ADDIS ABABA UNIVERSITY

Addis Ababa Institute of Technology
School of Chemical and Bio Engineering

Investigation of Acid Treated Lemna minor as Adsorbent for Removal of Cu and Pb from aqueous solution

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As members of Examining Board of the Final M.Sc. thesis open defense, we certify that we have read and evaluated the thesis prepared by Yalembrhan Debebe, entitled "Investigation of acid treated lemna minor as bio adsorbent for Removal of Heavy Metals from aqueous solution" and recommended that it can be accepted as fulfilling the thesis requirements for the Degree of Master of Science in Chemical Engineering (Environmental Engineering).

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I, the undersigned, declare that this thesis entitled **“***Investigation of acid treated lemna minor as adsorbent for removal of* Pb^{+2} *and* Cu^{+2} *from aqueous solution”* is my original work, and has not been presented by any other person for an award of a degree in this or any other University, and that all resources of materials used for this thesis have been duly acknowledged.

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This thesis has been submitted to the University with my approval as the University Advisor.

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Table of Contents

List of Tables

List of figures

List of Acronyms

AAS	Atomic absorption spectrophotometer
APHA	American Public Health Association
ATLM	Acid treated lemna minor
ASTM	American society for testing and materials
BOD	Biological oxygen demand
CCD	Central composite design
COD	Chemical oxygen demand
Ci	Initial metal concentration (mg/l)
Ce	Concentration of the sorbate at equilibrium (mg/L),
FTIR	Fourier transform infrared radiation
IQ	Intelligence quotient
K_f	Freundlich adsorption capacity (mg/g)
K_L	Langmuir equilibrium constant (l/mg).
k_f	Pseudo first order rate constant
ks	Pseudo second order rate constant
NMN	National mining association
pH PZC	pH of point of zero charge
XRD	X- ray diffraction
UV/Vis	Ultraviolet visible

Abstract

The release of wastewater with heavy metals of Cu, Pb, Zn, Cd etc. Particularly in areas with expansion of industries can pose a significant threat to human health and environment due to their toxicity. This paper deals with bio sorption of heavy metals (copper and lead) using acid treated dried lemna minor powder. The adsorbent after treated with hydrochloric acid was dried and ground to a particle size range of (820-850) µm. Characterization of the adsorbent such as proximate analysis, surface charge, FTIR spectroscopy, surface area and XRD was done prior to bio sorption process. A batch adsorption experiment was carried out using central composite design expert 6.0.8 and the effect of adsorption parameters: pH, initial metal concentration, and adsorbent dose was studied. The result indicated that maximum percentage removal of 92.45 % for Lead (II) was obtained at pH = 11. Similarly, 88.4 % removal was found for Copper (II) at pH = 9. Temperature effects showed that the adsorption process was endothermic with positive enthalpy and negative free Gibbs energy values. The Removal efficiency of lemna minor increased with increase in temperature. Data's from the adsorption were analyzed with Temkin, Langmuir and Freundlich isotherm models. Desorption of Cu^{+2} and Pb^{+2} were carried out by using a 100ml of 0.1M HCl in to 250 volumetric flasks with 0.1 g of 43.86 ppm of copper and 42.38 ppm of Lead loaded sun dried lemna minor. The percent desorption increased with time for both metals and maximum desorption observed at 60-minute contact time with 80% for Lead and 72.2% for Copper.

Key terms: Heavy metals, Lemna minor, Biosorption, Desorption, Percent removal, adsorption capacity

1. Introduction

At present, Water pollution as a result of industrial development has become a common problem. With increasing generation of heavy metal bearing effluents from various industries, many aquatic environments are prone to metal concentrations which exceed water quality criteria designed to protect the environment. Heavy metal containing wastewaters are mainly released from electroplating, metal finishing, metallurgical, chemical manufacturing, mining and battery manufacturing in considerable amounts (Fu F *et al*, 2011).

Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water and are toxic or poisonous even at low concentrations. Some well-known toxic metallic elements are arsenic, iron, chromium, cadmium, Copper, lead, and mercury. Due to expansion of industrial activities in developing country, the levels of heavy metals in water systems have greatly increased. They can easily enter the food chain because of their high solubility in water. These heavy metals being non-biodegradable can pose a serious problem, with human health concerns and environmental consequences (Hawari and Mulligan, 2006).

Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Allergies are common, and repeated long-term contact with some metals can cause cancer (Uluozlu O *et al*, 2008). Stringent policies with regard to the metal discharges are being enforced particularly in industrialized countries. Due to their high toxicity, industrial wastewaters containing heavy metals are strictly regulated and must be treated before being discharged in to the environment. This situation will require the development of simple and low-cost methods for the removal of heavy metals from waste water prior to discharge. The currently employed technologies for removal of heavy metals from industrial effluents appear to be inadequate and expensive. They often cause secondary problems with metal bearing sludge’s. It is therefore essential to develop alternative technologies to treat metal bearing effluents (Ushakumary, 2013).

Previous studies have showed that the adsorption onto activated carbon is a reliable and significantly effective technique for removal of heavy metals; however, the high cost of activated carbon limits its use as an adsorbent. (Balarak D *et al,* 2016; Dizge *et al,* 2008; Cengiz, S *et al* ,2008; Lata *et al*., 2014; Batzias *et al*, 2007). Recently, various materials such as fly ash, wheat straw, apple pomace, fungus, and orange peels, soy meal hull, eggshell membrane and etc has been applied to develop low-cost and effective adsorbent (Zazouli *et al*, 2014; Doulati *et al*, 2008; Arami *et al*, 2006). The *Lemna minor* is one of wide-spread aquatic plant that belong to duckweed species with special characteristics including rapid growth, high nutritional value, and high water purification. Therefore, the aim of present study was to assess the capacity of Lemna minor to remove the copper (II) and lead (II) and the effect of parameters such as pH, adsorbent dose and initial metal concentration was investigated.

1.1 Problem Statement

With increasing generation of heavy metals from industrial activities, many aquatic environments face metal concentrations that exceed water quality criteria (Abdelwahab, 2007). Heavy metals discharge from industries in Ethiopia are above allowable discharge limits and they cause toxicity to living beings and the environment (Ethiopia EPA, 2001). They have potential health risks associated with metal uptake via food chain, dermal absorption or inhaling. Copper and Lead are one of the common industrial effluents from electroplating, mining, pulp and paper etc , High levels of exposure to these metals have been proved to cause cancer, organ damage, joint diseases, and in extreme cases, death (Jarup, 2003). Several processes exist for removing dissolved heavy metals, including, ion exchange, precipitation, ultrafiltration, reverse osmosis, electro dialysis and activated carbon (Joshi, 2017). Many of these approaches demand high energy, high cost, advanced operational requirements, result in large amounts of sludge requiring treatment or difficult to treat and be disposed of in an environmentally sound manner, or do not enable recovery of metals or material.

From this point of view, there is a need to look for a low cost, simple, and efficient heavy metal removal technique. A number of biosorption studies were carried out by various researchers as biosorption is quite popular for its simplicity and efficiency (Rana *et al*, 2014). Bio sorption has potential advantages over conventional methods such as low cost, high efficiency, less use of chemicals, recovery of metals and reuse of the adsorbate, and minimum waste (Omran *et al*, 2015). The Lemna minor is one of wide-spread aquatic plant that belong to duckweed species with special characteristics including rapid growth, high nutritional value, and tolerate wide range of pH. These characteristics can make it as a promising adsorbent material. Therefore, the aim of present study was to evaluate the capacity of acid treated (HCl) Lemna minor to remove copper (II) and lead (II) and to investigate the effect of parameters such as pH, adsorbent dose and initial metal concentration on adsorption capacity. The purpose of treating the bio sorbent with acid is to enhance its adsorption capacity.

1.2 Research Questions

- ✓ Are there sustainable and available bio adsorbents such as lemna minor (duckweed) that can be used for the removal of heavy metals?
- ✓ Can the emerging bio adsorbents actually replace activated carbon which is very expensive adsorbent common today?
- ✓ What is the optimum Operating parameters for biosorption of metal ions under batch studies?
- ✓ Is it possible to desorb the lemna minor bio adsorbent to recover the metals after bio sorption is carried out?

1.3 Objective

1.3.1 General objective

In the aim of this study was Investigation of acid treated dried Lemna minor powder as adsorbent for removal of Cu^{2+} and Pb^{2+} ions from aqueous solutions.

1.3.2 Specific objectives

- To prepare and characterize lemna minor for bio sorption process
- To carry out batch adsorption of heavy metal ions particularly Pb^{+2} and Cu^{+2} and determine optimum values of adsorption parameters such as pH, initial metal ion concentrations and adsorbent dose on the efficiency and removal capacity of adsorbent
- To analyze the data obtained from the adsorption experiment using the sorption isotherm
- To determine the adsorption kinetics on the sorbent
- To study the recovery of used adsorbent

1.4 Significance of the study

The major importance for removing of heavy metal from contaminated waste water is to ensure safety on environment and health of humans. Bio sorption is a good treatment method in comparison with other techniques and it has advantages including low cost, high efficiency, minimization of chemical and or biological sludge, no additional nutrient requirement, and regeneration of bio sorbent and possibility of metal recovery (Kratochvil and Volesky, 1998). metals on acid treated lemna minor. It can provide basic information on adsorption of heavy metals on to lemna minor.

1.5 Scope of the study

This research generally covers bio sorption and desorption of heavy metals (Cu^{+2}, Pb^{+2}) ions from aqueous solution on to acid treated lemna minor powder. It includes preparation and characterization of the adsorbent by using proximate analysis and standard testing procedures (ASTM), determining specific surface area of adsorbent, determining functional groups of the adsorbent by using FTIR, and crystalline structure of the adsorbent with XRD, measuring initial and final concentration using UV/vis spectrophotometer. It also covers batch adsorption and desorption experiments on various factors including biosorbent dosage, initial metal concentration and pH. Result and discussion were made following the laboratory data.

2. Literature Review

Water is essential for the existence of all life forms. In addition to household uses, water is vital

For agriculture, industry, fishery and tourism. Increasing population, urbanization and industrialization has led to the decreased availability of water. The quality of water used is also being deteriorated as it is getting more and more polluted. Water pollution may be defined as the contamination of streams, lakes, seas, underground water or oceans by substances, which are harmful for living beings. A large amount of water is discharged back after domestic and industrial usage. This is contaminated with domestic waste and industrial effluents. When this contamination reaches beyond certain allowed concentrations, it is called pollution and the contaminants are called pollutants.

2.1 Health and Environmental Effects of Heavy Metals

Heavy metals are chemical elements which have a specific gravity at least 5 times the specific gravity of water and is toxic or poisonous even at low concentrations. Some well-known toxic metallic elements are arsenic, iron, Chromium, cadmium, lead, mercury, copper, nickel, lead, etc. Heavy metals are highly dispersed in a wide variety of economically important minerals. They are released to the environment during mineral extraction process. Therefore, mining activities are considered as the primary anthropogenic source of heavy metals. Heavy metal ions are discharged into water system from various industrial activities such as electroplating industries, electronic equipment manufacturing, and chemical processing plants. Due to rapid development of industrial activities, the levels of heavy metals in water systems have substantially increased.

Heavy metals can easily enter the food chain because of their high solubility in water. Cadmium, copper, chromium, lead and zinc are extremely toxic heavy metals of widespread use in many industries (ushakumary, 2013).

Heavy metal pollution is affecting the society with human health concerns and ecological consequences. It is essential to remove heavy metals from industrial waste waters. Numerous waste biomass sources are available in nature in which adsorption properties have been reported e.g rice husk, saw dust, tea and coffee waste, orange peel peanut shells, activated carbon, dry tree leaves and barks (Asma *et al*, 2005; Ferda and Selen, 2012; Kishore *et al*, 2008; Nuria *et al*, 2010). Adsorption of heavy metal ions occur as a result of physicochemical interaction, mainly ion exchange or complex formation between metal ions and the functional groups present on the cell surface.

2.1.1 Health effects of copper

Environmental contamination due to copper is caused by mining, printed circuits, metallurgical, fiber production, pipe corrosion and metal plating industries. The other major industries discharging copper in their effluents are paper, pulp, petroleum refining and wood preserving. Agricultural sources such as fertilizers, fungicidal sprays and animal wastes, also lead to water pollution due to copper. Copper may be found as a contaminant in food, especially shell fish, liver, mushrooms, nuts and chocolates. Any packaging container using copper material may contaminate the product such as food, water and drink. Copper has been reported to cause neurotoxicity commonly known as “Wilson’s disease” due to deposition of copper in the ventricular nucleus of the brain and kidney failure. In some instances, exposure to copper has resulted in jaundice and enlarged liver.

2.1.2 Health effects of lead

Lead has a significant role in many industries because it is ductile and easily shaped. It has been used in many sectors and products: batteries, petrol additives, chemical compounds, pigments, and cables (National Mining Association, NMA, 2009).

Overdoses of lead and long term exposure can tend to severe impacts especially on infants. High concentrations of lead may cause problems in the synthesis of hemoglobin, effects on the kidney, gastrointestinal tract, joints and reproductive system, and acute or chronic damage to the nervous system. According to the Environmental Protection Agency (EPA) the long-term exposure of lead can be severe and tends to decreased growth, hyperactivity, impaired hearing, and brain damage. Recent studies have stated that lead may have an impact on mental and psychological developments in children; for instance, children may lose up to 2 Intelligence Quotient (IQ) points if the blood lead level rises from 10 to 20 μg/dl. Lead mainly can be found in foods from the deposition of dust and rain containing lead on crops and soil. It can also accumulate in the human body from point source emissions. For example, lead can exist in drinking water from old lead piping and from illegal discharging of industrial waste water of high concentrations into surface fresh water.

Stricter regulations with regard to the metal discharges are being enforced particularly in industrialized countries. Toxicology of heavy metals confirms their dangerous impacts. Due to their high toxicity, industrial wastewaters containing heavy metals are strictly regulated and must be treated before being discharged in to the environment.

Table 2.1United States Environmental Protection Agency for electroplating Effluent standards (Frency Mathew, 2008)

Parameter	Daily maximum(mg/L)	4 day average (mg/L)
Total Cadmium	1.2	0.7
Total Chromium	7.0	4.0
Total Copper	4.5	2.7
Total Lead	0.6	0.4
Total Nickel	4.1	2.6
Total zinc	4.2	2.6
Total silver	1.2	9.7
Cyanides	1.9	1.0
Total toxic organics	2.1	-

2.2 conventional methods

Several technologies have been used to treat metal containing aqueous solution for the last few decades (Wang S *et al*, 2005). Commonly used methods for removing metal ions from aqueous streams include chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction. However, these methods proved either inefficient or expensive in case of low concentration (1-100 mg/l) of heavy metals prevailing in the environment and generate huge amount of sludge which are difficult to dispose. Major drawbacks of the conventional processes can be summarized as follows (Suhag A *et al*, 2011):

Reverse Osmosis: It is a process in which heavy metals are separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by the dissolved solids in wastewater. The disadvantage of this method is that it is expensive.

Electro dialysis: In this process, the ionic components (heavy metals) are separated through the use of semipermeable ion selective membranes. Application of an electrical potential between the two electrodes causes a migration of cations and anions towards respective electrodes. Because of the alternate spacing of cation and anion permeable membranes, cells of concentrated and dilute salts are formed. The disadvantage is the formation of metal hydroxides, which clog the membrane.

Ultra-filtration: They are pressure driven membrane operations that use porous membranes for the removal of heavy metals. The main disadvantage of this process is the generation of sludge.

Ion-exchange: In this process, metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin. The disadvantages include: high cost and partial removal of certain ions.

Chemical Precipitation: Precipitation of metals is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers. The large amount of sludge containing toxic compounds produced during the process is the main disadvantage.

the demerits in the above-mentioned techniques like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require careful disposal has led to look for cost-effective treatment method that is capable of removing heavy metals from aqueous effluents.

2.3 Bio sorption of heavy metals

Biosorption is defined as the capability of biological materials to accumulate heavy metals from wastewater through metabolically inactive or dead biomass. It is a process with some unique characteristics. It can effectively sequester dissolved metals from very dilute complex solutions with high efficiency. This makes biosorption a promising alternative for the complex waste-waters. Algae, bacteria, fungi and yeasts have proved to be potential metal bio sorbents. The major pros of bio sorption over conventional treatment methods include low cost, high efficiency, minimization of chemical or biological sludge, No additional nutrient requirement, and regeneration of bio sorbent and possibility of metal recovery. It has emerged as promising technique for metal removal. The processes can occur at an interface between any two phases, such as, liquid-liquid, gas-liquid, or liquid-solid interfaces (Barakat M. A, 2011).

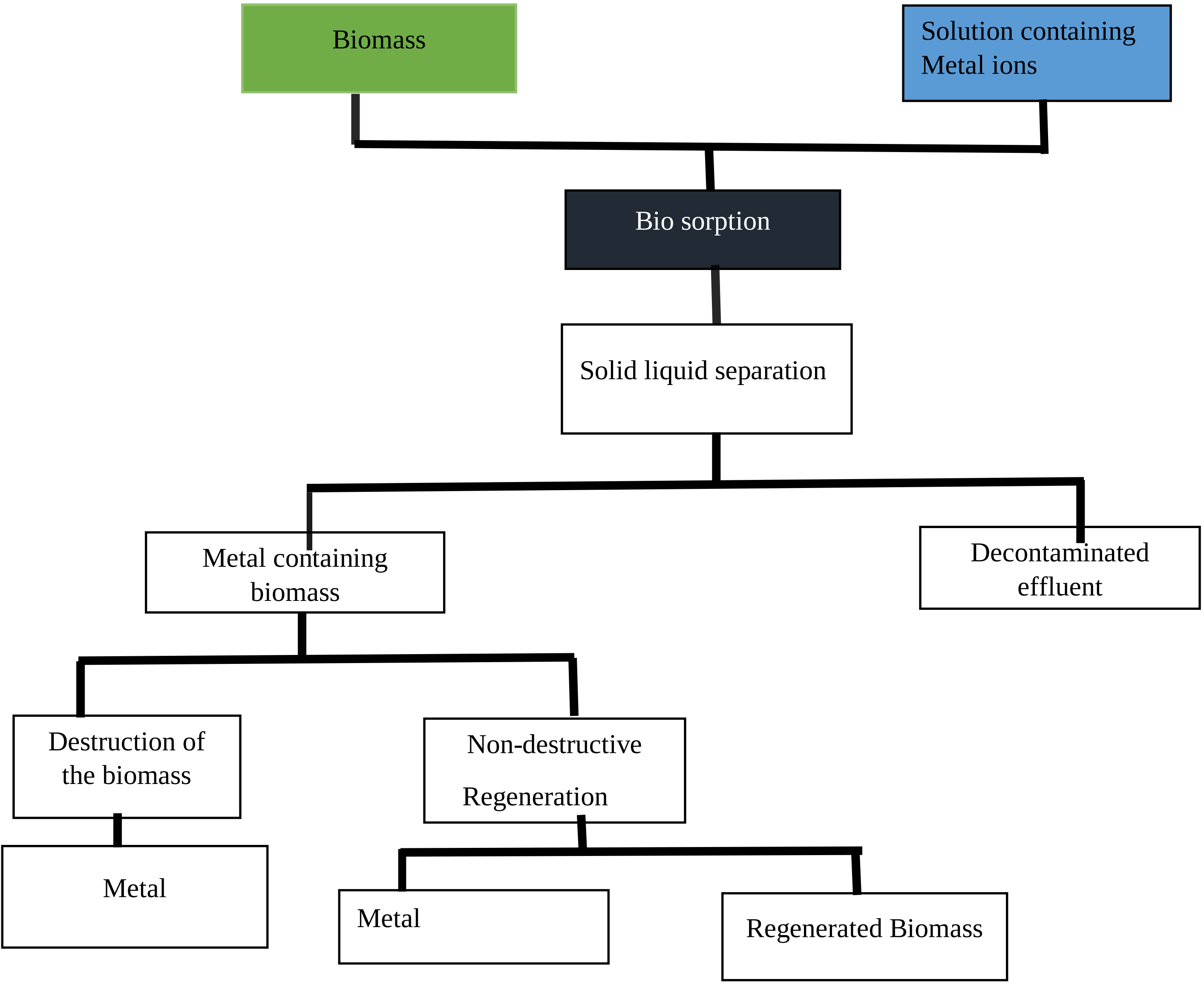


Figure 2.1 schematic diagram of bio sorption process of metal ions

2.3.1 Merits of Biosorption

Bio sorption is growth-independent process.i.e. e, non-living biomass is not subjected to toxicity limitation of cells. Costly nutrients are not required for the growth of cells in feed solutions Thus, there is no be problem with the disposal of surplus nutrients or metabolic products. The process is not governed by the physiological constraint of living microbial cells. Since non-living biomass behave as an ion exchanger; the process is very rapid and takes place between few minutes to few hours. Metal loading on biomass is often very high, leading to very efficient metal uptake. Because cells are non-living, processing conditions are not restricted to those conducive for the growth of cells. In other words, a wider range of operating conditions such as pH, temperature and metal concentration is possible. Metal can be desorbed readily and then recovered (Abida Z *et al*, 2015).

2.3.2 Bio sorbent Materials

I. Microorganisms

Microbial biosorbents are basically small particles, with low density, poor mechanical strength and little rigidity. Even though they have merits, such as high biosorption capacity, rapid steady state attainment, less process cost and good particle mass transfer, they often suffer several drawbacks. Biosorbents for the removal of metals mainly come under the following categories: bacteria, fungi, algae, industrial wastes, agricultural wastes and other polysaccharide materials. In general, all types of biomaterials have shown good biosorption capacities towards all types of metal ions. These Chromium (VI), Nickel (II), Copper (II) and Cobalt (II) heavy metal ions are approach with Bacillus subtilis, Pseudomonas aeruginosa and Enterobacter cloacae. It is a novel genus and species to the biosorption process for particular metals, this is very inaccessible theme to get definite references. Potent metal biosorbents under the class of bacteria include genre of Bacillus (Nakajima and Tsuruta, 2004), Pseudomonas (Chang *et al*,1997; Uslu and Tanyol 2006) and Streptomyces (Selatnia *et al*, 2004) etc. Important fungal biosorbents include Aspergillus (Kapoor and Viraraghavan 1997; Jianlong et al 2001; Binupriya et al 2006), Rhizopus (Bai and Abraham, 2002; Park *et al*, 2005) and Penicillium (Tan and Cheng, 2003), etc. Since these micro-organisms are used widely in different food/pharmaceutical industries, they are generated as waste, which can be attained free or at low cost from these industries.

II. Fungi

During past few decades, biosorption has been studied extensively using various biomasses such as white-rot fungus (Yetis *et al,* 2000; Bayramoglu *et al*, 2002), Saccharomyces cerevisiae (Goksungur *et al*, 2005; Ohnuki *et al,* 2005). Fungi and yeasts are easy to grow, produce high yields of biomass and can be manipulated genetically and morphologically. The fungal organisms are widely used in a variety of large-scale industrial fermentation processes. For example, strains of Aspergillus are used in the production of ferrichrome, kojic acid, gallic acid, itaconic acid, citric acid and enzymes like amylases, glucose isomerase, pectinase, lipases and glucanases; while S.cerevisiae is used in the food and beverage industries. The biomass can be cheaply and easily procured in rather substantial quantities, also as a byproduct from the established industrial fermentation processes, for the biosorption of heavy metals and radio nuclides, which made the fungi of primary interest as a raw material serving as a basis for formulating suitable biosorbents. The use of biomass as an adsorbent for heavy-metal pollution control can generate revenue for industries presently wasting the biomass and at same time ease the burden of disposal costs associated with the waste biomass produced.

III. Waste materials

Agricultural biomass mainly consists of lignin, cellulose, hemicellulose and some proteins which make them effective biosorbent for heavy metal cations. Various investigated biomasses for their possible application in wastewater treatment for heavy metal removal include peapod, cotton and mustard seed cakes (Iqbal *et al,* 2002), sawdust (Garg *et al*, 2004), Ocimum basilicum seeds (Melo and Dsouza *et al*, 2004), waste tea (Mahvi *et al,* 2005), Cicer arientinum husk (Ahalya *et al,* 2005), sugarcane bagasse, maize corncob and Jatropha oil cake (Garg *et al*, 2007).

The utilization of agricultural waste materials is increasingly becoming a vital concern because these wastes represent unused resources and in many cases present serious disposal problems. Numerous waste biomass sources are available in different parts of the world, on which some experimental adsorption properties have been reported e.g. rice husk (Kumar and Bandyopadhyay, 2006; Wong *et al*, 2003; Zulkali *et al*, 2006).

IV. Algae

Higher uptake capacity has been found in brown algae than red and green algae (Brinza *et al*, 2007). The reason seems to be that they offer better sorption than red or green algae (Romera *et al*, 2006). Researchers have employed mainly brown algae (treated in different ways to improve their sorption capacity (Romera *et al*, 2006).The micro algae include Chlamydomonas reinhardtii, Chlorella salina, Chlorella sorokiniana, Chlorella vulgaris, Chlorella miniata, Cyclotella cryptica, Lingpa taylorii, Phaeodactylum tricornutum, Porphyridium purpureum, Spirogyra sp., Spirulina platensis, Stich coccus bacillaris and Stigeoclonium tenue. These algae were reported to be able to adsorb one or more heavy metal ions, including Co, Cu, Mn, Ni, Zn, Cd, with good metal uptake capacity (Brinza *et al*, 2007). Chojnacka *et al* (2005) reported the biosorption performance of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue–green algae Spirulina.

Table 2.2 Comparison of bio sorption maximum capacity of Zn (II) on different bio sorbent

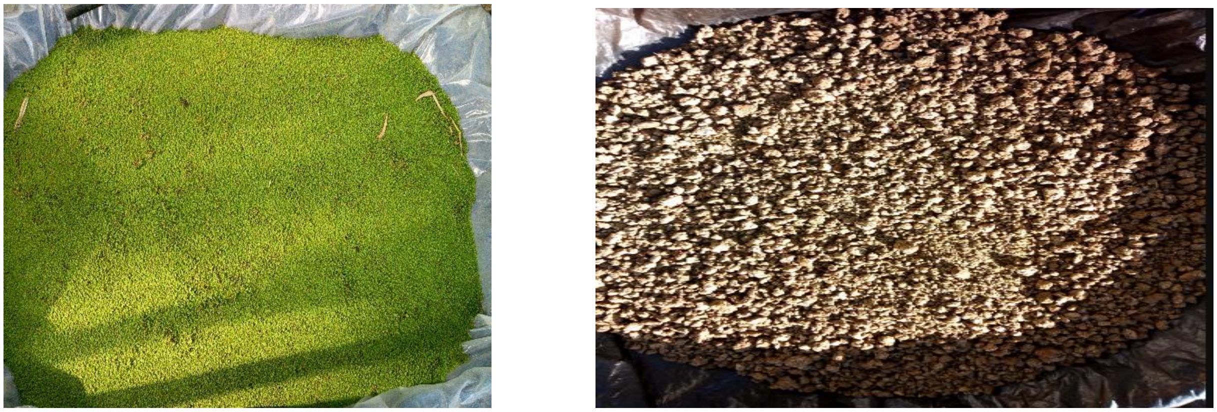
Bio adsorbent	pH	T (^{o}c)	q_{max} (mg/g)	Reference
Tobacco dust(cultural waste)	7	25	25.1	Qi B.C, Aldrich C, 2008
Chlorella vulgaris (fungi)	5	20	17	Javaid A *et. al*,2011
Cunningghamellaechinulata(fungi)	6	25	18.8	Javaid A.*et. al*,2011
Mucrrouxii(fungi)	5	25	16.6	Javaid A.*et. al*,2011
Geobacillustoebiisub.sp. Decanicus(bacteria)	5	80	21.1	Vijayaraghavank *et.al*, 2008
Acenitobacterlwoffii(bacteria)	6	25	36	Tabaraki *et.al*,2013
Streptomyces rimosus(bacteria)	7.5	20	30	Mameri N. *et.al*,1999
Delftiatsuruhatensis(bacteria)	6	25	14	Bautista-hernandez,2012
Azllafiliculoides(Algae)	5	25	34	Javaid A.*et. al*,2011
Fucusvesiculses (Algae)	4.5	25	52.3	Davis T.A *et.al*, 2003
Luminaria japonica(Algae)	4.5	225	91.5	Davis T.A *et.al*, 2003

2.3.3 Gaps in the Literature

Literature review indicates that different forms of biomass can be used for the removal of heavy metal pollutants in the industrial effluents. Investigation has been carried out to evaluate the performance of easily available adsorbent for removal of metals from aqueous solution. Undoubtedly, such low-cost adsorbents offer a lot of promising benefits for their commercial use in the future. However, only few researches were done on biosortpion studies using lemna minor as bio adsorbent. Thermodynamic effects on biosorption process have been insuffiently discussed. Interaction effects that could occur among process variables were neglected. The desorption of the used adsorbent which is essential for possible metal recovery was not given emphasis (Handojo *et al*, 2016, Tasrina *et al*, 2015, Balarak *et al*, 2015); therefore, the current research was conducted with the main objective of filling the identified gaps. The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to natural biomass. They have also proven useful in removing organic wastes from waste water. Some examples are cattails, calla lilies, arrowhead, ginger lilies, pickerelweed, water hyacinths, Lemna minor (Duckweed), water lettuce, water spinach, aquatic mosses and liverworts. The use of Lemna minor as a bio adsorbent has a lot of merits over other bio adsorbents. It is fast to grow (twenty times faster than corn), renewable, and tolerate wide range of pH (Suhag A *et al*, 2011).

2.4 Characteristics of Lemna minor

Duckweed is a stem less, small, and free floating aquatic plant that belong to Lemnaceae family (Cheng *et al*, 2002). The *Lemnaceae* family consists of four genera (Lemna, Spirodela, Wolffia and Wolffiella) and 40 species have been identified. They are green and have a small size (1-3mm), with short but dense roots (1-3cm) (Sooknah R *et al*, 2004). Duckweed is a small and free floating aquatic plant and grows on the surface of still or slow-moving water. Duckweed fronds grow in colonies that, in particular growing conditions, form a dense and uniform surface mat (Hasar *et al*, 2000). Duckweed are found world-wide on the surface of nutrient rich fresh and brackish waters (Zimmo, 2003) but the greatest diversity appears in subtropical and tropical areas. Their habitat comprises still or slowly moving fresh or polluted waters of only a few mm to 3m depth. In particular nutrient-rich and sheltered small ponds, ditches and swamps, e.g. down-stream from sewage works, often contain duckweed.



A B

Figure 2.2 lemna minor leaves A (fresh lemna minor), B, acid treated, ground and dried)

2.4.1 Previous study of Bio sorption on to lemna minor

Tasrina *et al* (2015) investigated the removal of Arsenic (III) from ground water by adsorption onto lemna minor. The study showed that maximum arsenic (III) removal was obtained under the following conditions: initial As (III) concentration, 100 µg/L; Duck weed amount, 3 g; average particle size, 0.595 mm, and pH, 5.5, respectively.

Balalark *et.al* (2015) studied the effect of pH, initial dye concentration, adsorbent dose and contact time on adsorption capacity of lemna minor (duck weed) biomass. It was found that lemna minor was able to remove 98% acid red 88 dye from aqueous solution. The resulting adsorbent was characterized its ultimate analysis, morphological structure and its surface area. The ultimate analysis of a dry duckweed was as the following (%): C − 39.11; H − 6.13; O − 37.74; N − 5.52; S − 0.67; balance − mineral matter. Scanning electron microscopy (SEM) images of modified lemna minor showed the adsorbent had heterogeneous surface structure with deep pores. The specific surface area of modified *Lemna minor* was determined in size of 30 m2/gr.

2.5 Adsorption mechanism

The cell wall is the first component that comes into contact with metal ions, where the solutes can be deposited on the surface or within the cell wall structure. The solute uptake by live/dead cells is extracellular, the chemical functional groups of the cell wall play vital roles in bio sorption. Due to the nature of the cellular components, several functional groups are present on the bacterial cell wall, including carboxyl, phosphonate, amine and hydroxyl groups (Van der Wal *et al*, 1997).

Biomass possess an abundance of functional groups that can passively adsorb metal ions. The term adsorption can be used as a general term and includes several passive, i.e. non-metabolic, mechanisms such as: complexation; chelation; co-ordination; ion exchange; precipitation; reduction. For adsorption to occur, there must be forces that attract the adsorbate to the solid surface in a solution. This mechanism or forces which attract the adsorbate to the solution of the solid interface can either be physical or chemical (Mckay, G. 1996).

Physical Adsorption

Physical adsorption (physisorption) is a reversible method in which there is the attraction of molecules by mechanical forces when the molecules come in contact with the adsorbent. The reversible process depends basically on the force of attraction between the adsorbate and adsorbent. This type of adsorption is multilayer which means that each molecule layer forms on the top of previous with the number layers being proportional to the contaminate concentration (Chiron N et al, 2003).

Chemical Adsorption

In comparison to physical adsorption, chemical adsorption is an irreversible process which is caused as a result of the reaction taking place between molecules of the adsorbed substance and the adsorbate. It involves the formation of covalent or ionic bonds, consumes high energy and it can occur over wide range of temperature. "Due to its irreversibility, monolayer is expected to form chemisorption while multilayer is encountered in physisorption" (Mckay G, 1996).

Complexation

As noted above complex formation of metal ions with organic molecules involves ligand centres in the organic species i.e. the presence of an atom or atoms having lone pair electrons to donate. Complexation may be electrostatic or covalent and the simplest case is complexation by a mono-dentate ligand such as RNH2. To approach and elucidate biosorption mechanisms, a significant part of the recent advances in biosorption are based on the classification of elements according to the hard soft acid-base classification (Pearson's classification). "Hard acids", metals such as Na, K, Ca, Mg, often essential nutrients for microbial growth, bind preferentially to oxygen containing "hard bases", ligands such as OH^- , $HPO4^{2-}$, $CO3^{2-}$, $R\text{-}COO^-$, and =C=O. Soft acids, metals such as the precious metals Ag, Au, Pt, Pd are bound covalently to the cell wall by "soft bases", ligands containing nitrogen or sulfur. As noted earlier several mechanisms might be involved in the immobilization of metals and it is now evident and confirmed by several researchers, that the biosorption of precious metals is a two-step mechanism comprising first covalent bonding and then in-situ reduction (Beveridge *et al*, 1976). Borderline ions on the "hard/soft" classification such as the transition elements: Mn^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+} exhibit a significant degree of covalent bonding with nitrogen or sulfur containing "soft" ligands (Davis *et al*, 2003).

Chelation

Organic molecules containing more than one functional group with donor electron pairs can simultaneously donate these to a metal atom. This can result in the formation of a ring structure involving the metal atom a process termed 'chelation'. In general, since a chelating agent may bond to a metal ion in more than one place simultaneously, chelated compounds are more stable than complexes involving mono dentate ligands. Stability tends to increase with the number of chelating sites available on the ligand. Thus chelation of metals by donor ligands of biopolymers leads to the formation of stable species. Co-ordination Metal atoms have preferences for specific donor atoms ("hard/hard" / "soft/soft") and the stereo chemical arrangements that play an important role in the binding with the available ligands on the microbial cell. Limited information of surface complexation models, based on the theory of surface co-ordination chemistry, is available to describe metal bio sorption

Anion exchange

Anion exchange on biopolymers can take place on a variety of organic-nitrogen-based groupings. In proteins, amino (lysyl side chain and N-terminal) imidazole (histidyl) and guanidine (arginyl) groupings are common centres of positive charge. Centres of positive charge in nucleic acids will occur with protonation of amino groups on purine or pyrimidine rings or with protonation of heterocyclic nitrogen atoms. Polysaccharides as a group are acidic or neutral macromolecules with basic functional groups being rare and arising as un-acetylated amino sugars. Chitin is the notable example where a proportion of glucosamine residues are reportedly un-acetylated and which provides on deacetylation chitosan with a high proportion of protonisable positive charge centres. Many examples have established the existence of an ion exchange mechanism in metal ion removal by biosorption (Vasudevan, 2002). However, it has been suggested by many researchers that ion exchange is neither the sole nor the main mechanism for metal biosorption (Brady, 1995). Precipitation Metal precipitation is also involved in biosorption. The precipitates may be formed and remain in contact with or inside the microbial cells or may be independent of the solid phase of the microbial cell. In the later case, the presence of the solid phase-microbial cell or biofilm also plays a favorable role in the phenomenon of precipitation. The term precipitation in most cases refers to the formation of insoluble inorganic metal precipitates (Remoundaki, 2003). However, in the case of metal biosorption by microbial cells, organic metal precipitates may also be formed. This may be more easily understood when metals are bound to Extracellular Polymeric Substances (EPS) excreted by some prokaryotic (bacteria, archaea) and eukaryotic (algae, fungi) microorganisms. Purified products from isolated cells such as glucan, mannan, and chitin accumulate greater quantities of cations than the intact cells, proving that biomolecules can form metal precipitates.

Reduction

The removal of toxic hexavalent chromium from aqueous solution by biosorption by different biomass types has been extensively reported. This removal is often associated with the simultaneous reduction of Cr (VI) to Cr (III), thus inactivated fungal biomass e.g. Aspergillus niger, Rhizopus oryzae, Saccharomyces cerevisiae and Penicillium chrysogenum remove Cr (VI) from aqueous solutions by reduction to Cr (III) when contacted with the biomass (Donghee, 2005). Also soft metals like gold and palladium are first bound on sites on and within the cell wall and these sites act as nucleation points for the reduction of metals and growth of crystals and elemental gold and palladium have been obtained. The biosorption mechanism is a two-step process: initiation of the uptake at discrete points by chemical bonding, then reduction of the metal ions (Lin *et al*, 2005).

2.6 Factors affecting biosorption

The most important factors that should be taken into account when considering biosorption are: the type and nature of the dosage, initial solute concentration, adsorbent dosage and physicochemical factors like temperature, pH, and ionic strength (Chimie, 2014).

Apart from the physicochemical factors such as pH, the presence of other anions and cations, metal speciation, pollutant solubility and form, etc. may also have an influence (Omran, 2015).

pH

The pH of the metal ion solution is an important parameter for adsorption of metal ions because it affects the solubility of the metal ions, concentration of the counter ions on the functional groups of the adsorbent and the degree of ionization of the adsorbate (Ii *et al*, 2011).Therefore, pH of solution influences the nature of biomass binding sites and metal solubility; Metal adsorption has frequently been shown to be strongly pH dependent in almost all systems examined, including bacteria, cyanobacteria, algae, and fungi (Holan & Volesky, 2007).

It has been generally reported that in highly acidic medium (pH≈2) the removal of metal ions is almost negligible and it increases by increasing the solution pH up to a certain limit (Abdelghani & Elchaghaby, 2014).

The decrease of adsorption levels by lowering pH can be due to competition between protons and metal ions for capturing same sites (Igwe, 2006). On the other hand, too high pH value can cause precipitation of metal complexes, so it should be avoided during experiments. For different adsorption system of metal ions, the optimal pH is different (Wang & Chen, 2006).

Temperature

The effect of temperature is fairly common and increasing the mobility of the metal cation (Malkoc & Nuhoglu, 2005). Temperature influence has more effect in a situation where by metal uptake increases within a temperature range of about 20-30 ^{0}C, but decreases with an increase of temperature above a critical value. (Sulaiman, 2015) Increase in temperature probably weakens the bond formed between the metal ions and the adsorption sites on the adsorbent thereby resulting in an increase in the amount of metal ions adsorbed on the adsorbent. This implies that increase in temperature creates a wider surface area for adsorption at the adsorbent (Okafor *et al*, 2012). At high temperature, the thickness of the boundary layer is expected to decrease due to the increased tendency of the metal ion to escape from the surface of the adsorbent to the solution Phase hence there was bound to be weak adsorption interactions between the adsorbent and the adsorbate (Ojedokun & Solomon, 2016).

Contact time

Adsorption is also affected by contact time between biomass and the solution containing metals. Adsorption proceeds fast and most metals are adsorbed at the very beginning of the process (Zabochnicka & Krzywonos, 2014). In adsorption systems, contact time plays a vital role irrespective of other experimental parameters affecting the adsorption kinetics. The determination of the optimum contact time needed to achieve the highest removal of metal ions is very important in batch adsorption experiments. The study on removal of iron, nickel and zinc was analyzed by (Elmaghrabi, 2014) that the time-dependent behavior of metal ions was measured by varying the equilibrium time in the range of 30–240 min. Results showed that the equilibrium is reached quickly (~30 min), indicating that, the adsorption sites are well exposed.

Initial solute concentration

It is generally agreed that the adsorption capacity increases as the initial metal ion concentration in the solution increases, whereas the metal removal percentage (also called removal efficiency) decreases by increasing the metal ion initial concentration (Islam M *et al*.2015). As a rule, increasing the initial metal concentration results in an increase in the adsorption capacity because it provides a driving force to overcome mass transfer resistance between the adsorbent and adsorption medium (Pahlavanzadeh *et al*, 2010).

Adsorbent dosage

The amount of biomass in the solution also affects the specific metal uptake. In principle, with more adsorbent present, the available adsorption sites or functional groups also increase (Mosbah & Sahmoune, 2013). At low biomass dosage, the number of ions adsorbed per unit adsorbent weight is high. Adsorption capacity is reduced when the biomass dosage increases as a result of lower adsorbate to binding site ratio where the ions are distributed onto larger amount of biomass binding sites (Ojedokun & Solomon, 2016).

2.7 Adsorption equilibrium model

Adsorption equilibrium is the basic requirement for designing adsorption system (Chimie, 2014). The adsorption isotherms describe the relationship between the mass of the adsorbed component per adsorbent mass and the concentration of this component in the solution ((Islam M *et al*, 2015). Modeling equilibrium sorption is important for industrial applications of adsorption; it yields data that facilitates designing and optimizing the process. During adsorption, a rapid equilibrium is established between adsorbed metal ions and adsorbent. The equilibrium metal uptake is calculated using the following equation:

Where q is the amount of metal biosorbed by biomass (mg/g); Co is the initial concentration of metal (mg/L); Ce is the concentration of metal (mg/L) at equilibrium; V is the volume of the metal solution(L); and M is the mass of adsorbent (g) (Can and Jianlong, 2007).The sorption uptake can be expressed in different units depending on the purpose of the exercise: for example, milligrams of solute sorbed per gram of the(dry) biosorbent material (the basis for engineering process–mass balance calculations), or mmol/g (when the stoichiometry and/or mechanism are to be considered). A biosorption isotherm, the plot of uptake (q) versus the equilibrium solute concentration in the solution (C_f), is often used to evaluate the sorption performance.

$$q_e = \frac{c_o - c_e}{\mathrm{w}} * \mathrm{v} \quad \text{.........2.1.}$$

2.7.1 Adsorption Isotherms

Adsorption process is usually studied through graphs known as adsorption isotherm. The equilibrium adsorption isotherm is important in the design of adsorption systems. Although several isotherm equations are available, but important isotherms including Langmuir, Freundlich and Temkin isotherms was studied. Langmuir and Freundlich sorption isotherm models consider sorption by free binding sites rather than ion exchange. Sorption isotherms are useful in quantitatively evaluating and predicting the process performance of the binding capacity and affinity for different metal concentrations and sorbent dosages (Bohli T *et al*, 2015). These models can be applied only at a constant pH. These models are mostly used for the modeling of adsorption equilibrium in the presence of one metal. Langmuir model is based on the simplest model of adsorption with the basic assumptions including 1) molecules are adsorbed at discrete active sites on the surface, 2) energetically uniform adsorbing surface, 3) each active site adsorbs one molecule only, 4) no interaction among the adsorbed molecules (Dada A *et al*, 2012). The Langmuir isotherm model can be represented by equation 2.2. Sorption isotherms are useful in quantitatively evaluating and predicting the process performance of the binding capacity and affinity for different metal concentrations and sorbent dosages (Isabel *et al*, 2014).

The values K_L and q_m are computed from the slope and intercept of the Langmuir plot of $1/q_e$ versus $1/C_e$, respectively.

The Freundlich isotherm is one of the most popular isotherm which gives reasonable description of the adsorption of liquid molecules on solid surfaces. It is derived by assuming that the amount of substance adsorbed at equilibrium has a power law dependence on the concentration of the solute (Dada, A et al, 2012). Freundlich Isotherm model can be formulated by equation 2.3 Where q_e = amount of heavy metal ion removed at equilibrium (g), C_e = concentration of the adsorbate at equilibrium (mg/l), q_m = initial amount of heavy metal (g), X/m = adsorption per gram of adsorbent which is obtained be dividing the amount of adsorbate (x) by the weight of the adsorbent (m), K_L and K_f are constants.

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{q_m k_l c_e} \dots\dots\dots\dots..2.2$$

$$\log \mathrm{qe} = \log \mathrm{Kf} + \frac{1}{\mathrm{n}} \log \mathrm{Ce} \dots\dots\dots.2.3$$

Temkin isotherm contains a factor that explicitly takes into the account adsorbing species-adsorbent interactions. This isotherm assumes that (i) the heat of adsorption of all the molecules in the layer decreases linearly with coverage due to adsorbent-adsorbate interactions, and that (ii) the adsorption is characterized by a uniform distribution of binding energies, up to some maximum binding energy (Mall *et al*, 2005). A plot of *qe* versus ln *Ce* enables the determination of the isotherm constants B_1 and K_T from the slope and the intercept, respectively. K_T is the equilibrium binding constant (l/mol) corresponding to the maximum binding energy and constant B_1 is related to the heat of adsorption. Temkin isotherm is given in equation 2.4.

$$qe = \frac{RT}{b} \ln(KTCe)\text{……..2.4}$$

This can be linearized as:

$$qe = B1lnkt + B1lnCe\text{……2.5}$$

$$\text{Where } B1 = \frac{RT}{b}$$

2.7.2 Adsorption Kinetics Models

The prediction of adsorption rate gives important information for designing batch adsorption systems (King P *et al*, 2007). The kinetics of adsorption was studied by using pseudo first order, pseudo second order and intraparticle diffusion models as the most important kinetic models (Balarak *et al*, 2015). Adsorption kinetics describes the rate of solute uptake, which is also responsible for the residence time needed for an adsorption study. Therefore, it is an important characteristic in defining how fast the sorption processes is carried out.

Pseudo first-order kinetics model

The Pseudo first-order rate kinetics model based on adsorption capacity of adsorbent is generally expressed as: (Zazouli *et al.*, 2014):

$$\log(qe - q) = \log \mathrm{qe} - \frac{k_1 \mathrm{t}}{2.303} \ldots\ldots\ldots..2.4.$$

The plot of log (q_e – qt) versus t should give a linear relationship from which K1 and qe can be determined from the slope and intercept of the plot, respectively.

Pseudo second-order kinetics model

The Pseudo-second order kinetics model is tested in the same way by rewriting the equation in its linear form and plotting the appropriate variables. The equation that describes the pseudo-second order model is given in the following linear form (Zazouli *et al*, 2014)

$$\frac{\mathrm{t}}{q_t} = \frac{1}{\mathrm{K_2 qe^2}} + \left(\frac{1}{\mathrm{q_e}}\right)\mathrm{t} \ldots\ldots\ldots..2.5$$

The constants, q_e and K_2, are obtained from the slope and intercept of t/qt versus t linear plot, respectively.

Intraparticle diffusion model

The nature of the rate-limiting step in adsorption system could be assessed from the properties of the adsorbate and adsorbent as the metals were probably transported from its aqueous solution to the adsorbent by intra-particle diffusion (Santhy *et al*, 2006). Therefore, to investigate whether there was a possibility of adsorption of the adsorbate to diffuse into the interior pores of the adsorbent or not, intra-particle diffusion was explored by using Weber and Morris equation. (Weber and Morris, 1963).

$$q_t = \mathrm{K_i}\,(\mathrm{t})^{0.5} + \mathrm{C} \ldots\ldots 2.6$$

Where, q_t is the amount of metal adsorbed at time‘t’ (mg g^{-1}), Ki is intra-particle diffusion rate constant (mg g^{-1} $min^{-1/2}$) and C is intercept. The value of K_i and C were obtained from slope and intercept of the linear plot of q_t versus ($t^{0.5}$) at different initial metal concentrations and room temperature 25^0c. When the metal ion solution is mixed with the adsorbent, transport of the metal ions from the solution through the interface between the solution and the adsorbent occurs into pores in the particles. There are four main stages in the process of adsorption by porous adsorbents (i) solute transfer from the bulk solution to the boundary film that surrounds the adsorbent's surface, (ii) solute transport from the boundary film to the adsorbent's surface, (iii) solute transfer from the adsorbent's surface to active intra particular sites, and (iv) interactions between the solute molecules and the available adsorption sites on the internal surfaces of the adsorbent. One or more of these four steps controls the rate at which solute is adsorbed.

3.Materials and Methods

3.1 Equipment and Chemicals

The main chemicals used for the experimental works were lemna minor adsorbent, 0.1M sodium hydroxide, 0.1M hydrochloric acid, lead nitrate ($Pb(NO_3)_2$), Copper Sulphate ($CuSO_{4.}$ $5H_2O$), Sodium chloride (NaCl) and distilled water. All Chemicals were obtained from chemical stores in Addis Ababa. The bio sorption process has different stages and hence uses a number of instruments and equipment. Lemna minor leaves after dried was ground using a grinder. The samples of the bio adsorbent were weighed using analytical balance. Samples were placed in a conical flask for measuring the volume and were titrated using pipette. Both adsorbate and adsorbent were well mixed by using incubator shaker. Samples were heated in an oven and were kept in glass beakers. The functional groups present in the bio adsorbent were analyzed by FTIR spectra. The specific surface area of the adsorbent was measured by using sears method. The concentration of the metal content in the solution was measured using UV VIS spectrophotometer through calibration curve. Chemicals such as salts of copper and lead were used to make an aqueous solution that is ready for adsorption. 0.1M Hydrochloric acid was used to treat the bio adsorbent and adjust the pH of the solution. 0.1M Sodium hydroxide (NaOH) was used for adjusting the pH; Sodium chloride was used for making a solution to be used in surface area determination by sears method. Tong and stop watch were needed for handling hot beakers and time keeping respectively. Masks, safety cloth and glove were used for safety.

3.2 Experimental methods

3.2.1 Preparation of acid treated Lemna Minor powder

Lemna minor was collected from around Hawasa called "Tikur haik". They were washed with distilled water several times to remove dust and fines until the color of the wash water became transparent. After that, the washed duck weed was completely dried in order to reduce moisture content. Then, it was grounded with the grinding mill to form a powder. The ground duckweed was sieved through a fine mesh (820-850μm) (Davoud et al, 2016). Activation of the biomaterial was carried out by soaking the lemna minor powder in 0.1 M HCl solution for 24 hr under slow stirring. The solution pH was kept constant at 5.0 using 0.1 M of NaOH. The acid treated biomaterial was washed with distilled water to remove excess HCl. The activated samples were dried at 60°C for 24 hrs and stored at an airtight plastic bottle for later use (Zazouli et al, 2014).

3.3 Characterization of Lemna minor

3.3.1 Proximate Analysis of Lemna Minor

Proximate analysis ash content, volatile matter, fixed carbon and moisture content of the duck weed was measured using the method of ASTM.

Determination of moisture content

The moisture contents of the duck weed samples was determined using ASTM, E-871 (2013) procedure. A powdered sample of duckweed with particle size in the range of 820-850 μm weighing 10 g was taken in a pan and placed inside a hot air oven at a temperature of 105±3°C. The sample was weighed at regular intervals and once the weight observed became constant, the moisture content was calculated using the formula:

$$\text{moisture content}, \% = \frac{\text{weight(g)}_{\text{sample before drying}} - \text{weight(g)}_{\text{after drying}}}{\text{weight(g)}_{\text{of sample before drying}}} * 100 \ldots\ldots\ldots..3.1$$

Determination of ash content

The ash content of the duck weed biomass was determined following the ASTM D 482-13 standard procedure. Biomass sample of 2g size from the oven dried sample was taken in crucible and placed in a furnace at 575±25°C for a period of 4 hr then it was cooled to room temperature in a desiccator and its weight was recorded. Then again it was placed in furnace and was dried to a constant weight. The percentage of ash in the sample was determined using the expression.

$$\text{Ash content}(\%) = \frac{\text{weight(g)}_{\text{of crucible with ash}} - \text{weight(g)}_{\text{of crucible}}}{\text{weight(g)}_{\text{of sample}}} * 100 \ldots\ldots\ldots\ldots\ldots.3.2$$

Determination of volatile matter

1 g of dried sample was weighed into a crucible and incinerated at 850°C for 10 minute in furnace. This procedure is undertaken without contact with air in closed crucible. The crucible

was then cooled in desiccators and reweighed (ASTM, 1989). The % volatile of the sample was calculated as follows:

$$\%, \text{volatile mater} = \frac{\text{weight sample,g before incineration} - \text{weight of saple afeter incineration}}{\text{weight of sample befroe incineration}} * 100 \ldots 3.3$$

Determination of fixed carbon content

The fixed carbon (ASTM D3172 – 13) of samples was calculated by subtracting the sum of ash content (%) and volatile matter (%) from 100. The fixed carbon is the residue left after removing the volatile matter and the ash from the substance.

Fixed carbon (%) = 100 – % [ash content + volatile matter +moisture content] …….3.4

3.3.2. FTIR analysis of Lemna Minor

FTIR analysis was carried out in order to identify the functional groups that might be involved in the binding of heavy metal ions. The functional groups of raw, treated and metal loaded lemna minor were determined using FTIR Spectrometer (Spectrum 65 FT-IR, PerkinElmer) at wave number range of 4000- 400 cm^{-1}. First, the dried adsorbent was mixed with KBr particles to make it suitable to infrared analysis. The mixture was then pressed to a small thickness, slightly below 1 mm, required for FTIR analysis. FTIR analysis was done at Addis Ababa University, College of Natural Science, chemistry Laboratory.

3.3.3 Point of zero charge

A total of ten 200 ml of 0.1M NaCl solution conical flasks were prepared and their initial pH values were adjusted to 2, 3, 4, 5,6 7, 8, 9, 10 and 11 by adding 0.1M NaOH or 0.1M HCl solution by using a pH meter. 0.2 g of lemna minor adsorbent was added into each solution. The solution mixtures were allowed to equilibrate in an incubator shaker at 200 rpm and 25^0c for 1 hr. Then the solution was filtered and the filtrates final pH was measured. The results were plotted with ΔpH against pH final. The point at which ΔpH = 0 is known as pHPZC (Yadav O *et al*, 2012).

3.3.4 Specific surface area

The Lemna minor specific surface area was determined using the method described by Yadav et al, 2011. 1.0 g of adsorbent was mixed with 100 ml of distilled water and 20 g NaCl. The mixture was shaken for five minutes. Its final pH was adjusted to 4 with 0.1 M HCl. It was then titrated against 0.1M NaOH to raise the pH from 4 to 9 and the volume (ml) of 0.1M NaOH used was measured in replicate and the average value was taken for the surface area calculation by sears method. Specific surface area of adsorbent was calculated using the formula Sears method (Yadav O *et al*, 2012):

S = 32 x V-25 ………3.1

Where, S is surface area of bio adsorbent per gram (m^2 g^{-1}), V is volume (ml) of 0.1M NaOH required to raise the pH of the sample from 4 to 9. The numbers 32 and 25 are physical constants.

3.4 Preparation of aqueous solution

Copper sulphate - $CuSO_4.5H_2O$ and Lead nitrate - Pb $(NO_3)_2$ salts were used in the preparation of stock solutions. 1000 ppm of Stock solutions Pb (II) and Cu (II) were prepared by dissolving accurately weighed amounts of each salts, 1.599g of Pb $(NO_3)_2$ and 3.601g of $CuSO_4.5H_2O$ in 1000 ml distilled water. Working solutions of different concentration were prepared from the stock solution by serial dilution with distilled water. All chemicals used were of analytical reagent grade and bought from different chemical stores in Addis Ababa.

3.5 Batch adsorption experiments

Batch adsorption experiments were carried out by shaking a fixed amount of the adsorbent (Lemna minor), along with the selected metal ion solution. Copper and lead are the common effluents of metal finishing and coating, dyes manufacturing, paper mill, fine chemicals (Ram S et al, 2011). 100, 150 and 200 ppm of Copper and Lead solutions were taken in 250 ml conical flasks. They were shaken at 200 rpm, for two hours of time and room temperature of 25^oc using an incubator shaker. The solutions were filtered using filter paper prior to analysis, to separate the adsorbent from the solution mixture. The concentration of metal ions in the filtrate was analyzed using uv/vis spectrophotometer and standard calibration curves by measuring their absorbance at a wave length 550nm for lead and 600 nm for copper. Each experiment was repeated to get average values.

The data obtained in these batch studies were used to calculate the percentage removal of the metal ion and adsorption capacity of the adsorbent.

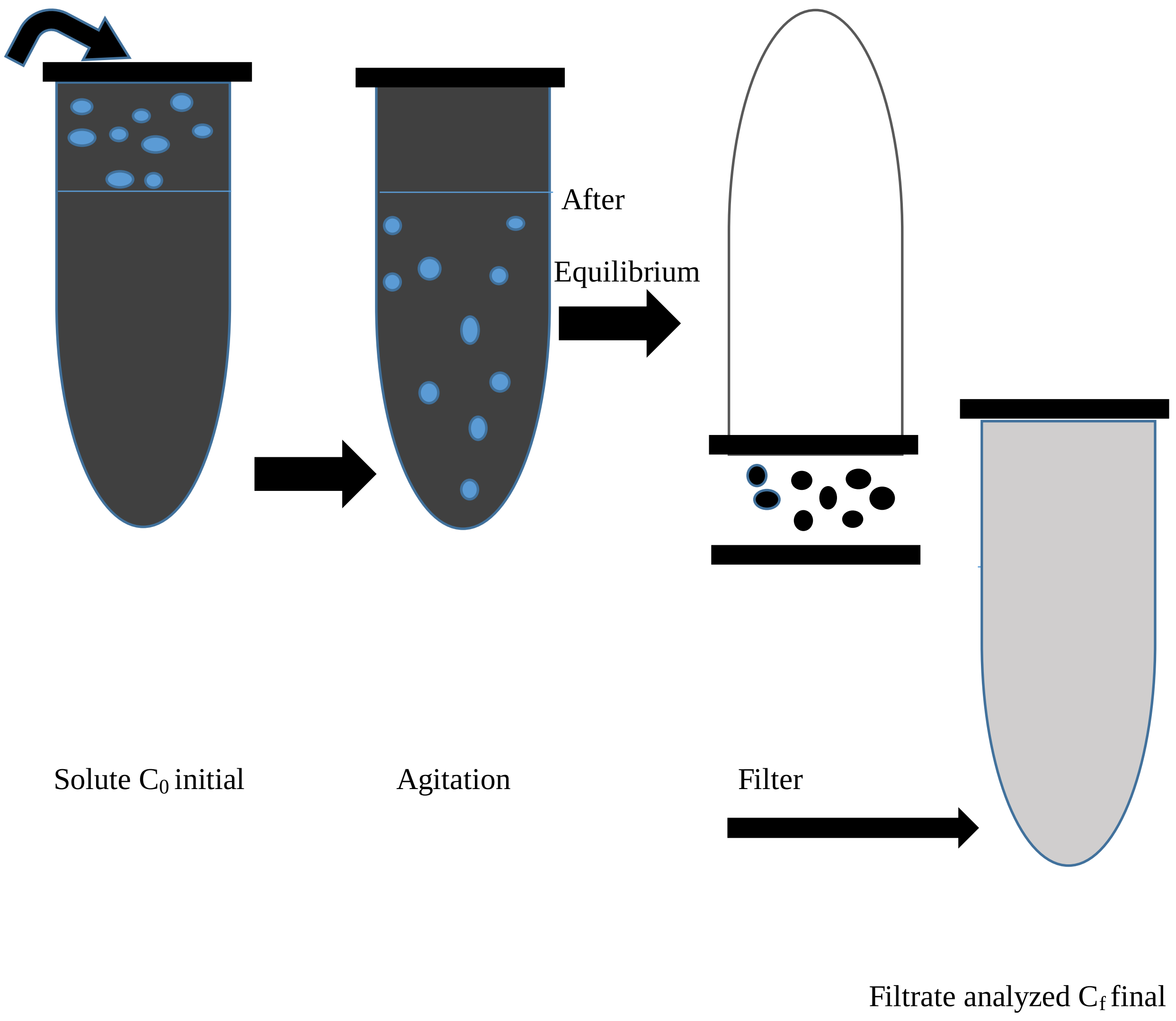


Figure 3.1 schematic representation of batch biosorption process

3.5.1 Effect of pH

The effect of the solution pH on Copper and Lead metal ions removal using Lemna minor as an adsorbent was conducted, using a 100 mL solution of 100 ppm of metal ion solution in the pH range of 3.0 to 11 at 25^{o}C and adsorbent dosage used was 0.2 g per 100ml solution. Flasks were shaken on an incubator shaker for 200rpm shaking speed and 120 min contact time to make sure equilibrium is reached. After equilibrium was reached, the solutions were filtrated and analyzed to determine the metal ion concentration.

3.5.2 Effect of initial metal ion concentration

Batch adsorption experiments were carried out by contacting 0.2 g of the lemna minor adsorbent with 100 mL of the metal ion solution of different initial concentrations (100 - 200 ppm) at pH 7.0 and at a temperature of 25 ^{o}C. The solutions were shaken for 120 min at a speed of 120 rpm and then filtered. The filtrates were analyzed for the residual metal ion concentration using the spectrophotometer.

3.5.3 Effect of adsorbent dose

Batch adsorption experiments were carried out in different adsorbent doses, from 0.2 to 1.0 g/l in a 100 mL solution containing 100 ppm of both metal ions at pH 7.0, for a contact time of 120 min and temperature of 25 ^{o}C. The samples were then shaken at 200 rpm, filtered, and the filtrates were analyzed using the spectrophotometer.

3.5.4 Thermodynamics studies

The following procedure was followed to determine the effect of temperature on bio sorption rate and to evaluate the enthalpy of adsorption (ΔH), entropy of adsorption (ΔS) and Gibbs free energy (ΔG). 100 mL of 100 ppm metal solution was taken in five conical flasks (Mafra *et al*, 2013, Bhadusha N and Ananthabaskeran, 2012). 0.3 g of adsorbent (lemna minor) was added in each of these flasks. The above five conical flasks were shaken in an orbital shaker at 200 rpm for equilibrium agitation time for five different temperatures (283, 293, 303, 313 and 323) K. These samples were filtered. The metal concentrations of the filtrates were determined by using UV Vis spectrophotometer.

3.6 Adsorption isotherms

When a solution is contacted with a solid adsorbent, molecules of adsorbate get transferred from the fluid to the solid until the concentration of adsorbate in solution as well as in the solid phase are in equilibrium. At equilibrium, equal amounts of solute eventually are being adsorbed and desorbed simultaneously. This is called adsorption equilibrium. The equilibrium data at a given temperature are represented by adsorption isotherm and the study of adsorption is important in a number of chemical processes ranging from the design of heterogeneous chemical reactors to purification of compounds by adsorption. Adsorption isotherm models such as Temkin, Langmuir and Freundlich models were used to fit the experimental data. It helps to find out the best fitting isotherm model in order to evaluate the efficiency of the prepared adsorbent and to develop a suitable batch adsorber design.

The adsorption capacity at equilibrium, qe (mg/g) was calculated by the following relationship:

$$\mathrm{qe} = \frac{c_o - c_e}{\mathrm{w}} * \mathrm{v} \quad \ldots\ldots\ldots..3.2$$

Where, C_o and C_e (mgL^{-1}) are the initial and equilibrium metal ion concentrations respectively.

3.7 Kinetic studies on metal ion Adsorption

Batch adsorption experiments were carried out by varying the contact time from 15 to 90 min at 25^{o}C, keeping other parameters such as pH, adsorbent dose and initial metal ion concentration constant at 7.0, 0.4 g/L and 100 ppm respectively. The samples were shaken at 200 rpm and withdrawn at 15 min time intervals. The filtrates were analyzed to determine their final concentrations by measuring their absorbance at 550nm for Pb and 600nm for Cu by using UV VIS spectrophotometer. Kinetic rate constant and order of adsorption were determined by using Pseudo first order, pseudo second order and intra particle diffusion (Yuh-Shan H, 2004).

3.8 Experimental design for biosorption study of Copper and Lead

Several experimental operating parameters have been found to influence the bio sorption process. The most important factors include the solution pH, bio sorbent dose and metal ion concentration (Nour T *et al*, 2014). For finding optimum conditions of the parameters for removal efficiency of lemna minor, data analysis was performed by Design Expert 6.0.8 software using CCD design. The experiment was designed to investigate the single and interaction effects of selected parameters. The analysis resulted in 40 experiments and the experiment were repeated again to improve reliability of the data. The factors and levels are illustrated below.

Table 3.1 Experimental factors and levels

Factors	**Levels**		
Initial metal ion concentration, ppm	100	150	200
PH	3	7	11
Adsorbent dose, g	0.2	0.3	0.4

Response variable: percentage removal efficiency of adsorbent

3.9 Desorption of the metals

Desorption of copper and lead from used lemna minor (duck weed) was studied using a 0.1M HCl solvent. Once the adsorption was carried out, the used adsorbent was sun sundried. The dried duck weed (1g) was taken in 250 ml conical flasks and 100 ml of solvent was added to it. After that, the solution was shaken at 120 rpm and the samples were withdrawn after every 30 minutes for analysis (Sunil J. Kulkarni, 2015).

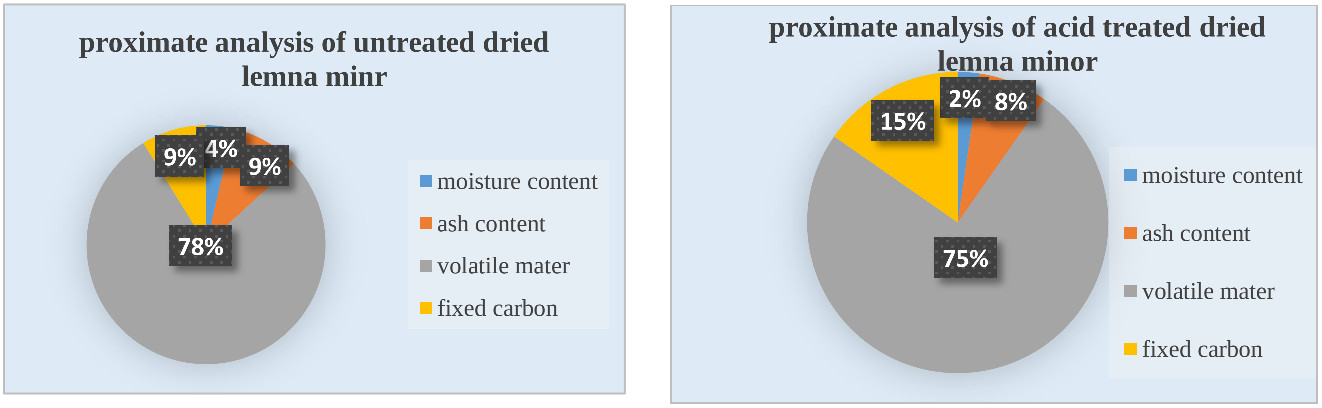
4. Result and Discussion

4.1Proximate Analysis

The proximate analysis of both acid treated and untreated Lemna minor are shown in figure 4.1 (a) and 4.1(b). A clear difference was observed on the calculated proximate analysis of the adsorbent. The moisture content, ash content and volatile matter values reduced significantly after treating the adsorbent with HCl acid. The reduction in moisture content might be due to the evaporation of water during the acid soaking process.

The reduction in ash content is attributed to the leaching out of some of the non-combustible mineral content of the lemna minor predominantly oxides of alkali and alkali earth metals during acid treatment. The reduction in value of ash content was favorable because the ash content serves as interference during the adsorption. The lower the ash content the better the starting material for adsorption (Khan *et al,* 2009).

The volatile mater is usually a mixture of short and long chain hydrocarbons, aromatic hydrocarbons and some sulfur (Khan A.A *et al*, 2009). The lower volatile content of the acid-washed lemna minor showed that some of organic molecules of the material was attacked and it is stable for adsorption experiment of Cu and Pb ions from aqueous solution. The increased value of fixed carbon is directly due to the reduction of moisture, ash and volatile mater content of lemna minor.



Figur4.1 Proximate analysis of unteated and acid treated lemna minor

4.1.1 Point of zero charge

An important indication whether the adsorbent surface is likely to become negatively or positively charged as a function of the pH is that the pH value at which the net electric charge on the surface of lemna minor is zero. This value is called pH of point of zero charge 'pHPZC' (Geethakarthi and Phanikumar, 2011). At pH value lower than the pHPZC (pH < pHPZC) the adsorbent surface has

a net positive charge, while at pH value higher than the pHPZC (pH > pHPZC) the surface has a net negative charge (Al- Degs *et al*, 2000) In Figure below the results were plotted with Δ pH against pH final and pH of point zero charge was determined at the point at which Δ pH = 0. The pHPZC of lemna minor adsorbent is **7.5**. Therefore, the adsorption of the metal ions at pH < pHPZC was not flavored, whereas at pH > pHPZC the adsorption of copper and lead was favored Venkatraman *et al.* (2011).

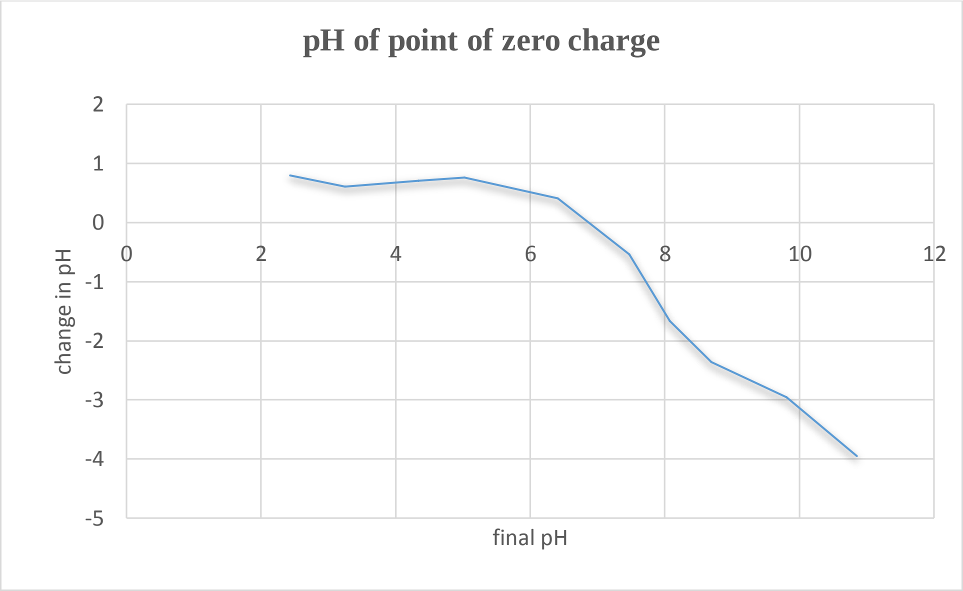


Figure 4.2 pH of point of zero charge of lemna minor

4.2 FTIR analysis

The FTIR spectrum plots of the lemna minor is shown in figure 4.3, displaying a number of adsorption peaks indicating the complex nature of the material examined. The broad, intense adsorption peaks around 3,440cm^{-1} are indicative of the adsorption of water molecules, resulting from the O-H stretching mode of hydroxyl groups characteristic of adsorbed water, while the bands at 2,900 cm^{-1} were attributed to C-H interactions with the surface of the adsorbent (Al-Qodah et al, 2009). The peak around 2300 cm^{-1} is due to the N-H stretching attributed to nitrile group's amine bond. The peak around 1,635 cm^{-1} is due to the C=C stretching that can be attributed to the aromatic C-C bond, and the peak at 1600 cm-1is as a result of Aromatic rings C-C=C symmetric stretch. Ethers C-C-O stretching and - COOR ester stretching were observed at peaks around 1400 cm^{-1} and 1250 cm^{-1} respectively. The carbon- sulfur bond C-S stretching is associated with the peak 600cm^{-1}. The O-O stretching is due to the peak at 800cm $^{-1}$.

As it can be seen from the graph, there is a slight difference between the untreated and treated lemna minor. The untreated adsorbent has a weak and broad peak than the acid treated lemna minor because the molecules in the untreated one transmit most of the light and absorbs less. The acid treated adsorbent is however with a sharp and strong and intense peak absorbing high amount of light. At peak around 3400 cm^{-1}, the acid treated lemna minor showed a stronger and sharp peak attributed to the Alcohol group of - OH bonds while the untreated one being a weak and broad peak was associated with a carboxylic acid of hydrogen bonded OH stretching.

Similarly, the sharper and very intense peak of acid treated adsorbent at 1600cm^{-1} is attributed to the alkene groups while the weak and somewhat broad peak at the same wave number of the untreated one is due to the C=O stretching. There was no significant intensity change after heavy metal adsorbed onto lemna minor. A slightly difference in their vibration band intensity was observed at 2900 cm^{-1} and the graph confirmed the participation of functional groups in adsorption process mainly by carboxylic acid and amines (Adewumi O *et al*, 2017). A small difference was also observed between copper loaded and lead loaded lemna minor particularly at peak around 2800 cm^{-1}. The copper loaded adsorbent showed a double broad and strong peak due to the CH_3-CH_3 methyl symmetry alkane groups while the lead loaded adsorbent showed a relatively sharp peak which is due to the presence of aldehydes C-H stretch off C=O bonds. A similar result has been found by Handojo *et al*, 2016.

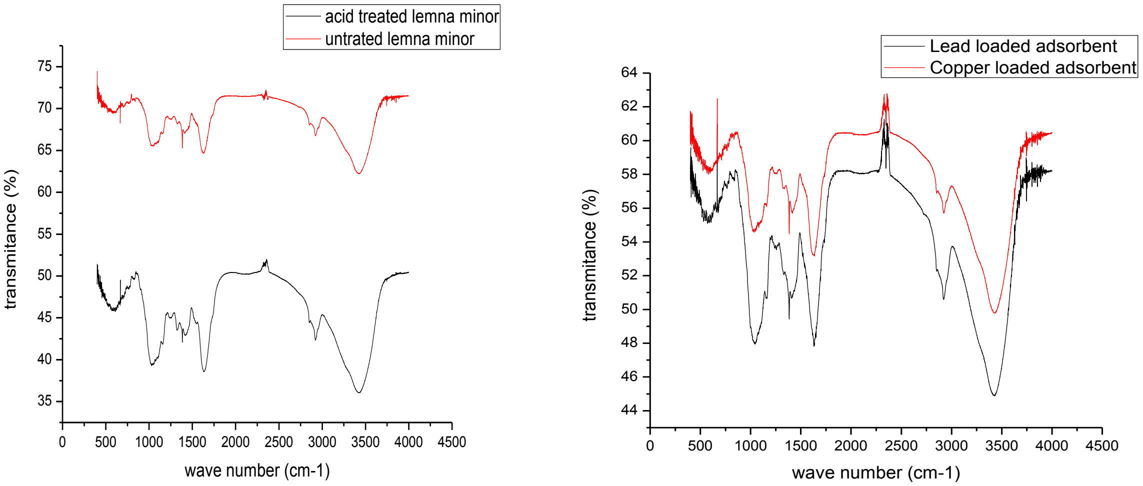


Figure 4.3 FTIR spectra of untreated, acid treated, lead loaded and copper loaded fine lemna minor powder

4.3 Surface area analysis

The surface area was calculated using equation 3.1 under the materials and methods section. The titrated volume of NaOH required to raise the pH of the solution from four to nine was 1.

6ml.The resulting specific surface area was 31m^2 per gram. Similar result was obtained by (Balarak et al 2015).

4.4 Effect of pH

The graph in figure 4.4 showed that the percentage removal of both copper and lead increased with pH. This was due to the fact that the Point of zero charge of the lemna minor was at pH = 7.5. The cationic adsorption is favored at pH greater than pHpzc. Minimum percent removal for Cu^{+2} = 33 % and for Pb $^{+2}$ = 44% was observed at pH = 2. Maximum removal for copper was 92.95 % at pH 9 and maximum removal for lead was 89.11% obtained at pH 11. At low pH, H_3O^+ ion competes with Cu^{2+} and Pb^{+2} for binding and surrounded hydronium ions (H$^+$) preventing the copper ions from approaching the binding sites and it could be responsible for low adsorption capacities (Karthikeyan *et al*, 2007). Protonated adsorption sites were incapable of binding metal ions due to electrostatic repulsion between positively charged metal ions and positive charged sites. Hence, only low percentage of Pb^{2+} and Cu^{2+} ions were adsorbed. As the pH increased, there were fewer H+ ions present in the solution and consequently more negatively charged sites were made available and this facilitated greater metal ions uptake by electrostatic attraction. It also can be due to precipitation of copper and lead on the surface of the adsorbent (Wang *et al*, 2005; Sheng *et al*, 2004; Memon *et al*, 2008).

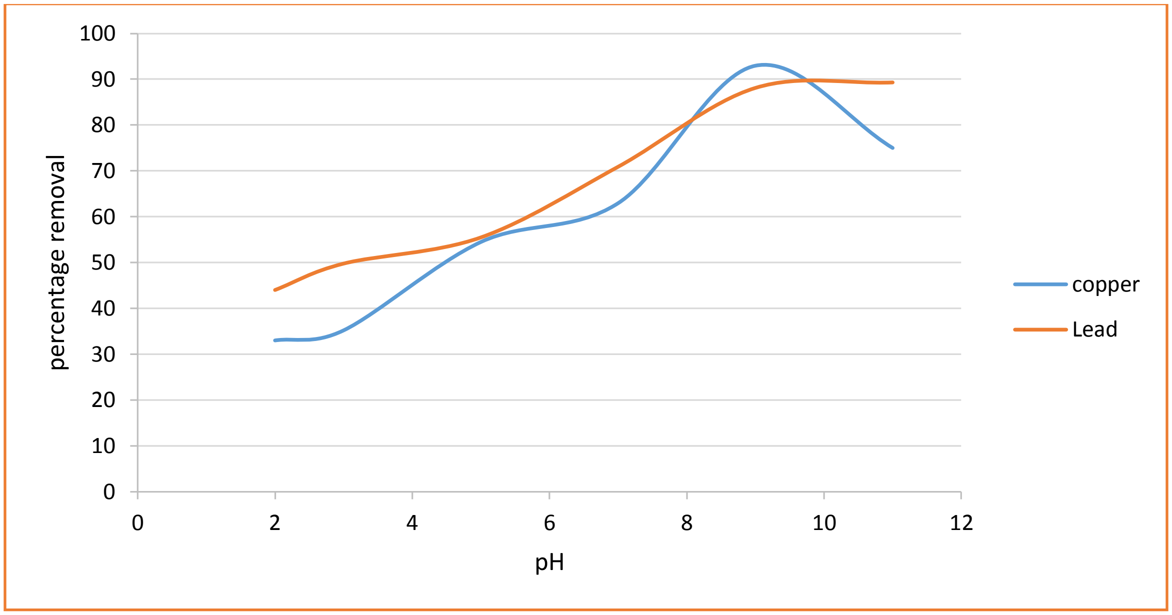


Figure 4.4 Plot of percent removal of Cu^{+2} and Pb^{+2} versus pH on ATLM at contact time = 2hr, initial concentration = 100 ppm, adsorbent dosage = 0.2 g in 100 ml of metal ion solution, agitation speed = 200 rpm, particle size 820- 850 μm and temperature = 25^0c

4.5 Effect of initial metal concentration

Figure 4.5 indicates the removal of both Cu and Pb decreased with increase in metal concentration from 50 – 250 ppm. The % removal at 50 ppm was 92.38 and 87.72 for Pb and Cu respectively. This can be due to the interaction of all metal ions present in the solution with binding sites. At higher concentrations, more metal ions are left un adsorbed in solution because of saturation of adsorption sites (Azouaou *et al*, 2010; Mausumi *et al*, 2006; Mohammad Mehdi *et al*, 2011). The initial Pb^{2+} and Cu^{+2} concentration constitutes a significant driving force allowing the ionic mass transfer between the aqueous and the solid phases (Adewumi O *et al*, 2017). On the other hand, the adsorption capacity q_e (mg/g) increased with an increase in metal concentration.

Maximum adsorption capacity(60 mg/g for Copper and 64 mg/g for lead) was obtained at 250 mg/l initial metal concentration. Particularly, the increase in adsorption capacity (qe) with an increase in concentration is due to increase in driving force of the concentration gradient to overcome all mass transfer resistance of metal ions between aqueous and solid phases and accelerate the probable collision between metal ions and sorbents, thus resulting in higher uptake of metal ions (Ushakumary,2013).

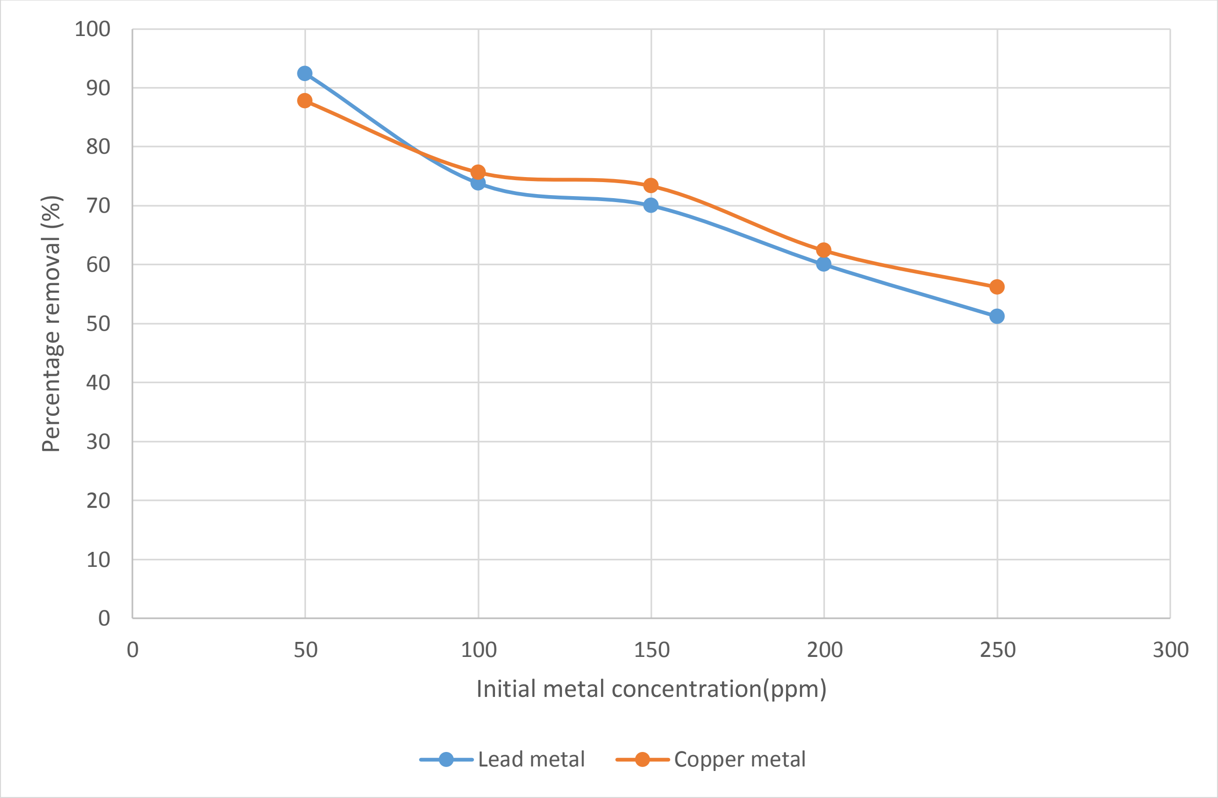


Figure 4.5 Plot of percent removal of Cu^{+2} and Pb^{+2} versus initial metal ion concentration on ATLM at contact time = 2hr, adsorbent dosage = 0.2 g in 100 ml of metal ion solution, pH=9 agitation speed = 200 rpm, particle size 820- 850 μm and temperature = 25^0c

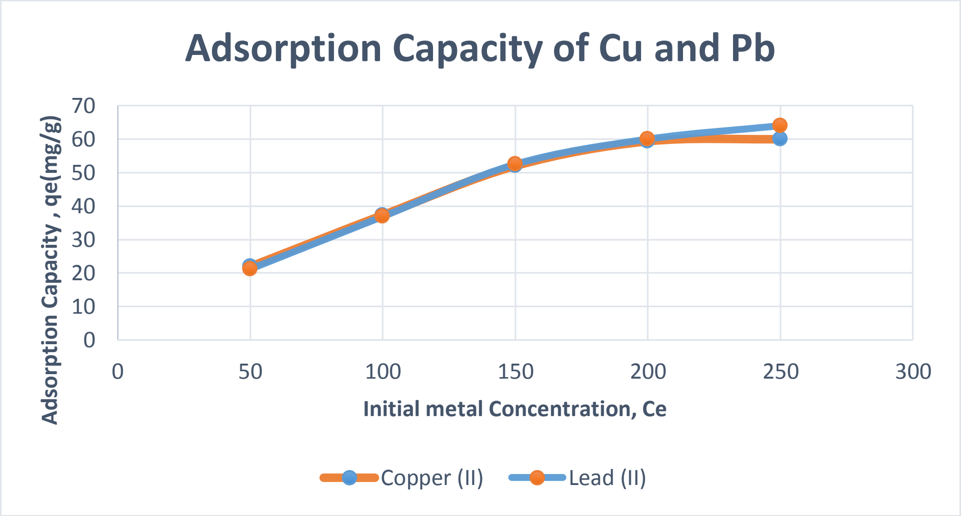


Figure 4.6 A plot of Adsorption Capacity versus initial metal concentration

4.6 Effect of adsorbent dose

The percentage removal of metal ions Cu^{+2} and Pb^{+2} against adsorbent dosage were plotted and shown in Figure 4.6. The figure indicated the percent removal of both tend to increase with an increase adsorbent dose. The increase in the weight of adsorbents increased the rate of adsorption due to availability of more adsorption surface sites. (Azouaou *et al*, 2010; EI-Said *et al*, 2010; Saifuddin and Kumaran 2005). Percentage removal of metal ions increased as weight of the adsorbent increases from 0.2 to 1.0 gm. Such behavior is obvious because the number of active sites available for metal removal become higher as the amount of the adsorbent increases. This may also be attributed to aggregation of adsorbent surface area available to ion and increase in diffusion path lengths (Yasemin B *et al*, 2007).

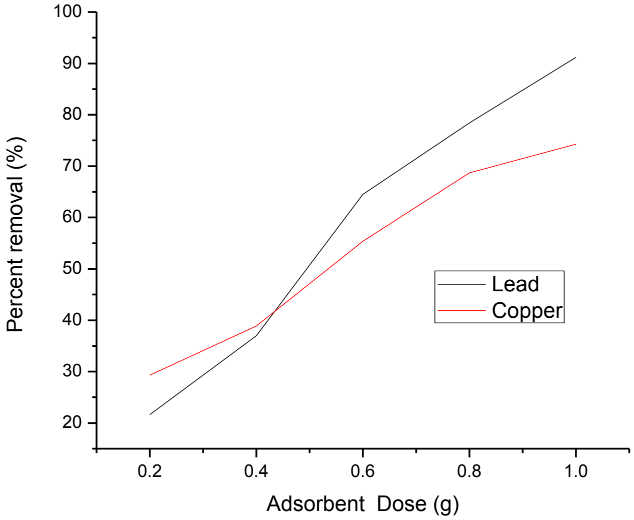


Figure 4.7 Plot of percent removal of Cu^{+2}(black) and Pb^{+2} (red)versus adsorbent dosage on ATLM at contact time = 2hr, initial concentration = 100 ppm, pH = 9 in 100 ml of metal ion solution, agitation speed = 200 rpm, particle size 820- 850 μm and temperature = 25^0c

4.9 Temperature effects

The figures from 4.8 - 4.11 indicates the effect of temperature on both adsorption capacity and percent removal of Cu^{+2} and Pb^{+2} ions. The result showed that the percent removal and adsorption capacity of the adsorbate increased with increase in temperature on a temperature range of 20^0c – 30^0c. This increase in adsorption is mainly due to increase in number of adsorption sites caused by breaking of some of the internal bonds near the edge of the active surface sites of the adsorbents (Xia *et al*, 2011; Panda *et a*l, 2009; Jain and Sikarwar, 2008; Wang and Wang, 2008).

This can be attributed to increase in the number of molecules attaining sufficient energy to undergo chemical reaction which shows chemisorption (s.v.Mohan *et al*, 2008). It also can be due to increase in the mobility of the large metal ions with temperature since sufficient energies were acquired to interact a large number of Cu and Pb molecules with active sites at the surface. Moreover, increasing temperature may produce a swelling effect within the internal structure of the adsorbent enabling large metal ion to penetrate further (Dogan and Alkan, 2003). The increment of the adsorption capacity of the adsorbent at higher temperatures was attributed to the enlargement of pore size and activation of the adsorbent surface. A further temperature increment above 30 ^{0}C resulted in exothermic nature of adsorption. This could be attributed to the formation of big crystals during the adsorption process.

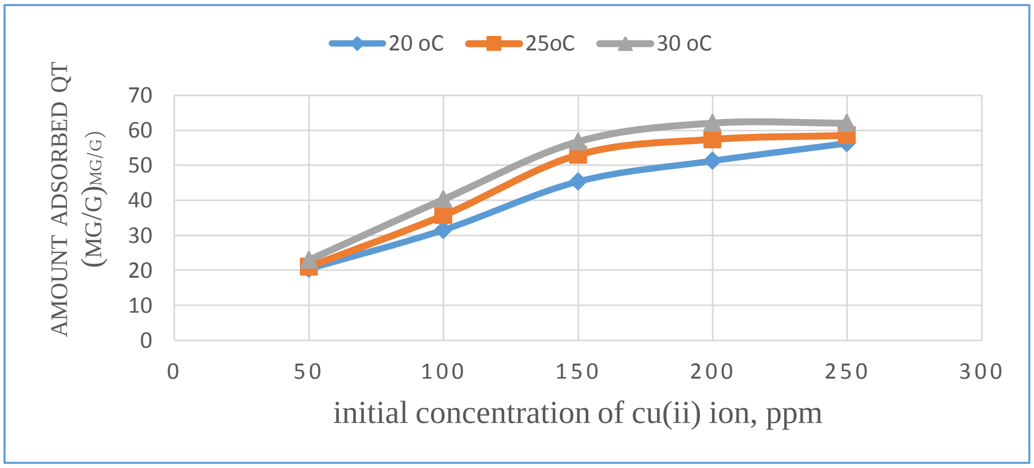


Figure 4.8 a Plot of adsorption capacity of Cu^{+e2} versus initial metal concentration on ATLM at contact time = 2hr, initial concentration = 100 ppm, Adsorbent dosage= 0.2, pH = 9 in 100 ml of metal ion solution, agitation speed = 200 rpm, particle size 820- 850 µm

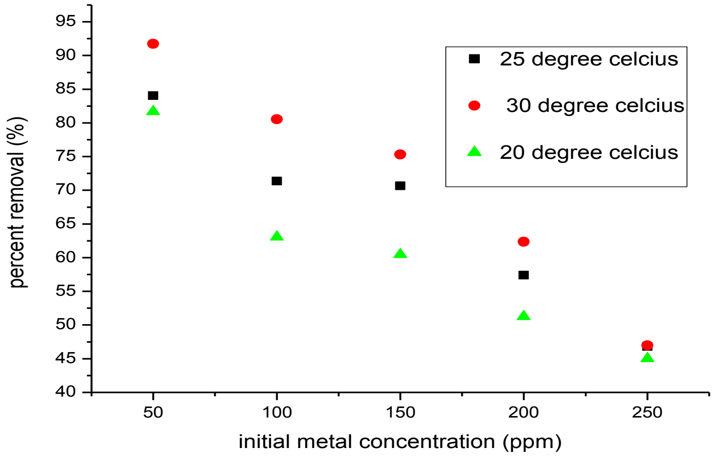


Figure 4.8b Plot of percent removal of Cu^{+2} versus initial metal concentration on ATLM at contact time = 2hr, pH = 9 in 100 ml of metal ion solution, agitation speed = 200 rpm, particle size 820- 850 µm and temperature = 25^0c

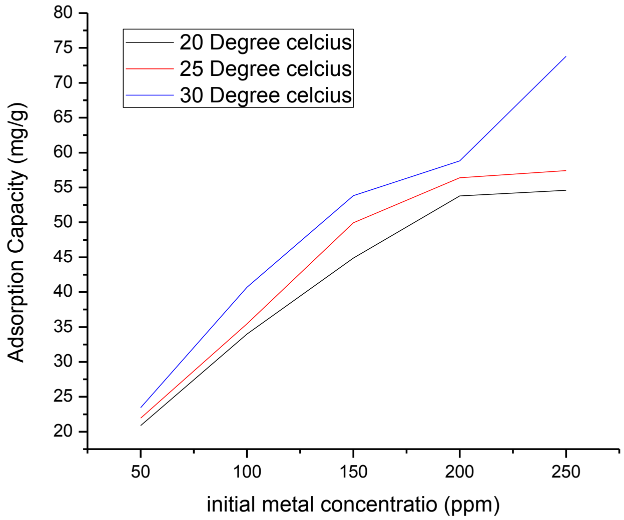


Figure 4.8c Plot of adsorption capacity of Lead (II) ion versus initial metal concentration on ATLM at contact time = 2hr, Adsorbent dosage= 0.2, pH = 9 in 100 ml of metal ion solution, agitation speed = 200 rpm, particle size 820- 850 µm and temperature range 20^0c,25^0c,30^0c

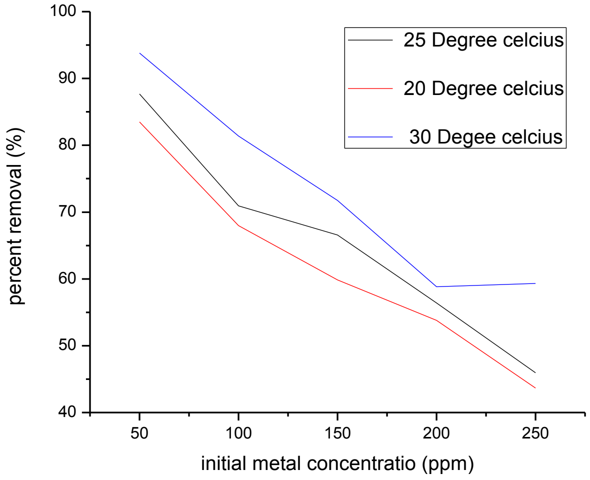


Figure 4.8 d Plot of percent removal of Lead (II) ion versus initial metal concentration on ATLM at contact time = 2hr, Adsorbent dosage= 0.2, pH = 9 in 100 ml of metal ion solution, agitation speed = 200 rpm, particle size 820- 850 µm and temperature range 20 ^{0}c,25 ^{0}c,30 ^{0}c

4.10 Adsorption equilibrium studies

Adsorption isotherms are mathematical models that describe the distribution of the adsorbate species among liquid and adsorbent, based on a set of assumptions that are mainly related to the heterogeneity/homogeneity of adsorbents, the type of coverage and possibility of interaction between the adsorbate species. Adsorption isotherms indicate the distribution of solute between the liquid and solid phases. Adsorption data are usually described by adsorption isotherms, such as Langmuir, Freundlich and Temkin isotherms. These isotherms relate metal uptake per unit mass of adsorbent, *qe*, to the equilibrium adsorbate concentration in the bulk fluid phase *Ce*. The linearized Langmuir, Freundlich and Temkin adsorption isotherms were used in order to check the best fitting model. Langmuir and freundlich model fitted very well to the sorption data in the studied concentration range with a higher correlation coefficient R^2 value (0.9948 and 0.9944 for Cu^{+2} and Pb^{+2}) Langmuir; (0.9919 for copper and 0.99 for lead) freundlich respectively. The Langmuir model assumes that maximum adsorption occurs when a saturated monolayer of solute molecules is present on the surface of the adsorbent, the energy of adsorption is fixed and molecules of the adsorbent will not migrate in the surface plane (P. Senthil *et al*, 2009). The q_m and K_L values of the Langmuir constants were calculated as 3.4 mg/g, 0.0422 L/mg for Copper and 4.468 mg/g, 0.066 L/mg respectively. R_L a separation factor given as $1/(1+K_LC_0)$ also showed a result between 0 and 1 which explains a favorable adsorption (N. rajamohan *et al*, 2013).

Table 4.2 Langmuir separation factor R_L for copper and Lead

Concentration (ppm)	Langmuir Separation factor RL	
	Copper (II) ion	Lead (II) ion
50	0.32	0.23
100	0.19	0.13
150	0.136	0.09
200	0.105	0.07
250	0.08	0.057

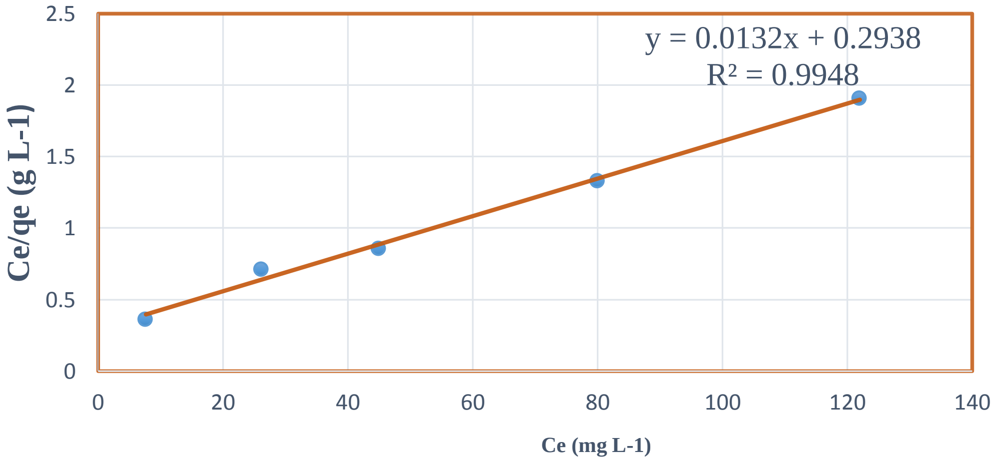


Figure 4.9a Langmuir isotherm of Cu^{+2}

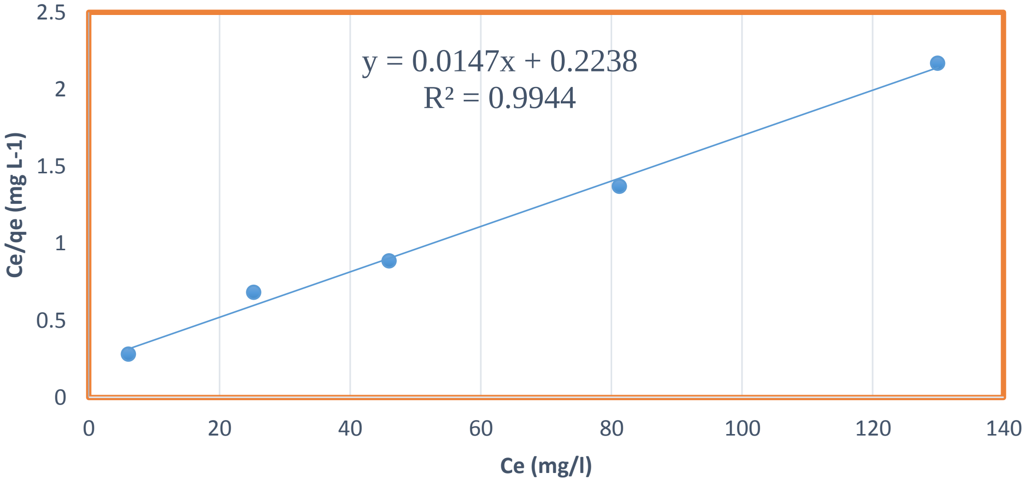


Figure 4.9b Langmuir isotherm of Pb^{+2}

Frendulic isotherm model was plotted as log Ce versus log qe on figure 4.9a and 4.9b to test the validity generate the values of freundlich constants K_f indicator of adsorption capacity and" n" adsorption intensity. The freundlich constants K_f and n were 10.5 mg $g^{-1}(mg\ L^{-1})^{1/n}$, 2.411 for Copper and 9.52 mg $g^{-1}(mg\ L^{-1})^{1/n}$, 2.18 for Lead respectively. The correlation coefficient R^2obtained was 0.9919 for Copper and 0.99 for Lead. Even if these values are satisfactory, they are less than those obtained by Langmuir models.

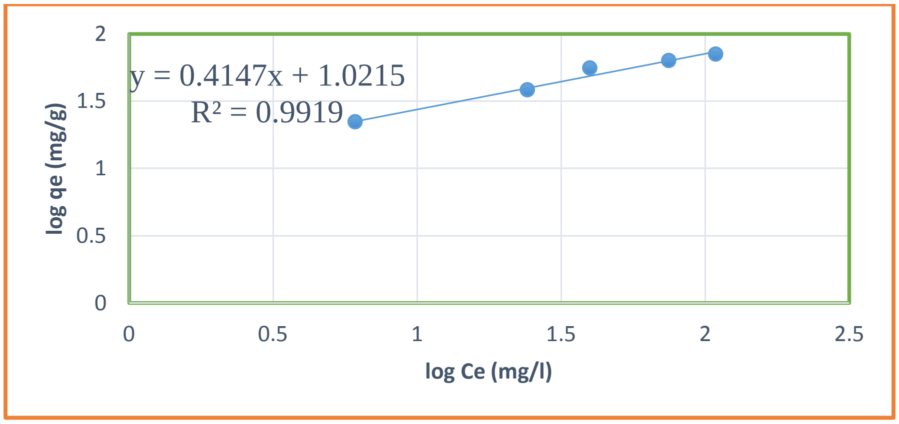


Figure 4.9c Frendulic isotherm of Cu^{+2}

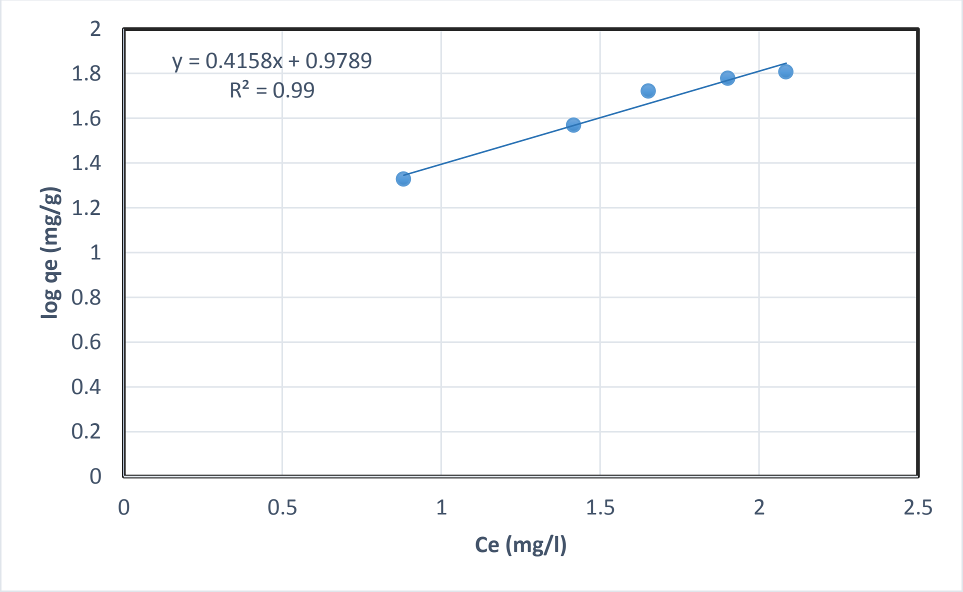


Figure 4.9b Frendulic isotherm of Pb^{+2}

A plot of *q*e versus ln *C*e is depicted on figure 4.10a and 4.10b to test the validity of Temkin isotherm and determine the equilibrium constants.B_1 and $K_{T.}$ K_T is the equilibrium binding constant (l/mol) corresponding to the maximum binding energy and constant B_1 is related to the heat of adsorption. As seen on the graph below the R^2 values obtained are almost same with those obtained from freundlich isotherm.it can be concluded that the adsorption process followed all the adsorption isotherms but best fits to the Langmuir isotherm model and freundlich isotherm.

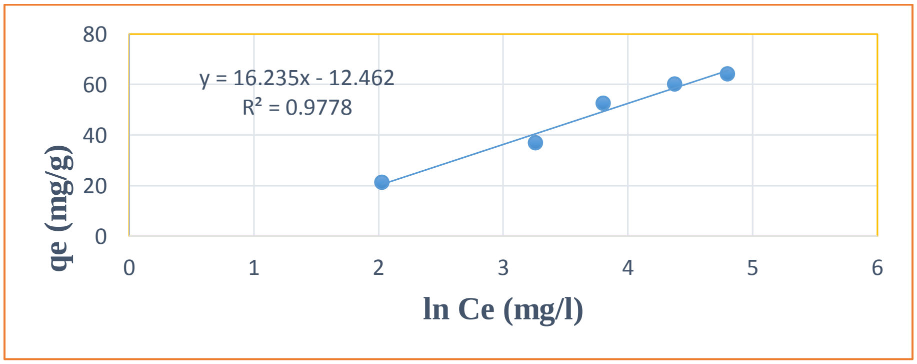


Figure 4.9E Temkin isotherm of Pb^{+2}

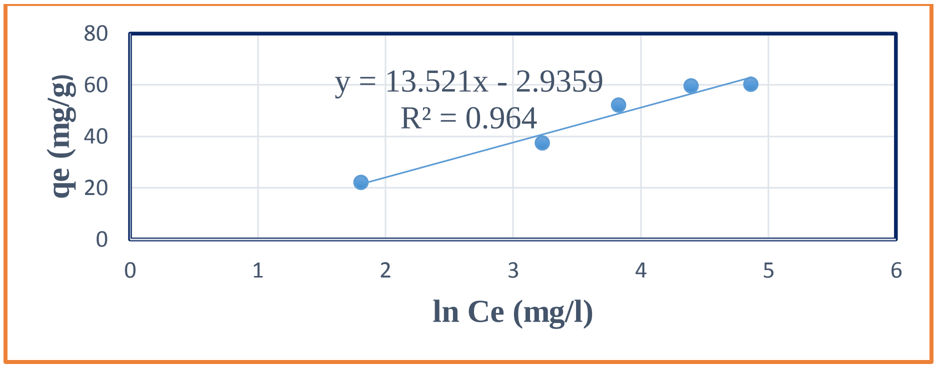


Figure 4.9 F Temkin isotherm of Cu^{+2}

Table 4.3 summarizes the results of the isotherm equilibrium constants for the three different equilibrium isotherms evaluated. Based on their respective correlation coefficients (R^2) values, Langmuir and freundlich isotherm seemed to represent the equilibrium adsorption with higher correlation coefficient for both metals.

Table4.3 summary equilibrium parameter values of Pb^{+2} and Cu^{+2} with different isotherms

Heavy Metal ions	Langmuir Isotherm			Freundlich Isotherm			Temkin Isotherm		
	q_m (mg/g)	$K_L min^{-1}$	R^2	n	K_f g/(mg*min)	R^2	K_i mg g^{-1} $min^{1/2}$	B_1 (RT/b)	R^2
Pb^{+2}	4.468	0.066	0.994	2.18	1.25	0.991	2.153	13.521	0.978
Cu^{+2}	3.4	0.042	0.995	2.41	10.5	0.99	0.804	16.235	0.964

4.11Thermodynamics studies

The adsorption equilibrium constants were calculated from the best fit Langmuir model K_L at 20 ^{0}c = 21.6L/mg, K_L at 25°c = 16.33 L/mg, K_L at 30^{0}c = 13.24L/mg. These values again were used for determining the free Gibbs energy $\Delta G = - RT \ln K_c$, were R is universal gas constant = 8.314 Joule/mole K. -ΔG = 7.4889 Kjoule/mol, 6.923 Kjoule/mol, 6.51 Kjoule/mol at 20^{0}c, 25^{0}c and 30^{0}c respectively. ΔH and ΔS values were determined from the slope and intercept the vant 'hoff equation. $\ln Kc = \Delta H^*/RT + \Delta S^*/R$ by using figure 4.11

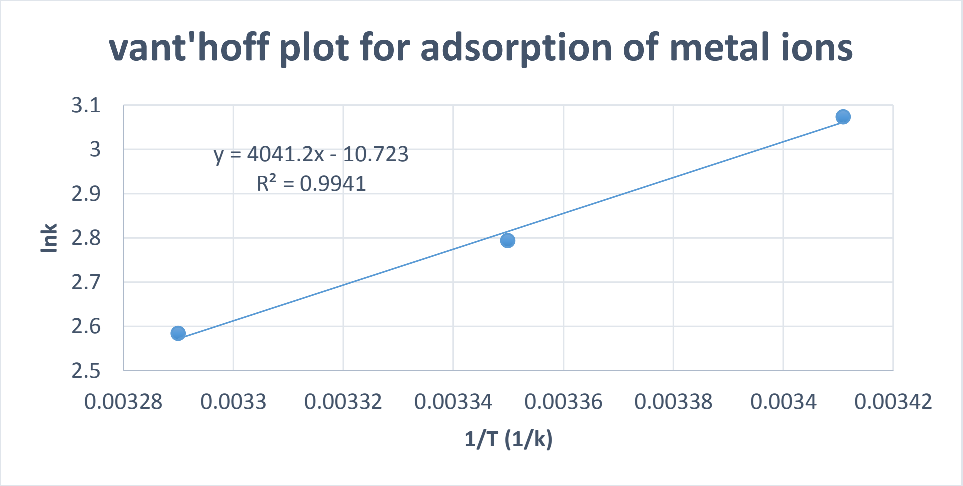


Figure 4.10 Van't Hoff Plot for adsorption of Cu and Pb onto ATLM

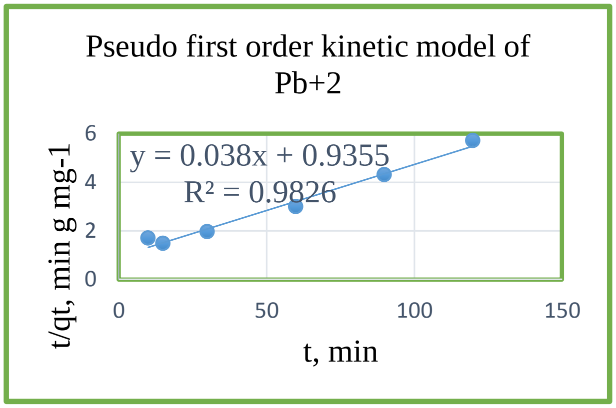
The change in enthalpy ΔH and entropy ΔS were calculated as 3.359 Kjoule/mol and -89.15kjoule/mol respectively. The result of thermodynamic parameters showed that the values of free Gibs energy ΔG increased with increase in temperature. These is attributed to a higher driving force resulting in a greater adsorption affinity at higher temperatures (Neamtu, M, 2004). The negative values the Gibbs free energy for both metals showed that the adsorption process was spontaneous and that the degree of spontaneity of the reaction increases with increasing temperature (Dong X *et al*, 2014). The positive value of enthalpy ΔH reveals the endothermic nature of adsorption. The negative entropy indicated the decreased randomness at the solid liquid interface during the adsorption of metal ions on to ATLM (Aman *et al*, 2008).

4.12 Kinetic studies

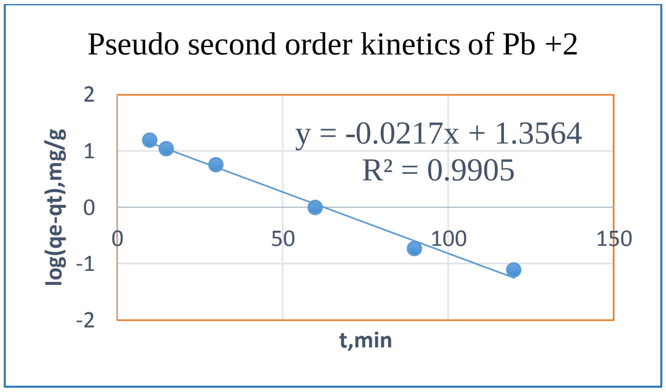
The kinetic models pseudo first order, pseudo-second-order and intra-particle diffusion were used to analyze the kinetic behavior of the metal sorption process. The kinetic rate constants, adsorption capacities and correlation coeffient were calculated from the slope and intercept of their corresponding graphs (table 4.4). The calculated, q_{cal} values derived from the pseudo second order model for sorption of metal ions were very close to the experimental (q_{exp}) values. The pseudo second order equation appeared to be the best fitting model than both first order and intra particle diffusion equations because it has highest R^2 value (Yuvaraja, G, 2014). This kinetic study confirmed that biosorption of Cu^{+2} and $Pb^{+}2$ on acid treated dried *Lemna* minor was a multistep process involving adsorption on the external surface (diffusion of metal ions from the bulk solution to the surface of the adsorbent) and diffusion into the interior (diffusion of metal ions from the surface to internal sites. (Nguyen T. *et al*, 2013). Therefore, the adsorption process can be approximated more favorably by the pseudo-second order kinetic model, suggesting the adsorption process to be a chemisorption process involving exchange or sharing of electrons between adsorbent and adsorbate .(Lakshmi *et al*, 2009). stated if a plot of qt versus $t^{1/2}$ is linear and passes through the origin, then intra-particle diffusion is the sole rate-limiting step. However, Figure 4.12 c and 4.13b revealed the linear plots did not pass through the origin for each metal. Although intra-particle diffusion was involved in the adsorption process, it was not the sole rate-controlling step.

Table 4.4 pseudo first order, pseudo second order and intraparticle diffusion parameters

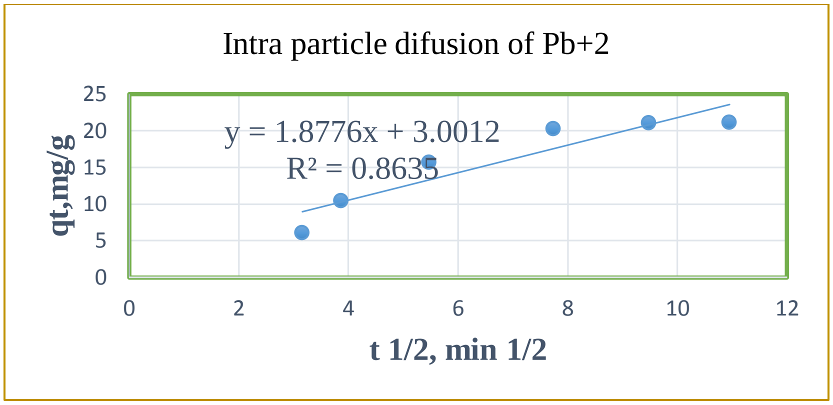
Heavy Metal ions	First order kinetic model			Pseudo Second order			Intraparticle Diffusion		
	q_o (mg/g)	K_1 min^{-1}	R^2	q_o(mg/g)	K_2 g/(mg*min)	R^2	K_i mg g^{-1} $min^{1/2}$	C	R^2
Pb^{+2}	22.92	0.049	0.983	26.315	0.0015	0.995	1.877	3.001	0.864
Cu^{+2}	40.15	0.006	0.921	25.18	0.002	0.999	1.606	5.745	0.994



A

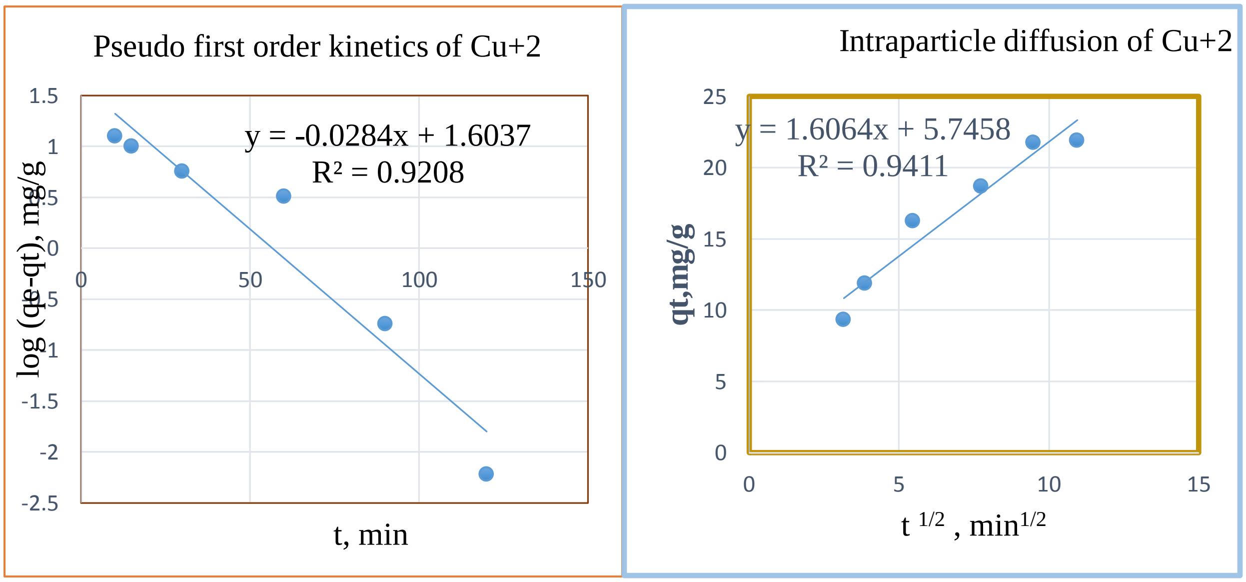


B

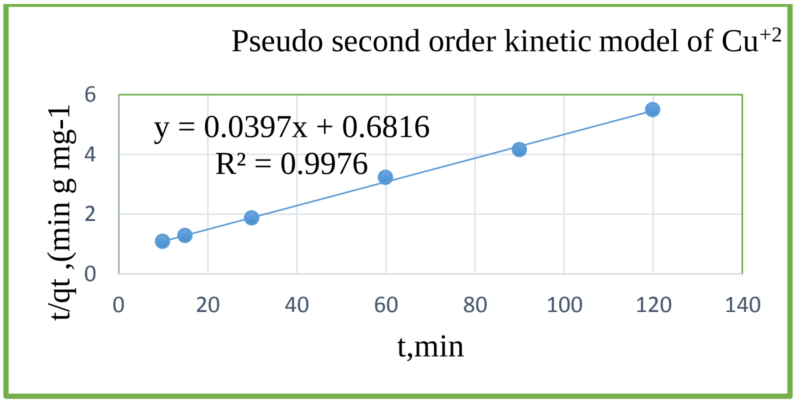


C

Figure 4.11 Pseudo second order **A**, pseudo first order **B,** intra particle diffusion **C** of Lead



A B



C

Figure 4.12 pseudo first order A, intraparticle diffusion B, and pseudo second order of Cu (II)ion

4.13 Desorption study

Desorption efficiency is defined as the percent extraction of heavy metal ions that are loaded onto the biomass. Recovery of metal ions from the loaded adsorbents is necessary for disposal as well as for reuse of adsorbate (Chen et al., 2007). Desorption of Cu^{+2} and Pb^{+2} were carried out by using a 100ml of 0.1M HCl in to 250 volumetric flasks with 0.1 g of 43.86 mgl^{-1} of copper and 42.38 mgl^{-1} of Lead loaded sun dried lemna minor. The samples were withdrawn at 15 minutes' time interval and their concentration was measured. The calculated percent desorption is shown on the table below. The percent desorption increased with time for both metals and maximum desorption observed at 60-minute contact time with 80% for Lead and 72.2% for Copper.

Table 4.5 Desorption of Cu^{+2} and Pb^{+2} by using a 100ml of 0.1M HCl in to 250 volumetric flasks with 0.1 g of 43.86 mgl^{-1} of copper and 42.381of lead loaded sun dried lemna minor

Time (minute)	Absorbance Of Copper	Absorbance Of lead	C_t Cu^{+2} mg L^{-1}	C_t Pb^{+2} mg L^{-1}	Percent desorption of Copper (%)	Percent Desorption of Lead (%)
15	.215	.101	7.88	6.83	18.6	16.1
30	.225	.209	20.26	17.71	46.16	41.7
45	.213	.217	31.45	33.59	71.7	79.25
60	.231	.223	31.69	33.92	72.2	80.03

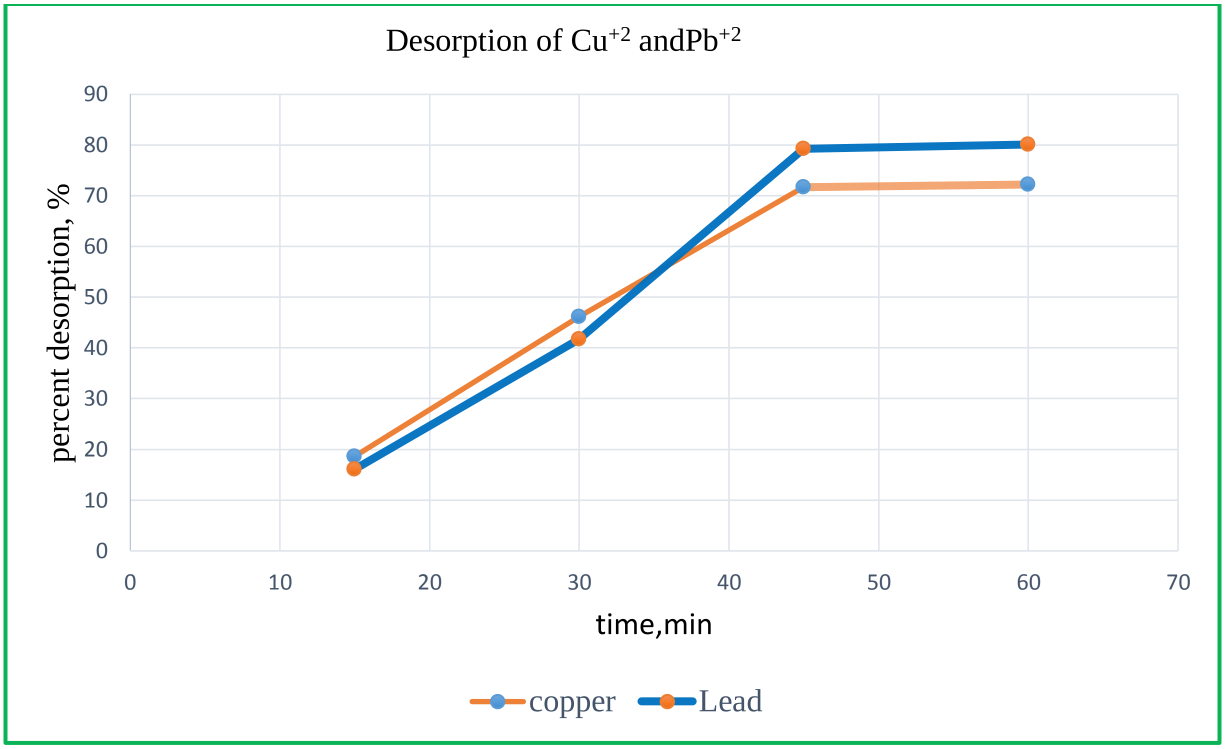


Figure 4.13 percent desorption of cu^{+2} and Pb^{+2} with 0.1M HCl

4.14 Data analysis using Design Expert 6.0.8

Effect of concentration, pH and adsorbent dose on percentage removal was analyzed using Design-Expert 6.0.8 software to check the significancy of the model and the factors using the datas obtained from the experiment.

The ANOVA summary for copper and lead percentage removal are given in Table 4.6a, 4.6b.The parameters with probability value less than 0.05 are said to be significant. The probability of the model for both metals was <0.0001 showing the sugested model was highly significant. The factors A(adsorbent dose), B(initial metal concentration), C(pH), and C^2 for copper percentage removal , were the only statistically significant ($P<0.05$) model terms at the 95 % confidence level. Unlike the ANOVA for copper removal, all the factors and termsA, B, C, A^2, B^2, C^2, AB, AC, BC under Percentage removal of lead were significant.This showed that the model better fitted to lead removal .

Table 4.6a, 4.6b analysis of variance of Cu $^{+2}$

Source	Sum of Squares	DF	Mean Square	F value	Prob>F
Model	1228.42	9	136.49	36.56	<0.0001 significant
A	433.10	1	433.1	115.99	<0.0001
B	60.12	1	60.12	16.10	0.0025
C	627.90	1	627.90	168.17	<0.0001
A^2	6.33	1	6.33	1.7	0.2219
B^2	0.20	1	0.20	0.053	0.8232
C^2	30.54	1	30.54	8.18	0.0170
AB	0.45	1	0.45	0.12	0.7353
AC	16.82	1	16.82	4.50	0.0598
BC	0.051	1	0.051	0.014	0.9091
Residual	37.34	10	3.73		
Lack of Fit	30.72	5	6.14	4.64	0.0587 not significant

Source	Sum of Squares	DF	Mean Square	F value	Prob>F
Model	1608.87	9	178.76	30.11	<0.0001 significant
A	127.66	1	127.66	29.82	0.0003
B	111.16	1	111.16	18.73	0.0015
C	35.38	1	35.38	5.96	0.0348
A^2	643.98	1	643.98	108.48	<0.0001
B^2	183.46	1	183.46	30.91	0.0002
C^2	430.98	1	430.88	72.58	<0.0001
AB	59.08	1	59.08	9.95	0.0103
AC	47.63	1	47.63	8.02	0.0178
BC	79.76	1	79.76	13.44	0.0043
Residual	59.36	10	5.94		
Lack of Fit	44.48	5	8.90	2.99	0.0043 not significant

A = adsorbent dose B = initial metal concentration C = pH

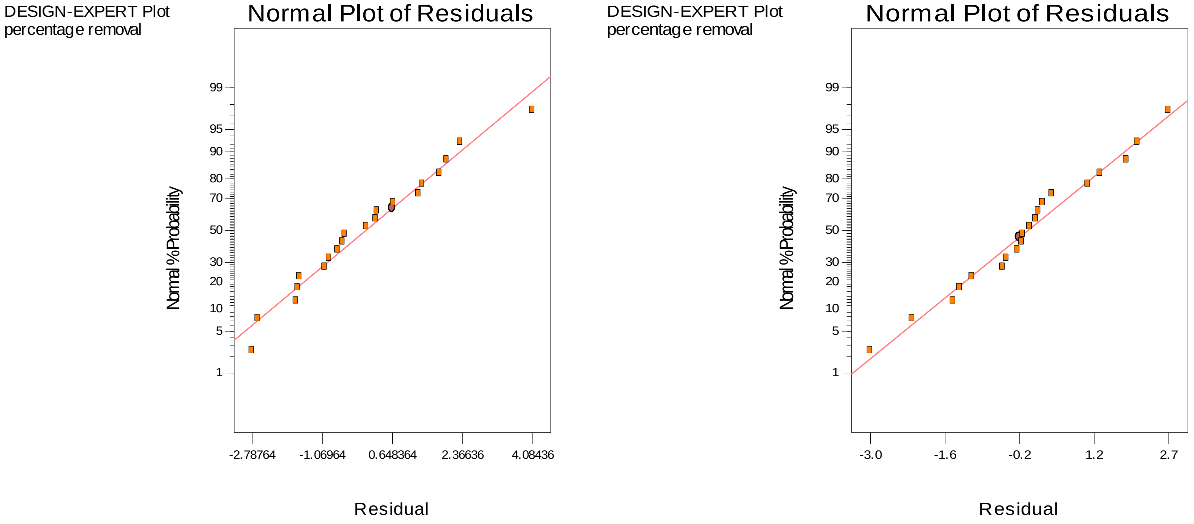
According to the ANOVA results, adsorbent dose, initial metal concentration and pH were dominant factors affecting bio sorption of copper and lead. Previous literatures also confirmed that bio sorption of heavy metals is strongly affected by the above three factors (yusra safa et al, 2010). Unlike to the single effect of the factors where all appeared to be significant, the interaction effects for lead and copper were different. All the three interactions AB AC, and BC for lead removal were highly significant showing the bio sorption of lead as a result of the interaction of all the factors. However, only the interaction of adsorbent dose and pH were significant for copper removal. The other two combinations of the factors were not significant terms.

4.14.1Model adequacy check

Model adequacy was checked by analysis of variance. The regression model was found to be significant with the correlation coefficients R-Squared, adjusted R-Squared and predicted R-Squared having a value of closer to unity for both metals. Acording to the values of R squared ,96.44 % for Lead and 97.05% for Copper of the data can be explained,analysed, examined by this model .The diference between the adjusted R squared and pridicated R - squared is less than 0.2 meaning that Pred R-Squared is in reasonable agreement with the Adj R-Squared as recommended by the software. The Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio obtained for both metals indicates an adequate signal.This model can be used to navigate the design space.Therfore,the model is found to be adequate for biosortption of copper and lead.The actual versus predicted graph is constructed by using the actual values from the experiment and those pridicted values calculated from the model equations.the point are all scatered along the line.the scatered of the data is perfectly matched showing the model is designed very well.the grph of normal plot of residuals also showed that the points followed the striaghtline meaning they are best fitted to the model.

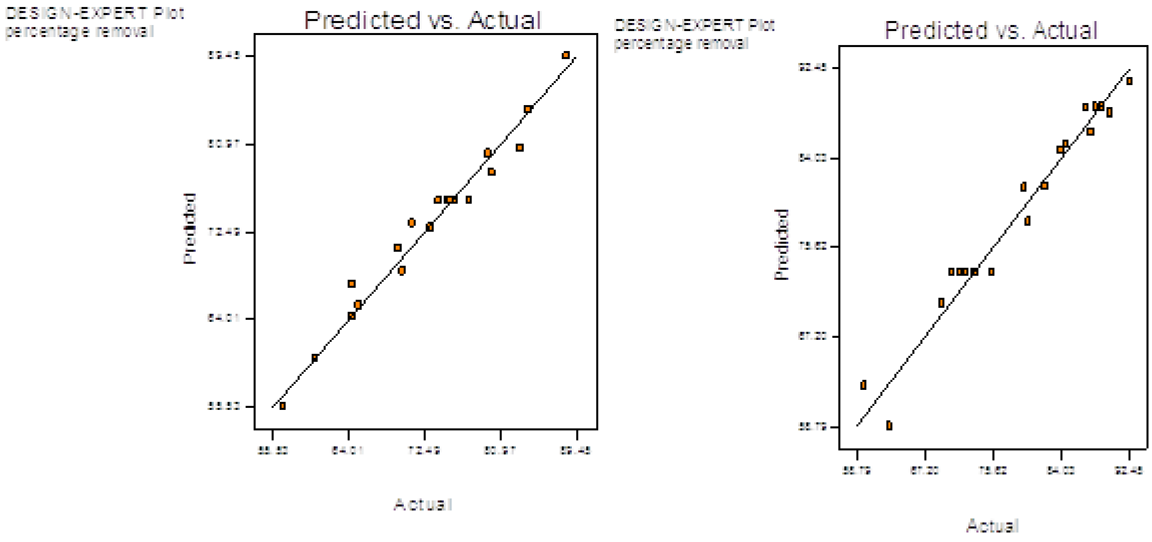
Table 4.7 model adequacy parameters for Copper and Lead biosorption

Copper (II)				**Lead(II)**			
Std.Dev	1.93	R-Squared	0.9705	Std.Dev	2.44	R-Squared	0.9644
Mean	73.17	Adj R-Squared	0.9440	Mean	78.66	Adj R-Squared	0.9324
C.V.%	2.64	Pred R-Squared	0.8054	C.V.%	3.10	Pred R-Squared	0.7589
PRESS	246.27	Adeq Precision	24.821	PRESS	402.19	Adeq Precision	18.738



A B

Figure 4.14 normal plot of residuals versus % probability for A Lead (II) and B Copper (II)



A B

Figure 4.15 Predicted versus actual experimental values for copper (A) and lead (B) bio sorption

4.14.2 Single and interaction effects

Single effects

The main and interaction effects observed on the analysis of variance were furtherly studied with the help of single and interaction graphs of design expert. The percentage removal of metals increased with increasing adsorbent dose. Minimum percent removal, 58.8 % was obtained at 0.2g adsorbent dose and maximum removal of 71.99% was at 0.4g. Conversely, the % removal of copper decreased with increasing initial metal ion concentration. Maximum removal of 60.32% occurred at 100 ppm concentration and minimum removal of 53.9% was obtained at 200 ppm. Similar to the adsorbent dose, the percentage removal increased with pH. 74.6% and 53.9 % metal removal were observed at pH = 11 and at pH= 3 respectively. These values generally agreed with findings under the literature.

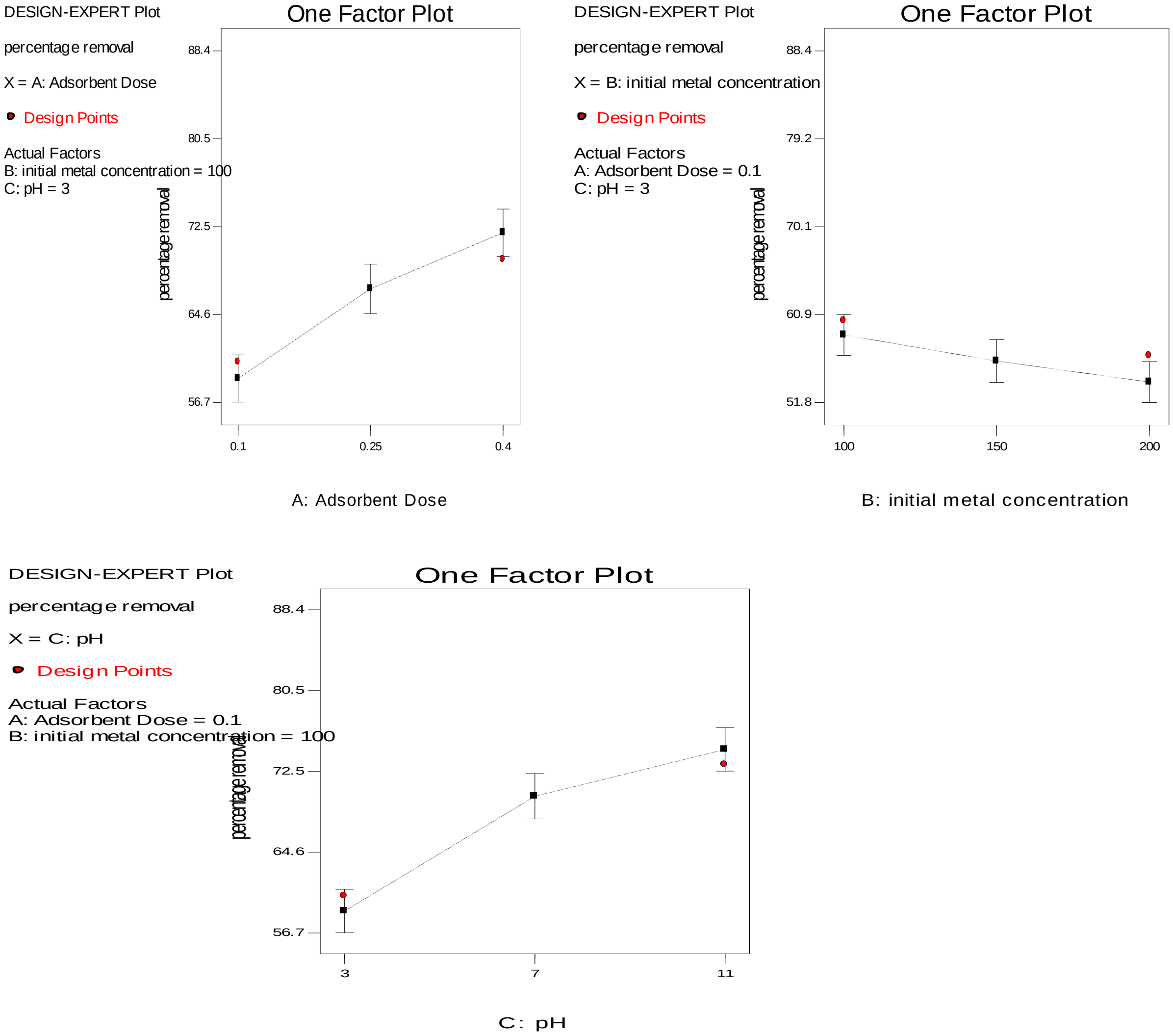


Figure 4.16 Single effect of adsorbent dose, initial metal ion concentration and pH on % removal of Copper

Interaction effect

The percentage removal of both metal ions was plotted as a function of the interactions of two factors putting the third factor at average value.

1. Adsorbent dose versus initial metal concentration

The interaction of adsorbent dose and metal concentration indicated that maximum removal of lead occurred at high adsorbent dose and low initial metal concentration. Lower percentage removal of lead was obtained at low adsorbent dose and high metal concentration. Their interaction strongly affected the bio sorption of lead. The Percent removal of copper however was not affected by the interaction of adsorbent dose and metal concentration since the lines in the plot were far apart.

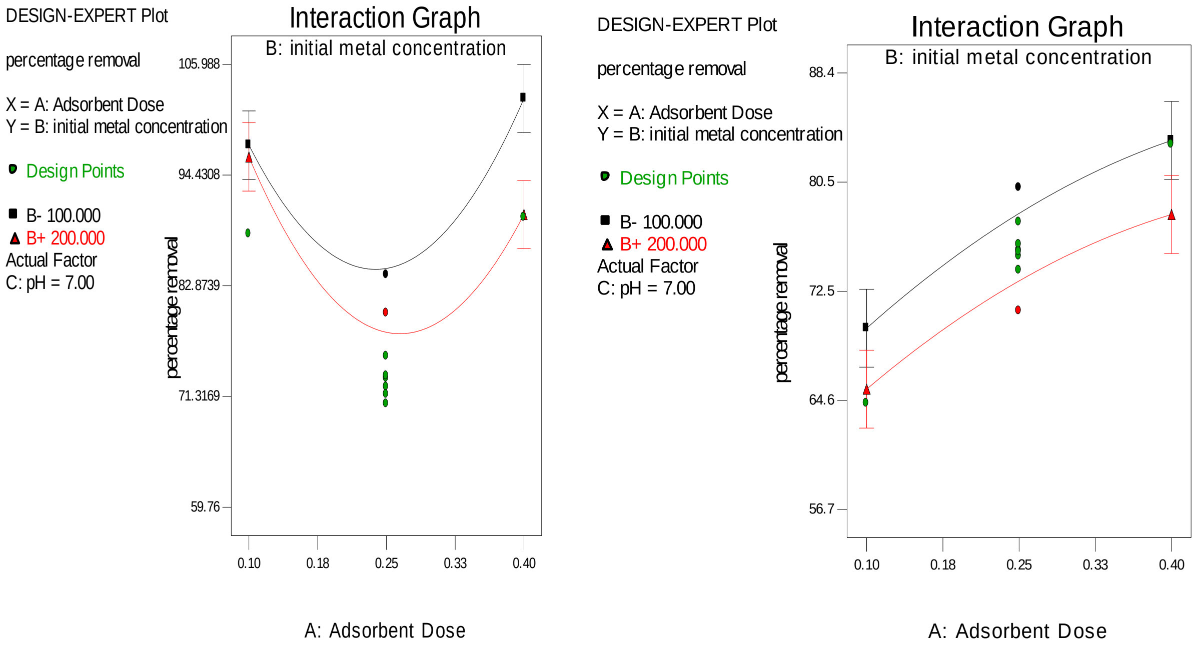


Figure 4.17 interaction effect of initial metal concentration versus adsorbent dose Pb (II), Cu (II) (left to right)

2. pH versus adsorbent dose

The plot of pH and Adsorbent dose confirmed that there exist interaction effects on the removal of lead. Here, maximum removal was obtained at both higher values of pH and adsorbent dose and decreased at lowest value of the factors. The figure also showed the removal of lead was highly dependent on pH than adsorbent dose. The percent removal of copper was highly dominated by pH with insignificance interaction effect.

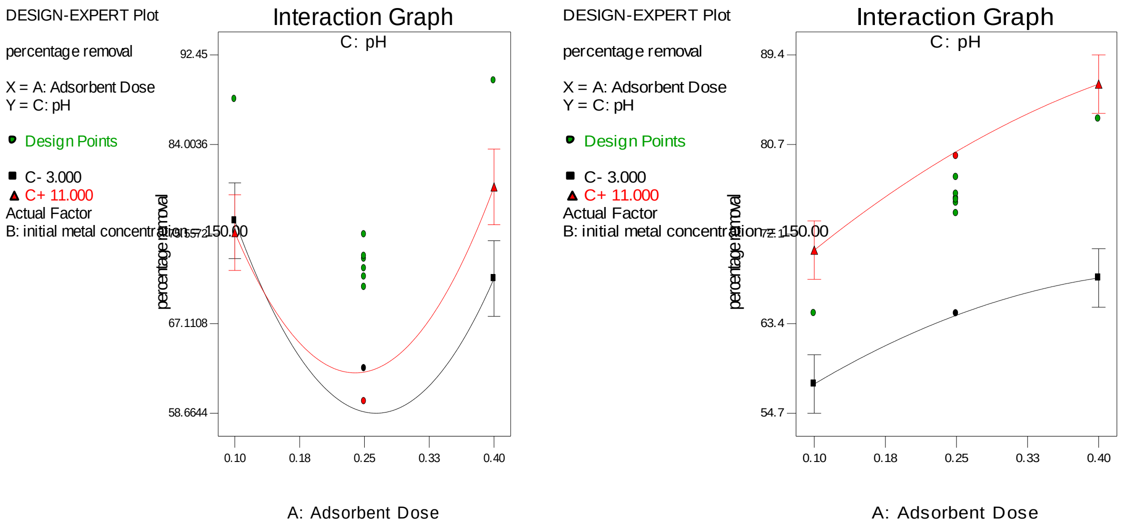


Figure 4.18 interaction effect of pH versus adsorbent dose Pb (II), Cu (II)

pH versus initial metal concentration

As can be seen on the figure, a low value of initial metal concentration and high pH contributed to percentage removal 82.703 %. The third factor adsorbent dose were kept constant at an average value. The effect of pH was higher than the effect of metal ion concentration in copper removal. It can be concluded that there was high interaction effect during the biosorption of lead but no/very insignificant interaction effects of copper were observed in all the plots. This might be due to the dominance of one factor over the other.

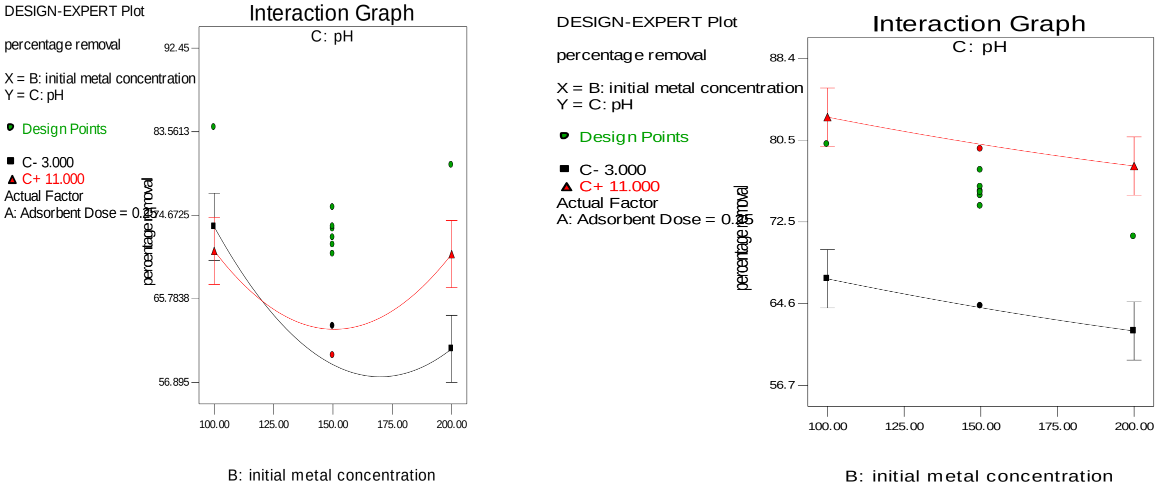


Figure 4.18 interaction effect of pH versus initial metal concentration of Pb (II), Cu (II)

4.15 Optimization of percentage removal of metal ions

Optimization of the output response (% removal) of coper and lead was carried out by numerical optimization method. Optimum process variables corresponding to maximum percentage removal are given in table. The table indicated highest percentage removal of both metals were obtained at similar adsorbent dosage and metal ion concentration while their pH values were different i.e. pH = 7.88 for Lead and pH = 10.97. This confirmed pH was the dominant factor throughout the biosorprtion process.

Table 4.8 optimum values of factors and responses for biosorption of Cu^{+2} and Pb^{+2}

Heavy metals	Maximum percentage removal	Optimum values of process variables		
		pH	metal concentration, ppm	Adsorbentdosage, (g)
Pb^{2+}	99.2587	7.88	150.91	0.4
Cu^{+2}	89.2	10.97	103.63	0.4

5.Conclusion and Recommendation

5.1 Conclusion

Biosorption of Copper (II) and Lead (II) from aqueous solution onto acid treated lemna minor adsorbent was investigated. A batch adsorption experiment was carried out using one factor at a time and central composite design expert 6.0.8 to investigate the effect of adsorption parameters: pH, initial metal concentration, and adsorbent dose. Maximum percentage removal 92.45 % for Lead (II) at pH = 11 and 88.4 % for Copper (II) at pH = 9 was obtained. pH was found as the most dominant factor. Temperature effects showed that the adsorption process was endothermic with positive enthalpy values and negative free Gibbs energy values. The Removal efficiency of lemna minor increased with increase in temperature. Data’s from the adsorption were analyzed with Temkin, Langmuir and Freundlich isotherm models. Langmuir and freundlich model fitted very well to the sorption data in the studied concentration range with a higher correlation coefficient R^2 value 0.9948 and 0.9944 for Cu^{+2} and Pb^{+2} (Langmuir); 0.9919 for copper and 0.99 for lead (freundlich) respectively. Kinetic behavior of the metal sorption process was analyzed through pseudo first order, pseudo second order and intraparticle diffusion. The pseudo second order equation appeared to be the best fitting model than both first order and intra particle diffusion because it has highest R^2 value. The data analysis showed the adsorption capacity of lemna minor forPb^{+2} found to be higher than Cu^{+2}. From this, it can be concluded that the affinity of lemna minor for Pb (II) is greater than that for Cu^{+2}

Desorption of Cu^{+2} and Pb^{+2} were carried out by using a 100ml of 0.1M HCl in to 250 volumetric flasks with 0.1 g of 43.86 ppm of copper and 42.38 ppm of Lead loaded sun dried lemna minor. The percent desorption increased with time for both metals and maximum desorption observed at 60-minute contact time with 80% for Lead and 72.2% for Copper. To conclude, the occurrence of various functional groups: hydroxyl, alcohol, alkene, carboxylic and amine groups in lemna minor helped the biomass to adsorb heavy metals of Cu and Pb from synthetic waste water. Because of their adsorptive properties and quick adsorption capacity, lemna minor can be a potential method for post-treatment of wastewater at minimum cost.

5.2 Recommendation

The following recommendations was made for future work and application of lemna minor.

- Batch adsorption should be made at various factors such as particle size and temperature
- Activation of the bio materials should be studied using various acid such as nitric acid, phosphoric acid in order to compare which acid contribute to the best removal capacity
- Desorption of the used adsorbent need to furtherly studied at different metal loaded concentration to understand more the regeneration ability of the adsorbent
- Column adsorption can be developed to move laboratory batch adsorption results into practical real wastewater treatment containing heavy metal ions. The development of the biosorption processes by Lemna minor requires further investigation in the direction of modeling, of regeneration of bio sorbents material and of testing immobilized raw biomasses with industrial effluents.
- The efficiency of this material should also be investigated for the removal of dyes, BOD and COD from aqueous solution and also the application of the material on real wastewater containing all sorts of pollutants.

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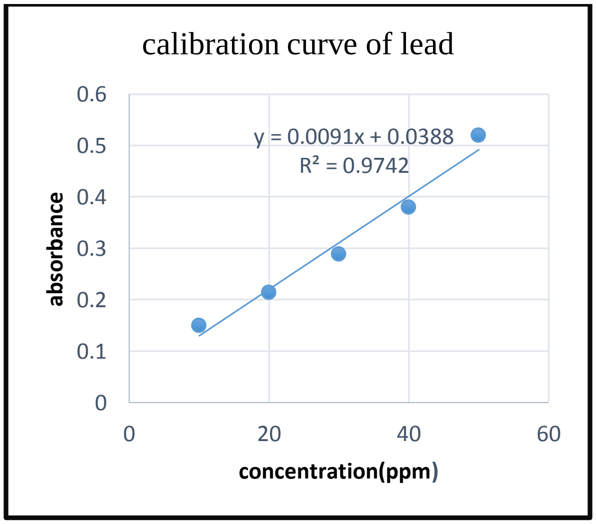
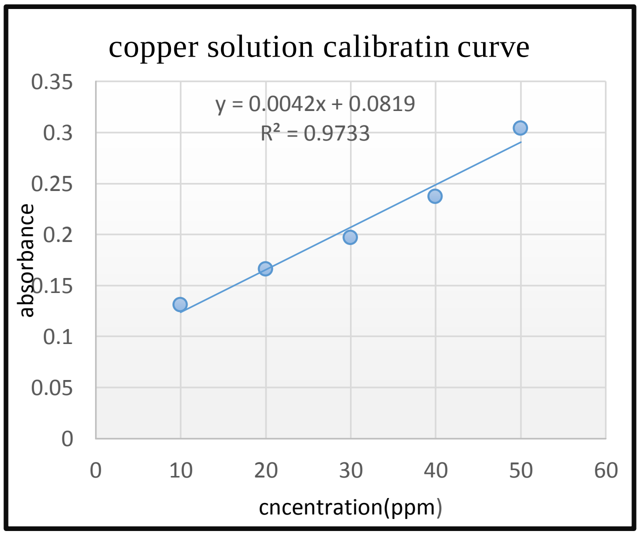
APPENDIXES

APPENDIX A: standard calibration curves of copper (II) and lead (II)

APPENDIX A1

Calibration curve of Copper and Lead for determining concentration of the supernatant of both metals





APPENDIX A2

pH of Point of zero charge values

Initial pH	Final pH	ΔpH

2	2.43	0.8
3	3.25	0.61
4	4.34	0.71
5	5.02	0.76
6	6.41	0.41
7	7.47	-0.54
8	8.07	-1..67
9	8.69	-2.36
10	9.81	-2.96
11	10.85	-3.95

APPENDIX B Fourier Transform Infrared Spectroscopy (FTIR) Correlation Table

Functional Group names	Absorption Ranges (cm^{-1})	Type of vibration causing IR absorption
Alkane	3000-2000	H-C-H asymmetric & symmetric strech
	1500-1440	H- C - H bend
	3100-3000	C=C-H asymmetric strech
Alkene	1675-1600	C - C=C symetric strech
Alkyne	3300-3200	≡ C - H strech
	2200-2100	C ≡ C strech
Aromatic Rings	3100-3000	C=C-H assymetric strech
	1600-1580	C - C = C symetric strech
	1500-1450	C - C = C assymetric strech
Phenols & Alcohols	3600-3100	Hydrogen bonded – OH strech
Carboxylic Acids	3400-2400	Hydrogen bonded – OH strech
	1730-1650	C= O strech
Ketone	1750-1625	C= O strech

Aldehyde	1750-1625	C= O strech
	2850-2800	C- H strech of C= O
	2750-2700	C-H strech of C= O

APPENDIX C: Photos Taken At Different Stages Of Laboratory Works

Sieving of ground adsorbent

preparation of 0.1M HCl solution

Aqueous solution preparation

Mixing of adsorbent to metal ion solution

Adjusting pH values

flasks with desired pH, adsorbent dosage, metal ion

Shaking of the adsorbent at the desired time, temperature and rpm

APPENDIX D: Results for Different Isotherm Values

APPENDIX D1: Langmuir, Frendulic and Temkin isotherm values for lead

Initial metal concentration	Final concentration C_e	Ln ce	% removal	qe(mg/g)	Ce/qe	Log ce	Log qe
50	7.619	2.031	92.381	21.19	0.359	0.882	1.326
100	26.2	3.265	73.8	36.9	0.71	1.418	1.567
150	45	3.806	70	52.5	0.857	1.653	1.72
200	80	4.382	60	60	1.33	1.903	1.778
250	122	4.804	51.2	64	1.906	2.086	1.806

APPENDIX D 2 Langmuir, Frendulic and Temkin parameter isotherm values for copper

Initial metal concentration(mg/l)	Final concentration	Ln ce	Qe	Ce/qe	Log ce	Log qe	% removal
50	6.14	1.814	21.93	0.279	0.788	1.34	87.72
100	25.36	3.233	37.32	0.679	1.386	1.577	75.64
150	46.01	3.828	51.99	0.884	1.602	1.740	73.326
200	81.3	4.398	59.35	1.369	1.876	1.795	62.375
250	130	4.867	60	2.166	2.039	1.845	56.16

APPENDIX E: Analysis Result Values for Kinetic Parameters

APPENDIX E1: Kinetic parameter values of copper

Time (minute)	Time$^{1/2}$	C initial	Concentration Ct	Qe	Qt	qe-qt	Log(qe-qt)	t/qt
10	3.162	50	31.301	21.93	9.349	12.581	1.099	1.069
15	3.872	50	26.216	21.93	11.892	10.038	1.001	1.261
30	5.477	50	17.512	21.93	16.244	5.686	0.754	1.846
60	7.745	50	12.625	21.93	18.687	3.243	0.511	3.211
90	9.486	50	6.504	21.93	21.748	0.182	-0.7399	4.138
120	10.95	50	6.151	21.93	21.924	0.006	-2.221	5.473

APPENDIX E2: Kinetic parameter values of lead

Time Minute	Time$^{1/2}$ (minute)	C initial	Ct final	Qe	qt	t/qt	qe -qt	Log (qe-qt)
10	3.162	50	37.974	21.19	6.013	1.658	15.177	1.181
15	3.872	50	29.146	21.19	10.427	1.438	10.763	1.031
30	5.477	50	18.752	21.19	15.624	1.920	5.566	0.745
60	7.745	50	9.556	21.19	20.222	2.967	0.968	-0.014
90	9.486	50	7.983	21.19	21.008	4.284	0.1815	-0.741
120	10.95	50	7.777	21.19	21.115	5.683	0.075	-1.124

APPENDIX F: Analysis Results of temperature effects on percentage removal

APPENDIX F1

Temperature effects on adsorption efficiency of Lead ,50, 100, 150, 200 and 250 mg L^{-1} of lead (Pb^{+2}) solution, o.2 g of adsorbent dosage, contact time, 1 hr., agitation speed = 200 rpm and pH optimum

C_o (mg L^{-1})	C_t (mg L^{-1})			q_t (mg g^{-1})			% Removal of copper		
	Temperature (°c)								
	20	25	30	20	25	30	20	25	30
50	8.25	6.15	3.10	20.87	21.92	23.45	83.5	87.7	93.8
100	32.03	29.05	18.61	33.98	35.47	40.69	67.97	70.95	81.39
150	60.23	50.14	42.37	44.88	49.93	53.81	59.84	66.57	71.75
200	92.39	87.18	82.35	53.80	56.41	58.82	53.80	56.41	58.82
250	140.82	135.13	102.42	54.59	57.43	73.79	43.67	45.94	59.32

APPENDIX F2

Temperature effects on adsorption efficiency of copper ,50, 100, 150, 200 and 250 mg L^{-1} of copper ion solution ($Cu^{+2)}$ solution, 0.2 g of adsorbent dosage, contact time, 1 hr., agitation speed = 200 rpm and pH optimum

C_o (mg L^{-1})	C_t (mg $^{L-1}$)			q_t (mg g^{-1})			% Removal of copper		
	Temperature (°C)								
	20	25	30	20	25	30	20	25	30
50	9.15	7.97	4.13	20.42	21.02	22.93	81.7	84.06	91.74
100	36.94	28.63	19.47	31.53	35.68	40.26	63.06	71.37	80.53
150	59.29	44.04	37.05	45.35	52.98	56.75	60.47	70.64	75.3
200	97.45	85.18	75.29	51.27	57.41	62.05	51.27	57.41	62.35
250	137.42	132.93	125.51	56.29	58.53	62.03	45.03	46.82	46.97