can pollute the environment.

1.4 Regeneration Studies

Regeneration of sorbents can give higher efficiencies compared to the untreated material dose, this could be explained by the fact that all the impurities of the sorbent are removed after regeneration and therefore increasing surface area for binding (Shukla *et al*., 2002). In some other cases removal efficiency can decrease after a couple of cycles of regeneration, since a small fraction of adsorbed metals not recoverable by regeneration represents the metals which are bound through stronger interactions. Regenerating biosorbents has the advantages of cheap raw material, low cost, economical, practical and easy operation.

It has been reported that desorbing agent mostly depends on the adsorbate used. Most metals are desorbed using dilute acids like HCl, HNO_3 and H_2SO_4 (Shiow-Tien *et al.,* 2013 ; Heng *et al.*, 2012; Mohamed *et al*., 2012; Ningchuan *et al.*, 2012) from various sorbents, including fruit peels, sawdust (Annadural *et al*., 2003). For example, in a study of *Moringa Oleifera* bark (MOB), as a biosorbent for the removal of Ni(II) from aqua solutions, the regeneration of the biosorbent was effected when 0.2 M HCl was used with a 98.02% recovery (Kumar *et al.,* 2011). In another study, *Moringa oleifera* leaf biomass showed feasibility of regeneration of the biosorbent for further use after treating with diluted HCl. The removal efficiency of Pb ions from wastewater was 89-95% after consecutive cycle (Kumar *et al.,* 2010). Except Cr(VI) which is desorbed from the sorbents with bases like NaOH, Na_2CO_3 or $NaHCO_3$ (Mishra, 2014; Annadural *et al*., 2003). The use of hydrogen peroxide acts as s strong oxidizing agent in acidic medium has been found to increase the desorption capacity for Cu ions using a corncob adsorbent (Khan and Wahab 2007).

1.5 Problem Statement

Water shortage has led to use of water from different sources that is prone to be contaminated. Use of possibly contaminated water for domestic and agricultural purposes may cause hazardous effects on crops, animals and human beings. Therefore there is need to come up with cheap and effective ways to solve the problem.

1.6 Significance of Research

Mining and industries are part of income generating sectors in Botswana. Due to such activities, heavy metals may find their way into the water sources that are being used in the various communities. Many people in the rural areas do not have access to technologies such as ion exchange, chemical precipitation etc. that can help in purifying of the water that they use in their households and farming. This could be attributed to the fact that these technologies are costly and complicated. Hence communities' health is at risk as a results of consuming water that could be contaminated with organic and inorganic substances. The findings of this research will be useful in developing the most efficient means of water purification. With this knowledge apparatus that are relatively cheaper and affordable may be developed to get rid of heavy metals that are in wastewater that is used in horticulture industries around Gaborone areas and also in the other different sources of water that are being used in the rural areas all over Botswana.

1.7 Aim and Objectives of study

The main aim of this study was to utilize non edible (normally treated as waste) biomass Moringa seed pods biomass and Morula nut shell biomass as possible biosorbents for metal removal from sewage waste waters and borehole water.

1.7.1 Specific Objectives

The specific objectives of the study were as follows:

- To characterize the Morula nutshells (MNS) and Moringa seed pods (MSP) using spectroscopic analyses which consist of identification of functional group with Fourier Transform Infra-red (FTIR) and morphology analysis using Scanning Electron Microscope (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDS).
- To investigate and compare the uptake of heavy metals from aqueous solutions by Moringa seed pods and Morula nutshells by batch adsorption experiments.
- To study the effects of varying parameters on sorption capacity of Pb, Mn, Cu, Cd, Fe, Zn and Mg by MSP and MNS. Particularly: pH, Contact time, adsorbent dose, particle size, temperature and initial metal concentrations.
- To optimize the parameters that affect sorption capacity of MNS and MSP for heavy metal removal
- To apply the optimized parameters to remove selected heavy metals from sewage waste water and borehole samples.
- To compare the efficiency of metal removal between acid treated sorbents and untreated sorbents.

2.0 Analytical methods for heavy metal determination

In analytical chemistry, different techniques are used to determine the concentration of a particular element (analyte) in a sample to be analyzed. Proper selection requires basic understanding of each technique since each has its individual strengths and limitations. In order to make a decision on which technique to use, several factors have to be considered (Elemental, 2001; Elmer, 2008; Piccolo and O'Connor, 2009). Such as sample type (for example solid, liquid or gaseous), analytical performance of the technique for example detection limits, working range and precision, a technique that can offer multi-elemental determination and minimal operating cost. It should reduce overall cost of analysis due to rising costs of instrument supplies, power, labor costs etc.

Several techniques have been employed to determine the amount of metal in different sample matrices. For example Cu, Fe and Zn were determined in rocks, wastewater and human milk using Flame Atomic Absorption Spectroscopy (FAAS)(Ma 2012; Silvestre et al., 2000; Ferreira 2000); Pb content in tilapia and pine pollen was determined using Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) (Ni and Qu 2010; Zhong and Fan 2011); Cd, Co, Cr, Cu, Hg, Ni and Pb were determined in leather and fur by Micro-wave Plasma Atomic Emission Spectroscopy (MP-AES) (Y. Zhao *et al*., 2015); alloying elements like Mn, Ni, Cr and Cu were determined in silicon steel and stainless still using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Zhang *et al*., 2012; Ding and Pan 2013); Pb, Cd, As and Hg content in medicinal material using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Huang *et al*., 2006). These techniques have been used for metal determination in food samples such as vegetables (Maeda and Tanimoto 1988; Znidarsic-Pongrac and Hodnik 2003), animal products like milk and meat (Ruut and Past 1994; Temerdashev *et al*., 2000), fish (Zhong and Fan 2011). In pharmaceuticals such as ciprofloxacin, amoxicillin and diclofenac sodium (Albero *et al*., 2002; Lewen *et al*., 2004;

Lewen 2011; Wollein *et al*., 2015), petrochemical industries for example crude oil (Bettmer *et al*., 2012; Kawada 2007) and petrochemical sludge (Kumar *et al*., 2013), environmental samples such as wastewater (Cosgrove and Bracco 1973; Marshall *et al*., 1998; He 2008), sediment (Hall 1997; Yao *et al*., 2011; Liu *et al*., 2012; Liang *et al*., 2015), agricultural soils and crops (Akan *et al*., 2013; Yan *et al*., 2015; Huang *et al*., 2015; Aidarkhanova *et al*., 2015; Cao *et al*., 2015; Alrawiq *et al*., 2015), mining samples such as rocks (Ma 2012) and effluents from mines (Smolders *et al*., 2003; Kemper and Sommer 2002; Dzhuvelikyan 2002; Qiu *et al*., 2007), chemical industries for example in paints (Duffer 1972; Michalke 2002). The principles and application of these techniques will be discussed in Sections 2.1 to 2.5.

Prior to analysis, sample has to go through sample preparations so as i) to degrade and solubilize the matrix, ii) to release all metals for analysis, ii) to extract metals from the sample matrix into a solvent more suitable for the analytical method used, iv) to bring metals present at very low levels into a concentration range suitable for analysis, v) to separate a single analyte or group of analyte from other species that may interfere in the analysis (Lubomir *et al*., 2010). Sample preparation depends on the analytical techniques to be employed and their capabilities. For metal analysis, the sample preparation steps mainly include homogenization (size reduction in case of solid samples), extraction and concentration. For solid samples, sample digestion is normally done as follows i) wet digestion using strong acids like HCl, HF, HNO_3; ii) wet digestion with mixture of strong acids with oxidizing agents like royal water, $HClO_4$ or H2SO_4 with H_2O_2; iv) dry-ashing: calcinations followed by solving the ash in an acid; v) Microwave digestion: a modern technique combining pressure and temperature. Extraction of metal from solid samples can be quite tedious, involves a lot of random and systematic errors, loss of sample during the numerous sample preparations steps, possibility of contamination and its time consuming. Liquid samples do not require homogenization and can be aspirated directly to the instrument without need of extraction therefore low contamination of sample and save time. However, in a case where the total metal concentration is of interest, then the digestion can be done using acids like HNO_3, HCl or extracting metal from the matrix using chelating agents (Lubomir *et al*., 2010; Mitra 2003).

2.1 Atomic Absorption Spectroscopy (FAAS and ETAAS)

Figure 2.1 shows a schematic diagram of Atomic Absorption Spectroscopy (AAS) when using either a flame or graphite furnace as a source of heat.

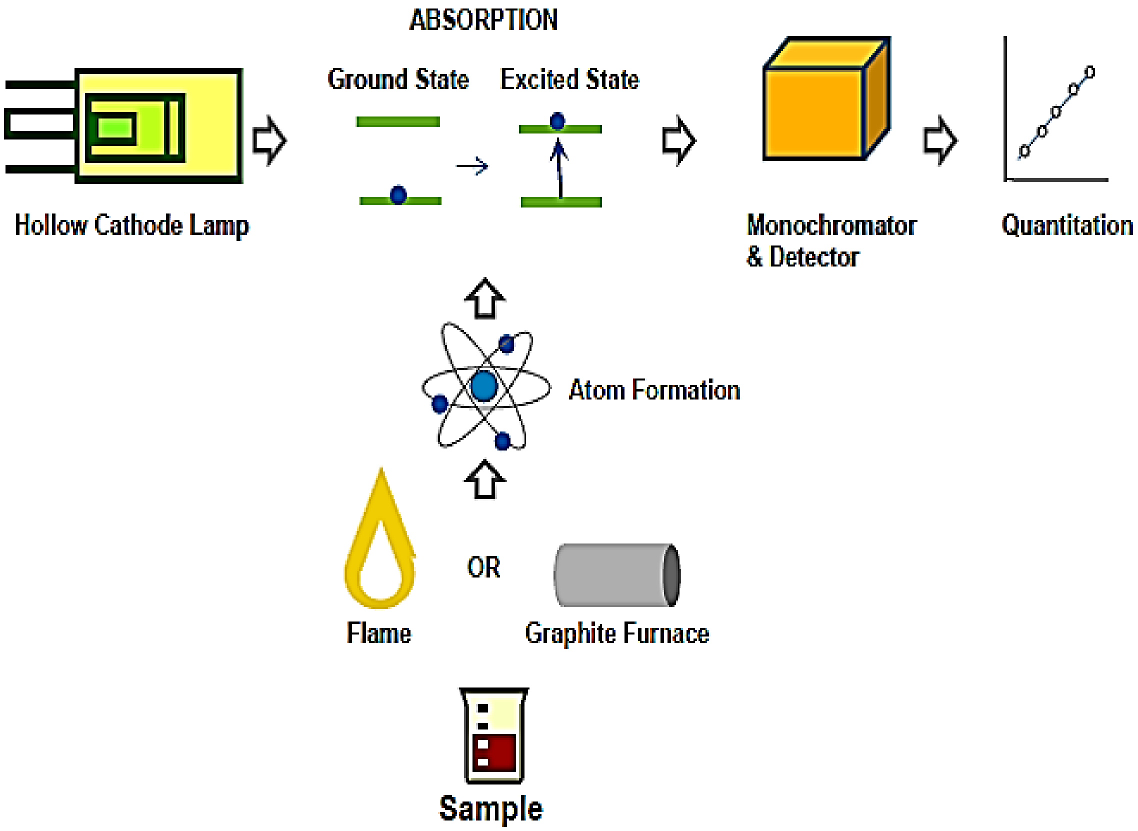

Figure 2.1: Schematic diagram of Atomic Absorption Spectroscopy (Elmer 2008)

There are five basic components of an atomic absorption spectroscopy:

- The light source: that emits the spectrum of element of interest for example hollow cathode lamps

- Atom source- how atoms are generated from a sample e.g air/acetylene or nitrous-oxide/acetylene flame

- A monochromator: it isolates the specific wavelength of light to be measured and disperses it.

- A detector: measures the light intensity and amplifies the signal e.g photomultiplier tube

- A display that shows the reading after it has been processed by the instrument.

2.1.1 Flame atomic absorption spectroscopy (FAAS)

This is a spectro-analytical procedure that uses absorption of light by free atoms in the gaseous state. The analyte concentration is calculated based on the Beer-Lambert law by comparing absorbance of sample against calibration curve (Garcia and Baez 2012).

In the flame AAS, a nebulizer and a spray chamber introduces the sample as an aerosol into the flame. The high temperature of the flame atomizes and the aerosols are reduced to gaseous metal atoms. Quantifying the amount of light absorbed determines the amount of analyte in the sample (figure 2.1). The gases used to produce the flame (2600^0C) are either air/acetylene or Nitrous Oxide/acetylene.

FAAS is the cheapest technique for determination of metals in different samples. It is easy to operate, has low maintenance costs and offers great method recoveries (Ferreira 2000; Silvestre *et al*., 2000; Knap *et al*., 2007). However, this technique shows poor efficiency as only 5-10% of the aerosol reaches the flame (Welz and Sperling 1999) and residence time of analyte atoms in the flame is short (Matusiewicz 1997; Davies and Berndt 2003). It is time consuming since only one element can be determined at a time, samples having high concentrations beyond the working range of the instrument have to be diluted prior to the analysis and this may increase analytical errors, cannot quantify some metals and it is prone to ionization interferences (Garcia and Baez 2012).

2.1.2 Graphite furnace Atomic Absorption Spectroscopy (ETAAS)

This is also known as Electrothermal Atomic Absorption Spectrometry (ETAAS). This technique works almost like FAAS except that the source of heat is replaced by graphite tube which is heated to a temperature of up to 3000^0C (Elemental 2001). In GFAAS, samples are deposited in a small heated graphite or pyrolytic carbon that is coated with graphite tube, the sample vaporize and the analytes atomized (see figure 2.1). The free atoms absorb light at wavelengths characteristic of the element of interest. The light absorbed is linearly correlated to the concentration of analyte present. The main three steps of GFAAS are (i) evaporation of solvents, (ii) removal of matrices such as hydroxides, carbonates and sulfates, and (iii) atomization of the analytes (García *et al*., 2008). Some of advantages of this technique is that it offers greater sensitivity and lower detection limit compared to FAAS. It can accept liquid, slurries or solid samples (Miller-Ihli 1992), has low spectral interferences (Belarra *et al*., 2002), ETAAS has been applied in a wide range of matrices, for example Ni, Co, Cu, Cd, Cr(III) and Pb were determined in wastewater (Yang *et al*., 2009; Mirzaei *et al*., 2011); Cd, Pb, Cu and Zn in sediment and fish (Dalman *et al*., 2006); Cd, Zn and Cu in mushroom (Tüzen 2003); Ni in human urine (Wang and Hansen 2001; Saracoglu 2003), Cu, Zn, Cr, Ni and Pb content in soils (Cao et al., 2015), Cd, Cr, Mn, Pb and Cu in vegetables (Bakkali *et al*., 2009). Palladium content was determined in a bulk pharmaceutical drugs, the sample were digested with concentrated nitric acid and compared to microwave digestion. Better recoveries were observed with microwave digestion (Jia 2001). Use of concentrated nitric acid is simple, reduces cross-contamination, and is very reliable. However, dissolving organic compounds with concentrated nitric acid can and sometimes, present a potential explosion hazard. In addition, concentrated nitric acid shortens the lifespan of graphite furnace tubes, and accelerates the corrosion of the metal components of the instrument (Wang *et al*., 1997). The concentration of mercury, lead, cadmium, tin and arsenic were determined in canned tuna fish and the good recoveries (91.7% to 99.3%) were observed using spiked samples were between (Emami Khansari *et al*., 2005). In spite its numerous application, this technique has limitations for example chemical interferences, therefore matrix modifier such as hydrogen peroxide (Viñas *et al*., 1997), iridium (Pedro *et al*., 2006) and nickel-containing chemical modifiers (Acar *et al*., 1999) are needed to facilitate removal of interferents and increase sample throughput. GFAAS also has limited dynamic range and atomized particles may jump to higher energy levels thus lowering precision (Garcia and Baez 2012)

2.2 Microwave Plasma-Atomic Emission Spectrometer (MP-AES)

MPAES employs microwave energy to produce a plasma discharge using nitrogen, helium or argon (Figure 2.2). Use of nitrogen extracted from ambient air eliminates the need for sourcing gases in remote locations. The microwave plasma torch (MPT) can be sustained at atmospheric pressure and at very low rate (10 mL/min) and low forward power (40–500 W). Samples are typically nebulized prior to interaction with the plasma in MP-AES measurements. The atomized sample passes through the plasma and electrons are promoted to the excited state. The light emitted electrons return to the ground state and light is separated into a spectrum and the intensity of each emission line measured at the detector. Most commonly determined elements can be measured at trace level concentration range (ppm). The sample aerosol can be introduced into the plasma with or without desolvation. Since the analyte is forced to pass through the central channel of the microwave discharge, it is efficiently vaporized, atomized, excited and ionized; and causes minimal perturbation to the plasma (Kilicgedik, 2012; Jin *et al*., 1991).

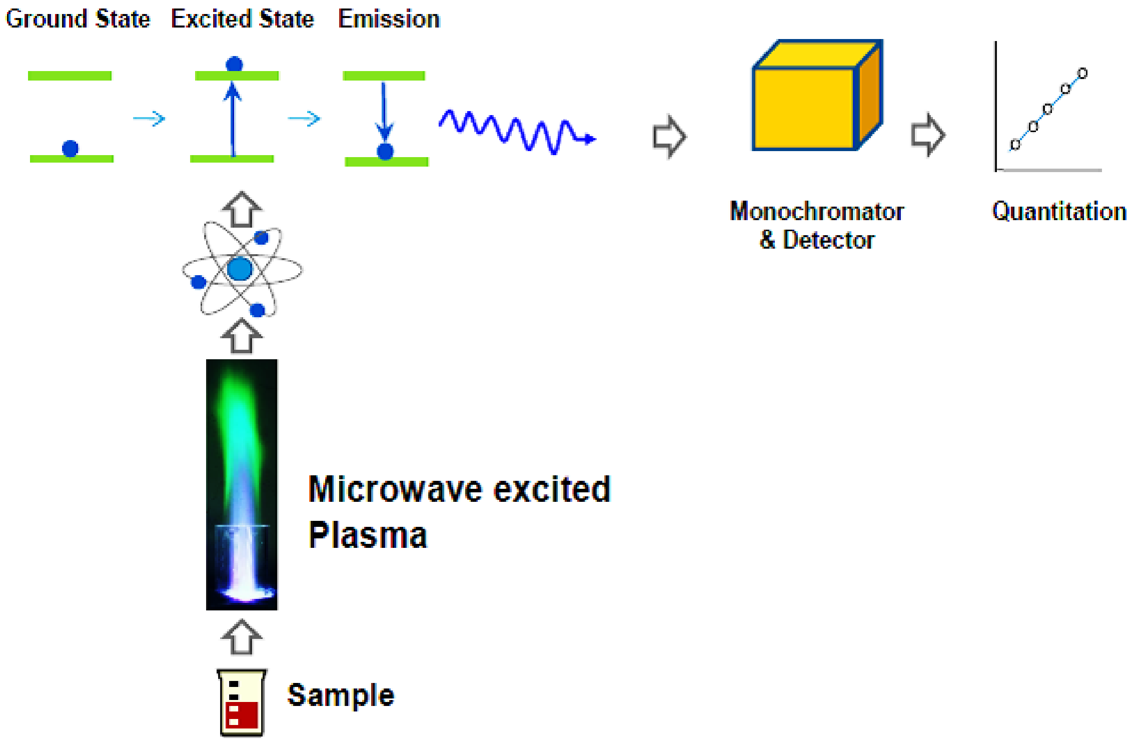

Figure 2.2: Schematic diagram of Micro-wave Plasma Atomic Emission Spectroscopy (Elemental 2001)

This is an analytical technique for elemental analysis that has been designed to improve performance and productivity while decreasing operating costs. Some of the advantages (Elmer 2008) of this technique include:

- Higher performance compared to flame AAS: It has superior detection limits and improved dynamic range, higher sample throughput with fast sequential measurements
- Easy to use: It has software for automated optimization and one torch that requires no alignment.
- Reduced operating costs: It eliminated need for acetylene, Nitrous Oxide and Argon since it runs on air. It also eliminates need for light sources like hollow cathode lamps.
- Improves safety in the labs since flammable gases and cylinder handling is not required.

While the technique is mature, there are a limited number of manufacturers supplying commercial MP-AES instruments. The performance of MP-AES was compared to that of ICP-AES, for the determination of Cd, Co, Cr, Cu, Hg, Ni and Pb in leather and fur (Y. Zhao et al., 2015). Under optimum conditions, recoveries with MP-AES were in the range of 98.1% to 102.6% and the relative standard deviations were 0.7% to 3.0% and low detection. Multi-elemental analysis was done on Chinese herbal medicines(Wu *et al*., 2012). The recoveries of the method were between 90 and 110% and the method detection limits for most elements were less than 3 µg/mL in the solid sample. The recovery and precision were also excellent. MP-AES is a rapid, simple and accurate technique that offers low analysis costs and improved operational safety with use of nitrogen for plasma generation (Wu *et al*., 2012; Kamala C T *et al*., 2014; Lima *et al*., 2015).

2.3 Inductively Coupled Plasma Atomic emission Spectroscopy (ICP-AES)

This is a multi-element analysis technique that uses an inductively coupled plasma (ICP) source to dissociate the sample into its constituent atoms and ions, exciting them to a level where they emit light of a characteristic wavelength. This technique is based upon spontaneous emission of photons from atoms and ions that have been excited in a Radio Frequency discharge. Samples are injected directly into the instrument; the sample is converted into an aerosol by a nebulizer and directed into the central channel of the plasma. The aerosol are quickly vaporized into free atoms by the plasma that has a temperature of approximately 10,000K (Hou Xiandeng 2000). High energy converts the atoms to ions and subsequently promotes ions to excited states. Both atomic and ionic excited species may relax to the ground state via emission of a photon. The photons emit characteristic wavelength that can be used to determine the element from which they originated. Total number of photons is directly proportional to the concentration of the originating element in the sample (Murray *et al*., 2000). The photons are collected with a lens or a concave mirror, an image of the ICP on the entrance aperture of a monochromator. The exiting wavelength is converted to an electrical signal by the photodetector. The signal is amplified and processed by the detector electronics, then displayed and stored by a computer (Figure 2.3).

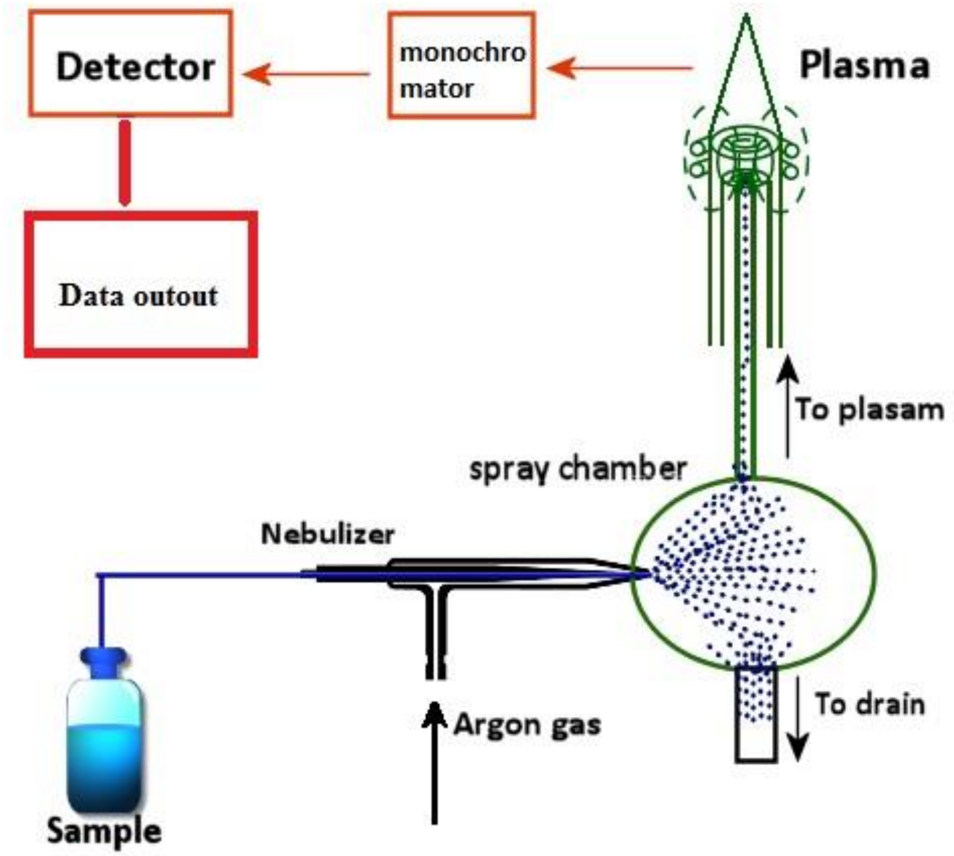

Figure 2.3: Schematic diagram of an ICP-AES technique (Hou Xiandeng 2000)

ICP-AES has been used in multi-elemental (Cd, Pb, Zn, Al, Cr, Cu, Fe, Ni, Ba, Ca, Mg, Ti, Se, V, B and Co) analysis in different matrices such as dusts, ashes, sediments (Paudyn and Smith 1992); seawater (Willie and Iida 1998); coal fly ash (Iwashita *et al*., 2006);Tungsten in bulk drugs (Wang *et al*., 1999). Good recoveries were obtained for boron in soils, (after microwave extraction using ICP-AES (de Abreu *et al*., 1994). In a multi-elemental analysis of copper, iron and nickel in edible oils, the recoveries ranged from 90% to 110% and low detection limits in the range of µg/l were found (Murillo *et al*., 1999). Cu, Fe, Mn, Zn, Cd and Pb were determined in wine samples and

ICP-AES was found to be very fast in multi-elemental analysis and the due to its ability to determine concentration even at µgL^{-1} levels, it was found to be more sensitive than atomic absorption spectroscopy (Aceto *et al.*, 2002). However this technique has limitations such as matrix effects and interferences. For example, to minimize matrix effect, Wang *et al*., (1999) reported that the calibration blanks and standards are prepared in the same way as the sample. Spectral interferences are monitored by using the 224.875 nm and 220.448 nm W-lines simultaneously in ICP-AES. Another method was developed to compensate for the effects of varying acid and salt concentrations on the efficiency of sample introduction into the plasma that does not involve the use of internal references, precise matrix matching, or standard additions (Botto 1985). The method uses the intensity of a hydrogen emission line at 486.133 nm (H-beta). The H-beta intensity is proportional to sample introduction rate and to the hydrogen content of the sample solution. Interference calibration curves are prepared for a specific acid/salt matrix relating observed H-beta intensities and apparent concentrations for each of the elements (and wavelengths) in the analytical program. These calibration curves are used to perform the interference corrections for samples having that acid/salt matrix (Boumans, Tielrooy, and Maessen 1988; F. Zhao, McGrath, and Crosland 1994). ICP-AES also has high operational and maintenance cost and require a trained personnel (Elemental 2001).

2.4 Inductively Coupled Plasma mass Spectrometry (ICP-MS)

This technique was commercially introduced in 1983 and has gained popularity in many types of laboratories (Wolf, 2005). It is a multi-element technique that also uses inductively coupled plasma (ICP) source to dissociate the sample into its constituent atoms or ions. In ICP-MS, Inductively Coupled Plasma is combined with a mass spectrometer. Unlike in ICP-AES, where the photons emitted from ions are detected by a photodetector, in ICP-MS the ions are detected themselves by a mass spectrometer. The ICP plasma uses argon gas and has a temperature of around 6000-10000 K. The ions formed from ICP plasma are typically positive ions, M^+ and M^{+2} therefore it can be difficult to analyse samples with negative ions such as I, F Cl using ICP-MS (Michalke 2002).

A sample is introduced into the ICP plasma as an aerosol, by aspirating a liquid or dissolved solid sample into a nebulizer. In the ICP torch, all the solids in the sample are desolvated, then the aerosols are vaporized, the elements are atomized and at the end of the plasma the gaseous atoms are ionized (Wolf 2005; Thomas 2001). The ions are extracted from the plasma and passed into the mass spectrometer via the interface cones, where they are separated on their atomic mass-to-charge ratio by a quadruple or magnetic sector analyzer (Elmer 2008). Figure 4 shows a schematic representation of the ICP-AES technique

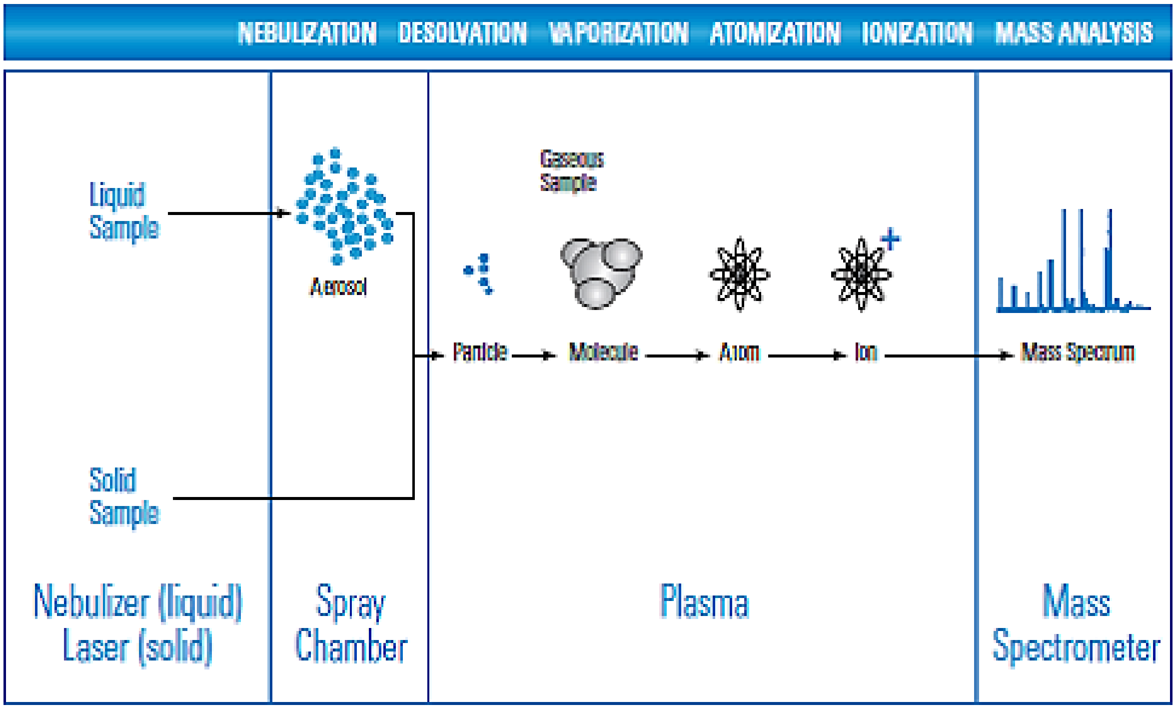

Figure 2.4: Schematic diagram of processes in ICP-MS from sample introduction to mass analysis (Agilent Technologies 2005)

The described method has been in use for several years and its versatility has been demonstrated by successfully applying it to a wide variety of sample matrices. Analysis on numerous sample matrices investigated indicates that there is no dependence of the various chemical functionalities contained in the sample matrices on the individual element recoveries. The average recovery for selected metals (arsenic, selenium, cadmium, indium, tin , antimony, lead, bismuth, silver, palladium, platinum, mercury, molybdenum and ruthenium) from the various sample matrices investigated ranged from 89 to 102% (Lewen *et al*., 2004). ICP-MS has excellent detection limits (1-10 ppt), it's a very economic for analyzing many samples and many elements, its sample throughput is very fast, has wide dynamic range (Gustavsson and Larsson 1992). However it has high operational and capital costs, has spectral interferences and may require a trained personnel to operate it (Huang *et al*., 2006).

2.5 Characterization of sorbents

The physical and chemical properties of a sorbent plays a key role in determining its efficiency. For instance, the porous nature of the sorbent is an evidence of its adsorptive ability. Therefore, studying the sorbent's morphology, porosity and chemical structures assist in evaluating how the sorbent interacts with analyte of interest. Frontier transform infrared and Scanning electron Microscope have been used to characterize the sorbents. Their principles and applications are discussed in Section 2.5.1.and 2.5.2

2.5.1 Frontier transform infrared Spectroscopy (FTIR)

Infrared Spectroscopy belongs to the group of molecular vibrational spectroscopies which are molecule-specific, and give direct information about the functional groups, their kind, interactions and orientations. It is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information (Simonescu 2012). Its sampling requirements allow the gain of information from solids, and in particular from solid surfaces. FTIR offers quantitative and qualitative analysis for organic and inorganic samples. It measures the frequencies at which the sample absorbs and also the intensities of the absorptions. The infrared radiation is absorbed by the sample and while some of the radiation is transmitted. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The spectrum created represents the molecular absorption and transmission thus creating a molecular finger print of the sample (Nicolet and All 2001) The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule (Stuart 2004).

Using this technique, carbonyl, carboxylic, alkyl, aromatic, amine, and hydroxyl groups can be analysed and have been found to be involved in metal uptake by different biosorbents (Taha *et al.*, 2013). In order to understand the sorption process, it is important to characterize the biosorbent materials. These materials consist of cellulose, hemicellulose, lignin, protein, lipids etc. (Foidl e*t al.*, 2001). Metal removal using plant sorbents is possible through ion exchange or complexation phenomena which occur on the surface of the plant material through interaction of the metal with the functional group present. The shift of the bands and the changes in signal intensity allow the identification of the functional groups involved in metal sorption. For example, in removal of lead from wastewater using plant biomass, a decrease in intensity and shift in asymmetric stretching frequencies of hydroxyl, carbonyl and carbonyl groups is presumably due to coordination of lead to the functional groups. The alkyl, aldehyde, ketonic and carboxylate groups shifted due to coordination of zinc (II) to the different functional groups. (Kalavathy and Miranda 2010; Kumari *et al*., 2006; Reddy *et al*., 2010).

2.5.2 Scanning Electron Microscope-Energy dispersive X-ray Spectroscopy (SEM-EDAX)

SEM is a useful technique in the study of both the natural sorbent morphology and its modification derived from sorbate interactions. SEM is an electron microscope, which provides images of the sample surface by scanning it with a high-energy beam of electrons. It is comprised of an electron generating component known as the gun, a column through which electron beam travels, a series of lenses to shape the electron beam, a chamber where the sample is housed and a series of pumps to keep the system under vacuum as shown in Figure 2. 5. The electron interactions with the atoms of the sample produce signals that contain information about topography, morphology, and composition of the sample surface. The samples must be electrically conductive, at least on their surface, for conventional SEM imaging. Nonconductive samples are coated with an ultra-thin layer of electrically conducting material; this coating prevents the accumulation of static electric charges on the sample surface during electron irradiation. Magnification of the imaging can be controlled over a range of up to 6 orders of magnitude from about x25 to 250,000 times (Ammrf 2014). When coupled with energy dispersive X-ray analysis (EDAX), the atom concentrations on the sorbent surface can be determined. This enables the confirmation of a mechanism of ion exchange, generally investigated by determining the concentration of alkaline and alkaline-earth metal ions released from the sorbent after metal sorption (Nixon 1971). SEM-EDX has been used to determine the morphology of plant biomass. Such as ceiba pentadra (L) Gaertn (Abdullah *et al*., 2010), grace waste (Hameed 2009), rice (El-Shafey 2010), corncobs(Khan and Wahab 2007), Botrytis cinerea (Akar, *et al*., 2005), *Moringa oleifera* seeds (Kalavathy and Miranda 2010), wheat (Farooq et al., 2010). It has significant advantages like good resolution at high magnification; it can do microanalysis such as chemical composition, crystallography, magnetic and electrical characteristics of a sample. However its major drawback is that it operates under vacuum and therefore in many analytical procedures, the sample must be rendered conducive in order to view. This is done by coating the sample with either a metal or carbon (Ammrf 2014)

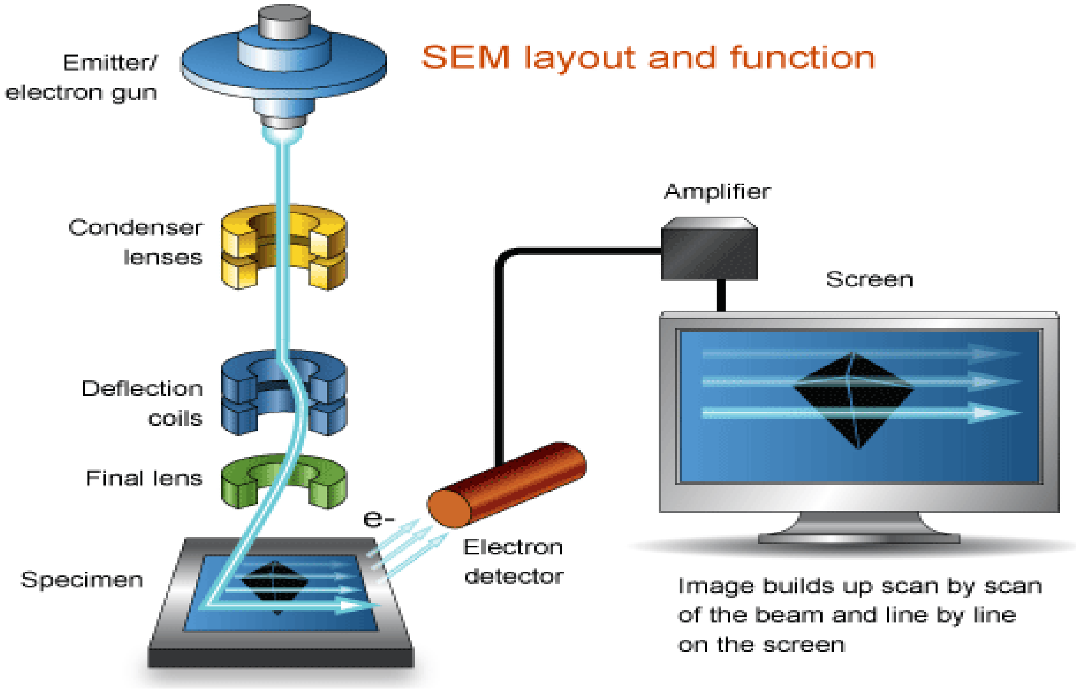

Figure 2.5: Schematic representation of a SEM layout and function (Adopted from Philips XL 30 ESEM manual)

Energy dispersive x-ray (EDX) is used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-ray excitation and a sample. To stimulate the emission of characteristic X-rays from a specimen, a high-energy beam of charged particles such as electrons or protons or a beam of X-rays, is focused into the sample being studied. At rest, an atom within the sample contains ground state electrons in discrete energy levels or electron shells bound to the nucleus. The incident beam may excite an electron in an inner shell, ejecting it from the shell while creating an electron hole where the electron was. An electron from an outer, higher-energy shell then fills the hole, and the difference in energy between the higher-energy shell and the lower energy shell may be released in the form of an X-ray. The characteristic X-ray lines are named according to the shell in which the initial vacancy occurs and the shell from which an electron drops to fill that vacancy. There are three electron shells K, L and M. If the initial vacancy occurs in the K shell and the vacancy filling electron drops from adjacent (L) shell, a K x-ray is emitted and if an L-shell electron is ejected and an electron from M-shell fills the vacancy, L radiation will be emitted (Hafner 2006). Figure 2.6 shows how different X-rays are emitted.

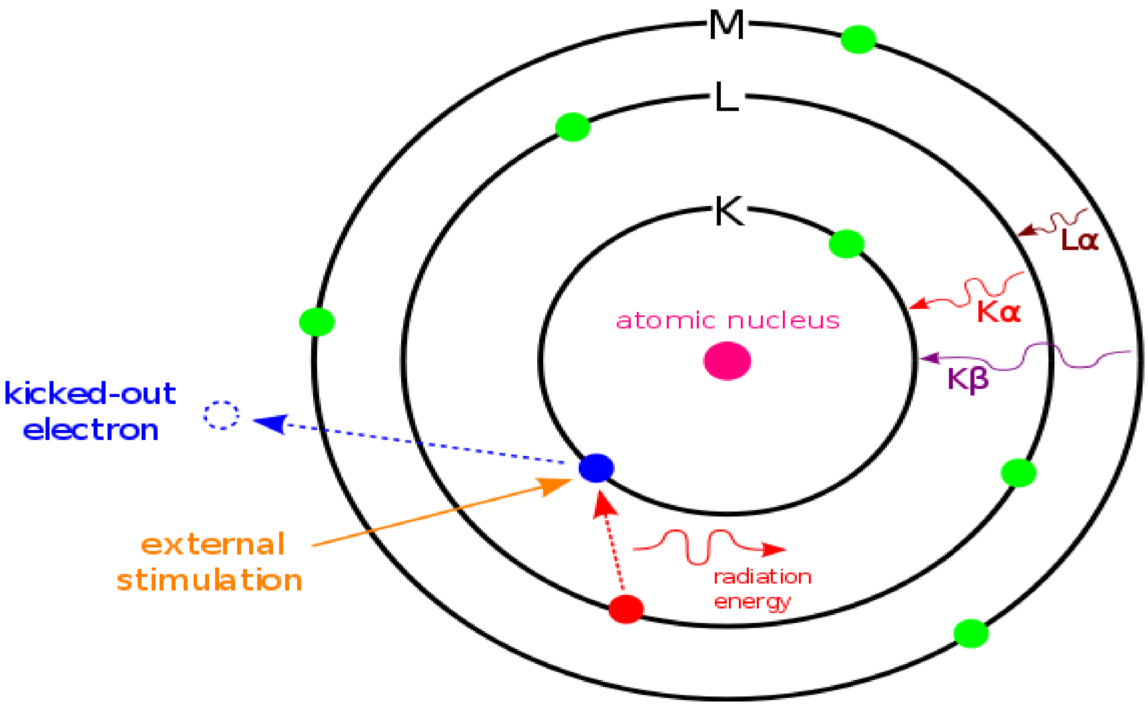

Figure 2.6: X-ray emitted from different electron shells (https://en.wikipedia.org/wiki/Energy-dispersive_X-ray_spectroscopy

3.0 EXPERIMENTAL

3.1 Instrumental analysis

A Varian 220FS Atomic Absorption spectrometer operated with air/acetylene was used for determination of seven selected metal ions which included Pb, Mn, Fe, Zn, Cu, Mg and Cd. Varian hollow cathode lamps for each of the analysed metals were used as radiation source. 1000 mg L^{-1} ppm stock solutions of metals were used to prepare working standards (in the range 0.0 to 5.0 mg L^{-1}) in deionized water. The instrument was calibrated manually by aspirating the prepared working standards of the cations of interest one by one into the flame. The samples were then also aspirated manually into the flame for atomization. The instrumental condition applied were according to AAS manual (PerkinElmer 2006)

Perkin Elmer System, Spectrum two Fourier transform infrared (FTIR) spectroscopy was used to determine the functional groups of sorbents. The FTIR spectra were recorded in the wavenumber range 400-4000 cm^{-1} on a Perkin Elmer system 2000 FTIR. The sorbents were kept at ambient temperature. 1mg seed powder per 200 mg of KBR was weighed. The powder was pressed into pellets by using a 15 ton hydraulic press. The data were collected at 2.0 cm^{-1} resolution, and each spectrum was a result of 256 scans. A Philips XL 30 ESEM model from USA is an Environmental Scanning Electron Microscope (ESEM), which was used to determine the surface morphology and determine elemental composition of the sorbents. The instrument resolution was about 142eV at amplification time of 10microseconds, it uses an Electron Dispersive X-ray (EDX) detector and Philips GSR v, 3.2 software. The ESEM worked at beam voltage of 20kV, low vacuum (typically 2 to 6 Torr), and utilizes a chamber gas (H_2O) for imaging, charge suppression and sample humidity.

A stainless steel Thomas-Wiley laboratory model 4 grinder purchased from Arthur H Company, USA was used for grinding the sorbents (Moringa Oleifera seed pods and Morula nut shells). Hanna Instruments HI 991001 pH meter from USA, used for adjusting the pH of the solutions and a ZNCL-S Intelligent hot plate was used to heat while digesting samples.

3.2 Materials and Reagents

All reagents were of analytical grade. Acids were used for digesting and adjusting pH. HNO_3 (69%) purchased from Skylabs and HCl (32%) from Minema Chemicals, South Africa. Glasswares such as conical flasks, measuring cylinders, volumetric flasks and filter funnels were purchased from Pyrex companies. They were pre-cleaned, then soaked in water acidified with HNO_3 (1:9 v/v) and rinsed with deionized water prior to use. Double deionized water (Millipore-Q Millipore 18.2 MΩ/cm resistivity) was also used for dilutions of samples to be analysed with FAAS. Elemental standards solutions used for calibration were prepared from 1000 ppm of stock solutions supplied by Minema Chemicals, South Africa (Pb and Fe) , Merck Pty Ltd, USA (Cu, Mn, Mg and Zn) and Sumitomo Metal Mining Instruments (pty) Ltd, South Africa (Cd). NaOH pellets for adjusting pH were supplied by Rochelle chemicals, South Africa. An agate pestle and mortar was also used for reducing the particles further. A 100 µm, 200 µm, 500 µm stainless steel sieves were used for sieving the sorbents. The filter papers used were ash less Whatmann filter paper no.1 from England. The filter papers were used to filter the mixtures of sorbents and solution.

3.3 Sampling and treatment of sorbent materials

The Moringa Oleifera seed pods (deseeded) and Morula nut shells were obtained from various sampling points in Block 9 Gaborone, Botswana.

Dried Moringa Oleifera seed pods and Morula nut shells were washed with double deionized water, further dried in an oven at 105^0 C for 24 hr. The dried samples were ground then sieved to different mesh sizes (100, 200, and 500 µm) and stored in glass bottles until further analysis.

3.3.1 Characterization of Morula nutshells and Moringa seed pods

Fourier transform infrared (FTIR) spectroscopy was used to characterize the two sorbents for possible functional groups responsible for metal uptake. 0.1 mg of sorbent was placed on the plate of the FTIR spectrometer and analysis conditions were as shown in Section 3.1. The spectra were observed for each sorbent before being used for removal of metal ions and after exposure to sample. The spectra are provided and the peak shifts are shown in results and discussion (Chapter 4, Section 4.1.3).

Environmental Scanning Electron Microscope (ESEM), was used to determine the surface morphology of MSP and MNS and determine elemental composition of the sorbents. The sorbent was placed on the SEM grid and coated with carbon at high pressure (see Section 3.1).

3.3.2 Determination of metal concentration in sorbent material

2 g of each ground sorbent was weighed and then digested with an aqua regia solution of HCl: HNO_3 in a ratio of 3:1 v/v. Each mixture was placed in 100 mL conical flasks and heated at 85°C until the volume was reduced to 1 mL. The resulting volume was filtered using a whatman No. 1 filter paper and put into 50 mL volumetric flasks. Deionized water was added to the flasks and filled up to the mark. The samples were analyzed using FAAS following a modified method (Mustafa, 2003).

3.3.3 Acid treatment of MSP and MNS sorbents

15 g of each sorbent was weighed and put in a conical flask, 200 mL of 0.1M HNO_3 was added and the mixture was soaked for 24 hours as in literature (Annadural *et al*., 2003.). The mixture was then filtered and the sorbent washed several times with deionized water until the pH of the sorbent was neutral (around pH 7). The sorbent was then dried in an oven at 50^0C overnight and then put in glass bottle until further use.

Using the optimized sorption parameters (section 3.4), the sorption capacity for the acid treated and untreated sorbents were compared to remove selected heavy metals from wastewater samples.

3.4 Batch biosorption studies for metal removal using the two sorbents

All experiments were carried out in batches and done in triplicates. The procedures for optimizing the various parameters were modified from literature as indicated in the text. A 20 ppm standard mixture of Mg, Mn, Pb, Fe, Zn, Cu, and Cd was prepared from 1000 ppm stock solution of each of the seven metals. The sorbent used were ground and sieved Moringa Oleifera Seed Pods and Morula nut shells. One variable at a time method of optimization was used.

3.4.1 Optimization of parameters

3.4.1.1 Effect of extraction time

0.5 g of sorbent was added to 50 mL of the 20 ppm standard mixture at pH 8. The mixture was shaken for 8 hours using a water bath shaker at an interval of 10 minutes for the first hour and 30 minutes interval for the rest of the time. This was adopted from a modified procedure from literature (Amuda, *et al*., 2007). The mixture was then filtered using whatman No. 1 filter paper and the filtrate was transferred into a 50 mL volumetric flask and filled with deionized water to the mark. The unbound metals were analysed in the filtrate using FAAS as described in Section 3.1.

3.4.1.2 Effect of pH

To study the effect of initial pH on the sorption, a procedure by Akhtar *et al*., (2007) was modified. The pH of a 20 ppm standard mixture was adjusted using 0.1M HCl and 0.1 NaOH in the range 2 to 10. 0.5 g of sorbent was added to 50 mL of each of standard mixture (different pH). The resultant optimized (see section 4.2.3) filtrate mixture was treated and analysed as in Section 3.4.1.1.

3.4.1.3 Effect of Initial metal concentration

A 50 mL standard mixture of the seven metals (Mg, Mn, Pb, Fe, Zn, Cu, and Cd) was prepared with concentration ranging from 1ppm to 20 ppm in a 100 mL conical flask (Amuda *et al.,* 2007). The resultant optimized (see section 4.2.4) filtrate mixture was treated and analysed as in Section 3.4.1.1 for each of the different concentration mixture.

3.4.1.4 Effect of temperature

To study the effect of temperature, 0.5 g of sorbent was added to 50 mL of the 20 ppm standard mixture solution at pH 8. The mixtures were put into four different water baths in the range 25°C to 60°C. Each optimized (see section 4.2.6) filtrate mixture (at varying temp) was treated and analysed as in Section 3.4.1.1.

3.4.1.5 Effect of particle size

Particle sizes of 100 µm, 200 µm and 500 µm of each of the sorbents were obtained by sieving the ground plant material using different sieve mesh. 0.5 g of each of the sorbents (of varying particle sizes) was weighed and added to 50 mL of the 20 ppm standard mixture solution at pH 8 in a conical flask. The optimized (see section 4.2.5) filtrate mixtures were treated and analysed as described in Section 3.4.1.1.

3.4.1.6 Effect of adsorbent dose

To study effect of sorbent dose, a modified method by Garg, *et al*., (2007) was used. Briefly, a sorbent dose in the range of 0.5 to 2.5 g was added to 50 mL of the 20 ppm standard mixture solution at pH 8. The optimized (see section 4.2.2) filtrate mixture was treated and analysed as in Section 3.4.1.1. Figure 3.1 shows summary of the optimization procedure.

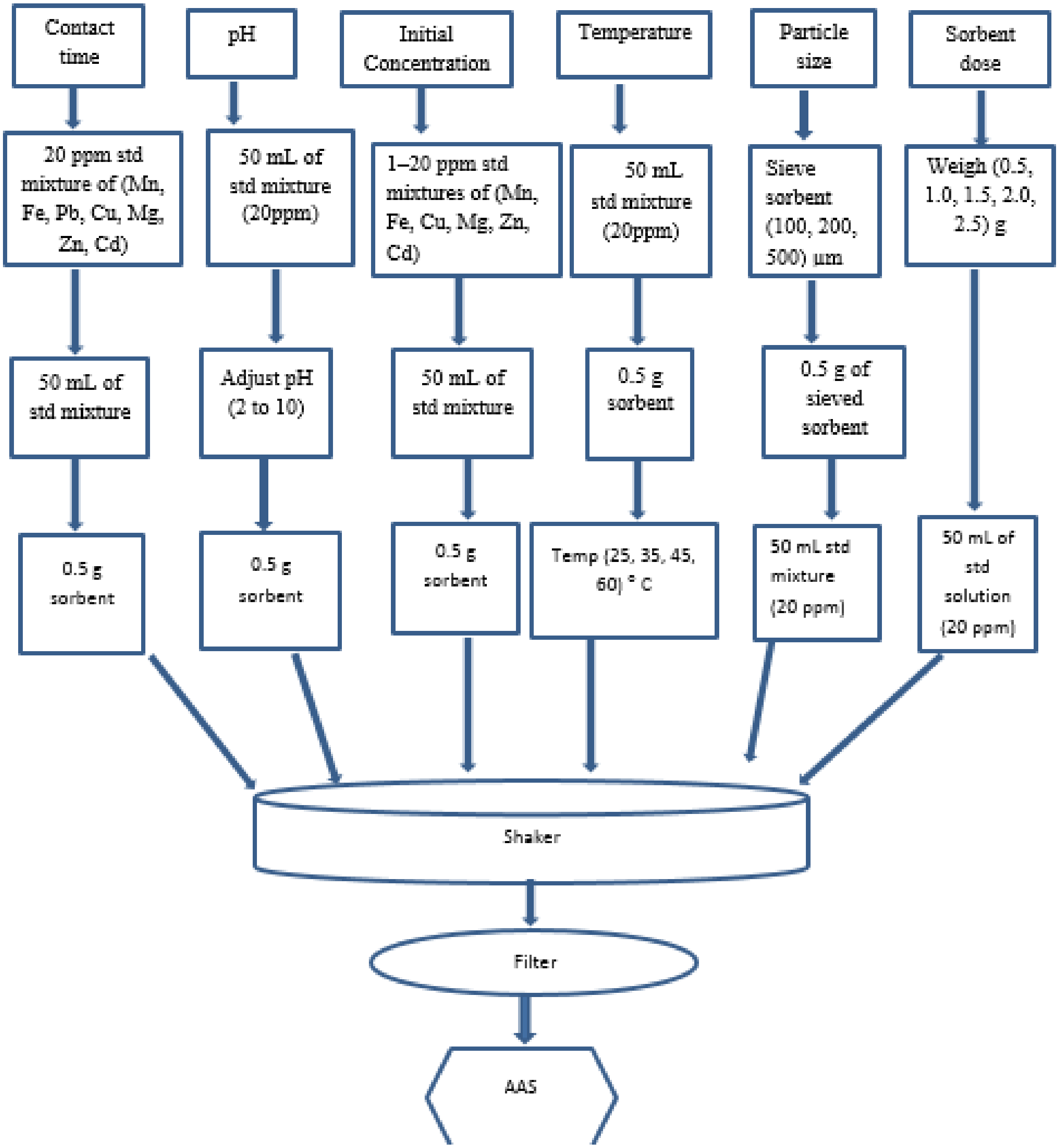

Figure 3.1: Summary of the optimization procedures for biosorption of metals on morula nut shells and moringa seed pods

3.5 Application to real samples

3.5.1 Sampling and sample treatment of water samples

Water samples were obtained from Glen valley wastewater treatment plant in Gaborone, Botswana (Figure 3.2) on the 20th February, 2015. At the treatment plant five sampling sites were identified (Inlet point, primary settling, secondary settling point, aeration point and effluent point) as shown by figure 3.2.

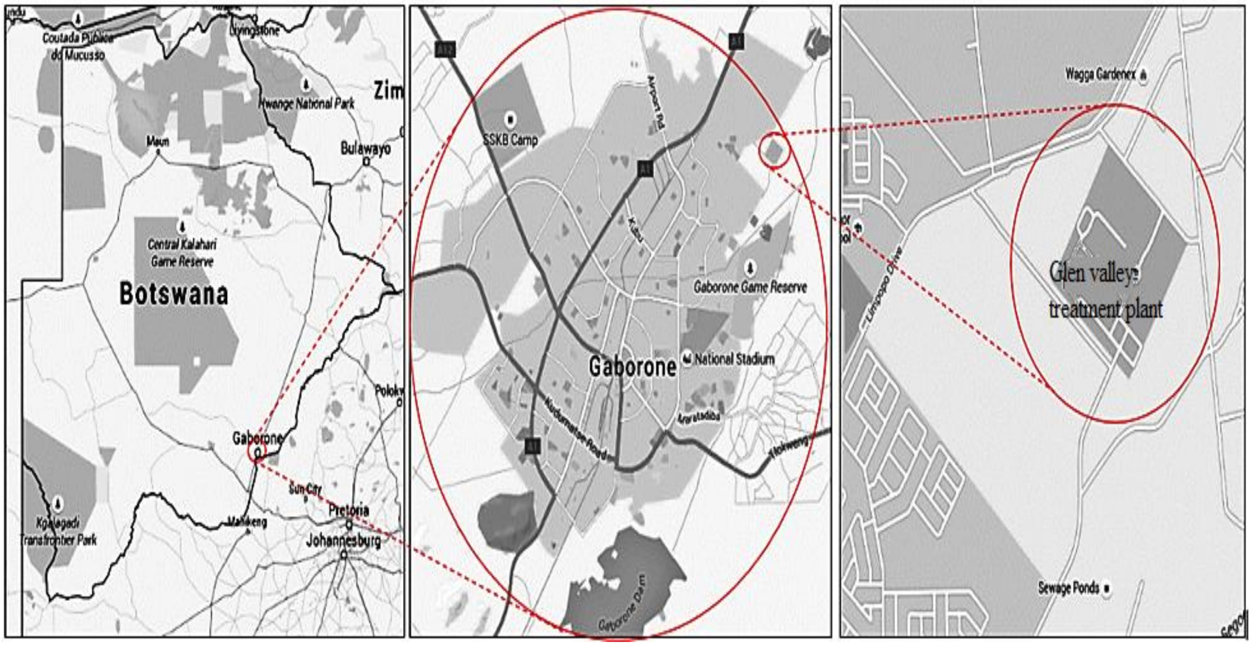

Figure 3.2: Map showing location of Glen valley Waste water Treatment Plant Gaborone, Botswana (24.609085 S, 25.965977 E) (Water Utilities Corporation, 2014. Annual report 2012/13)

Grab sampling technique was used to obtain the water samples from individual sampling sites. Each sample was put in a labeled amber glass bottle and acidified to pH < 2 for preservation and also to prevent metal ions from adsorbing on the glass container. The water sample was placed in the cold room at 4^0C until analysis.

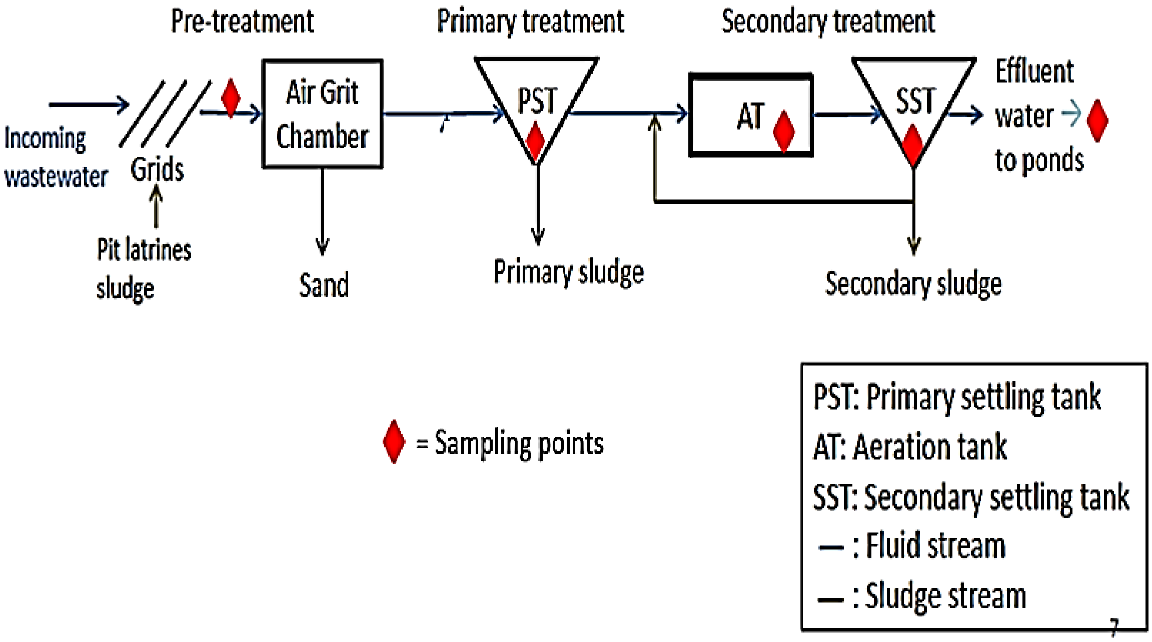

Figure 3.3: Wastewater treatment stages in Glen valley wastewater treatment plant, Gaborone Botswana (Water Utilities Corporation, 2014. Annual report 2012/13)

3.5.2 Determination of initial metal concentration in water samples

Water samples collected from inlet, primary, secondary, aeration and effluent points were acid digested following a method adopted from EPA 1999 method 200.2 for analyzing wastewater samples 50 mL water sample was placed in five 250 mL conical flasks. 1mL of 69% HNO_3 and 0.5 of 37% HCl was added to each flask to digest the samples. The mixture was heated until the initial volume was reduced to approximately 0.5 mL. It was then filtered into a 50 mL volumetric flask and deionized water added to the mark. The samples were then analyzed with FAAS to determine the concentration of seven selected metals (Pb, Fe, Mn, Mg, Zn, Cu, and Cd). This was done in triplicate.

3.5.3 Application of the optimized biosorption method to real samples

The extraction efficiency for the acid treated sorbents and the untreated sorbents were compared by applying the optimized parameters (Section 3.4) to water samples collected from sewage treatment plant (section 3.5.1) and borehole water collected from a borehole in Artesia, Botswana. Both Moringa Oleifera seed pods and Morula nut shells biomass were used for the metal removal in the samples. 50 mL of water samples were used and the optimized conditions applied to both water samples. The analysis was done in triplicates, and the analytes remaining in the water sample after sorption were determined with FAAS.

3.6 Performance of the developed method

The analytical parameters studied were linearity, detection limits, method precision and recovery studies

3.6.1 Linearity

The linearity of the analysis was determined using the matrix matched standard mixtures of varying concentrations (0.0 mgL^{-1} to 5.0 mgL^{-1}) prepared using de-ionized water. Each concentration was determined in triplicates. These were analysed using AAS and the linearity of the calibration curves were obtained and are discussed in chapter 4 Section 4.3.1.

3.6.2 Detection limits

Limit of detection (FAAS) is the lowest quantity of an analyte that can be distinguished from the absence of a blank value within a stated confidence limit. The LODs are calculated as three times standard deviation of seven replicates of water samples spiked with low concentration of analytes (2 mgL^{-1}).

LOD= 3 X SD

The limit of quantification (LOQ), which is the lowest concentration at which the analyte can reliably be detected for precise quantification, was calculated from the equation below (miller and Miller, 2010). LOQs were calculates as ten times of standard deviation of the replicates (n)

LOQ= 10 X SD

Where:

SD is the standard deviation of the replicates (n)

Water samples were spiked with 2 mgL^{-1} of each analyte and were taken through the sorption stage using the optimized parameters. Then the samples were aspirated into the FAAS to determine the concentration. The standard deviation was calculated from the concentration of seven spiked water less the initial concentration of analyte.

3.6.3 Removal efficiency of the sorbents

The samples were spiked with 2 mgL^{-1} standard of each of the metal ions (Pb, Mn, Mg, Fe, Zn, Cu and Cd) and the optimized parameters were applied to each. Analysis were done in triplicate using FAAS. The removal efficiency were calculated using the equation:

$$\% \text{ Removal } = \frac{\text{amount added} - \text{amount found}}{\text{added amount}} \times 100 \quad \text{Eqn 1}$$

4.0 RESULTS AND DISCUSSION

4.1 Characterization of the sorbents.

4.1.1 Metal determination using FAAS

The concentration of the metals (Mg, Mn, Fe, Pb, Cu, Cd, and Zn) in moringa seed pods and morula nut shells was determined from an external calibration curve (see appendix B), obtained using standards of known concentration. Figures 4.1 and 4.2 shows the concentrations (mgg^{-1}) of the selected metals as was determined in the sorbents, morula nutshells and moringa seed pods after acid digestion.

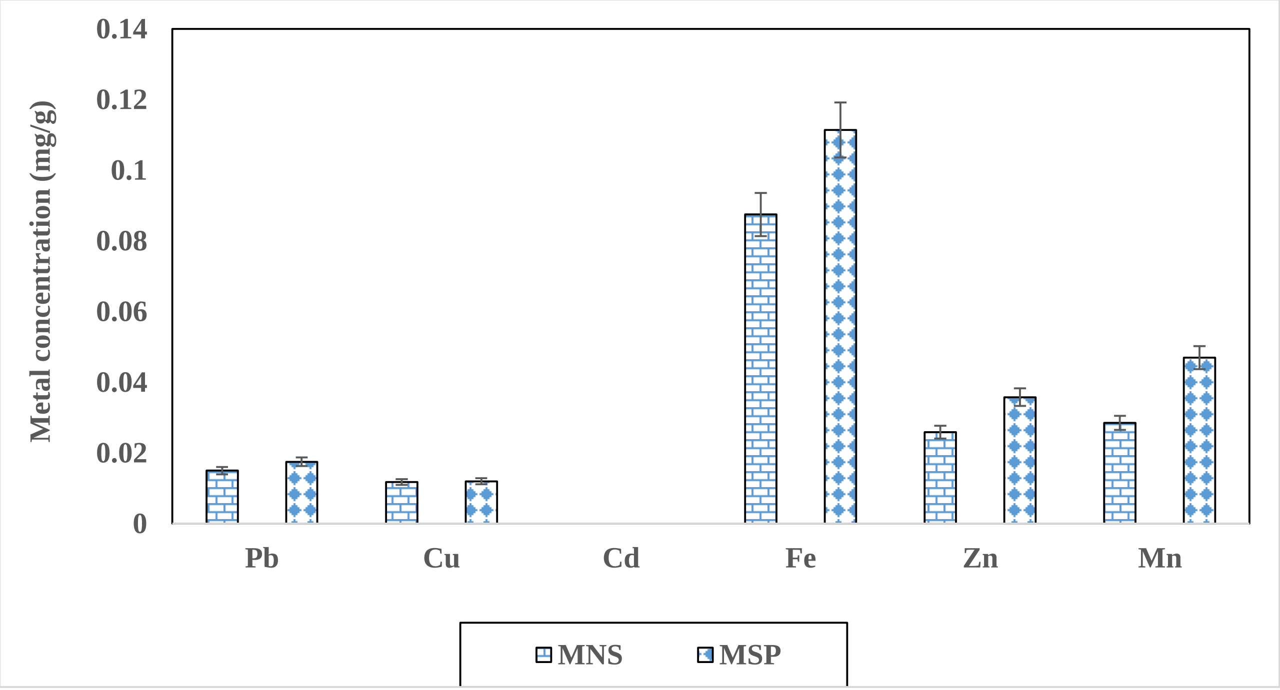

Figure 4.1: Concentration (mg/g) of selected metals in Moringa seed pods and Morula nut shells

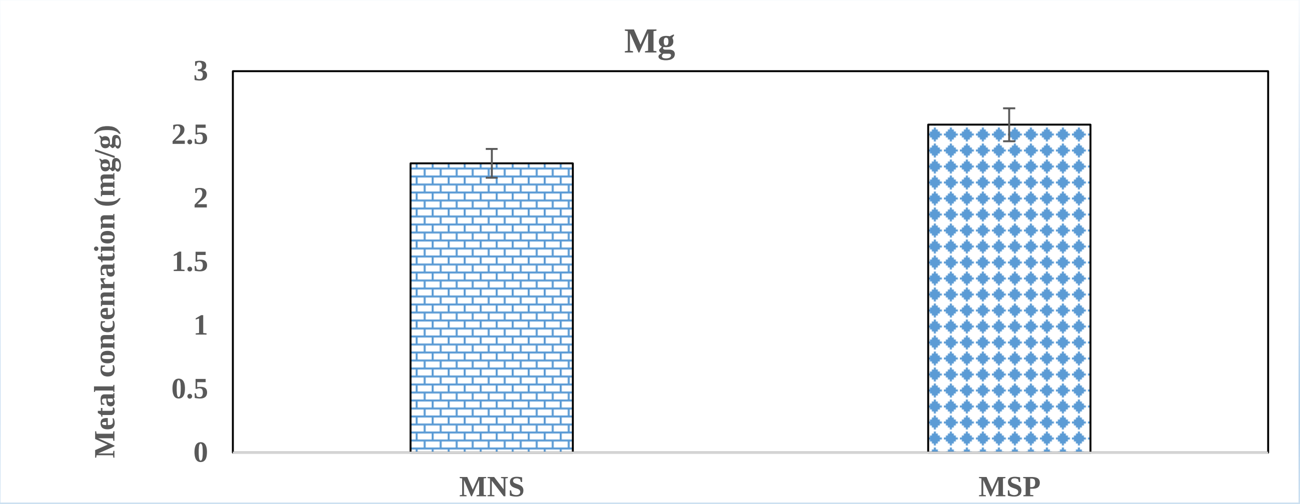

Figure 4.2: Concentration (mg/g) of magnesium in moringa seed pods and morula nut shells

It was observed that the concentrations of essential elements such as magnesium and iron were higher. The other metals were in trace levels and cadmium was not detected. All the concentrations were within the recommended limits for edible plants set by FAO 2012 and WHO 1999. Although moringa seed pods and morula nut shells are normally considered as waste, they can also be a source of the mentioned essential elements (Akan *et al*., 2013).

4.1.2 SEM-EDX analysis

Scanning electron Microscope (SEM) is used to determine the morphology of a sample. It produces images of a sample by scanning it with focused beam of electrons and produces information about the sample surface topography and composition (see Section 2.4). Figure 4.3 and 4.4 SEM images revealing the surface texture and morphology of both MSP and MNS respectively.

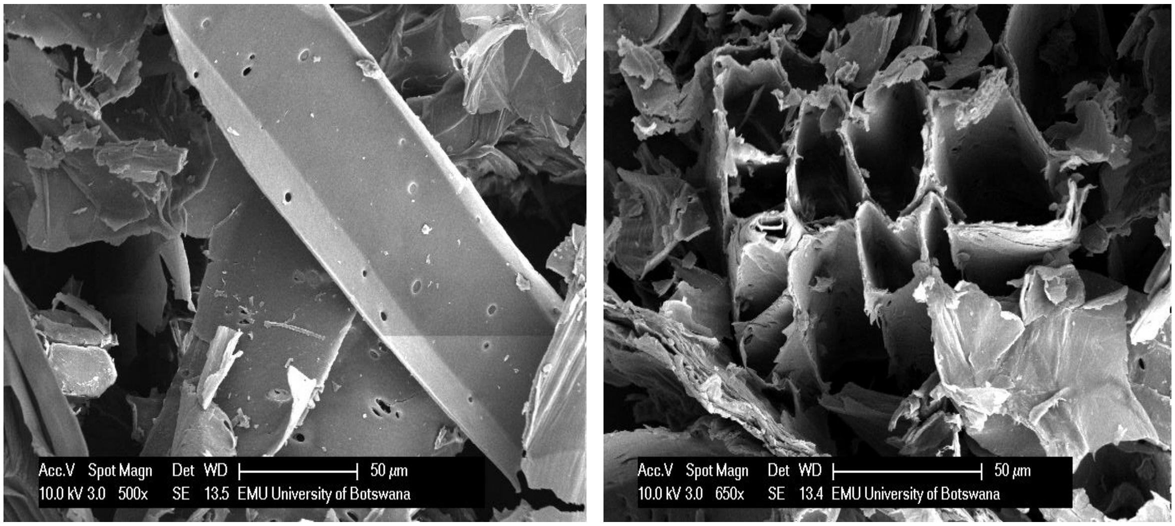

Figure 4.3: SEM micrograph of Moringa seed pods (MSP)

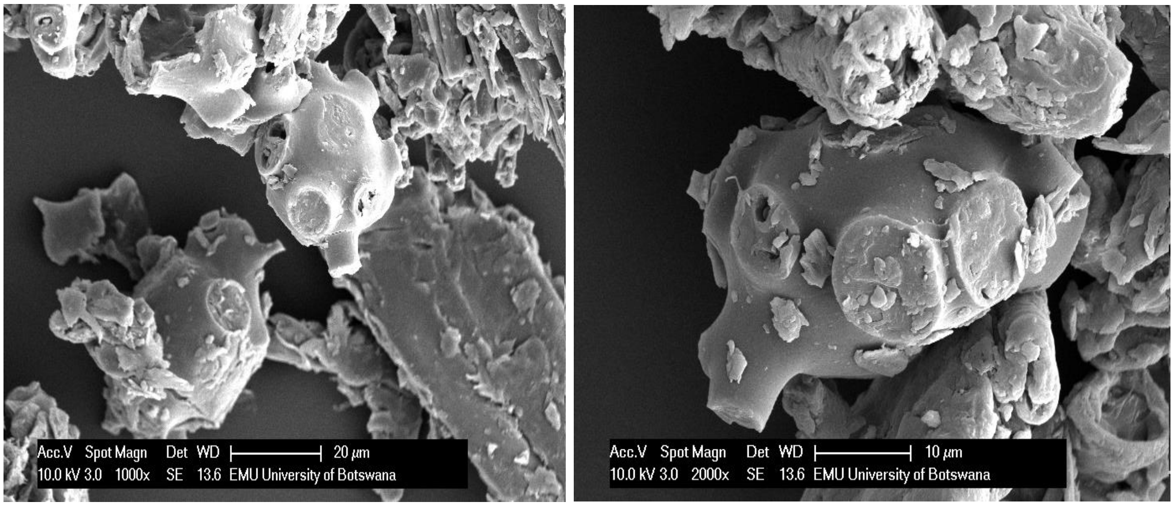

Figure 4.4: SEM micrograph of Morula nutshells (MNS)

The results show that the materials have relatively porous surface. The micrographs of MSP (Figure 4.3) reveals cylindrical structures with different diameters. This surface (physical) characteristics would result in high metal removal due to available binding cavities for the metal ions. MNS (Figure 4.4) micrographs shows that the structures are spherical and have pores on the surface offering binding sites for higher adsorption capacity of metal on the sorbent. The pores are evidence that these materials are efficient for metal removal. The efficiency of a sorbent depends on available number of pores, pore size and the surface area (Srivastava and Hasan 2011; Ofudje *et al*., 2015). The particles with higher surface area and have many pores is expected to be more efficient for metal removal. Other sorbents have shown similar characteristics for example almond shells (Demirbas *et al*., 2004), *Moringa oleifera* wood (Kalavathy and Miranda 2010) and fruit peels (Annadural *et al*., 2003). However, the efficiency of the sorbent can be increased by increasing the number of pores and the surface area. This is done by treating the sorbents with acids or activated with carbons (Gupta *et al*., 2010)

Figure 7.1 (see appendix 3) shows the EDX results of MNS and MSP. The amount of the selected elements in SEM-EDX may show disparities from those of FAAS results. This is because the SEM-EDX analysis is based on a single particle as compared to that of FAAS that is based on the total sample. The data on Table 7.8 (appendix 3) shows the elemental composition of MSP and MNS. The elemental composition of MNS shows that Carbon and Oxygen had higher percentages (73.06% and 20.69 % respectively), which is expected of plant material. Elements such as Na, Mg, Al, Si, P, K, Ca, Ba, Fe and Si were in traces. Carbon and oxygen have a higher percentage in MSP with a 62.53% and 31.98% respectively. *Moringa Oleifera* tree in general has been reported to have many essential elements. Foidl *et al*., (2001) reported that Moringa seed pods had Ca, P, Mg, Na and K in macro levels while Fe, Mg, Zn and Cu in micro levels. The leaves, back, fruits and roots of morula tree have also been reported to have high amounts of Ca, Mg and Na and other metals such as Fe, Cu and Zn are in trace levels (Belemtougri *et al*., 2001; Adel 2002). As observed from the elemental composition of the sorbents (Table 7.8), essential elements such as Na, Mg, K, Ca and Fe are present in MNS and MSP. There is no reported work on the quantities of the essential elements in morula nutshells but it would be expected that the nutshells may have some of the essential elements found in the other parts of the morula tree. Hence these can be source of the mentioned essential elements instead of being thrown away.

4.1.3 FTIR Analysis

A different way to determine the sites responsible for metal cation interaction with the MSP and MNS is to check the behavior of the functional groups in the sorbent before and after metal ion removal. FTIR spectra were used to determine the functional groups responsible for interaction. The spectrum observed for MNS and MSP were in the range of 500-4000 cm^{-1} are as shown (Figure 4.5 and 4.6).

The IR spectra of MSP as shown on Figure 4.5a shows broad bands around 3333 cm-1 attributed to the surface hydroxyl group (Araújo *et al.*, 2013). The bands between 2917 cm^{-1} and 2840 cm^{-1} were assigned to stretching vibration of C-H alkene group (Helen Kalavathy and Miranda 2010; Kumari *et al.*, 2006), The bands between 1736 cm^{-1} and 1640 cm^{-1} were attributed to strong C=O ketonic and aldehyde group. Small peaks observed at 1510 cm^{-1} and 1468-1300 cm^{-1} can be assigned to COO- Carboxylate groups respectively (Bejarano *et al.*, 1994). Around 1250 cm^{-1} the band can be assigned to COOH group (Dyer 1991). The bands between 1000 cm^{-1} and 1250 cm^{-1} were assigned to be C-O carboxylic acid ether or ester groups (Lin and Rayson, 1998). Bands below 800 cm^{-1} can be considered as the finger print regions and any shift in this region could be due to change in the nature of binding (stretching or bending) (Pradhan, Singh, and Rai 2007).

Figure 4.5b shows the spectrum of MSP after being metal removal (metal loaded MSP). It shows that some peaks decreased in intensity and also shifted.

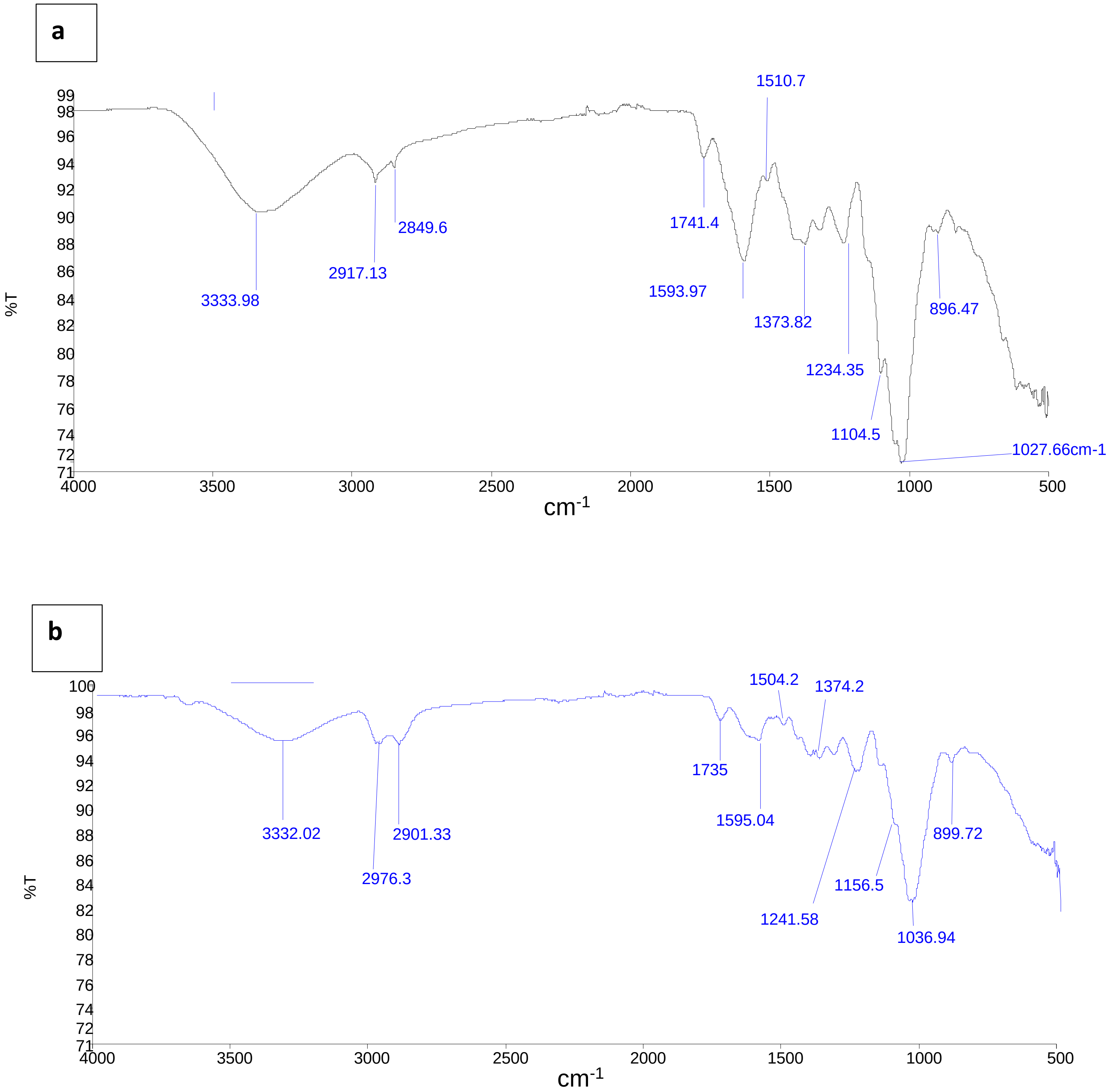

Figure 4.5: Spectrum of MSP (a) before metal ion removal (b) after metal ion removal

Decrease in intensity and shift of the above mentioned peaks as shown in Figure 4.5b is due to interaction of the metal with the specific functional groups. These interaction could be due to complex formation of metal with the surface functional groups. Complexation of the metal ions with the different functional groups is responsible for the removal of metal from the water samples. Metals forms coordinate bonds with the different functional groups and hence cause a decrease in peak intensity and also shift in peaks. Altering pH helps in changing the surface charge of the sorbents thereby improving on the removal efficiency of the sorbent. The functional groups such as amines, carboxylic, hydroxyl, carbonyls form anionic sites as pH is raised from acidic to basic conditions. The anionic sites are responsible for binding with the metal cations. The functional groups responsible for metal removal in MSP are therefore hydroxyl (-OH), C-H of the alkenes, C=C alkenes and C-O from the carboxylic acids. The peaks from moringa seed pods were assigned some functional groups and the shifts of the peaks after the sorbent was used for metal removal were determined as shown in Table 4.1.

Table 4.1: Moringa seed pods peaks from FTIR before and after metal removal, peak shifts and group assignment of the peaks

Moringa seed pods			
Peak wavenumber(cm^{-1}) for Powder MSP alone	Peak wavenumber(cm^{-1}) for metal loaded MSP	peak shift	group assignment
3333.9	3332.0	1.9	O-H Alcohols, Phenols
2917.1	2976.3	-59.2	C-H Alkenes,
2849.6	2901.0	-51.4	C-H Alkenes
1741.4	1735.0	6.4	C=O Esters
1593.9	1595.0	-1.1	C-C Aromatics
1510.7	1504.2	6.5	C=C alkenes
1234.3	1241.5	-7.2	C-O Carboxylic acid and esters
1104.5	1156.5	-52.0	
1027.6	1036.9	-9.3	
896.5	899.7	-3.2	C-H Aromatics

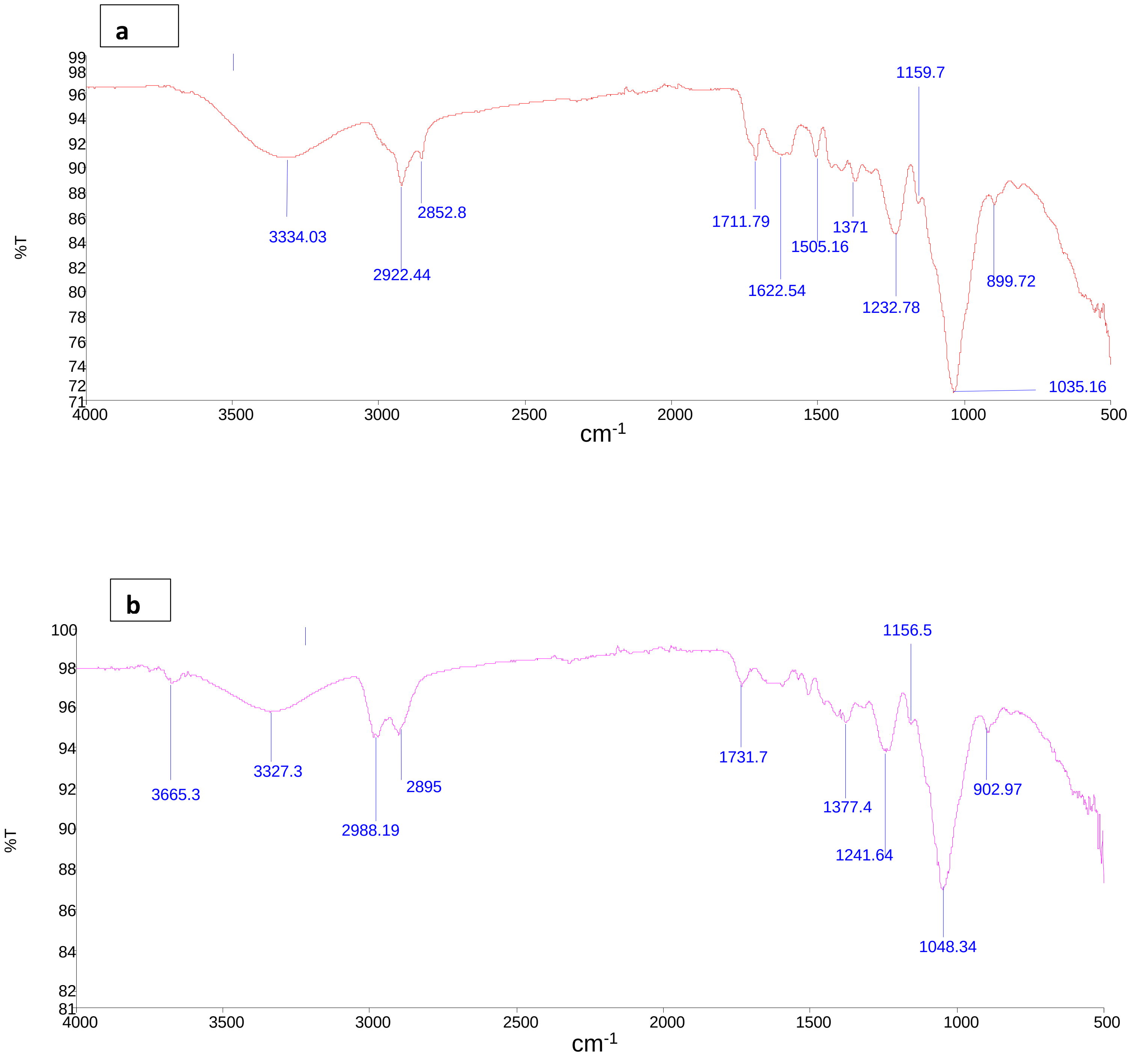

Figure 4.6: Fig: Spectrum of MNS (a) before metal ion removal (b) after metal ion removal

Figure 4.6a shows the spectrum of MNS before being loaded with the metal and Figure 4.6 b is after loading with the metals. There is a significant change in intensity of peaks and shift of the peaks on the metal-loaded sorbent as shown in Figure 4.6b using MNS. The bands around 3300 cm^{-1} assigned to O-H group in the metal-loaded sorbents decreased in intensity and shifted as shown in Table 4.1 and 4.2. This is attributed to the fact that the metals were forming bonds with the OH group and fewer OH groups are present on the surface (Cox *et al.*, 2000). The peak shift at around 2900 cm $^{-1}$ assigned is due to metals forming coordinates bonds with the C=C bonds of the alkenes. Likewise the peaks around 1593cm^{-1} and 1027cm^{-1} in metal-loaded MNS is respectively attributed to stretching of C-C from aromatic rings and C-O from carboxylic acids, ester and ethers. The peaks around 1711cm^{-1}, 1232cm^{-1} and 1035cm^{-1} were respectively attributed to the stretching of C-O from Ketones, C-N from aliphatic amines and C-O from either carboxylic acid, esters or ethers(Pradhan *et al*., 2007;Araújo *et al*., 2013; Helen Kalavathy and Miranda 2010; Kumari *et al*., 2006).

Table 4.2 below shows the assigned peak wavenumber for MNS without the metal, those of MNS metal loaded, peak shifts and the assigned functional groups

Table 4.2: Morula nutshell peaks from FTIR before and after metal removal, peak shift and functional group assignment.

morula nut shells			
Peak wavenumber(cm^{-1}) for Powder MNS alone	Peak wavenumber (cm^{-1})for metal loaded MNS	peak shift	group assignment
3334.9	3327.3	7.6	O-H Alcohols
2922.4	2988.2	-65.8	C-H alkenes
2852.8	2895.0	-42.2	C-H alkenes
1711.8	1731.7	-19.9	C-O Ketones
1371.0	1377.4	-6.4	COO- Carboxylate
1232.8	1241.6	-8.9	C-N aliphatic amines
1159.7	1156.5	3.2	

1035.2	1048.3	-13.2	C-O Carboxylic acids, esters, ether
899.7	903.0	-3.3	C-H Aromatic

The functional groups such as amines, carboxylic, hydroxyl, carbonyls form anionic sites as pH is raised from acidic to basic conditions. The anionic sites are responsible for binding with the metal cations. It can be speculated that combination of the following could be the principal mechanism for metal removal i) electrostatic interactions between the conjugate base of either carboxyl group or amine group (at pH 8) reacting with metal ions, (ii) Coordination or complex formation (using empty d-orbitals of metal ions) to interact with the electron pairs from the oxygen in the carboxyl, hydroxyl and nitrogen of the amine groups, (iii) Ion exchange processes in which the ionisable hydrogen on the carboxyl and amine groups exchange with metal cations, (iv) Microprecipitation (especially at higher pH) where the OH- ions forms insoluble hydroxides with the metal ions and the sorbent here then acts as a filter, (v) Acid-base interactions. Most of these metal ions are hard acids and the interact with atoms on the functional groups such as oxygen on the carboxyl and hydroxyl group and nitrogen on the amine groups (vi) Adsorption for example with the presence of alkenes, the concentration of electron density on them actually creates dipole moments with negative dipole moment concentrated on the center of the double bond and the positively charged metal ions will sit on these areas with high electron density. It can be concluded that in the MSP, the functional groups significant for metal removal is the carbonyls, alcohols and alkenes groups. While with MNS, the active groups for metal removal are the alcohols, carbonyls, alkenes, amines and carboxylic acids.

4.2 Optimization of parameters.

Different parameters such as pH, biosorbent dosage, initial metal concentration, temperature, particle size, and contact time affect the sorption capacity of heavy metals onto the sorbent. The Table 4.3 below shows a summary of the effect of the major factors affecting adsorption of heavy metals (Chojnacka 2010)

Table 4.3: A summarized table of effects of factors affecting sorption capacity.

Factors	Effect
pH	Increase in pH enhances biosorptive removal of cationic metals, but reduces that of anionic metals.(Akhtar *et al*. 2007)
Biosorbent dosage	Increase of sorbent dose increases percentage removal, due to increase in the number of binding sites.(U. Garg *et al*., 2008)
Initial metal concentration	It affects the quantity of biosorbed pollutant per unit weight of biosorbent up to a point of sorbent saturation then remains constant.(Akar, Tunali, and Kiran 2005)
Temperature	It usually enhances biosorptive removal of adsorptive pollutant by increasing surface activity and kinetic energy of the adsorbate, but may damage physical structure of biosorbent. (M. Nadeem *et al*., 2006).
Agitation speed	It enhances biosorptive removal rate of adsorption pollutant by minimizing its mass transfer resistance, but may damage physical structure of biosorbent.

Ionic strength	It reduces biosorptive removal of adsorptive pollutant by competing with the adsorbate for binding sites of biosorbent.
Particle size	Small particles are favorable for batch process due to higher surface area of the biosorbent, but not for column process due to its low mechanical strength and clogging of its column.
Other competing metal concentration	If coexisting metal competes with a target pollutant for binding sites or forms any complex with it, higher concentration of other pollutants will reduce biosorptive removal of the target pollutant.

Each parameter was varried while others were kept constant.

The removal efficiency was determined by computing the percentage removal using the formula in equation (1) below

$$\% \text{ Removal} = \frac{Ci - Cf}{Ci} \times 100 \qquad \text{Equation 2}$$

Where,

C_i – is the initial concentration of metal in water sample before sorbent is added

C_f – is the concentration of metal in water sample after removal of metal ion

4.2.1 Effect of Contact time

Contact time is an important parameter for determining the equilibrium time required for the sorption of metal ions on a sorbent as it is directly proportional to amount of metal ions removed from aqeous solution. In other studies moringa seeds and seed pods have shown maximum removal at optimum time of 60minutes (Obuseng *et al*., 2012; Akhtar *et al*., 2007) and 40 minutes (Sharma *et al*., 2006; Adelaja *et al*., 2011). It was observed that when MSP sorbent was used for metal removal, the highest removal was reached within 60 min (Figure 4.7) and for MNS sorbent was 120 min (Figure 4.8), the equilibrium was reached and further increase in time did not show any significant change. More time was required with MNS and this may be attributed to charactreristics (particles shape, pore size and the fuctional groups) of the MNS that are different to those of MSP as seen in section 4.1.2 and 4.1.3. Akhtar *et al* (2007) found 60 minutes to be the equilibrium time and while Reedy *et al*., (2010) used 30 minutes. The extraction efficiency of the selected metals by MSP was directly proportional to the sizes of the metal ions i.e the order was Pb>Cd>Fe>Cu>Mn>Zn>Mg (largest to smallest), with exception of Cu and Mg. This trend could be attributed to the fact that the sorbents have pores of different sizes, (see Figure 4.1 and 4.2) and therefore offer different binding sites to the different metal ions as explained by Gurgel and Gil (2009). However, larger metal ions were removed efficiently compared to smaller ions. In sorption studies, affinity of cations to the sorbent is greater for larger ions compared to smaller ions (Lee 2008). Similar trend has been observed in previous studies (Obuseng *et al.,* 2012). The trend in metals removal by MNS from the aqueous samples was in the order Cu>Fe>Pb>Zn>Cd>Mn>Mg. Percentage removal of the metal ions on the MNS increases with increase in contact time. More time allows the adsorbent particle to intract with the metal ion and increase chances of more adsorption(Jimoh, 2012). MNS has greater affinity for the metal with higher electronegative values, i.e Cu (2.00 Pauling), Fe (1.91 Pauling), Pb (2.33 Pauling), Zn (1.65 Pauling), Cd, (1.69), Mn (1.55 pauling) and Mg (1.31 Pauling). Higher biosorption of the metals on the sorbents can also be explained by hard-soft-base theory (Fourest and Roux 1992). Metals belonging to hard acid like Fe may be removed effectively.

One way-analysis of variance (ANOVA) was used to determine that there was no significant change after the selected optimum time (see appendix A9) for the two sorbents. Therefore the subsequent experiments using MSP were performed at the optimum contact time of 60 minutes while using MNS was 120 minutes.

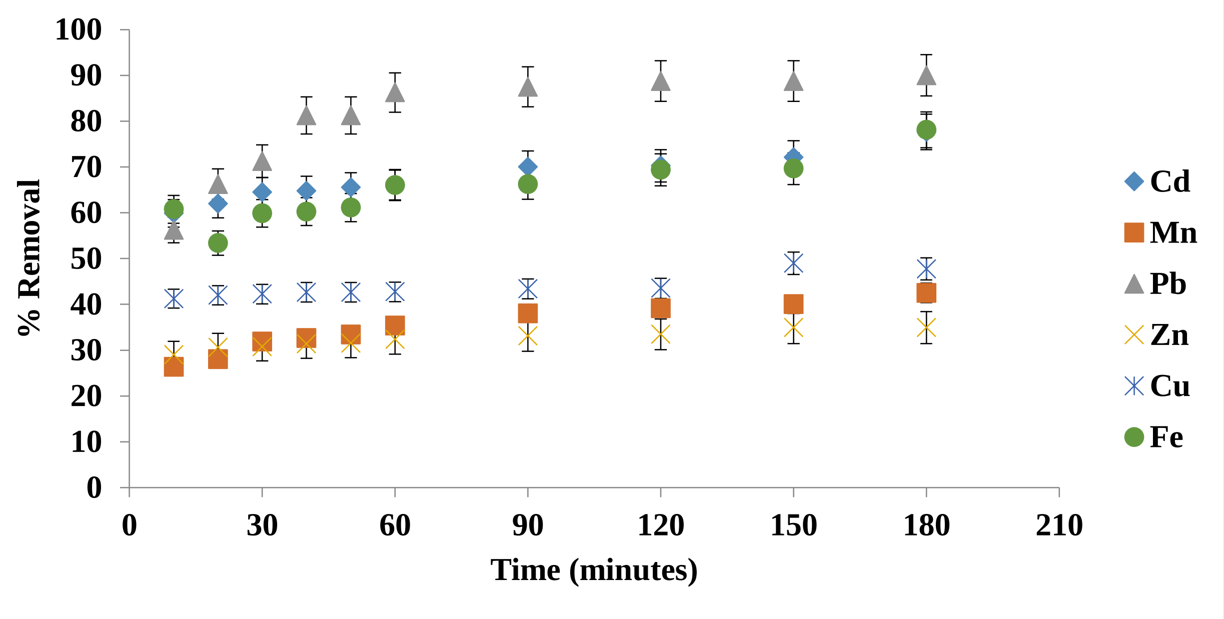

Figure 4.7: Effect of contact time on metal removal using MSP. (Other conditions kept constant)

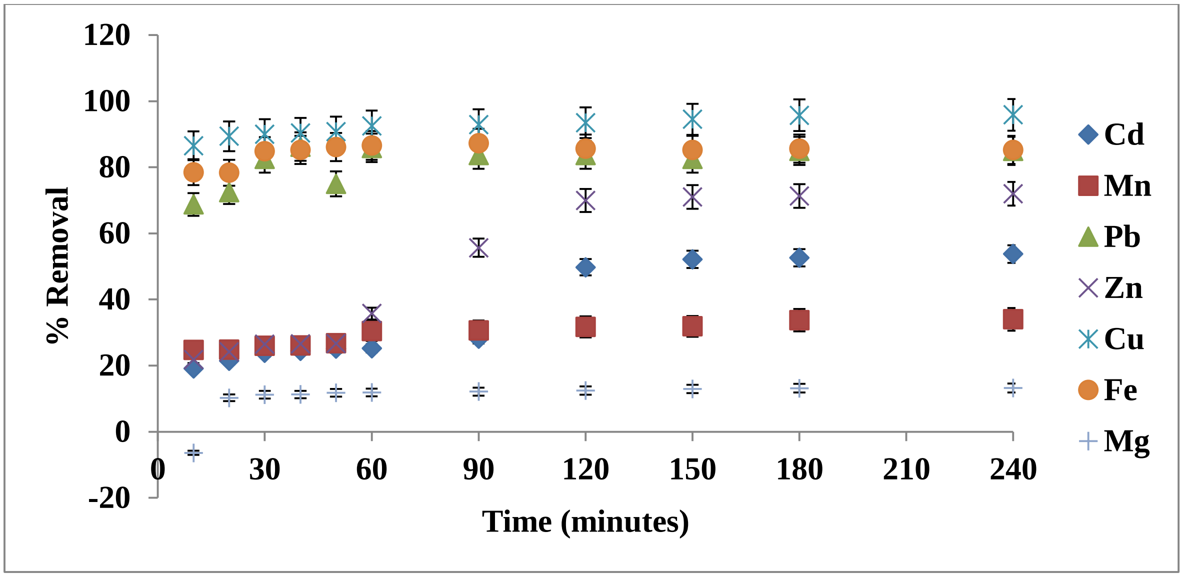

Figure 4.8: Effect of contact time on selected metal removal using Morula nut (MNS) sorbent. (Other optimized conditions kept constant)

4.2.2 Sorbent dose

It is expected that as the sorbent amount increases, the number of sorbent particles surrounding the metal ion or ratio of sorbent particle to metal ion increases (Iftikhar *et al*., 2009). These increases the surface area on which the metal ions binds to the surface of the sorbents and hence increasing the removal efficiency (Adelaja et al., 2011). However a further increase beyond the optimum sorbent dose, decreases the percentage removal and this is attributed to possibility of metal bbleeding from the material and also the fact that there is possibility of the particle overlapping and overcrowding resulting in a reduction of the total adsorbent surface area and hence decreases the percentage removal (Iftikhar et al., 2009; Rahman and Islam 2009; Pehlivan et al., 2009). Figure 4.9 shows that as the sorbent dose was increased from 0.5 g to 1.0 g of MSP sorbent, the percentage removal of metal ions increased and the optimum sorbent dose for the removal of Cd, Mn, Zn, Cu and Fe was 1g except Pb which had its highest removal using 1.5 g sorbent. However ANOVA showed that there was no significant difference when 1g was used to remove Pb ions (see appendix A5). The results for metal removal using MNS adsorbent at different dosing levels (0.5 g to 2.5g) showed a slight increase for Cd, Mn and Zn up to 2 g dosing level (Figure 4.10), but no significant difference was observed for copper,lead and iron at all dosing levels (based on ANOVA; see appendix A4 and A5). Similar studies have been reported with other low cost sorbents like moringa seeds (Sharma *et al*., 2006; Akhtar *et al*., 2007), sawdust (Shukla et al., 2002; Gupta and Babu 2009) and corncobs (Nasiruddin and Farooq 2007). While using 0.5 g and 1.0 g of MNS, Mg desorbed into the solution and therefore there was no removal of magnesium from the aqeous solution. 1.5g of sorbent showed highest removal for magnesium. However there was no significant difference when using 2.0g.

Therefore 1g of MSP and 2.0g of MNS was taken to be the optimum sorbent dose for the experiments.

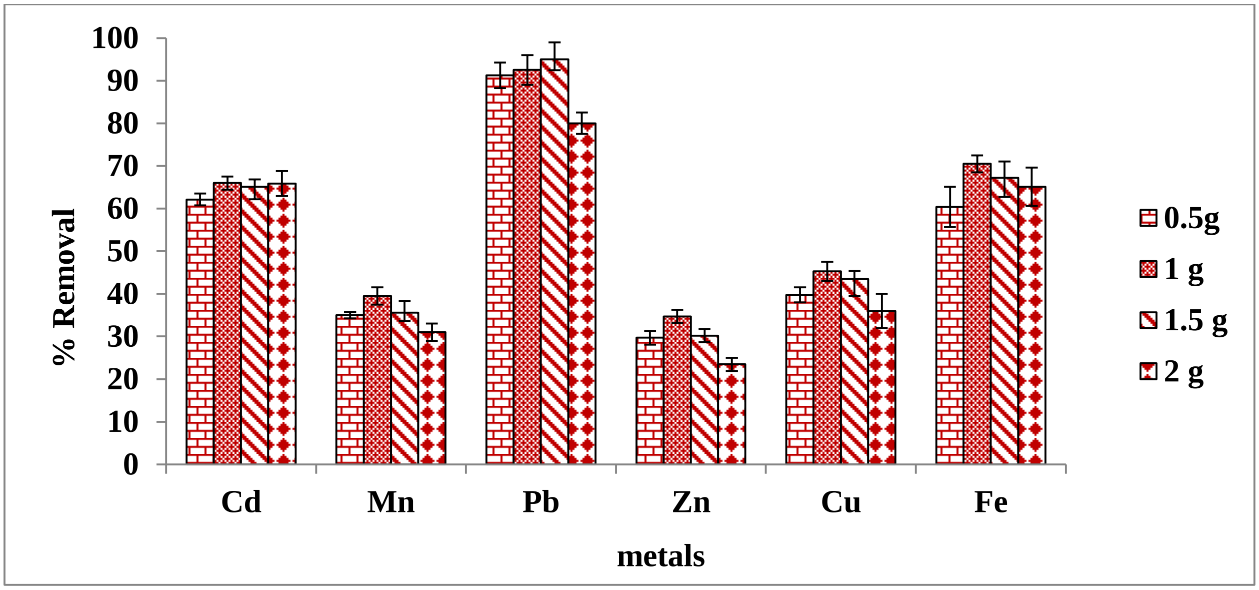

Figure 4.9: Effect of adsorbent dose on metal removal using Moringa seed pods. (Other optimized conditions kept constant)

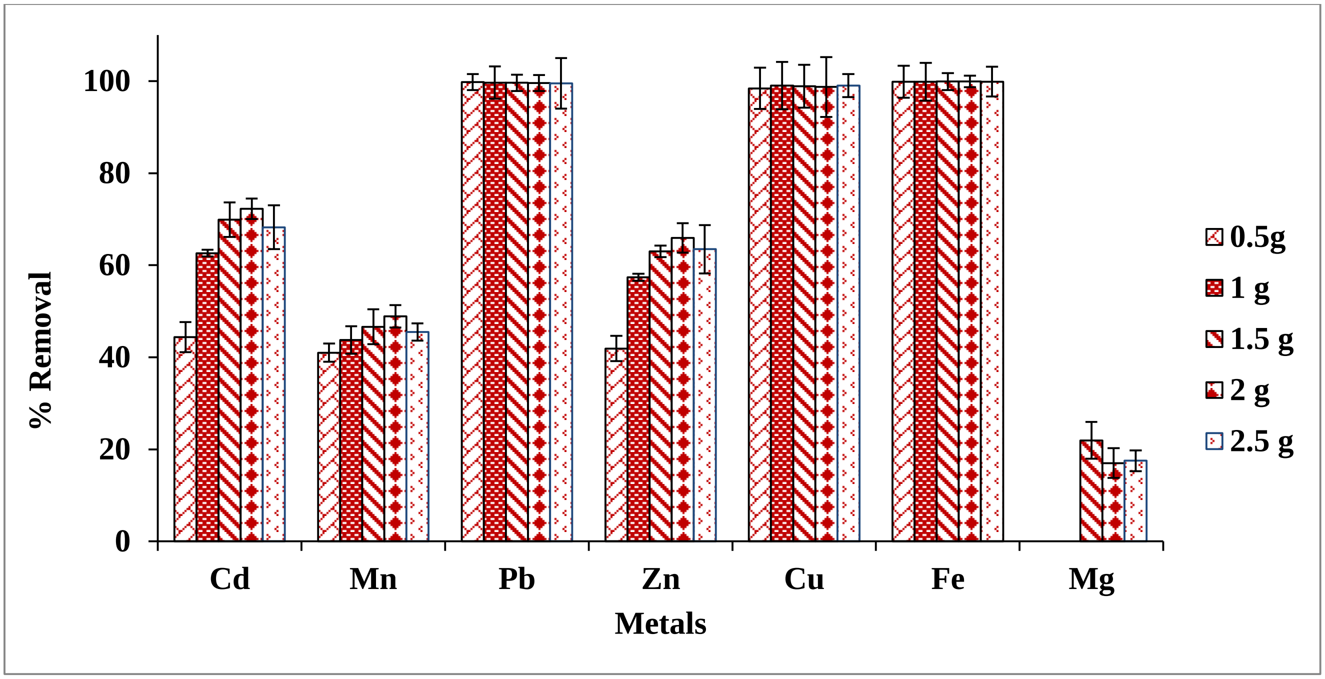

Figure 4.10: Effect of adsorbent dose on metal removal using MNS. (Other optimized conditions kept constant)

4.2.3 Effect of pH

The pH of aqeous solution is very important in sorption processes since it affects the solubility of metal ions, concentration of the counter ions on the functional groups of the adsorbent and the degree of ionization of the sorbate during the reaction (Amuda, Giwa, and Bello 2007). An increase in pH of solution while keeping all other factors (time, temperature,metal concentration, sorbent dose and particle size) constant, increased the percentage removal (rise of curves) of the selected metal ions using the MSP (Figure 4.11) and MNS (Figure 4.12). This could be due to the surface charge on the sorbent. The functional groups such as amines, carboxylic, hydroxyl, carbonyls (see Table 4.1 and 4.2) form anionic sites as pH is raised from acidic to basic conditions. The anionic sites could be responsible for binding with the metal cations. At low pH, the cations compete with the hydrogen ion in the solution for the active sites and therefore lower adsorption. But at higher pH, the surface of the adsorbent has a higher negative charge which aatracts more cations (Helen

Kalavathy and Miranda 2010). At pH > 8, precipitation of metal hydroxide is likely to occur hence could enhance metal removal, resulting in the sorbents acting like filtering materials. Removal of Pb was highest at pH 6, however ANOVA showed that there was an insignificant decrease in percentage removal as the pH was increased to pH 8 (see appendix A10 and A11). At higher pH the removal may decrease (fall of curves) due formation of metal oxides and higher amounts of hydroxyl which suppress sorption activities (Akhtar *et al*., 2007).Therefore the optimum pH was taken to be 8 for all metals using both sorbents since there was highest percentage removal before the precipitates could form.

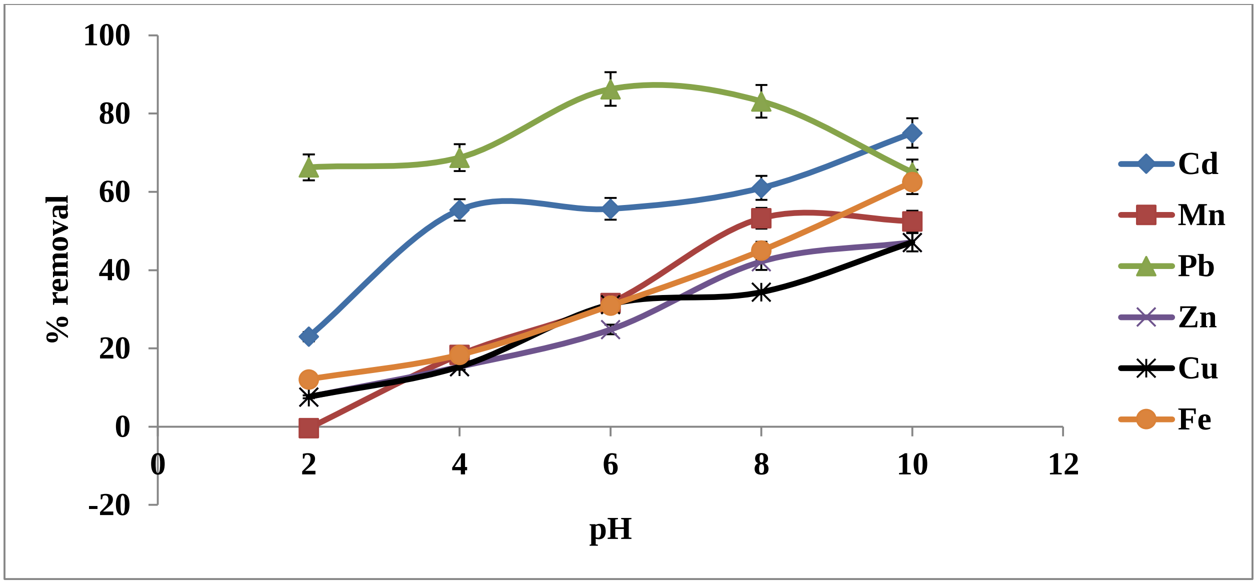

Figure 4.11: Effect of adjusting pH on metal removal by Moringa seed pods (MSP). (Other optimized conditions kept constant).

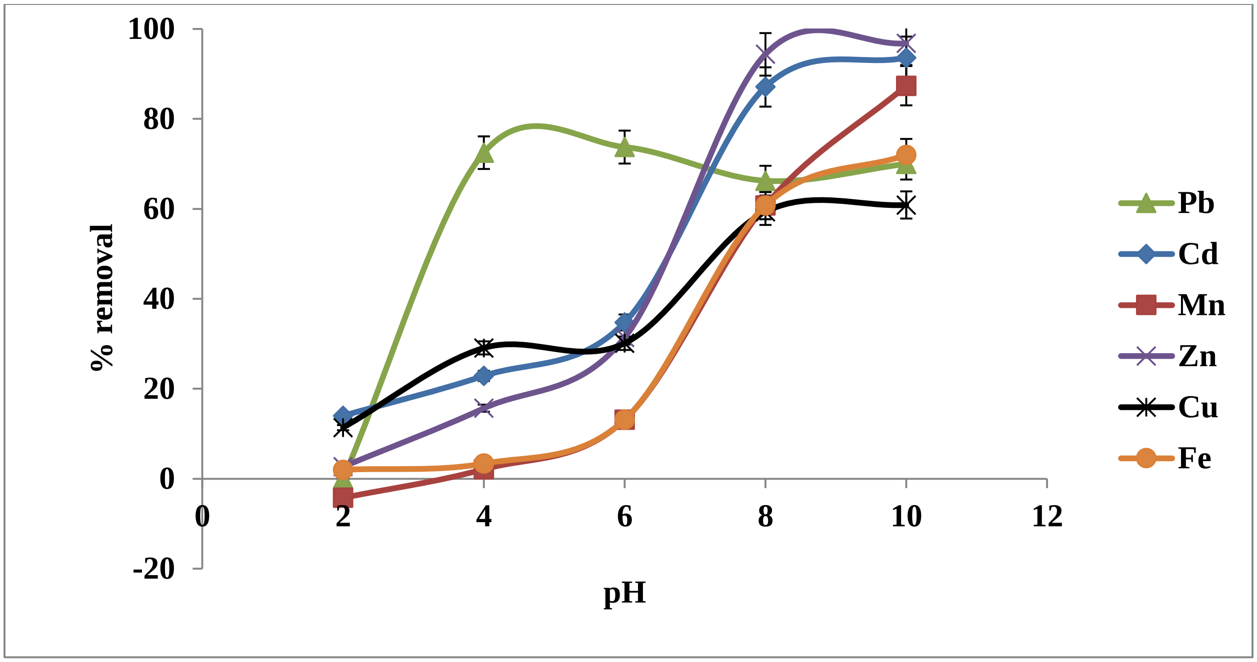

Figure 4.12: Effect of pH on metal removal using morula nut shells (MNS) while keeping other optimized condition constant

4.2.4 Effect of initial metal concentration

The initial concentration provides essential driving force to overcome all mass transfer resistance of metal ions between the aqeuous and the solid sorbent (Malkoc and Nuhoglu 2005). As the initial metal concentration is increased, the removal efficiency is increased and after saturation, the percentage removal is constant as shown in Figure 4.13 while using MNS. This could also be attributed to the saturation of the binding sites as more ions are added in the solution. As the concentration of metal ion increases, the number of metal ions competing for available binding sites on the sorbent increases. Therefore, binding sites become quickly saturated as the biomass amount remained constant (Bhatti *et al*., 2007; R. Nadeem *et al*., 2008). The same was observed using MSP, at lower concentration there was an increase in removal effciency, however after a threshold (different for each metal) any further increase in the metal concentration resulted in a reduced removal efficiency for Zn, Mn, Fe, Cu and Cd (See Figure 4.14). Removal of Mg by both sorbents was poor and these was attributed to limited loading capacity of the sorbents due to saturation of magnesium binding sites. This is because the concentration of magnesium ions in the sorbent was higher (see Section 4.1.1) in comparison to the other metals. Therefore increase in initial concentration led to a decrease in percentage removal of magnesium.

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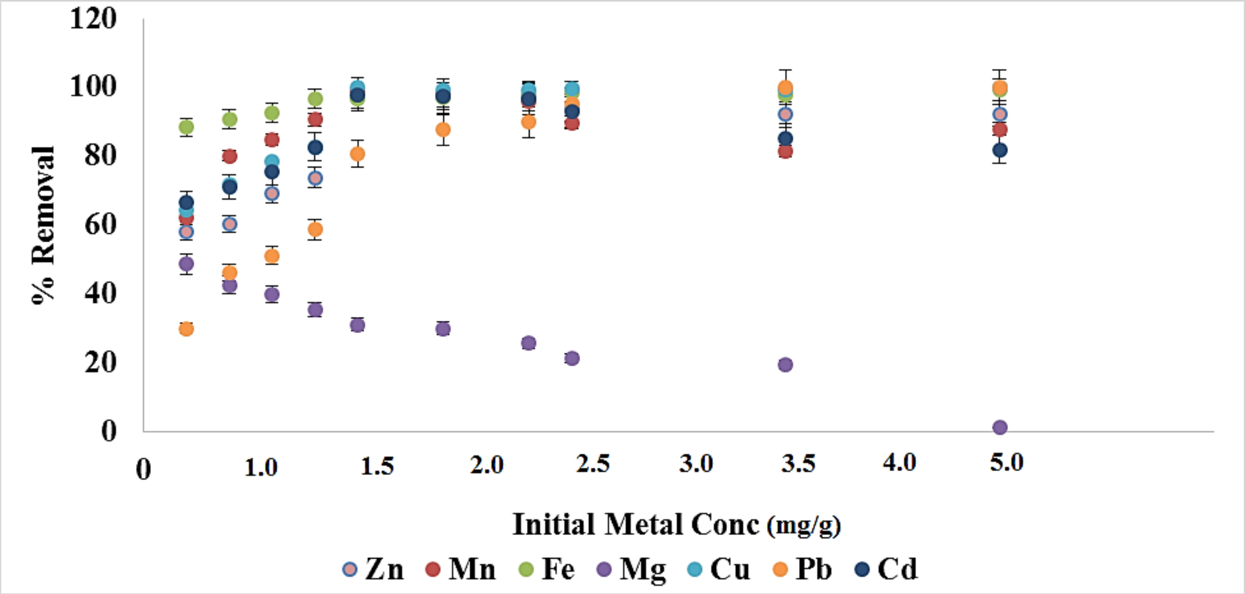

Figure 4.13: Effect of initial metal concentration on metal removal by MNS. (Other optimized conditions kept constant)

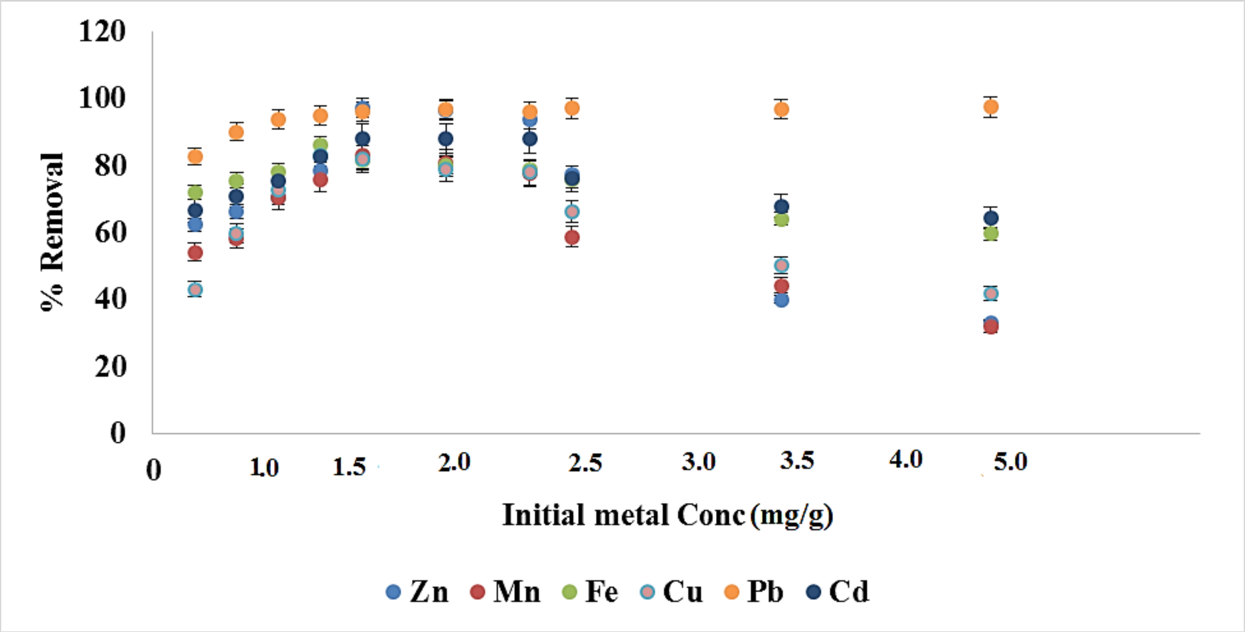

Figure 4.14: Effect of initial metal concentration on metal removal by MSP. (Other optimized conditions kept constant)

4.2.5 Effect of Particle size

Surface area of the sorbent is significant for sorption process. Exposure of the sorbent sites for solid-metal ion interaction is high if the surface area of the sorbent is high. As shown in the Figure 4.15 and Figure 4.16 using MSP and MNS respectively, the percentage removal of the metal was effective at sorbent particles size less than 100 µm as compared to larger particles. The smaller the particle size the higher the surface area per unit weight of sorbent and hence higher percerntage removal is expected (Bhatti *et al*., 2007). Similar trends have been observed by other researchers(Shukla *et al*., 2002; Nadeem *et al*., 2008; Obuseng *et al*., 2012). An exception was the Magnesium that showed higher removal at 500 µm while using MNS (Figure 4.16) and this may be attributed to the fact that magnesium may prefer to adsorbed internally as opposed to external adsorption. Beside adsorption at the outer surface of the sorbent, there is a possibility of intraparticle diffussion from the outer surface into the internal surface of the sorbent (Shukla *et al*., 2002). A large particle size has larger internal surface area than a small one, and hence Mg showed higher removal at 500 µm than at 100 µm. However ANOVA (analysis of variance) significant test was conducted to show if there was any significant change and showed that using 100 µm had no significant difference(see appendix A1 and A3) resulting to using 100 µm to be the optimum particle size using both sorbents. It was also observed that while using MSP, there was no removal of magnesium due to the fact that MSP had higher amounts of magnesium (see Section 4.1.1) and therefore the adsorbing sites for magnesium were saturated.

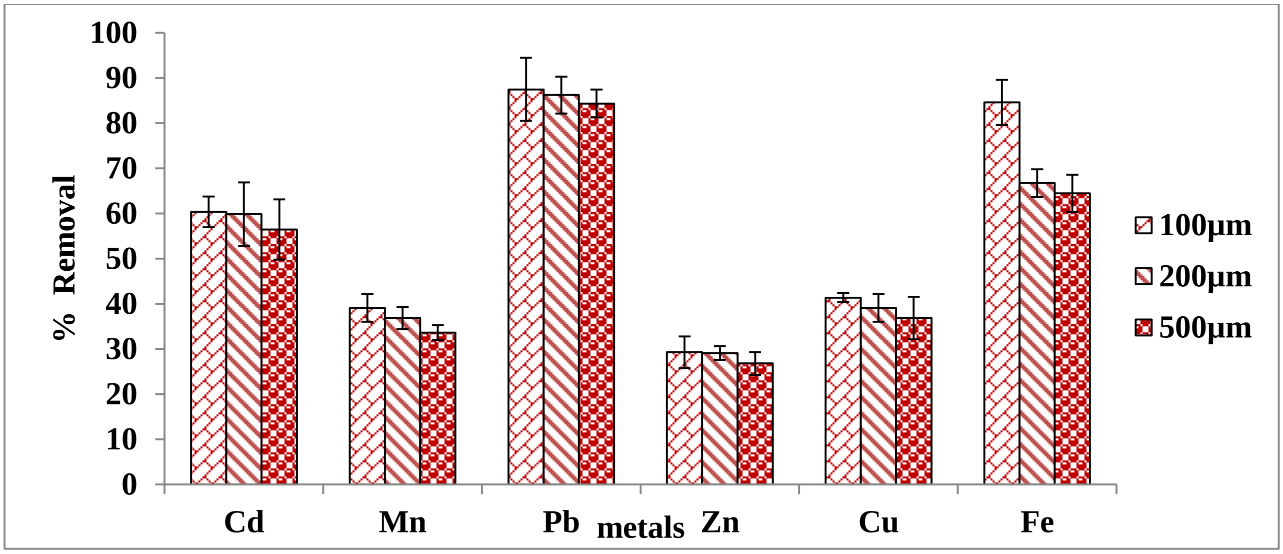

Figure 4.15: Effect of particle size on metal removal using MSP (Other optimized conditions kept constant)

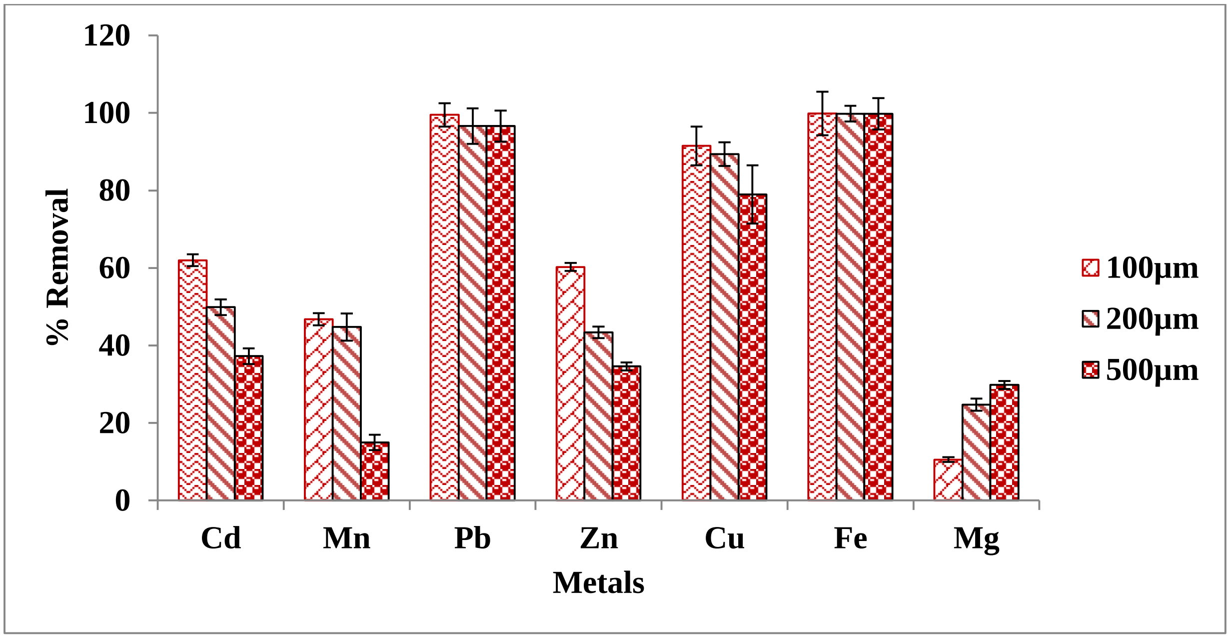

Figure 4.16: Effect of particle size on metal removal using MNS (Other optimized conditions kept constant)

The ionic sizes trend of the metals is Pb>Cd>Zn>Cu>Mg>Fe>Mn for divalent cations. The larger ions are expected to be removed first and the order of removal should decrease from Pb to Mn, but in some case it will be different depending on the environment of the interacting sites. This trend was not observed completely and this could be attributed to the fact that the metals were interacting differently with the MNS and MSP adsorbing sites.

4.2.6 Effect of Temperature

An increase of removal efficiency with increase in temperature has been attributed to two factors. Increase in temperature increases the mobility of the metal ions towards the sorbent and also it may cause a swelling effect within the internal structure of the sorbents and therefore enabling the metal ions to penetrate further or increases the surface area for the binding of the metal ions (Asku 2002; Malkoc and Nuhoglu 2005; Nadeem *et al*., 2006)

Temperature effect on metal removal by the sorbents was investigated using batch experiments conducted in water bath with temperature range from 25°C to 60°C. There was no significant change in percentage removal for other metals except for lead where there was increase at 35°C as depicted in figure 4.17 when using MSP. It was also observed that there was no significant change (see appendix A) when higher temperatures up to 60°C were used, hence 35°C was used as optimum temperature. Araújo *et al*., 2013 discussed that use of Moringa seed pods for removal of Zn and Pb worked well at a temperature of 30°C to 35°C. Figure 4.18 shows an increase in percentage removal from 25°C up to 35°C for removal of Zn, Cd, Mn and Mg using MNS. For removal of Fe, Cu, and Pb was not significantly different when temperature was altered (see appendix A6 and A7) and hence 35°C was therefore taken to be optimum extraction temperature.

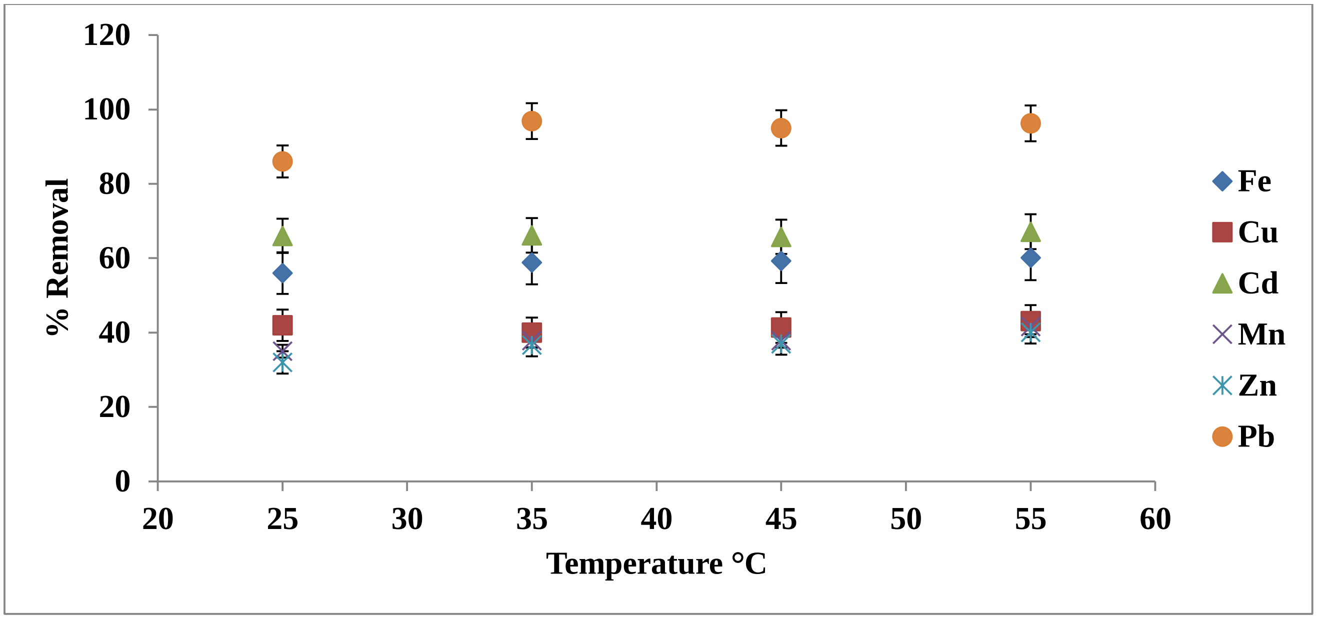

Figure 4.17: Effect of Temperature on metal removal using MSP (Other optimized conditions kept constant)

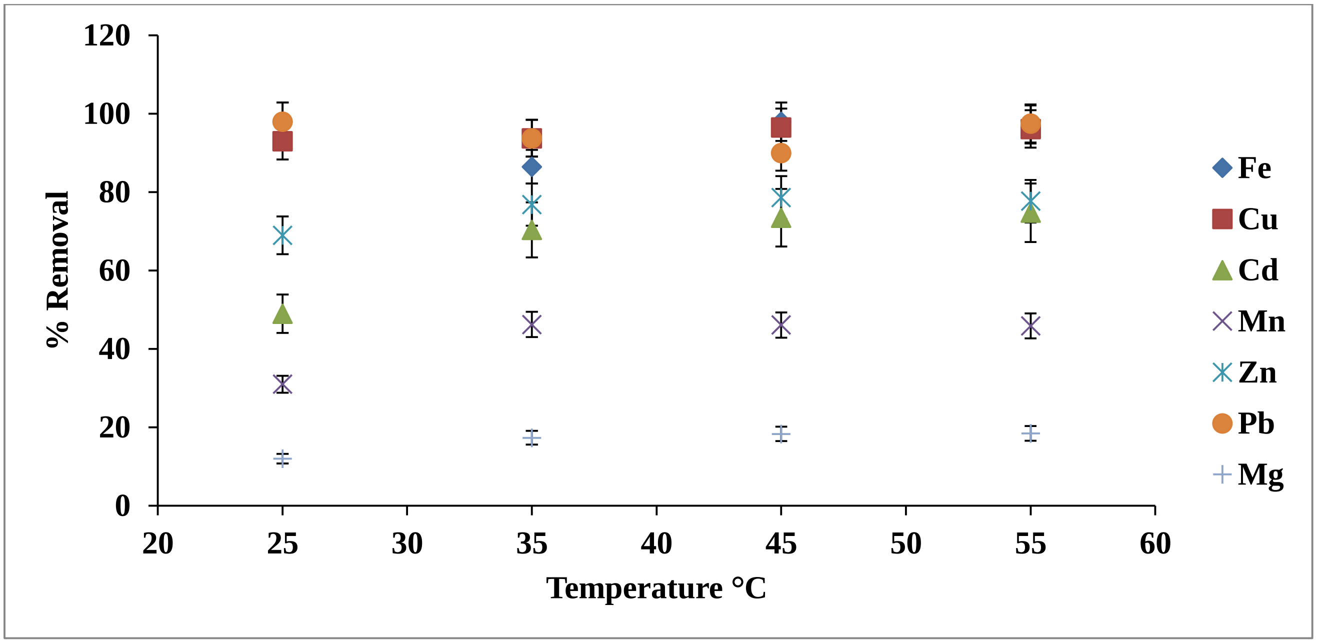

Figure 4.18: Effect of Temperature on metal removal using MNS (Other optimized conditions kept constant)

4.3 Performance of developed study

After optimization (see Table 4.8), the developed method was validated by studying linearity, recoveries and detection limits.

4.3.1 Calibration studies

The Table 4.4 below shows the regression equations and correlation coefficients of metal using FAAS. The linearity was determined from the matrix matched standards spiked at different concentrations, ranging from 0 to 5mgL^{-1}. The calibration curves were obtained from plotting ratio of peak areas of individual metal standards against concentration of the individual standard using Microsoft excel software (see appendix 2). All the metal showed a good linearity with the correlation coefficients greater than 0.99. These observations are in agreement with other work when extracting heavy metals from different matrices e.g wastewater (Balarama Krishna *et al*., 2005; Ghaedi *et al*. 2009) and sludge (Jamali *et al*., 2009).

Table 4.4: Linearity data for selected metals using FAAS

Metal	Regression equation	R^2
Mg	y= 0.241x + 0.0189	0.9970
Fe	y=0.0424x + 0.0027	0.9997
Cu	y= 0.1053x + 0.0059	0.9995
Cd	y= 0.1545x + 0.0388	0.9923
Pb	0.0241x + 0.0013	0.9996
Zn	y=0.1234x +0.0334	0.9922
Mn	y= 0.086x + 0.0092	0.9982

4.3.2 Removal efficiency of the sorbent

Table 4.5 shows the average metal removal (%) of the seven selected metals. The method show efficient removal from the range of 86.49 ± 4.33 to 99.63 ± 1.84%. The limits were within allowable limit 70-130% set by EPA method 40. Other studies have also shown better efficiencies in removal of Fe, Cu, Ni, Co, Cr and Zn from environmental samples was between 96.0 ± 1.4% to 106 ± 1.1%. (Ghaedi *et al*., 2007).

Table 4.5: Percentage removal of the selected metals

Metal	Removal (%) ± RSD (MSP)	Removal (%) ± RSD (MNS)
Pb	92.54 ± 1.85	89.01 ± 3.59
Cu	89.86 ± 2.01	90.33 ± 2.11
Cd	99.63 ± 3.36	93.61 ± 1.97
Fe	92.14 ± 1.84	87.06 ± 2.07
Zn	95.95 ± 1.92	91.66 ± 1.37
Mg	86.49 ± 4.33	88.04 ± 1.49
Mn	94.11 ± 1.88	95.07 ± 3.89

4.3.3 Detection Limits

The limit of detection (LODs) and Limit of quantification (LOQs) were investigated as per section 3.61. The limit of detection and quantification of the selected metals using FAAS is shown on Table 4.6

Table 4.6: Limit of detection and quantification of selected metals.

Metal	**LOD(mgL^{-1})**	**LOQ(mgL^{-1})**
Pb	0.031 ± 0.010	0.098 ± 0.01
Cu	0.010 ± 0.003	0.030 ± 0.003
Cd	0.015 ± 0.005	0.048 ± 0.005
Fe	0.036 ± 0.010	0.114 ± 0.010
Zn	0.015 ± 0.005	0.048 ± 0.005
Mg	0.067 ± 0.020	0.223 ± 0.020
Mn	0.011 ± 0.003	0.035 ± 0.003

The LODs were calculated as three times the standard deviation of 7 replicates runs of blank plant samples spiked with low concentration of analytes. For calculating the LOQs, the standard deviations were multiplied by ten. The detection limits were lower than 0.1 mgL^{-1}. Detection limits may vary depending on several factors such as; type of the matrix, instrument type and condition. Similar results were observed in different studies, detection limits for removal of Cu, Ni, Co and Pb were recorded to be ranging from 0.08 to 0.26 mgL^{-1} (Duran *et al.*, 2007). However other studies have shown lower detection limits and others higher detection limits(Balarama Krishna *et al.*, 2005)

4.4 Application of optimized conditions to real samples

4.4.1 Removal of metal ions from aqueous solution using optimized conditions

After determining the optimum parameters as shown in Table 4.7, the parameters were applied in a 50 mL aqeous solution in similar procedure as section 3.5.3. The percentage removal of metal ions was as shown in the Table 4.8

Table 4.7: The optimised extraction parameters using 20 ppm standard mixture solution.

MATERIAL	**CONTACT TIME (min)**	**PH**	**TEMP (°C)**	**ADSORBENT DOSE (g)**	**PARTICLE SIZE (µm)**
Morula nut shells	120	8	35	2	100
Moringa seed pods	60	8	35	1	100

Morula nutshell sorbent showed a higher metal removal compared to Moringa seed pods as shown in Table 4.8.

Table 4.8: Percentage removal of metal ions using MNS and MSP

Average (%) removal of metal ions							
	Fe	**Cu**	**Cd**	**Mn**	**Zn**	**Pb**	**Mg**
Moringa seed pods	70.5	45.3	66.0	32.6	34.4	92.5	-
Morula nut shells	99.0	98.7	72.5	48.9	65.9	99.6	21.9

Higher percentage removal using morula nut shells could be attributed to a number of factors like (i) possibility of more binding sites on the surface of morula nutshells as compared to Moringa seed pods; (ii) there was more interaction of the metal to the functional groups in Morula nutshells (See Table 4.2) as compared to those of Moringa seed pods (Table 4.1) and (iii) some functional groups present in Morula nutshells, were not available in Moringa seed pods for example C-N aliphatic amines (see Figure 4.5 and 4.6). Hence, Morula nut shell performed as a better sorbent. Similar trend was observed during optimization of the parameters affecting sorption process (see sections 3.2.1 to 3.2.6). Morula nut shells showed higher percentage removal as compared to Moringa seed pods during optimization process.

Magnesium desorbed from the MSP sorbent into the solution, therefore it was not possible to remove magnesium when using the Moringa seed pods biomass. However, a 21% removal was recorded when MNS were used. This could be attributed to higher initial concentration of magnesium in the sorbents in comparison to other selected metals (see Figure 4.2).

4.5 Determination of metal concentration in real water samples

The main water source for local people in Botswana is ground waters from local boreholes but most of the boreholes have low capacity (The World bank, 2009). The supply does not meet the demands for irrigation. Around the Glen Valley Wastewater treatment plant, irrigation schemes using the wastewater from the plant have been set up (Nkegbe, 2005). Due to these factors, borehole and wastewater samples were ideal to be used as the real water samples in this work. Five sampling sites from wastewater treatment plant were considered while analyzing for heavy metals in the wastewater samples. The samples were collected from inlet point, primary settling tank, secondary settling tank, aeration tank and effluent storage tank (Section 3.5.1). The initial total metal concentrations (mgL^{-1}) from the digested wastewater samples and borehole water samples were determined as shown in Table 7.9 (appendix 4).

The metal concentrations in all the sampling points in the wastewater treatment plant were found to be within the allowed levels which are set by waste water specification-BOBS 93:2012 in Botswana and US EPA (United States Environmental Agency, 1992), except for Mn at effluent point that were higher than the allowed limits of 0.1mgL^{-1} by BOBs and 0.2mgL^{-1} by the EPA,1992.

Figure 4.19, shows the trend of the concentration of the selected metals from the inlet point to the effluent point. It was noted that in the treatment plant, the concentration decreased from inlet point until the secondary tank, then increased at the aeration tank then decreased at the effluent tank. A decrease in concentration of metal from inlet point up to secondary tank could be due to the treatment processes at the plant, where the metal ions are removed from the water through adhesion of the ions on organic and inorganic waste that is screened and removed at the different stages. An increase at aeration tank could be due to continuous accumulation of the metal ions in tank over time. At aeration tank, air is pumped into the water to encourage bacteria to breakdown the tiny bits of sludge that escaped the sludge scrapping process. The breakdown of the sludge increases the particles and hence increasing the surface area onto which the metal ions can adsorb on. Therefore the waste water that is transferred from aeration to effluent tank has lower metal concentration as seen depicted by the trend below.

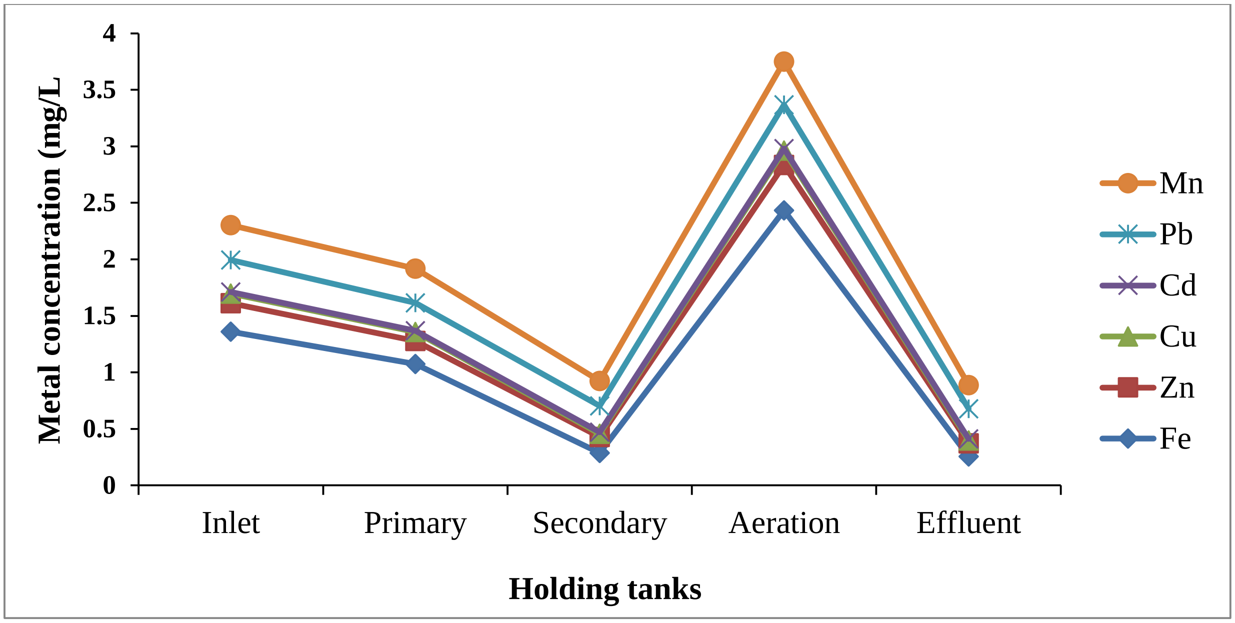

Figure 4.19: Concentration (mg/L) of metal in Sewage wastewater samples using FAAS

The results for the borehole water samples also showed that the water was not highly contaminated with the selected heavy metals, and therefore was safe for drinking according to WHO 1992 and BOBS 2009. Figure 4.20 shows the concentration of Mg and Fe to be higher compared to Pb. This metals are essential and expected to be abundant in borehole water since they are naturally occurring minerals. All other metals were below detection limit and therefore not detected as shown on Table 4.9

Table 4.9: Average concentration of metal ions in the borehole samples using FAAS

Average Conc (mg L^{-1}) of metal in borehole water and the standard deviations							
	Fe	Zn	Cu	Cd	Pb	Mn	Mg
	0.3440 ± 0.0036	Not detected	Not detected	Not detected	1.2877 ± 0.0321	Not detected	0.1133 ± 0.0079

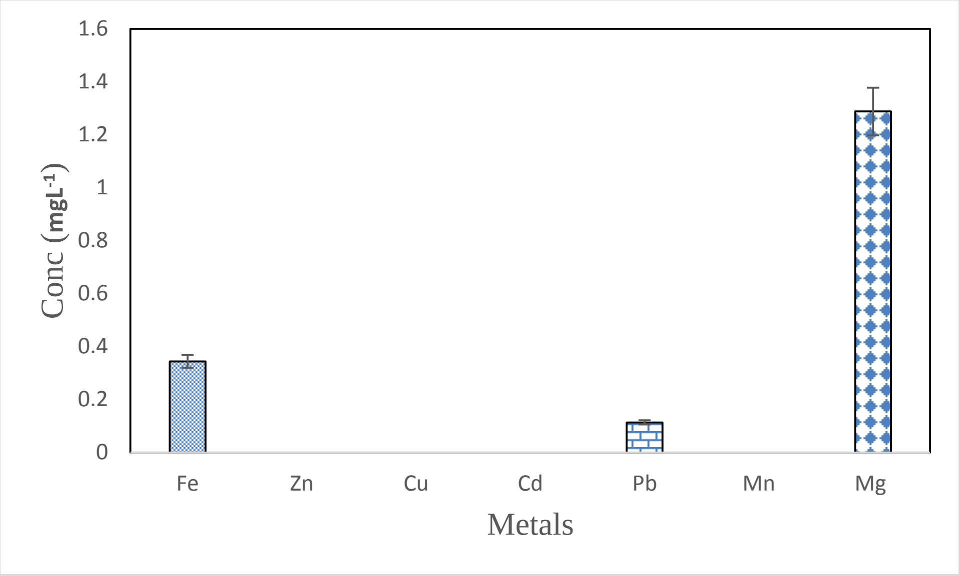

Figure 4.20: Average metal concentration (mgL^{-1}) in borehole water

4.5.1 Metal removal from wastewaters and borehole samples

The optimised sorption conditions for metal removal by MNS and MSP sorbents were applied to wastewaters and borehole water samples.

The percentage removal is as shown in Tables 4.10 and 4.11 when MNS and MSP were used respectively. MNS (Table 4.10) removal efficiency was relatively lower for Cd and Mg and this may be attributed to the possibly fewer binding sites due to presence of competing metal ions that were present in the wastewater beside the seven selected metals.

Table 4.10: Average percentage removal of selected metals from wastewater using MNS

Average percentage removal of metal ± %RSD							
	Mn	Cu	Zn	Fe	Pb	Mg	Cd
Inlet	64.7 ± 1.91	67.3 ± 5.33	58.1± 6.45	84.4± 2.30	67.4± 5.39	9.20 ± 1.74	31.2 ± 1.22

Primary	65.8 ± 8.37	62.8 ± 6.83	49.6 ± 4.90	80.3± 2.23	49.4± 2.04	13.4 ± 4.78	36.1 ± 5.07
Secondary	58.6 ± 6.69	61.8 ± 4.90	62.2 ± 4.55	67.6± 4.01	46.5± 4.17	7.60 ± 2.07	48.9 ± 5.46
Aeration	57.9 ± 4.24	77.1 ± 5.75	60.0 ± 2.63	87.8± 3.42	76.5± 3.16	10.9 ± 2.95	51.6 ± 5.75
Effluent	56.8 ± 2.96	60.4 ± 3.63	53.9 ± 5.65	64.6± 5.59	56.6 ± 4.77	8.00 ± 2.18	44.1 ± 5.44

Removal of Zn and Mg was impossible. It was noted that Zn and Mg were desorbed from MSP into the wastewater samples. This could be attributed to saturation of binding sites for Mg and Zn in the sorbents. However all the other metals were effectively removed at effluent point and the percentage removal was ≥ 52% as shown in Table 4.11 below.

Table 4.11: Average percentage removal of selected metals using MSP

Average percentage removal of metal ± %RSD					
	Mn	Cu	Fe	Pb	Cd
Inlet	34.9±2.09	76.7 ± 2.96	73.5± 3.95	78.9± 3.16	53.8 ± 2.60
Primary	35.9 ± 3.19	64.2 ± 2.98	74.0 ±2.01	67.3± 4.32	51.1 ± 3.13
Secondary	54.1 ± 5.38	72.6 ± 3.03	79. 1± 1.33	69.1± 4.27	51.9± 2.14
Aeration	45.8 ± 2.14	78.3 ± 1.94	84.0 ±2.32	76.4± 2.91	54.4 ± 1.59
Effluent	52.0 ± 1.82	73.5 ± 3.43	68.7± 1.64	69.8± 2.80	62.7 ± 3.89

Percentage removal of the metal using both sorbents was lower in comparison to optimization steps as compared to using the real samples. This is due to matrix effect while using the real samples. During optimization step, aqueous solution used were prepared using standards of the seven selected metals while the real samples contain other metals and other organic compounds and therefore the selected metals were competing with other analytes for the same binding sites and consequently reducing the percentage removal. The method was precise since the calculated percentage relative standard deviation (RSD) is < 10% in both cases as seen in Table 4.10 and 4.11.

A comparison of the metal concentration in the borehole sample before and after removal using MSP (Figure 4.21) and MNS (Figure 4.22) was done. Percentage removal of Fe, Mg and Pb was 54.94 ± 2.52, 64.47 ± 2.32 and 100 ± 1.69 respectively when using MNS. Using MSP the removal was 29.94 ± 3.01 and 91.17 ± 3.44 for Fe and Pb respectively. The method was precise (% RSD <4%) for all metals. However, Zn and Mg desorbed into the water samples resulting into an increase in the final metal concentration while using MSP as shown in Figure 4.21. This could be attributed to the high concentration of the metal ions in sorbent as compared to the borehole samples.

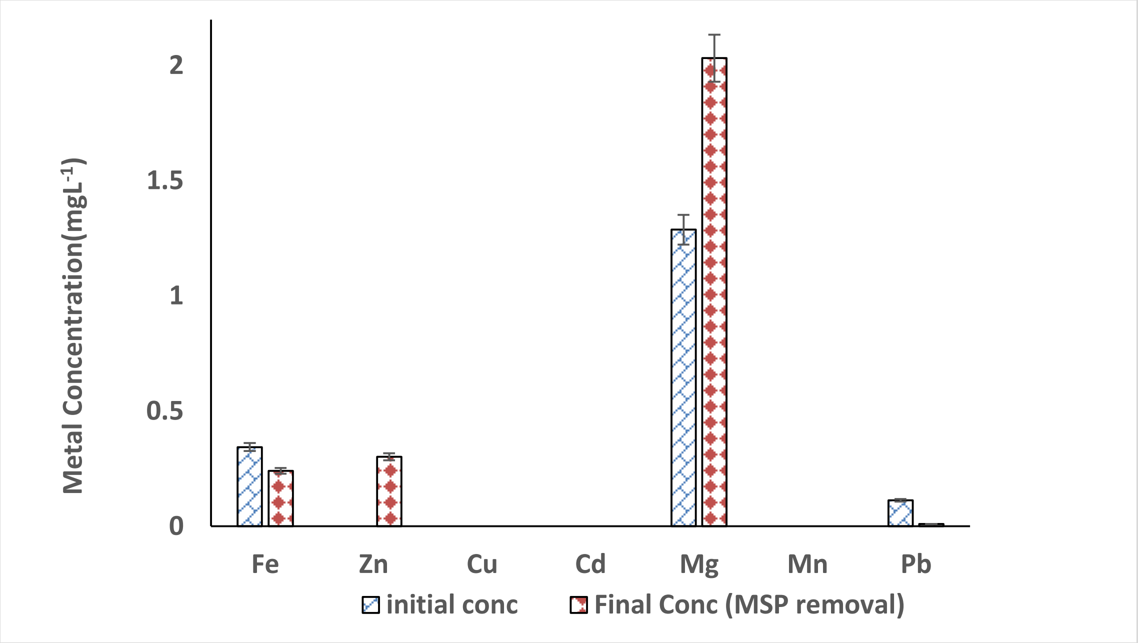

Figure 4.21: Metal removal from Borehole water sample using optimized sorption conditions with MSP

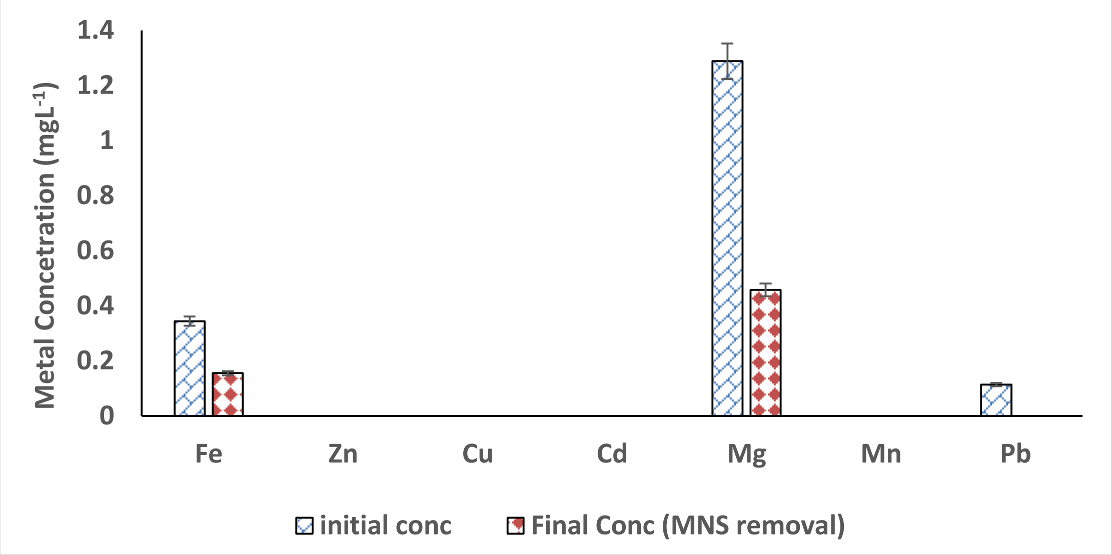

Figure 4.22: Metal removal from Borehole water sample using optimized sorption conditions with MNS

4.6 Use of treated MNS and MSP for metal removal from wastewater samples

Use of untreated plant biomass is capable of directly sorbing metal ionic species from aqueous solutions (Volesky and Holan 2003; Singh *et al*., 2006; Sarin & Pant 2006; Zwain *et al*., 2014) . This has also been confirmed in this work. To further enhance metal removal efficiency,sorbents can be chemically pre-treated using acid. Chemical treatments that are commonly employed are alkaline solutions, phosphoric and citric acids (Wayne E. Marshall and Johns 1996). This treatment normally removes organic and inorganic matter from the sorbent surface (G. Yan and Viraraghavan 2000). Treating the sorbents with acid helps to extract any metals cations on the sorbents, thereby creating new sorption sites and increasing the surface area of the sorbent and hence increasing the metal removal efficiency (Kurniawan *et al*., 2006a; Gupta and Babu 2009). Acid treated sorbents like maize bran(Singh *et al*., 2006), teawaste (Mahvi *et al*., 2005), rice husks(Singh *et al*., 2005) and Mucor rouxii (G. Yan and Viraraghavan 2000) have been reported to have high metal removal efficiency (>90%).

In this current work, acid (0.4M of HNO_3) and untreated MNS and MSP sorbents were compared in terms of extraction of heavy metals from the inlet and effluent wastewater samples. It was observed that treated sorbents showed better removal efficiency as compared to the untreated sorbents as shown by Figures 4.23 to 4.24 using MNS and MSP respectively.

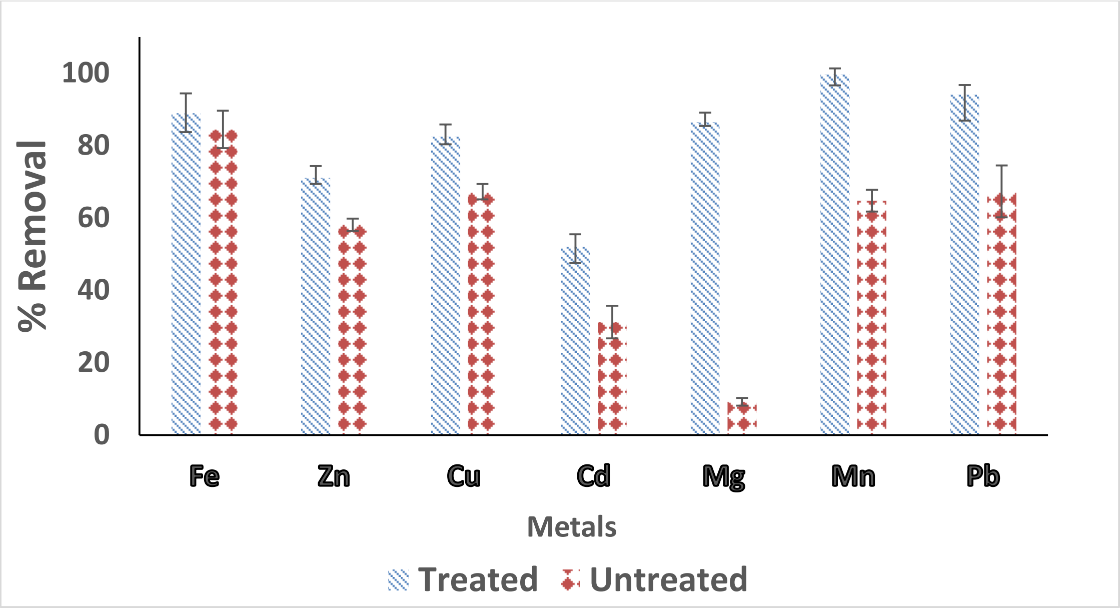

Figure 4.23: Comparison of treated and Untreated MNS for removal of metal from wastewaters at inlet point

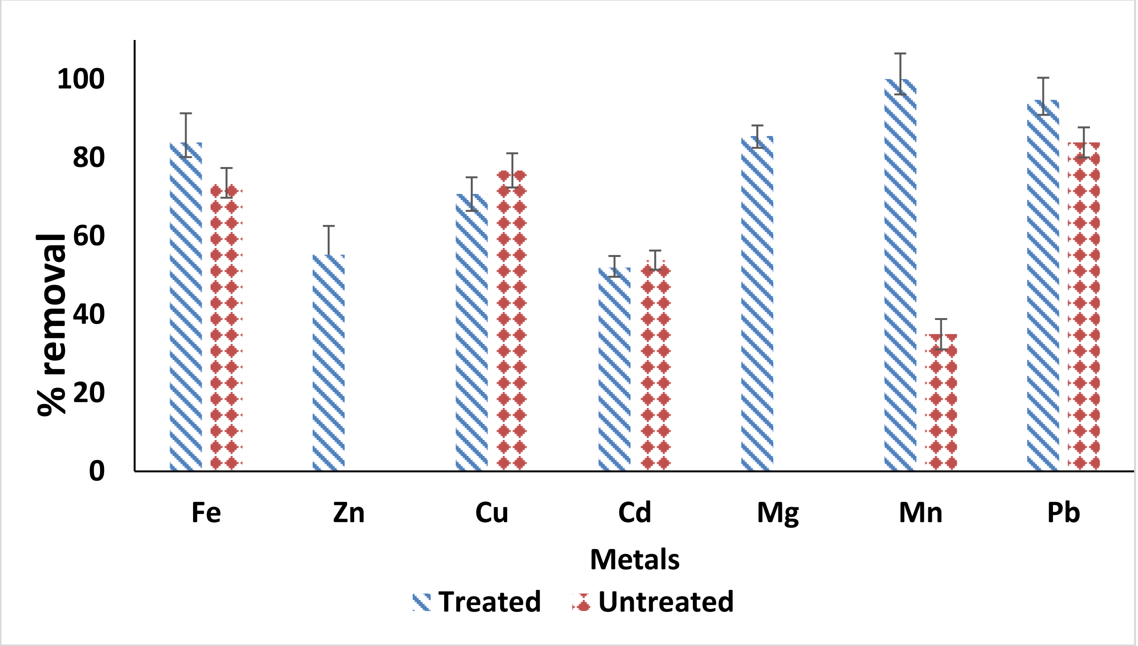

Figure 4.24: Comparison of treated and untreated MSP for removal of metal from wastewaters at inlet point

There was an increase in removal efficiency of all the metals. This increase was more notable for Zn and Mg ions which were found to be desorbing before the sorbents were treated but it was noticed that the removal increased up to 55.6% for Zn and 88.2% for Magnesium. This could be due to, more binding sites being available after treating the sorbents and hence more ions can bind to the many adsorption sites.

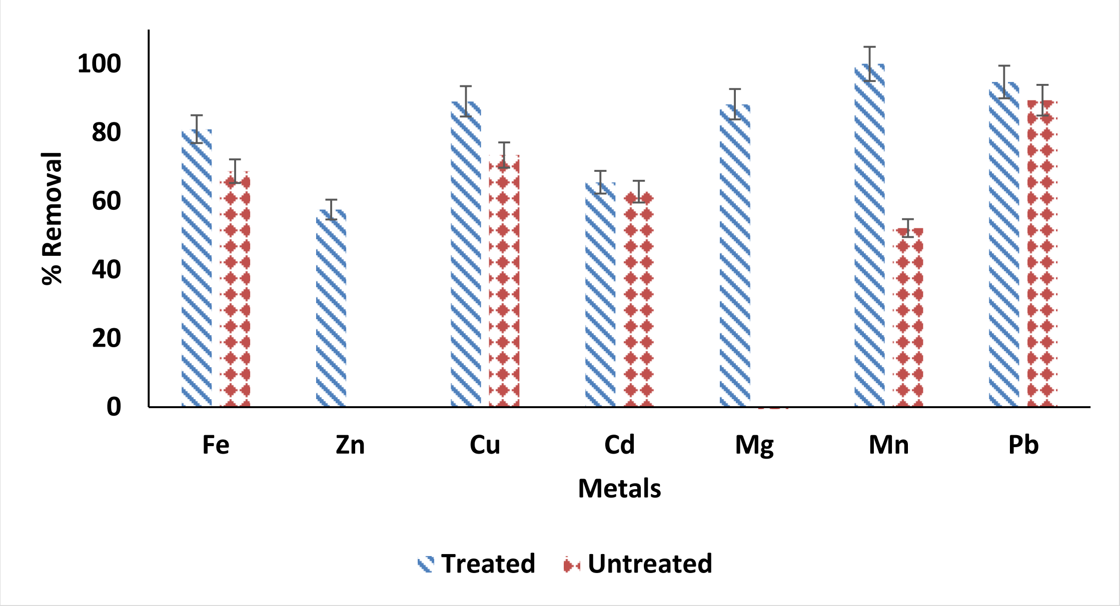

Figure 4.25: Comparison of treated and untreated MSP for removal of metal from wastewaters at effluent point

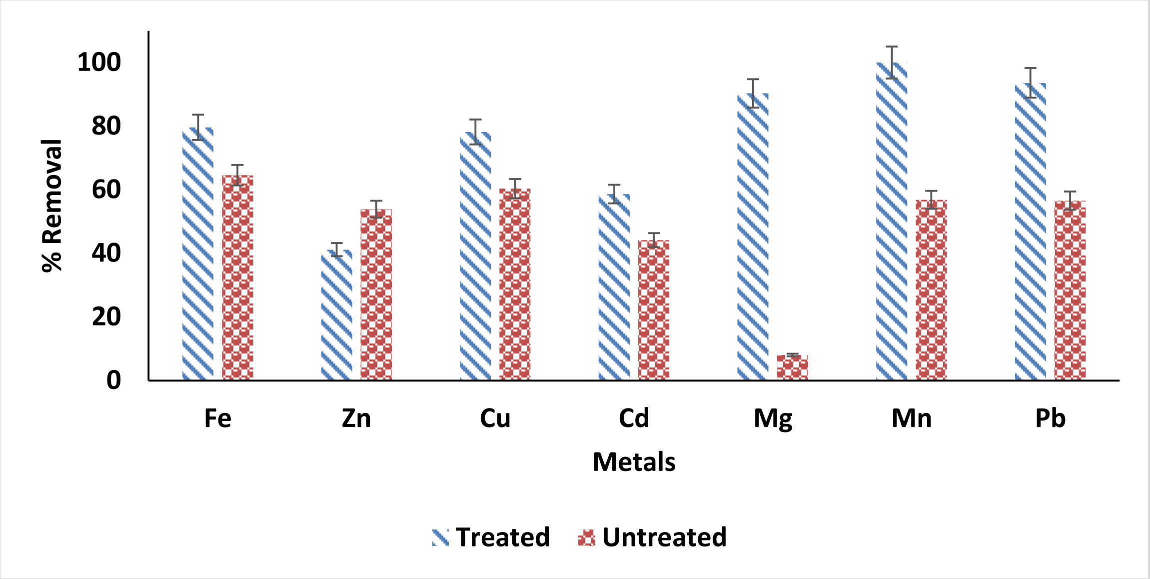

Figure 4.26: Comparison of treated and untreated MNS for removal of metal from wastewaters at effluent point

5.0 CONCLUSION

Use of nonedible plant parts such as Moringa seed pods and Morula nut shells were used to successfully remove selected heavy metals from water samples. Optimization of pH, initial metal concentration, sorbent dose, particle size and temperature helped to achieve desirable extraction efficiencies. To improve on efficiencies, both Moringa seed pods and Morula nutshells were acid treated with HNO_3 and the percentage removal was increased due to increase of surface area that ultimately increased the binding sites of the sorbents. The percentage removal of Fe, Zn, Cu, Cd, Mg, Mn and Pb from water samples using treated MNS was 79.6, 52.9, 78.2, 58.6, 90.3, 100.0 and 93.4 while using treated MSP was 80.9, 57.6, 89.0, 65.5, 88.2, 100.0 and 94.7 respectively. Moringa seed pods was a better sorbent in comparison to morula nut shells since the percentage removal of all metals was higher while using Moringa seed pods except magnesium that was relatively higher when using morula nut shells.

The developed method was found to be simple, cheap, environmental friendly and does not need trained personnel to use it. It emphasized on importance of recycling all unwanted and useless materials such agricultural waste for purification of water. It also introduced possibilities of recycling and reuse of black water for example from industries, wastewater treatment plants etc. in order to curb the problem of water scarcity. The developed method can be used in rural areas where there are no resources to obtain the conventional expensive techniques.

In order to determine the functional groups that are present in the sorbents and that could be responsible or participating in removal of metal ions, FTIR measurements were done. Removal of the selected metals from the water samples to the sorbents could be through adsorption, ion exchange or through precipitation where the sorbent would act like a filter. SEM-EDX was used to determine the morphology and to also give an elemental analysis of the sorbents. The surface of the sorbent had pores of different sizes and had higher surface area that increased their efficiency in metal removal. Metal ions such as K, Ca and Mg were found to be in higher concentration in Moringa seed pods and therefore the pods can be a source of these essential elements. Morula nut shells had relatively high concentration of Na, K, Mg and. Other metals such as Zn, Cu, Fe, Pb and Mn were in trace levels. All selected metals were within the set limits by WHO and FAO.

Wastewater and borehole samples were used as the real samples in this study. The use of Moringa seed pods and morula nutshells helped in removal of heavy metals to allowable limits set by US EPA and thus helping in making the contaminated water recyclable for agricultural and human consumption. Method detection limits, linearity and efficiency were also validated. The method was found to have low limits of detection ranging from 0.010 ± 0.003 to 0.067±0.020 mgL^{-1}, good linearity ($R^2>0.99$) and the removal efficiencies were in the range of 86.49 ± 4.33 to 99.63 ± 3.36%.

5.1 Recommendations

1. Moringa seed pods and Morula nut shells are in large quantities in most parts of Botswana. The author recommends use of these plant material for removal of heavy metals especially in rural areas where it may be difficult to acquire other expensive techniques.
2. Regeneration studies should be done to establish the possibilities of recycling the used sorbents and determine how efficient they can be after several cycles.
3. Multivariate optimization may be used to study the effect of interaction of the optimized parameters and if the interaction can increase the removal efficiency.
4. Further studies can be done to establish the possibilities of using the developed method on solid samples such as contaminated soil.

5. As much as these plant material are readily available and are efficient, research need to be done to compare the efficiency of these two sorbents with other biosorbents.

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7.0 APPENDICES:

Appendix 1: Analysis of variance (ANOVA)

Table 7.1: ANOVA of Mn using different Particle size of MSP

Anova: Single Factor		Mn	MSP			
SUMMARY						
Groups	Count	Sum	Average	Variance		
100	3	126.48	42.16	9.318675		
200	3	111.84	37.28	5.847075		
500	3	109.23	36.41	7.203675		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	57.6338	2	28.8169	3.864681	0.083465	5.143253
Within Groups	44.73885	6	7.456475			
Total	102.3727	8				
	F calc < F crit, therefore no significant difference					

Table 7.2: ANOVA of Cd using different Particle size of MSP

Anova: Single Factor		Cd	MSP			
SUMMARY						
Groups	Count	Sum	Average	Variance		
100	3	192.375	64.125	11.54688		
200	3	185.65	61.88333	4.050208		
500	3	183.6	61.2	20.77		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	14.04764	2	7.023819	0.57941	0.588748	5.143253
Within Groups	72.73417	6	12.12236			
Total	86.78181	8				
	F calc < F crit, therefore no significant difference					

Table 7.3: ANOVA of Mg using different Particle size of MNS

Anova: Single Factor		Mg				
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
100µm	4	34.28099	8.570248	28.59313		
200µm	4	75.72916	18.93229	150.0457		
500µm	4	87.3879	21.84697	192.9567		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	389.5184	2	194.7592	1.572348	0.259628	4.256495
Within Groups	1114.787	9	123.8652			
Total	1504.305	11				
	F calc < F crit, therefore no significant difference					

Table 7.4*: ANOVA of different sorbent dose using MNS*

Anova: Single Factor		Mg MNS				
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
1.5 g	3	65.6875	21.89583	0.880208		
2 g	3	56.175	18.725	4.27		
2.5 g	3	40.2	13.4	58.48172		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	110.589	2	55.2945	2.606922	0.153176	5.143253
Within Groups	127.2639	6	21.21064			
Total	237.8528	8				

Table 7.5*: ANOVA of different sorbent dose using MPS*

Anova: Single Factor		MSP Pb				
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
0.5g	3	276.75	92.25	1		
1 g	3	282.25	94.08333	2.270833		
1.5 g	3	282	94	1		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.430556	2	3.215278	2.258537	0.185682	5.143253
Within Groups	8.541667	6	1.423611			
Total	14.97222	8				

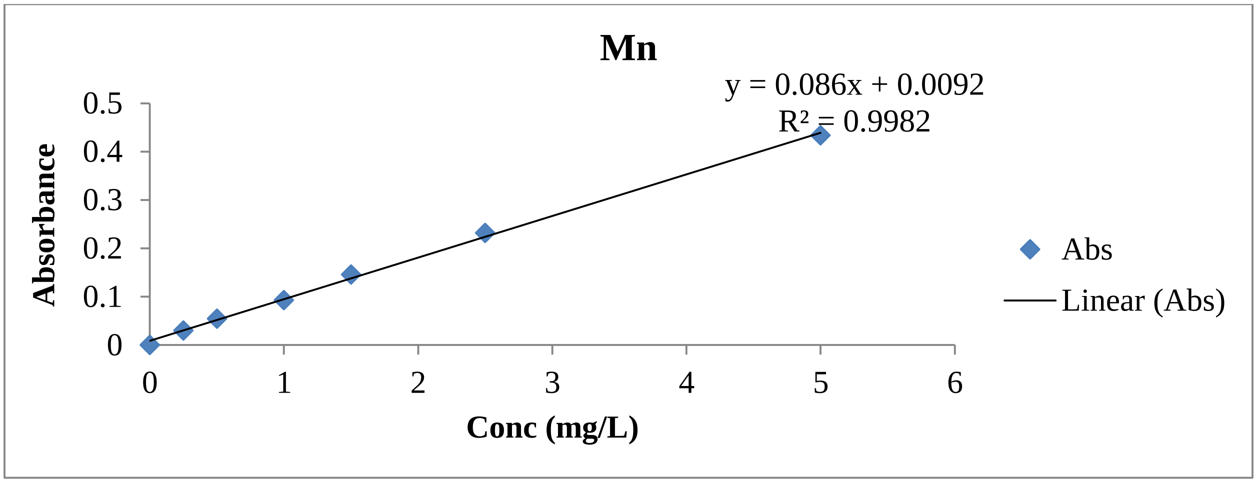
Table 7.6*: ANOVA 0f temperature using MPS*

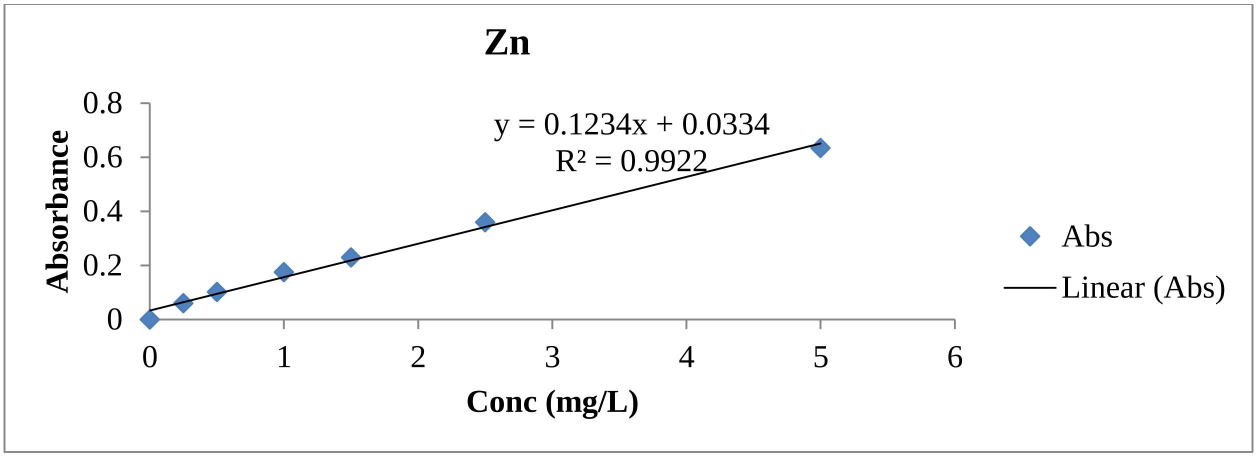
Anova: Single Factor		Cu MSP				
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
25	3	123.875	41.29167	0.380208		
35	3	122.25	40.75	4.9375		
45	3	121.875	40.625	0.4375		
60	3	127.125	42.375	0.4375		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.722656	3	1.907552	1.232128	0.359883	4.066181
Within Groups	12.38542	8	1.548177			
Total	18.10807	11				

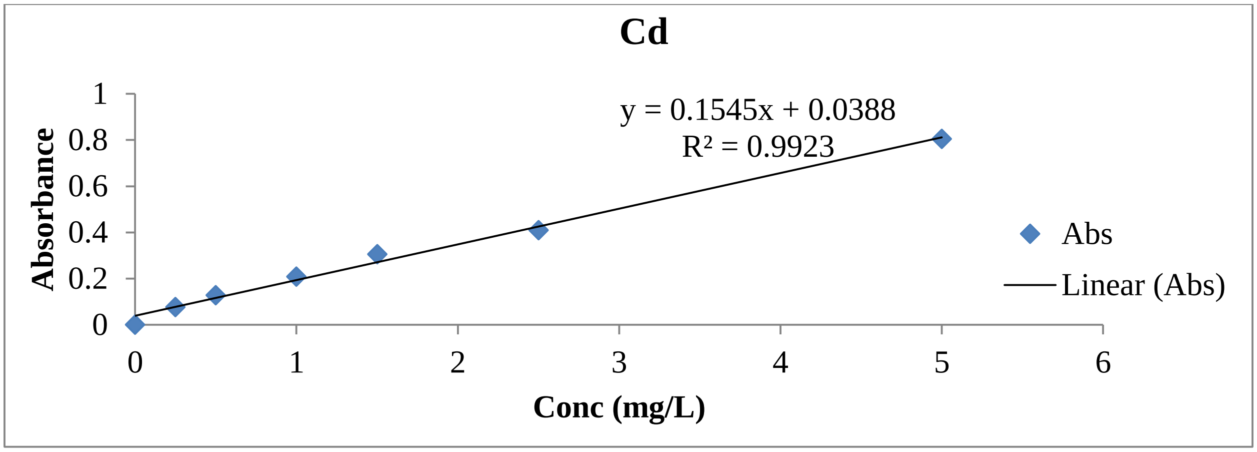
Table 7.7*: ANOVA 0f temperature using MNS*

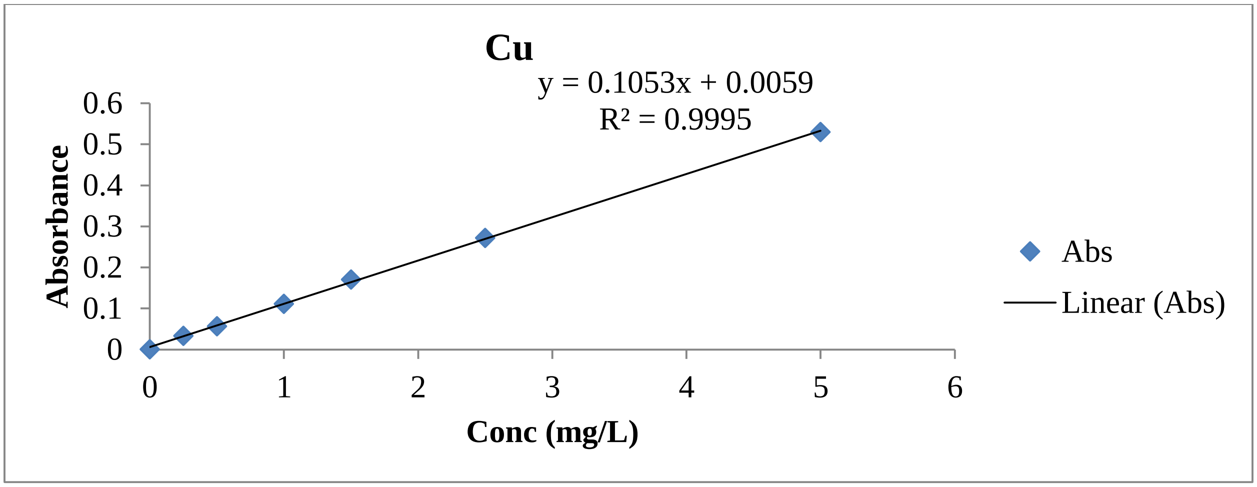
Anova: Single Factor		Mn MNS				
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
35	3	138.625	46.20833	0.098958		
45	3	137.3875	45.79583	0.634427		
55	3	135.125	45.04167	2.770833		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.100035	2	1.050017	0.898931	0.455541	5.143253
Within Groups	7.008438	6	1.168073			
Total	9.108472	8				

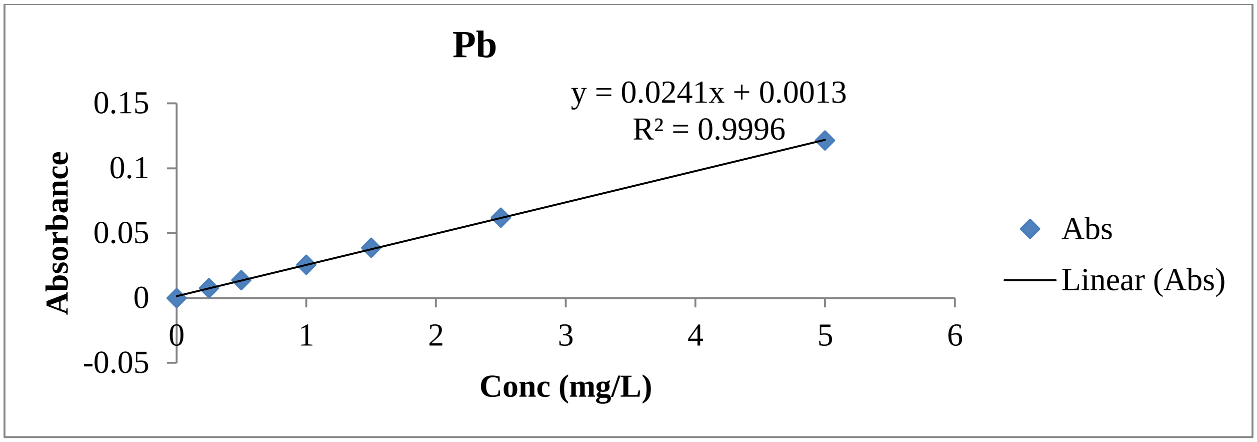
Appendix 2: Calibration curves of the selected metals

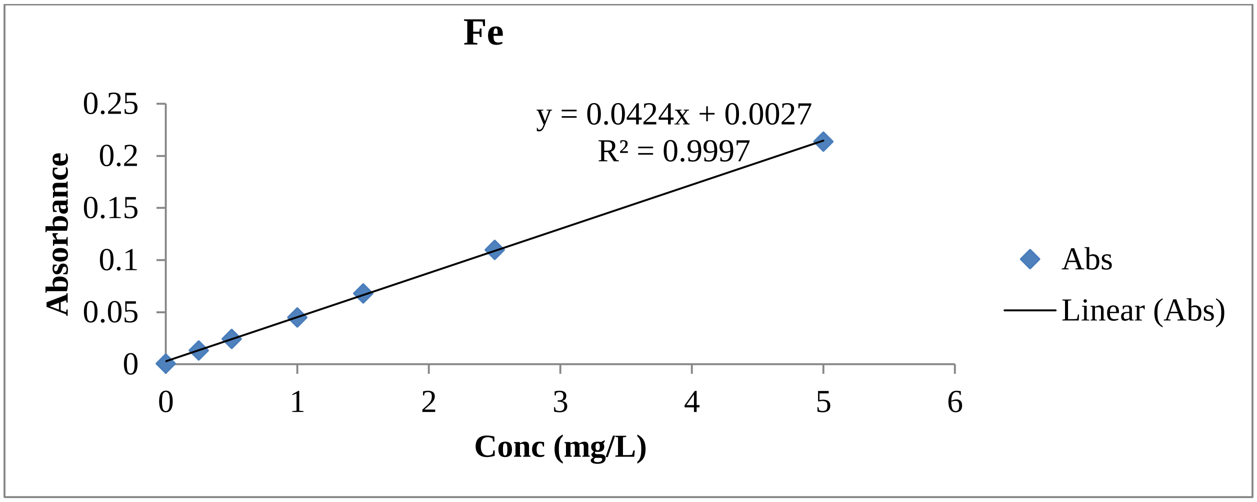


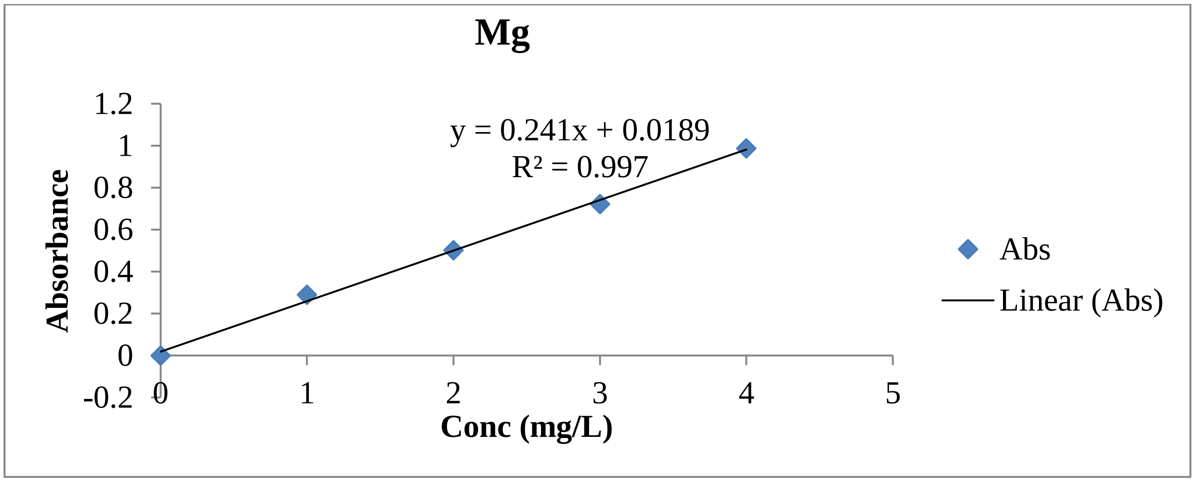

Zn
y = 0.1234x + 0.0334
R² = 0.9922
Absorbance
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
Conc (mg/L)
◆ Abs
— Linear (Abs)


Cd
y = 0.1545x + 0.0388
R² = 0.9923
Absorbance
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
Conc (mg/L)
◆ Abs
— Linear (Abs)


Cu
y = 0.1053x + 0.0059
R² = 0.9995
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
Conc (mg/L)
◆ Abs
— Linear (Abs)


Pb
y = 0.0241x + 0.0013
R² = 0.9996
Absorbance
0.15
0.1
0.05
0
-0.05
0
1
2
3
4
5
6
Conc (mg/L)
Abs
Linear (Abs)


Fe
y = 0.0424x + 0.0027
R² = 0.9997
Absorbance
0.25
0.2
0.15
0.1
0.05
0
0
1
2
3
4
5
6
Conc (mg/L)
Abs
Linear (Abs)


Mg
y = 0.241x + 0.0189
R² = 0.997
Absorbance
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
1
2
3
4
5
Conc (mg/L)
Abs
Linear (Abs)

Appendix 3: EDAX spectrum analysis

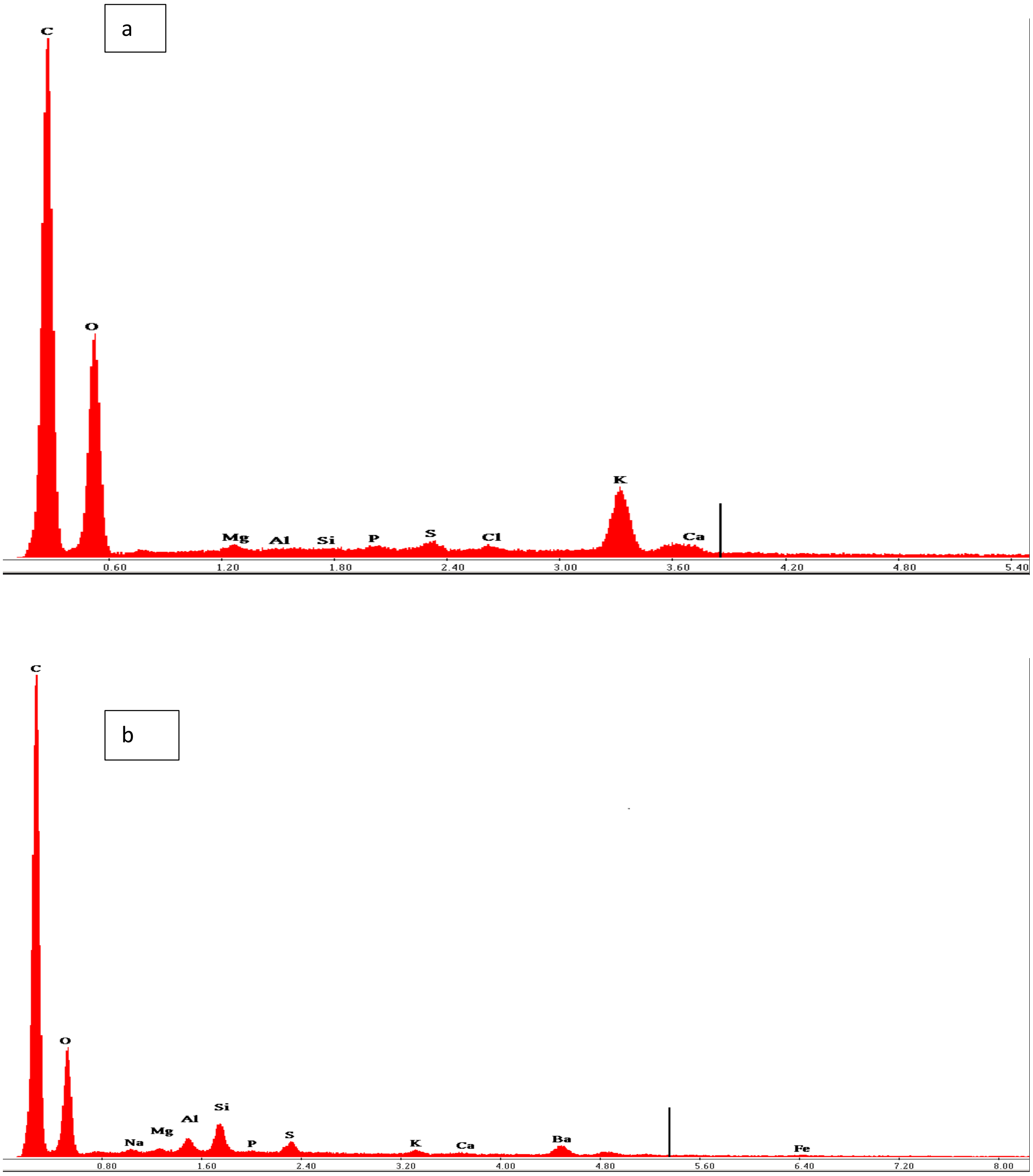


Figure 7.1: EDAX spectrum analysis of a) Moringa seed pods and b) Morula nutshell

Table 7.8: EDAX Elemental composition in Moringa see pods (MSP) and morula nut shells (MNS)

MSP		**MNS**	
Element	**Wt %**	**Element**	**Wt %**
C K	62.52	C K	73.06
O K	31.98	O K	20.69
MgK	0.27	NaK	0.22
AlK	0.1	MgK	0.2
SiK	0.1	AlK	0.63
P K	0.25	SiK	1.3
S K	0.41	P K	0.08
ClK	0.28	S K	0.52
K K	3.61	K K	0.23
CaK	0.48	CaK	0.09
Total	100	BaL	2.78
		FeK	0.19
		Total	100

Appendix 4:

Table 7.9: Average concentration of metals in wastewater samples

Average concentration of selected metals in water samples in mg/L and ± standard deviations.							
	Fe	**Zn**	**Cu**	**Cd**	**Pb**	**Mn**	**Mg**
Inlet	1.3610 ± 0.0202	0.2511 ± 0.0103	0.0855 ± 0.0049	0.0125 ± 0.0007	0.2850 ± 0.0212	0.3090 ± 0.0006	12.8667 ± 0.0093
Primary	1.0735 ± 0.023	0.2055 ± 0.0122	0.078 ± 0.0014	0.013 ± 0.0014	0.245 ± 0.0071	0.3040 ± 0.0006	11.5957 ± 0.0025
Secondary	0.2875 ±0.046	0.1419 ± 0.0632	0.0285 ± 0.0021	0.0147 ± 0.0012	0.23 ± 0.0424	0.2220 ± 0.0007	13.4873 ± 0.0021
Aeration	2.432 ± 0.0457	0.4045 ± 0.0866	0.1265 ± 0.0120	0.0155 ±0.0007	0.3900 ± 0.003	0.3800 ± 0.0007	13.4783 ± 0.0012

Effluent	0.2550 ± 0.0283	0.1158 ± 0.0183	0.0275 ± 0.0021	0.0145 ± 0.0007	0.2650 ± 0.0212	0.2110 ± 0.0054	13.6713 ± 0.0025