INVITRO STUDY OF THE REMOVAL OF COPPER, LEAD AND ZINC FROM THE HUMAN BLOOD PLASMA USING _ALLIUM CEPA_

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DECEMBER, 2016

DECLARATION

I declare that this research work entitled "*Invitro* Study of the Removal of Copper, Lead and Zinc from the Human Blood Plasma using *Allium cepa"* was carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and in the list of references provided. No part of this dissertation was previously presented for another degree at this or any other Institution.

JIBUNOR, UDOKA VICTOR

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Name of Student Signature Date

CERTIFICATION

This project entitled *INVITRO* STUDY OF THE REMOVAL OF COPPER, LEAD AND ZINC FROM THE HUMAN BLOOD PLASMA USING *ALLIUM CEPA* by VICTOR UDOKA JIBUNOR meets the regulations governing the award of Master of Science in Analytical Chemistry of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation

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Member supervisor committee Signature Date

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Dean, School of Postgraduate Studies Signature Date

DEDICATION

This work is dedicated to God Almighty the author and the finisher of our faith.

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I give all gratitude, praise and worship to the lover of my soul, the God Almighty for perfecting his strength in my weakness and for lavishing upon me abundance of grace beyond my comprehension to go on in life. May His name be praised and exalted forever.

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ABSTRACT

Dehydrated *Allium cepa* (Onion) biomass was used to adsorb Cu^{2+}, Pb^{2+} and Zn^{2+} from the human blood plasma *in vitro*. The samples were found to be free of Cu^{2+}, Pb^{2+} and Zn^{2+} after screening using AAS. Scanning Electron Microscope (SEM) and Fourier Transform Infrared (FTIR) analysis were carried out on the biosorbent before and after biosorption. Scanning Electron Microscope (SEM) analysis revealed physical disintegration in the surface morphology of biosorbent after biosorption while Fourier Transform Infrared (FTIR) analysis revealed a general shift in peaks of C-H stretch of alkane and alkyl groups, N-H symmetric amides, N-H bend amides, S=O sulfonyl, C-O stretch alcohols, O-H alcohols, ethers, esters and carboxyl functional groups after biosorption which is evidence of biosorption of these metal ions on the *Allium cepa* biomass. The optimisation of operational factors including solution pH, biosorbent concentration, initial metal (Cu^{2+}, Pb^{2+} and Zn^{2+}) ion concentration were investigated at physiological temperature. The optimum pH, initial metal ions concentration and biosorbent concentration for Cu^{2+} sorption was pH 6, 50 mgL^{-1} and 0.60 g respectively. The values obtained were used for the kinetic study and the highest percentage sorption of Cu^{2+} was 99.316 % at 70 min. The optimum of pH, initial metal ions concentration and biosorbent concentration for Pb^{2+} sorption was pH 4, 50 mgL^{-1} and 0.40 g respectively. The values obtained were used for the kinetic study and the highest percentage sorption of Pb^{2+} was 99.8914 % at 90 min. The optimum of pH, initial metal ions concentration and biosorbent concentration for Zn^{2+} sorption was pH 6, 50 mgL^{-1} and 0.80 g respectively. The values obtained were used for the kinetic study and the highest percentage sorption of Zn^{2+} was 97.8076 % at 80 min. The percentage removal of Cu^{2+}, Pb^{2+} and Zn^{2+} are in the order of Pb^{2+} (99.8914 %) > Cu^{2+} (99.316 %) > Zn^{2+} (97.8076 %). The experimental data obtained for Cu^{2+}, Pb^{2+} and Zn^{2+} sorption were treated using Langmuir, Freundlich and Temkin isotherm. Temkin isotherm provided the best fit with correlation coefficient (R^2) values of 0.508, 0.901 and 0.620 respectively. Simple kinetic models such as pseudo-first-order, pseudo-second-order and intra-particle diffusion model were employed to determine the biosorption mechanism. The kinetic study followed pseudo second order while the intra-particle diffusion model was observed not to be the only rate limiting step. This result suggests that chemical adsorption process was more dominant which involves the formation of chemical bonds between the metal ions (Cu^{2+}, Pb^{2+} and Zn^{2+}) and cell wall and cell membrane of *Allium cepa* respectively. This study demonstrated that *Allium cepa* biomass could be used as biosorbent for the detoxification of Cu^{2+}, Pb^{2+} and Zn^{2+} from human blood plasma.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
ABUTH:	Ahmadu Bello University Teaching Hospital
$A_{T:}$	Temkins equilibrium binding constant
ATSDR:	Agency for Toxic Substances and Disease Registry.
$b_{T:}$	Temkins isotherm constant
Cd:	Cadmium
CO_2:	Carbon dioxide
CPDA:	Oxalate Citrate Phosphate Dextrose Adenine
Cu:	Copper
DNA:	Deoxyribonucleic Acid
EDTA:	Ethylenediaminetetraacetic Acid
EPA:	Environmental Protection Agency
EPD:	Environmental and Process Instruments Division
FAAS:	Flame Atomic Absorption Spectroscopy
FTIR:	Fourier Transform Infrared Spectroscopy
g/dl:	gram per decilitre
HR-ICP-MS:	High Resolution Inductively-Coupled Plasma Mass Spectroscopy
ICP – AES:	Inductively Coupled Plasma Atomic Emission Spectroscopy
IQ:	Intelligent Quotient
K_1:	Pseudo first order rate constant
K_2:	Pseudo second order rate constant
$K_{f:}$	Freundlich constant measuring biosorption capacity

K_L:	Langmuir constant
Mg:	Magnesium
mgL^{-1}:	milligram per litre
NCI:	National Cancer Institute
Pb:	Lead
Q:	Quadrupole
q_e:	Amount of metal ion adsorbed per unit mass of biosorbent
Q^o:	Maximum metal uptake
q_t:	Amount of metal ion adsorbed per unit mass of biosorbent at time t,
t	Time
R^2:	Coefficient of determination
R_L:	Dimensionless constant
RNA:	Ribonucleic Acid
SEM:	Scanning Electron Microscope
WHO:	World Health Organisation
Zn:	Zinc
µg/L:	Micro gram per litre
$q$max:	Langmuir maximum monolayer biosorption capacity

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

People are exposed to a variety of potentially harmful agents in the air they breathe, the liquids they drink, the food they eat, the surfaces they touch and the products they use. An important aspect of public health protection is the prevention or reduction of exposures to environmental agents that contribute, either directly or indirectly, to increased rates of premature death, disease, discomfort or disability (WHO, 2000). Chemicals have become an indispensable part of human life, sustaining activities and development, preventing and controlling many diseases, and increasing agricultural productivity. Despite their benefits, chemicals may, especially when misused, cause adverse effects on human health and environmental integrity. The widespread application of chemicals throughout the world increases the potential of adverse effects. Heavy metals are among the chief constituents of these chemicals. Heavy metals are very harmful in reference to their non biodegradable nature, long biological half lives and their potential to accumulate in different body parts. Most of the heavy metals are extremely toxic because of their solubility in water. Even at low concentrations heavy metals can have damaging effects in human beings and animals as there is no good mechanism for their elimination from the body. The heavy metals are taken up faster than they are metabolized or excreted (WHO, 2000). Even those heavy metals which are considered to be essential can become toxic in case when present in excess. The heavy metals can impair important biochemical processes posing a threat to human health (Gupta and Rastogi, 2009).

These toxic metals tend to enter the human body through, inhalation and consumption of contaminated plant and animals as food. They accumulate in the human system dependent and independent of metabolism (Veglio and Beolchini, 1997). These toxic metals ions are transported to various parts of the human body through the human blood plasma (The main medium for excretory product transportation). With the aid of some biological activities in the human body and the ingestion of some chemical constituents into the human system, these toxic metals can be excreted, utilizing various elimination activities including biosorption (Christopher, 2005; Jaishankar *et al.,* 2014).

Biosorption is an emerging technology that uses biological materials to remove metals from solution through adsorption. Biosorbent from biological materials contains various types of functional sites such as amino, hydroxyl and carboxyl where it can react to form passive uptake of metals from aqueous solution (Bakircioglua *et al.,* 2010; Wang and Chen. 2009). Land plants, aquatic plants and herbs have all attracted considerable attention for the capacity to eliminate metals from the environment. Mechanisms responsible for biosorption, although understood to a limited extent, may be one or combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and microprecipitation (Veglio and Beolchini, 1997; Vijayaraghavan and Yun, 2008; Wang and Chen, 2006).

Allium cepa (Onion) is a member of *Allium* family. Studies have shown that this plant contains some powerful chemical constituent such as quercetine, flavonoids, capeanes, organosulphur compounds and dietary fibres. It is believed that these chemical constituent contains functional groups which are capable of binding the metals. These molecules as organometallic complexes, are further partitioned inside vacuoles to facilitate appropriate control of the cytoplasmic concentration of heavy metal ions, thus preventing or neutralizing their potential toxic effect (Cobbett and Goldsbrough, 2002).

1.2 Statement of Research

Due to the increasing amount of neuro-toxic chemicals and heavy metals in the environment, the resultant accumulation of heavy metals in the human body poses significant health risks (Adriano, 1986). Chronic exposure from occupational, environmental, dental amalgam, and contaminated food is a significant threat to public health. These metals bind with protein sites which are not made for them by displacing original metals from their natural binding sites causing malfunctioning of cells and ultimately toxicity leads to arygria, a blue-gray discoloration of the skin and other body tissues, chest pain, tightness of the chest, shortness of breath (Pattabhi *et al* 2008). These toxic metals are difficult to eliminate due to their persistent and cumulative nature. The development of new and more effective technologies becomes essential. This work was undertaken to study heavy metal detoxification of human plasma using *Allium cepa* as biosorbent. The result from this work will provide qualitative information on the effective use of *Allium cepa* as biosorbent for the detoxification of human plasma.

1.3 Justification of Study

The hazardous and adverse effects caused by heavy metals present in the environment emerging from different industries are of significant environmental concern. Heavy metals are non-biodegradable and bioaccumulates in living organisms thereby causing various damages, diseases and disorder. Hence, attention is currently on the use of cost effective biosorbent which studies have shown to exhibit high adsorption capacity of unwanted pollutants (Ahalya *et al.,* 2003). Several biological and chemical methods such as filtration, coagulation, oxidation, solvent extraction, and reverse osmosis have been used for heavy metal treatment in the environment. However, the increase in the variety and amount of hazardous chemicals present in the environment, makes these conventional methods inefficient. Consequently, the development of new and more effective technologies and biosorbents has becomes essential; therefore it is important to investigate the heavy metal adsorption capacity or efficiency of some herbs and vegetables from the human system. *Allium cepa* was investigated because of its high medicinal values and its commercial availability in the market. There has not been any known work in using them to detoxify the human plasma of the heavy metals under study.

1.4 Aim of the Research

The aim of this work is to study the metals (Cu^{2+}, Pb^{2+}, and Zn^{2+}) uptake capacity of *Allium cepa* from the human plasma.

1.5 Objectives of the Research

The aim of the study was achieved through the following objective

- Collection of healthy Human blood sample.
- Collection of sample of *Allium cepa* (Onion bulb).
- Centrifugation of the blood sample and collection of the human plasma.
- Screening of the human plasma to identify the concentration of metal ions (Cu^{2+}, Pb^{2+} and Zn^{2+}) present in it.
- Preparation of sample of dehydrated *Allium cepa* which was then used as biosorbent prior to analysis
- Screening of the prepared biosorbent to identify the concentration of metal ions (Cu^{2+}, Pb^{2+} and Zn^{2+}) present using AAS.
- Carryout FTIR analysis of the prepared native and metal ions treated biosorbent to identify the binding sites.

- Optimisation of the biosorption parameters at varying pH, varying concentrations of heavy metals (Cu^{2+}, Pb^{2+} and Zn^{2+}), and biosorbent concentration.
- Fit the experimental data obtained into Langmuir and Freundlich and Tempkin isotherms.
- Study the kinetics of Cu^{2+}, Pb^{2+}, and Zn^{2+} ion sorption using pseudo first order kinetic model, pseudo second order kinetic model and Intra-particle diffusion kinetic model.

CHAPTER TWO

LITERATURE REVIEW

2.1 Heavy Metals

Heavy metal is a member of a loosely defined subset of element that exhibit metallic properties. It mainly includes transition metals, some metalloids, lanthanides and actinides. Many different definitions has been proposed some based on density, some on the atomic number, atomic weight, and some on chemical properties or toxicity of the element. A few metals are not known to be essential to human health but may have some beneficial effects at low levels of exposure. These include silicon, nickel, boron, and vanadium (these metals are toxic at higher levels.) (NAS/IOM, 2003).

The term heavy metals have been called misinterpretation in the IUPAC technical report due to contradictory definition and its lack of coherence scientific base. There is an alternative term 'Toxic Metals', for which no contentious or exact definition exist either. Toxic metals can include metals lighter than carbon and exclude some of the heaviest metals (Duffus, 2002). Heavy metals occur in the ecosystem with large variation in concentration. In modern times, anthropogenic source of heavy metals has elevated their levels in the ecosystem. Waste derived from fuels are especially prone to certain heavy metals e.g. cadmium, arsenic, mercury, lead, chromium and manganese (Hogan, 2010).

Numerous industries produce and discharge wastes containing different heavy metals into the environment, such as mining and smelting of metalliferous, surface finishing industry, energy and fuel production, fertilizer and pesticide industry and application, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace, atomic energy installation and municipal wastewaters frequently contain metal ions that can be harmful to aquatic life and human health (Ucurum, 2009 and wang and Chen, 2006). Metals such as copper, chromium and zinc are micro-nutrients to plant development; however they can be toxic to plants when present at high concentrations. Plants are also able to absorb contaminants, such as heavy metals, and store them in roots and foliage, resulting in bioaccumulation in the food chain.

2.2 Copper

Copper is a chemical element with symbol Cu (from Latin: *cuprum*) and atomic number 29. It is a soft, malleable and ductile metal with very high thermal and electrical conductivity. A freshly exposed surface of pure copper has a reddish-orange color. The major portion of copper produced in the world is used by the electrical industries; most of the remainder is combined with other metals to form alloys (Prusty, *et al*., 1994). Important series of alloys in which copper is the chief constituent are brasses (copper and zinc) and bronzes (copper and tin) (Morse *et al.,* 1999). Copper resists the action of the atmosphere and seawater exposure for long periods to air however, results in the formation of a thin green protective coating (patina) that is a mixture of hydroxocarbonate, hydroxosulphate and small amounts of other compounds. Copper is a moderately noble metal, being unaffected by non-oxidizing or non-complexing dilute acids in the absence of air; it will however, dissolve readily in nitric acid and in sulphuric acid in the presence of oxygen (Frits *et al.*, 2000).

Copper is among heavy metals that are essential to life but could be toxic at elevated levels (Rogers *et al.*, 2009). It is toxic at low concentration in water and is known to cause brain damage in mammals (Georgopoulos *et al.*, 2001). Toxicity of Copper in plants as a result of high level in sewage treated agricultural soil has been reported. Contribution of copper to environmental burden could be by atmospheric deposition from metal industries, dumpsites and power plants that burn.

2.2.1 Health effect of Copper

Acute poisoning from ingestion of excessive copper can cause temporary gastrointestinal distress with symptoms such as nausea, vomiting, and abdominal pain. Liver toxicity was seen in doses high enough that resulted in death. High levels of exposure to copper can cause destruction of red blood cells, possibly resulting in anaemia (EPA, 2006).

Mammals have efficient mechanisms to regulate copper stores in the body as they are generally protected from excess dietary copper levels. However, at levels that are chronic over exposure to copper can damage the liver and kidneys (EPA, 2006).

Wilson's disease is an inherited (genetic) disorder in which copper builds up in the liver. Symptoms of liver toxicity (jaundice, swelling, pain) usually do not appear until adolescence (EPA, 2006).

Although some studies of workers exposed to copper have shown increased cancer risks, they were also exposed in the workplace to other chemicals with carcinogenic potential. Increased cancer risk has not been found in animal studies (EPA, 2006).

There are no reports of developmental effects occurring in humans exposed to elevated levels of copper. However developmental effects have been observed in a few studies of animals being given high doses of copper, including delayed growth and development, delayed bone formation, and decreased litter size and body weights (EPA 2006).

2.3 Lead

Lead (atomic number 82; relative atomic mass 207.19) is a soft, silvery grey metal, melting at 327.5°C. It is highly resistant to corrosion, but is soluble in nitric and hot sulphuric acids. The usual valence state in inorganic lead compounds is +2. Solubilities in water vary; lead sulphide and lead oxides being poorly soluble but the nitrate, chlorate and chloride salts are reasonably soluble in cold water. Lead also forms salts with organic acids such as lactic and acetic acids, and stable organic compounds such as tetraethyllead and tetramethyllead (WHO, 1989).

The level of lead in the earth's crust is about 20 $mgkg^{-1}$ (WHO 1989). It is seldom found in its elemental form; however, it is part of several ores including galena, PbS. Lead in the environment may be derived from either natural or anthropogenic sources. Natural sources of atmospheric lead include geological weathering and volcanic emissions and have been estimated at 19 000 tonne per year (WHO, 1989). Lead and its compounds may enter the environment at any point during mining, smelting, processing, recycling or disposal. Major uses are in batteries, cables, pigments, petrol (gasoline) additives, solder and steel products. Lead and lead compounds are also used in solder applied to water distribution pipes and to seams of cans used to store foods, in some traditional remedies, in bottle closures for alcoholic beverages and in ceramic glazes and crystal tableware. In countries where leaded petrol is still used, the major air emission is from mobile and stationary sources of petrol combustion (urban centres). Areas in the vicinity of lead mines and smelters are subject to high levels of air emissions. Airborne lead can be deposited on soil and water, thus reaching humans through the food chain and in drinking-water (LeCoultre, 2001; WHO, 1995).

2.3.1 Health effect of Lead

Chisolm, (1971) found that lead poisoning in young children are a major source of brain damage, mental deficiency and serious behaviour problems. It is difficult to diagnose, it is often unrecognized and until recently it was largely ignored by physicians and public health officials. Now public attention is finally being focused on childhood lead poisoning, although the difficult task of eradicating it has just begun.

WHO, (1989) reported that the adverse hematological effects of lead are mainly the result of its perturbation of the heme biosynthesis pathway. The activity of δ-aminolevulinic acid synthase (ALAD), an enzyme occurring early in the heme synthesis pathway, is negatively correlated with blood Pb levels between 50 and 950 μgl^{-1}. Although inhibition of ALAD occurs at very low exposure levels, there is some controversy as to the toxicological significance of a depression in ALAD activity in the absence of a detectable effect on haemoglobin levels.

Hertz-Picciotto and Croft, (1993) and WHO, (1995), pointed out that result of more recent epidemiology studies indicate that the lead contribution to elevated blood pressure is more pronounced in middle age than at younger ages. Lead poisoning in childhood has also been associated with hypertension during adulthood in the absence of clinically significant renal disease and discernable elevations in blood Pb level.

2.4 Zinc

Zinc (atomic number 30; relative atomic mass 65.38) is a metallic element belonging to group II and the fourth period of the Periodic Table. Zinc is a chalcophilic element like copper and lead, and a trace constituent in most rocks. Zinc rarely occurs naturally in its metallic state, but many minerals contain zinc as a major component from which the metal may be economically recovered. Sphalerite (ZnS) is the most important ore mineral and the principal source for zinc production (WHO, 2001).

The fate and transport of zinc (Zn^{2+}) in the environment is dependent on cation exchange capacity, pH, organic matter content, nature of complexing ligands, and the concentration of the metal in the soil. As pH increases, there is an increase in the negatively charged binding sites on the soil particles, which facilitates the adsorption of zinc ions and removal from solution. Studies have shown that the uptake of zinc by terrestrial plants is significantly increased at a low soil pH, but reduced when there is a high content of organic matter. Normal levels of zinc in most crops and pastures range from 10 $mgkg^{-1}$ to 100 $mgkg^{-1}$. Some plant species are zinc accumulators, but the extent of the accumulation in plant tissues varies with soil properties, plant organ and tissue age (Adriano, 1986).

Zinc is an essential element in the human diet thus zinc deficiency in diet may be more detrimental to human health. The human health effects associated with zinc deficiency are numerous, and include neurosensory changes, oligospermia, impaired neuropsychological functions, growth retardation, delayed wound healing, immune disorders and dermatitis. These conditions are generally reversible when corrected by zinc supplementation. A disproportionate intake of zinc in relation to copper has been shown to induce copper deficiency in humans, resulting in increased copper requirements, increased copper excretion and impaired copper status.

2.4.1 Health effect of Zinc

Chronic exposure to zinc have been associated with effects ranging from leukopenia and/or hypochromic microcytic anaemia to decreases in serum high-density lipoprotein concentrations. These conditions were reversible upon discontinuation of zinc therapy together with copper supplementation (Institute of Medicine, 2001).

The effects of inhalation exposure to zinc and zinc compounds vary somewhat with the chemical form of the zinc compound, but the majority of the effects seen will occur within the respiratory tract. Following inhalation of zinc oxide, and to a lesser extent zinc metal and many other zinc compounds, the most commonly reported effect is the development of “metal fume fever.” Metal fume fever, a well-documented acute disease induced by inhalation of metal oxides, especially zinc, impairs pulmonary function but does not usually progress to chronic lung disease. Symptoms generally appear within a few hours after acute exposure, usually with dryness of the throat and coughing. The most prominent respiratory effects of metal fume fever are substernal chest pain, cough, and *dyspnoe*. The impairment of pulmonary function is characterized by reduced lung volumes and a decreased diffusing capacity of carbon monoxide. Leukocytosis persisting for approximately 12 hours after the fever dissipates is also a common manifestation of metal fume fever. In general, the symptoms of metal fume fever resolve within 1–4 days after cessation of exposure and do not lead to long-term respiratory effects.

The exact mechanism behind the development of metal fume fever is not known, but it is believed to involve an immune response to the inhaled zinc oxide. It has been suggested that the zinc oxide causes inflammation of the respiratory tract and the release of histamine or histamine-like substances. In response, an allergen-antibody complex is formed that may elicit an allergic reaction upon subsequent exposure to the allergen. In response to the allergen-antibody complex, an anti-antibody is formed. The anti-antibody dominates with continued exposure to the zinc oxide, thereby producing a tolerance. When the exposure is interrupted and re-exposure occurs, the allergen-antibody complex dominates, producing an allergic reaction and symptoms of metal fume fever (Lindahl *et al*., 1998; Malo *et al*., 1990).

Nausea has been reported by humans exposed to high concentrations of zinc oxide fumes (300–600 mgm^{-3}) and zinc chloride (~120 mgm^{-3}) smoke, as well as following oral exposure to zinc chloride and zinc sulphate. Other gastrointestinal symptoms reported in cases of excess zinc exposure include vomiting, abdominal cramps, and diarrhea, in several cases with blood. In general, oral exposure levels associated with gastrointestinal effects of zinc have not been reliably reported, but the limited available data suggest that oral concentrations of 910 mgL^{-1} or single-dose exposures of ~140–560 mg (acute oral doses of 2–8 $mgkg^{-1}$ day^{-1}) are sufficient to cause these effects. The noted effects are consistent with gastrointestinal irritation. It is unclear in the majority of human studies whether the gastrointestinal effects seen following zinc inhalation were due to systemic zinc or were the result of direct contact with the gastrointestinal tract following mucociliary clearance of inhaled zinc particles and subsequent swallowing (Kobayashi *et al.*, 2008; WHO, 2001).

High-dose zinc administration has also resulted in reductions in leukocyte number and function. Some studies have also found decrease in high-density lipoprotein (HDL) levels in humans exposed to increased levels of zinc; however, not all studies have confirmed this observation. Long-term consumption of excess zinc may also result in decreased iron stores, although the mechanism behind this effect is not presently clear. In most cases, dermal exposure to zinc or zinc compounds does not result in any noticeable toxic effects (Hughes and Samman, 2006).

Available studies have not presented evidence of reproductive or developmental effects in humans following inhalation of zinc compounds. Effects on reproductive or developmental end points have been noted in oral-exposure animal studies, but generally only at very high doses (>200 $mgkg^{-1}day^{-1}$). Available studies of zinc-induced carcinogenic effects in humans following both oral or inhalation exposure have not adequately demonstrated an increase in cancer incidence. The EPA currently classifies zinc and compounds as carcinogenicity group D (not classifiable as to human carcinogenicity) (WHO, 2001).

The recommended dietary allowance (RDA) for zinc is 11 $mgday^{-1}$ in men and 8 $mgday^{-1}$ in women; these correspond to approximately 0.16 $mgkg^{-1}day^{-1}$ for men and 0.13 $mgkg^{-1}day^{-1}$ for women. Higher RDAs are recommended for women during pregnancy and lactation (Marsee *et al.*, 2006).

2.5 Heavy metal accumulation in the human body

Heavy metals can enter our bodies via food (fish, meat, and farm produce) and drinking water, and children can be exposed to lead through ingesting paint chips. People are exposed to a variety of potentially harmful agents in the air they breathe, the liquids they drink, the food they eat, the surfaces they touch and the products they use. One primarily concern is that marine animals which can readily absorb those heavy metals in wastewater and directly enter the human food chains present a high health risk to consumers.

Chemicals have become an indispensable part of human life, sustaining activities and development, preventing and controlling many diseases, and increasing agricultural productivity. Despite their benefits, chemicals may especially when misused, cause adverse effects on human health and environmental integrity. The widespread application of chemicals throughout the world increases the potential of adverse effects. The growth of chemical industries, both in developing as well as in developed countries is predicted to continue to increase (WHO, 1999). Heavy metals are not biodegradable and can lead to accumulation in living organisms, causing various diseases and disorders (Bailey *et al*., 1999).

2.6 Description of *Allium Cepa* (Onion)

Plate 2.1: Cross section of *Allium cepa*

Allium cepa (Plate 2.1), is a member of the *allium* family, the same as garlic, leeks, shallots and scallions. These smelly, but powerful veggies contain literally dozens of chemical compounds that have been used for centuries for their amazing healing properties. The Chinese, East Indians, Ancient Greeks, Romans and even Egyptians revered the onion, believing it to help with infections, digestion as well as issues with the eyes and joints among other things (Christopher, 2005). Today, we know the plant has potent diuretic, antibiotic and even anti-inflammatory properties. Studies also show it to be an effective expectorant, which makes it very useful in cases of infections like colds, flu and persistent coughs.

Allium cepa and others in the allium family are high in flavonoids, powerful antioxidants known to prevent disease by attacking harmful free radicals within the body (Christopher, 2005). In particular, *Allium cepa* is very rich in the flavonoid and quercetin, a compound shown in studies to help prevent heart disease by not only preventing cholesterol from attaching to arterial walls, but also by preventing blood platelets from sticking together and forming clots Diplock *et al*., (1999), shows people who consume the most quercetin-containing foods (onion soup in this study) have a reduced risk of thrombosis and cardiovascular disease. Quercetin is also shown to stop the progression of tumours, one reason this compound is often used for cancer prevention. Researchers at the University of Utah have further found that quercetin may in fact help to reduce blood pressure (Flora *et al* 2012).

2.6.1 Chemical constituents of *Allium cepa*

Allium cepa not only provide flavour; they also provide health-promoting phytochemicals as well as nutrients. *Allium cepa* contains an acrid, volatile principle that stimulates the tear glands and the mucous membranes of the upper respiratory tract. Of all the healthy compounds contained in onions, two stand out: sulphur and quercetin, both being strong antioxidants. They have each been shown to help neutralize the free radicals in the body, and protect the membranes of the body's cells from damage. Antioxidants are compounds that help delay or slow the oxidative damage to cells and tissue of the body (Christopher, 2005).

Studies have indicated that quercetin helps to eliminate free radicals in the body, to inhibit low-density lipoprotein oxidation (an important reaction in the atherosclerosis and coronary heart disease), to protect and regenerate vitamin E (a powerful antioxidant) and to inactivate the harmful effects of chelate metal ions (Young *et al* 2001).

White *Allium cepa* contain very little quercetin, so it is better to stick with the yellow and red varieties. Most health professionals recommend eating raw onions for maximum benefit, but cooking makes them more versatile and do not significantly reduce their potency (Olsson *et al* 2010). In fact, unlike sulphur compounds, quercetin can withstand the heat of cooking as long as it is a low heat. The strong smell of the *allium cepa* and its relatives contain thioallyl compound or alliins, and alliins are an amino acid. When cut or crushed, the alliin within the onion is converted by an enzymatic reaction into allicin, this breaks down into sulphide compounds. Sulphide compounds are aromatic and this is what gives the onion, and all the plants in the onion family, their distinctive smell.

In addition to quercetin, *allium cepa* contain the phytochemicals known as disulphides, trisulphides, and vinyl dithiins. These compounds have a variety of health-functional properties, including anticancer and antimicrobial activities (Christopher, 2005). *Allium cepa* is also a source of vitamin C, potassium, dietary fibre and folic acid. They also contain calcium, iron and have a high protein quality, ratio of mg amino acid/gram protein. *Allium cepa* are low in sodium and contain no fat. They are low in calories with only 30 calories per serving, yet it adds abundant flavour to a wide variety of foods. *Allium cepa* is also cholesterol free, and provides dietary fiber, vitamin C, vitamin B6, potassium, and other key nutrients (Pearson, 1997).

2.6.2 Therapeutic Potentials of *Allium cepa*

Allium cepa have been valued for their medicinal qualities by many cultures around the globe. Numerous health benefits have been attributed to the vegetable, including prevention of cancer and cardiovascular disorders. Scientific studies have shown a positive relationship between vegetable intake and risk for these common diseases. This has led many researchers to test whether the proposed medicinal attributes of *allium cepa* are valid:

Epidemiological data both support (Gao *et al*., 1999; Hu *et al*., 1999) and refute (Dorant *et al*., 1995) the concept that higher intake of *allium cepa* is positively related to lower risk for carcinoma. In a review on the effects of quercetin, (Katan *et al.*,1998) noted that persons in the highest consumption category versus the lowest had a 50% reduced risk of cancers of the stomach and alimentary and respiratory tracts.

Organosulphur compounds such as diallyl disulphide (DDS), S-allylcysteine (SAC), and S-methylcysteine (SMC) have been shown to inhibit colon and renal carcinogenesis (Fukushima *et al*., 1997; Hatono *et al*., 1996). Mechanisms of protection ranged from induced cancer cell apoptosis and gene transcription inhibition (Miodini *et al*., 1999) to protection against UV-induced immunosuppression (Steerenberg *et al.*, 1998).

Significant research has been carried out on the effect of *allium cepa* consumption on diabetic conditions. The organosulphur compounds S-methylcysteine sulphoxide (SMCS) and S-allylcysteine sulphoxide (SACS) were linked to significant amelioration of weight loss, hyperglycemia, low liver protein and glycogen, and other characteristics of diabetes mellitus in rats (Sheela *et al*., 1995).

An *In vivo* analysis of the effects of quercetin on human diabetic lymphocytes showed a significant increase in the protection against DNA damage from hydrogen peroxide at the tissue level (Lean *et al*., 1999).

It has been shown that both organosulphur compounds (Shenoy and Choughley *et al*., 1992) and flavonoids (Vinson, 1998) found in *allium cepa* have a protective effect against NOCs.

Muhlbauer and Li (1999) demonstrated that *allium cepa* intake by rats was responsible for increasing bone mass, bone thickness, and bone mineral density. *Allium cepa* inhibited bone resorption by 20% when consumed at a rate of 1.00g per day per kg body weight.

Research on *in vivo* effects of *Allium cepa* consumption in rats showed significant inhibition of serum thromboxane, an inducer of platelet aggregation, levels with high doses (500mg/kg) (Bordia *et al*., 1996).

Researchers found that presence of quercetin significantly reduced LDL oxidation *in vitro* from various oxidases including 15-lipoxygenase, copper-ion, UV light, and linoleic acid hydroperoxide (Nègre-Salvayre and Salvayre, 1992; da Silva *et al*., 1998; Aviram *et al*., 1999; Kaneko and Baba, 1999).

2.7 Blood

Blood is a circulating tissue composed of fluid plasma and cells (red blood cells, white blood cells, platelets). Anatomically, blood is considered as connective tissue because of its origin in the bones and its function. Blood as the transport system of the body, transports elements (e.g nutrients, waste products and heat) from one location in the body to another via blood vessels. Blood is a fluid in the human body that has the major task of transporting oxygen, salts, hormones, carbon dioxide and many other components from one place to another in order to maintain a healthy life. Blood composition is made of a fluid called "plasma" and three additional types of cells which include red blood cell (picks up oxygen from the lungs and carries it to the body's organs), white blood cell (assist the body in fighting infections and other diseases) and platelets (assist in forming blood clots to control bleeding) (Peter, 2013).

2.7.1 Human Blood Plasma

Plate 2.2: Human Blood Plasma

Blood plasma (Plate 2.2) is the pale-yellow liquid component of blood that normally holds the blood cells in whole blood in suspension. It makes up about 55% of the body's total blood volume (Dennis, 1999). It is the intravascular fluid part of extracellular fluid (all body fluid outside of cells). It is mostly water (up to 95% by volume), and contains dissolved proteins (6-8%) (i.e.—albumins, globulins, and fibrinogen), glucose, clotting factors, electrolytes (Na^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^- e.t.c. hormones and carbon dioxide (plasma being the main medium for excretory product transportation (Dennis, 1999).

Plasma also serves as the protein reserve of the human body. It plays a vital role in an intravascular osmotic effect that keeps electrolytes in balanced form and protects the body from infection and other blood disorders.

2.7.2 Importance of human blood plasma

Human blood plasma is a liquid medium in which cells and platelets can float. It helps in the transportation of CO_2 in solution, food materials in solution, urea in solution, hormones in solution, heat, substances needed for blood clotting, antibodies and unwanted materials and toxic substances from one part of the body to another (Peter *et al*., 2013).

2.8 Biosorption

Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). It can be described as the binding and concentration of heavy metals from aqueous solutions by certain types of inactive, dead, microbial biomass (Ahalya *et al* 2003). There are several applications of biosorption such as metal plating and metal finishing operations, mining and ore processing operations, metal processing (waste recovery), battery and accumulator manufacturing operations, thermal power generation (coal-fired plants in particular), and nuclear power generation.

Biosorption is a new technology for removing metal ions from aqueous solutions for replacing conventional methods such as reduction or oxidation, ion exchange, filtration, electrochemical treatment, membrane technology, evaporation recovery, chemical precipitation, chemical lime coagulation and solvent extraction. These conventional means has many disadvantages like extremely expensive, incomplete metal removal and generation of toxic compound. Thus, biosorption is a good method compare to others because it has major advantages include low cost, high efficiency, minimization of chemical and or biological sludge, no additional nutrient requirement, regeneration of biosorbent and possibility of metal recovery (Kratochvil and Volesky , 1998).

2.8.1 Factors affecting Biosorption

The investigation of the efficacy of the metal uptake by the microbial biomass is essential for the industrial application of biosorption, as it gives information about the equilibrium of the process which is necessary for the design of the equipment. The metal uptake is usually measured by the parameter 'q' which indicates the milligrams of metal accumulated per gram of biosorbent material and 'qH' is reported as a function of metal accumulated, sorbent material used and operating conditions. There are some factors that affect the biosorption process;

Temperature seems not to influence the biosorption performances in the range of 20-35^{0}C (Aksu *et al*., 2011), but pH seems to be the most important parameter in the biosorptive process: it affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions (Galun *et al*., 1987). Likewise biomass concentration in solution seems to influence the specific uptake: for lower values of biomass concentrations there is an increase in the specific uptake (Fourest and Roux, 1992; Gadd, 1993).

Gadd (1988) suggested that an increase in biomass concentration leads to interference between the binding sites. Fourest and Roux (1992) invalidated this assertion attributing the responsibility of the specific uptake decrease to metal concentration shortage in solution. Therefore this factor needs to be taken into consideration in any application of microbial biomass as biosorbent. Furthermore biosorption is mainly used to treat wastewater where more than one type of metal ions would be present; the removal of one metal ion may be influenced by the presence of other metal ions. For example: Uranium uptake by biomass of bacteria, fungi and yeasts was not affected by the presence of manganese, cobalt, copper, cadmium, mercury and lead in solution (Sakaguchi and Nakajima, 1991). In contrast, the presence of Fe^{2+} and Zn^{2+} was found to influence uranium uptake by *Rhizopus arrhizus* (Tsezos and Volesky, 1982) and cobalt uptake by different microorganisms seemed to be completely inhibited by the presence of uranium, lead, mercury and copper (Sakaguchi and Nakajima, 1991).

2.8.2 Biosorption mechanism

Adsorption and desorption studies invariably yield information on the mechanism of metal biosorption: how is the metal bound within the biosorbent. This knowledge is necessary for understanding of the biosorption process and it serves as a basis for quantitative stoichiometric considerations which constitute the foundation for mathematical modeling of the process.

The various metal-binding mechanisms that have been postulated to be active in biosorption are ion exchange, complexation, chelation, precipitation and coordination (Complex Formation).

2.8.2.1 *Ion Exchange*

Ion exchange is a reversible chemical reaction wherein an ion in a solution is exchanged for a similarly charged ion attached to an immobile solid particle. These solid ion-exchange particles are either naturally occurring inorganic zeolites or synthetically produced organic resins. Synthetic organic resins are the predominant type used today because their characteristics can be tailored to specific applications.

Ion exchange reactions are stoichiometric and reversible, and as such they are similar to other solution-phase reactions. For example, in the reaction

$$NiSO_4 + Ca(OH)_2 \longrightarrow Ni(OH)_2 + CaSO_4 \qquad (2.1)$$

The nickel ions of the nickel sulphate ($NiSO_4$) are exchanged for the calcium ions of the calcium hydroxide $Ca(OH)_2$ molecule (Norhaslin, 2008)

2.8.2.2 *Complexation*

Metal removal from a solution may also take place by complex formation on the cell surface after the interaction between the metal and the active groups. Aksu *et al*., (2011) hypothesized that biosorption of copper by C. *vulgaris* and Z. *ramigera* takes place through both adsorption and formation of coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides . Complexation was found to be the only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury accumulation by *Pseudomonas syringae.* Microorganisms may also produce organic acids (e.g., citric, oxalic, gluonic, numeric , lactic and malic acids), which may chelate toxic metals resulting in the formation of metallo-organic molecules . These organic acids help in the solubilisation of metal compounds and their leaching from their surfaces. Metals may be biosorbed or complexed by carboxyl groups found in microbial polysaccharides and other polymers (Norhaslin, 2008).

2.8.2.3 *Chelation*

The word *chelation* is derived from the Greek word *chele*, which means *claw*, and is defined as the firm binding of a metal ion with an organic molecule (ligand) to form a ring structure. The resulting ring structure protects the mineral from entering into unwanted chemical reactions (William *et al* 1982).

2.8.2.4 *Precipitation*

Precipitation may be either dependent on the cellular metabolism or independent of it. In the former case, the metal removal from solution is often associated with active defense system of the microorganisms. They react in the presence of toxic metal producing compounds, which favor the precipitation process. In the case of precipitation not dependent on the cellular metabolism, it may be a consequence of the chemical interaction between the metal and the cell surface. The various biosorption mechanisms mentioned above can take place simultaneously (Ahalya *et al*, 2003)

2.8.2.5 *Coordination (Complex Formation)*

A coordination complex is any combination of cations with molecules or anions containing free pairs of electrons. Bonding may be electrostatic, covalent or a combination of both; the metal ion is co-ordinately bonded to organic molecules.

Example of the formation of a coordination compound is:

$$Cu^{2+} + 4H_2O \longrightarrow [Cu\ (H_2O)]_4^{2+} \quad (2.2)$$

$$Cu^{2+} + 4Cl^{-} \longrightarrow [CuCl_4]^{2-} \quad (2.3)$$

Where coordinate covalent bonds are formed by donation of a pair of electrons from H_2O and Cl^{-} (Lewis bases) to Cu^{2+} (Lewis acid).

2.8.3 Biosorption Isotherms

Isotherm adsorption models have been used in waste stream treatment to predict the ability of a certain absorbent to remove a pollutant down to a specific discharge value. When a mass of adsorbent and a waste stream are in contact for a sufficiently long time, equilibrium between the amount of pollutant absorbed and the amount remaining in the solution will develop. For any system under equilibrium conditions, the amount of material adsorbed onto the media can be calculated using the mass balance of Equation 2.4:

$$q_e = V * \frac{C_o - C_e}{m_s} \quad (2.4)$$

where qe is equilibrium concentration of metal ions, C_0 and Ce (mg/L) are the initial and the final concentrations of adsorbate in flasks, respectively, V is the volume of the solution (in cm^3) and m_s is the weight of adsorbent used (in gram), (Wang *et al*., 2010).

Adsorption isotherms are important criteria in optimising the use of adsorbents as they describe the nature of interaction between adsorbate and adsorbent. Thus, analysis of experimentally obtained equilibrium data by either theoretical or empirical equations is useful for practical design and operation of adsorption systems. The Langmuir, Freundlich and Tempkin adsorption isotherms were applied to each metal under study.

2.8.3.1 *Langmuir Isotherm*

Langmuir equation relates the coverage of molecules on a solid surface to concentration of a medium above the solid surface at a fixed temperature. This isotherm based on three assumptions, namely adsorption is limited to monolayer coverage, all surface sites are alike and only can accommodate one adsorbed atom and the ability of a molecule to be adsorbed on a given site is independent of its neighbouring sites occupancy. By applying these assumptions, and a kinetic principle (rate of adsorption and desorption from the surface is equal), the Langmuir equation can be written as presented in equation 2.5 (Hashem, 2007).

$$Q_e = \frac{Q_m . K_L . C_e}{1 + K_L C_e} \qquad (2.5)$$

where:

• Ce – equilibrium concentration of metal ions in the liquid

• Qe – equilibrium concentration of metal ions in the solid phase

• Qm – Langmuir constant for maximum metal uptake

• K_L – Langmuir sorption equilibrium constant

The above Langmuir equation is often written in the linear form as follows:

$$\frac{C_e}{Q_e} = \frac{1}{Q_m} C_e + \frac{1}{K_L . Q_m} \qquad (2.6)$$

Qm and b can be calculated from the slope and intercept of the linear plot, Ce/qe versus Ce. That is, Qm = 1/slope and K_L = 1/(intercept. Qm).

2.8.3.2 *Freundlich Isotherm*

Freundlich isotherm is an empirical equation. This equation is one among the most widely used isotherms for the description of adsorption equilibrium. Freundlich isotherm is capable of describing the adsorption of organic and inorganic compounds on a wide variety of adsorbents including biosorbent. This equation as presented in equation 2.7:

$$Q_e = K_f C_e^{1/n} \tag{2.7}$$

where:

• Qe and Ce are the equilibrium concentrations of metal ions in the adsorbed and liquid phases

• Kf is the Freundlich constant characteristic of the system, indicating the capacity

• n is the Freundlich constant characteristic of the system, indicating the adsorption intensity

This equation can also be expressed in the linearized logarithmic form as presented in equation 2.8;

$$\text{Loq } Q_e = \text{Loq } K_f + \frac{1}{n} \text{Log } C_e \tag{2.8}$$

The plot of log *Q*e versus log *C*e has a slope with the value of 1/*n* and an intercept magnitude of log *K*f. Thus, the Freundlich constants Kf and n can be calculated from the intercept and slope of the linear plot of log qe versus log Ce. log *K*f is equivalent to log *Q*e when *C*e equals unity. However, in other case when 1/*n* 1, the *K*f value depends on the units upon which *Q*e and *C*e are expressed. On average, a favourable adsorption tends to have Freundlich constant *n* between 1 and 10. Larger value of *n* (smaller value of 1/*n*) implies stronger interaction between biosorbent and heavy metal while 1/*n* equal to 1 indicates linear adsorption leading to identical adsorption energies for all sites. This isotherm attempts to incorporate the role of substrate-substrate interactions on the surface. (Freundlich, 1906; Reddy *et al*., 2010).

2.8.3.3 *Tempkin isotherm*

The Tempkin isotherm model was also applied to the experimental data, unlike the Langmuir and Freundlich isotherm models, this isotherm takes into account the interactions between adsorbents and metal ions to be adsorbed and is based on the adsorption that the free energy of adsorption is simply a function of surface coverage (Wang and Qin, 2005).

The Temkin isotherm can be written as;

$$q_e = RT/b \text{ in}(A_T C_e) \qquad (2.9)$$

$$qe = Rt/b_T \text{ In } A_T + (RT/b) \text{ In } C_e \qquad (2.10)$$

$$B = RT/b_T \qquad (2.11)$$

AT =Temkin isotherm equilibrium binding constant (L/g)

bT = Temkin isotherm constant

R= universal gas constant (8.314J/mol/K)

T= Temperature at 298K.

B = Constant related to heat of sorption (J/mol)

The linear form of the Tempkin isotherm model is given in equation 2.12:

$$qe = B\ln A + B\ln Ce \qquad (2.12)$$

Where *b*= [RT/B] in (J/mol) corresponding to the heat of adsorption, *R* is the ideal gas constant, *T*(K) is the absolute temperature, *B* is the Tempkin isotherm constant and *A* (L/g) is the equilibrium binding constant corresponding to the maximum binding energy. This isotherm was applied by a linear plot of qe against lnCe and the constants *B* and *A* were calculated from the slope and intercept respectively.

2.8.3.4 *Separation Factor*

The shape of the isotherm can be used to predict whether adsorption system is favourable or unfavourable in a batch adsorption system. Accordingly, the essential feature of Langmuir isotherm was expressed in term of dimensionless constant called the separation factor. It is defined by the following relationship.

$$R_L = \frac{1}{(1+K_L C_i)} \qquad (2.13)$$

where R_L is the a dimensionless equilibrium parameter or separation factor, b, the constant from Langmuir equation and Ci the initial metal ion concentration. The parameter, R_L, indicates the shape of the isotherm and nature of the sorption process. If

$R_L > 1$ then unfavourable isotherm

$R_L = 1$ then linear isotherm

$R_L = 0$ then irreversible Isotherm

$0 < R_L < 1$ then favourable isotherm (Horsfall *et al*., 2003)

2.8.4 Sorption Kinetics

2.8.4.1 *Lagergren Pseudo first-order kinetic model*

The linearised form of the Lagergren pseudo first-order kinetic model (Abdelwahab *et al.*, 2006) was applied to the experimental data to describe the kinetics of Cu, Pb and Zn (II) ions sorption. This expression is presented in equation 2.11:

$$\text{Log}\,(q_e - q_t) = \text{Log}\,(q_e) - \frac{k_1}{2.303} \qquad (2.14)$$

Where q_t and q_e are the amount of solute absorbed per mass of sorbent (mgg^{-1}) at any time and equilibrium, respectively, and k_1 is the rate constant of first-order sorption (min^{-1}). The straight-line plot of log (qe - qt) against t gives log (qe) as slope and intercept equal to $k_1/2.303$. Hence the amount of solute absorbed per gram of sorbent at equilibrium (qe) and the first-order sorption rate constant (k_1) can be evaluated from the slope and the intercept. It is required that calculated equilibrium adsorption capacity values, qe (cal.), should be in accordance with the experimental qe (exp.) values (Febrianto *et al.* 2009). This will suggest that the biosorption follow pseudo-first-order kinetics.

2.8.4.2 *Lagergren Pseudo second-order kinetic model*

The Lagergren pseudo second-order kinetic model was applied to the experimental data to describe the kinetics of Cu, Pb, and Zn ion sorption. The model is represented in equation 2.11, (Ho and McKay, 1999):

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + (\frac{1}{q_e})t \qquad (2.15)$$

Where k_2 is the rate constant of pseudo-second-order biosorption ($gmg^{-1}min^{-1}$). The pseudo-second-order rate constant k_2 and q_e values were calculated from the slope and intercept of the plots t/q against t. The calculated q_e values must agree well with experimental qe values. This suggests that the experimental Data agrees with pseudo-second-order kinetic model where k_2 is the rate constant, qt is the metal uptake capacity at any time t.

2.8.4.3 *Intra-particle diffusion model*

Theoretical treatments of intra-particle diffusion yield rather complex mathematical relationships which differ in form as functions of the geometry of the sorbent particle (Crank, 1979). Although a mathematical treatment of the diffusion process will not be presented here, it is useful to describe one characteristic of the theoretical expressions in order to facilitate the presentation of the data. A functional relationship common to most treatments of intra-particle diffusion is that uptake varies almost proportionately with the half power of time, $t^{0.5}$, rather than t; nearly linear variation of the quantity sorbed with $t^{0.5}$ is predicted for a large initial fraction of reactions controlled by rates of intra-particle diffusion. Good linearization of the data is observed for the initial phase of the reaction in accordance with expected behaviour if intra-particle diffusion is the rate-limiting step. The intra-particle diffusion equation is the following:

$$qt = ki\ t^{0.5} \qquad (2.16)$$

Linearization of equation 2.16;

$$q = k_i\ t^{0.5} + C \qquad (2.17)$$

where:

k_{id} is the intra-particle diffusion rate constant, $mgg^{-1}min^{-0.5}$, C is the intercept.

According to this model, the plot of uptake, qt, versus the square root of time ($t^{0.5}$) should be linear if intra-particle diffusion is involved in the adsorption process and if these lines pass through the origin then intra-particle diffusion is the rate-controlling step (Arami *et al.*, 2008). When the plots do not pass through the origin, this is indicative of some degree of boundary layer control and this further show that the intra-particle diffusion is not the only rate-limiting step, but also other kinetic models may control the rate of adsorption, all of which may be operating simultaneously (Yakout and Elsherif, 2010).

2.9 Previous Research on Biosorption

There are varieties of plant product that maybe useful for removing heavy metal ions from blood Plasma.

Cha, (1987) pointed out that the protective effect of allium species is probably caused by the sulphur compounds combining with the heavy metals in the body and promoting excretion through bile to the faeces.

Cummings *et al.* (1998) reported that not only that *Allium Cepa* has long been used in traditional medicine but is one of the important *Allium* species commonly used in our daily diet and has recently been the source of much interest because of its anti-thrombotic, hypolipidaemic, hypertensive, diaphoretic, antibiotic, anti- diabetic and anti-cancer properties.

Vernet, (1991) studied the removal of heavy metals by fungal (*Aspergillus oryzae*) biosorption, other studies supported (Veglio and Beolchini, (1997); Vijayaraghavan and Yun, (2008); Wang and Chen, (2006), with the mechanisms responsible for biosorption, although understood to a limited extent, there maybe one or combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and microprecipitation.

Feng *et al.*,(1993) investigated Iron detoxification of human plasma with iron(III) -chelating resins, they reported that Iron detoxification of human blood plasma was studied with resins containing desferrioxamine B (DFO) or 3-hydroxy-2-methyl-4(1H)-pyridinone (HMP) as iron(III)-chelating groups. The behaviour of four resins was investigated; DFO-

Sepharose, HMP-Sepharose and crosslinked copolymers of 1-(/3-acrylamidoethyl)-3-hydroxy-2-methyl- 4(lH)-pyridinone (AHMP) with 2-hydroxyethyl methacrylate (HEMA) and of AHMP with N,Ndimethylacrylamidc (DMAA). The efficiency of iron detoxification of plasma of the resins was mainly dependent on the affinity of the ligands and the hydrophilicity of the resins. The results of a stability study in phosphate-buffered saline at a physiological pH indicated that AHMP-DMAA was the most stable resin, whereas the sepharose gels had a relatively lower stability. Experiments with the AHMP-DMAA resin showed that the resin was able to remove iron from plasma with different iron contents, and from plasma poisoned with $FeCl_3$, iron(iii) citrate or transferrin.

Koch *et al.,* (1996), reported a protective effect for garlic and onions against cadmium and mercury poisoning in rats. Oral co-administration of garlic and onion with cadmium or organic mercury compounds for 12 weeks resulted in a decrease in the accumulation of heavy metals in liver, kidneys, bone and testes. (These are the target organs of cadmium poisoning.) Histopathological damage and the inhibition of serum alkaline phosphatase were also decreased. The effective doses of fresh garlic and Onion were 3.35% and 6.70% of the diet, which provided 100 and 200 mgL^{-1} of allicin, respectively. The protective effect began to appear in the 3.35% group, and the heavy metal accumulation decreased more than 40% in the 6.7% group compared to the group exposed only to the metal.

Kamiñski and Modrzejewska, (1997) investigated the application of chitosan membranes for the separation of heavy metal ions. According to these authors, it is well-known that chitosan forms chelates with metal ions by releasing hydrogen ions. Therefore the adsorption of a metal ion on chitosan depends strongly on the pH of the solution. The results showed that chitosan membranes produced by the phase-inversion method can be applied successfully to the removal of metal ions. The metal ions Cu (II), Cd (II), Co (II), Zn (II) and Ni (II) were almost completely retained by the membrane. In the case of Cr (VI) and Mn (II) ions, the separation depended on the pH and the process conditions. Mechanisms of action include free radical scavenging, chelation of transition metal ions, and inhibition of oxidases such as lipoxygenase S described by (de Groot and Rauen, 1998; Lean *et al*, 1999).

Berthold *et al.*, (1998) and Blumenthal *et al.* (2000) in their studies pointed out the effects of aromatic herbs and spices in alleviating symptoms of the diseases, that are caused by heavy metal. The intervening actions, among the constituents of these plants as natural products, the toxicity of heavy metals and the biological activities of the living organisms were revealed. In particular, the use of *Allium cepa.* as a therapeutic remedy in the alternative medicine has recently, been focused.

Çeribasi and Yetis (2001), investigated the biosorption kinetics of Ni(II) and Pb(II) by the resting cells of a lignolytic white-rot fungus, *Phanerochaete chrysosporium*, from a binary metal system. Kinetic studies revealed that biosorption takes place in two stages: a rapid surface adsorption, within the first 30 min, and a slow intracellular diffusion till the end of the 3h contact time. In the first minutes the contact solution pH decreased sharply, parallel to the fast metal uptake, probably because of the protons released by the biosorbent. As sorption equilibrium was reached, solution pH also reached an equilibrium level. Metal biosorption capacities increased as the initial metal concentrations increased, independent of the initial pH of the solution. The results also show that some portion of the metal ions sorbed by *P.chrysosporium* was readily released into solution again with a decrease in pH. At equilibrium, the maximum total metal uptake of *P. chrysosporium* is 109.5 mg/g at a pH of 5. Under these circumstances Ni (II) and Pb (II) uptake capacities were 55.9 mg Ni/g and

53.6 mg Pb/g, respectively. The protective effect may be due to the combined effects of reduced absorption of the metal from the gastrointestinal tract and increased excretion. (Senapati, 2005).

Valdés *et al.*, (2008), studied the biosorption isotherms of garlic and onion skin at temperatures of 293 K, 303 K and 313 K and varying pH were also investigated. The study showed that both onion and garlic skin were efficient in Cu (II) ions detoxification of aqueous stream. Removal of Cu (II) ions from aqueous solution by onion skin and garlic skin was studied as a function of various experimental parameters. FTIR analysis and Boehm titration were used to identify and estimate the surface functional groups.

ESCOP, (2003) stated that garlic (*Allium* sativum) a member of the Alliaceae is related to onions (*Allium Cepa.*), chives (*Allium schoenoprasum*) and ornamentals like star of Persia (*Allium cristophii*), and it contains alliin as the main sulphur-containing amino acid. In the presence of the enzyme alliinase (for example, when the bulb is crushed), alliin is converted to allicin (an odourous compound), which then produces a range of other constituents including ajoenes, vinyldithiines and polysulphides, Garlic demonstrated a protective effect against heavy metal poisoning in rats. Oral co-administration of garlic with cadmium or organic mercury compounds for 12 weeks resulted in a decrease in the accumulation of heavy metals in liver, kidneys, bone and testes. (These are the target organs of cadmium poisoning.). In another series of tests, administration of garlic (6.4% of the diet) enhanced the excretion of cadmium, more through faeces than urine. In this test, administration of diallyl disulphide (30 mg/kg), a sulphur constituent of garlic, was inferior to whole garlic treatment. (In these tests, cadmium was administered by injection.)

Jeffrey and Herbert (2003) pointed out that among the two different extracts obtained from onion, cold water extract and fresh onion extracts, it was discovered that cold water extract could not exert any inhibitory effect on all the tested organisms isolated from ocular infection. This indicated the absence of the antibiotic property in cold water onion extracts, they suggested that it might be as a result of high temperature 121^0C used during autoclaving, which may denature the active component. However, the fresh onion extract was observed to inhibit the growth of the bacteria isolated from ocular infection as pointed out by Haley and McDonald (2016), indicating that extract from onion and garlic have antibacterial properties.

Pierre *et al.,* (2005), studied the inhibitory effect of aqueous extracts of some herbs on human platelet aggregation *in vitro*. The effect of the aqueous extract of several herbs on human platelet aggregation *in vitro* was investigated. Out of 28 herbs/ nutriceuticals investigated, camomile, nettle alfalfa, garlic and onion exhibited most significant anti-platelet activity (_45% inhibition). Garlic and Onion show to have the highest inhibitory properties 90.50+12.20% and 71.20+11.90% respectively as proposed by their study.

Gupta and Ratstogi (2008) reported that Biosorption has been found to be economically feasible, rapid, reversible, and ecologically friendly in the removal of heavy metals from aqueous solutions, especially when used in the treatment of high volumes and very low concentrations of solutions containing heavy metals.

Babarinde *et al.,* (2008) reported the removal of Zn (II) ions from dilute aqueous solution using maize (*Zea mays*) leaf as the adsorbent. The study showed that effects of pH, contact time, and initial metal ion concentrations were studied at 27 C. The analysis of residual metal ions was determined using atomic absorption spectrophotometer. The result of the pH study shows that the initial pH would play a vital role in the removal of the metal ions from solution. The optimum pH obtained was in the range pH 4-7. Kinetic studies show that the uptake of zinc ions increases with time and that maximum adsorption was obtained within the first 40 minutes of the biosorption process. The adsorption isotherms obtained at 27 C and optimum pH fitted well into both the Freundlich and Langmuir isotherms. The Freundlich and Langmuir equations are $\log \Gamma = 1.7833 \log C_e + 0.1316$ and $1/\Gamma = 0.0423/C_e - 0.3565$, respectively. The correlation factors are 0.9964 and 0.9974, respectively. The free energy change for the biosorption of Zn(II) at 27 C, initial concentration of 100 mgL^{-1} and pH 4 -7 is –5.4525 kJ mol^{-1}. These results indicate that maize leaf has potential for the removal of zinc ions from industrial wastewater.

Muráňová *et al.*, (2010) investigated cadmium sorption by dried plant biomass through a reversibility studies and reported that heavy metals such as cadmium are hazardous to biosystems and present possible human health risk. Thus, the removal of cadmium from liquid wastes is of great importance from an environmental and industrial point of view.

Csaba, (2011), investigated "Phytoremediation of heavy metals with cucumber, white poplar and aquatic plants" he reported that All three plants accumulated the heavy metals from their solution, which renders them a possible subject for phytoremediation/ phytoextraction experiments. The concentration of all three metals in all plants increased both when added solely and all together. With few exceptions, there were differences between the uptakes of heavy metals whether in the presence or absence of the other metals, indicating that there was no competition between the metals at uptake (this could be because there are specific uptake and binding sites for each of the metals, thus precluding interaction.

Ashraf *et al.*, (2011) in their study of low cost biosorbent for biosorption of heavy metals reported, that the efficacy of banana peel (*Musa sapientum*) biomass was tested for the removal of lead, copper, zinc and nickel metal ions using batch experiments in single and binary metal solution under controlled experimental conditions. It was found that metal sorption increases when the equilibrium metal concentration rises. At highest experimental solution concentration used (150 mg/L), the removal of metal ions were 92.52% for lead, 79.55% for copper, 63.23% for zinc and 68.10% for nickel while at lowest experimental solution concentration (25mg/L), the removal of metal ions were 94.80% for lead, 86.81% for copper, 84.63% for zinc and 82.36% for nickel. Biosorption equilibrium isotherms were plotted for metal uptake capacity (q) against residual metal concentrations (Cf) in solution. The q versus Cf sorption isotherm relationship was mathematically expressed by Langmuir and Freundlich models. The values of separation factor were between zero and one indicating favourable sorption for four tested metals on the biosorbent. The surface coverage values were approaching unity with increasing solution concentration indicating effectiveness of biosorbent under investigation.

Chowdhury and Datta *et al.* (2012) Investigated equilibrium, thermodynamic and kinetic studies for removal of copper (II) ions from aqueous solution by onion and garlic skin. Point of zero charge for the onion and garlic skin was between 4.25 to 4.45 and 4.55 to 4.75 respectively. Compared to garlic skin (66.7mg/g), the maximum adsorption capacity of onion skin (76.9 mg/g) was slightly higher (pH = 5.3, T = 303K). The pseudo second order kinetic model fitted the batch data adequately. The rate constant for onion and garlic scale in the absence of diffusional resistance was estimated to be 0.07 and 0.075 mg/g/min respectively at 303 K. The thermodynamic studies showed that the Cu^{2+} adsorption on the onion and garlic skin was spontaneous and endothermic. The high adsorptive capacity of these biosorbents implies that it can be looked as an alternative to costly adsorbents like activated carbon, resins, etc.

Adelaja *et al*., (2011)., in their study reported the adsorption of Pb^{2+} onto ground *Moringa oleifera* pods under various conditions of pH, contact time, adsorbent dosage, and concentration of adsorbate. The adsorption process was checked for conformity with the Freundlich and Langmuir isotherms. From the adsorption experiments conducted, it was observed that the adsorption was pH, contact time, and adsorbate concentration dependent. The adsorption process was partly dependent on adsorbent dosage. It was also discovered that the adsorption did not conform to the Freundlich and Langmuir adsorption isotherms. Maximum percent adsorption obtained after contacting the Pb^{2+} aqueous solution with ground *Moringa* pods for 180mins was 48.4% at pH7. This study revealed that *Moringa* pod is not a very good biosorbent for the removal of Pb^{2+} from wastewater at pH.

Salem *et al.*, (2012) carried out study to investigate the effectiveness of an inexpensive and eco-friendly loquat bark as a biosorbent for the possible removal of toxic lead, zinc, and cadmium ions from aqueous solutions. In this study, the effects of biosorbent dose, pH, initial concentration, contact time, and temperature were examined. The linear Langmuir and Freundlich models were applied to describe the equilibrium isotherms, and both models fitted well. The monolayer adsorption capacity of of Pb(II), Zn(II) and Cd(II) on loquat bark was found as 55.770, 29.447 and 28.802 mg g^{-1}, respectively, at pH 4 and 30oC. Dubinin-Radush Kerich (D-R) isotherm model was also applied to equilibrium data. The mean free energy of adsorption of Pb(II), Zn(II) and Cd(II) (5.596, 3.748 and 3.549 kJ/mol) onto loquat bark, respectively, may be carried out via physisorption mechanism. Loquat bark (LB) was found to remove Pb(II), Zn(II), and Cd(II) ions efficiently from aqueous solutions with selectivity in the order of Pb(II) > Zn(II) > Cd(II).

According to Elhadi *et al.,* (2013), heavy metal removal by herb plants has received the most attention, due to their medicinal properties, good performance, low cost and large available quantities. In this study, the performance of garlic cloves for removal of cadmium, lead and mercury ions from aqueous solutions and human plasma were examined by using Flame Atomic Absorption Spectrophotometer (FAAS). The effects of the amount of each additive, time interval of addition and pH, on the removal of heavy metals were evaluated. Based on the above results, concentrations of heavy metals in aqueous solutions, and human plasma have, therefore, been reduced by approximately 0.5% and 0.2 % with one gram of garlic per cm^3 of solution, in five minutes, respectively. A special technique to immobilize Allicins, is required.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Apparatus and Equipment

Volumetric flask (1000 cm^3), Measuring cylinder (50 cm^3 and 10 cm^3), Conical flask (250 cm^3), Polypropylene sample bottles (120 cm^3), Beakers (250 cm^3), Petri-dish, Watch glass, thermometer, refrigerator, wash bottles, Funnels, glass rod, Whatman No 11 filter papers, Analytical balance (A and D instrument GR-200EC model), pH meter (Crison Micro pH 2000 model), Buffer 7 solution, Mechanical Shaker (Gallenkamp BKS-300-010F model), Centrifuge (Labofuge 300 Hereaus model), Deionizer (Elgacan C115 model) and Atomic Adsortion Spectrophotometer (AA240FS Varian).

3.2 Reagent and Samples

Copper tetraoxosulphate (VI) pentahydrate salt ($CUSO_4.5H_2O$), Lead trioxonitrate(V) salt ($Pb(NO_3)_2$), Zinc trioxonitrate (V) hexahydrate salt ($Zn(NO_3)_2.6H_2O$), Nitric acid (HNO_3), Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Buffer 7, Deionise water, *Allium cepe* bulb, and Human plasma. All reagent used were of anular grade.

3.3 Sample Collection

3.3.1 Collection of *Allium cepa.*

The *Allium cepa* bulbs, used for heavy metal detoxification, were bought from a vendor at Samaru market, Zaria province. They were stored in a new clean polyethene bag prior to analysis.

3.3.2 Collection of human blood

The healthy Human blood from which the plasma was extracted was collected from haematology Department, Ahmadu Bello University Teaching Hospital, (ABUTH) Zaria in a clean blood collection tube prior to analysis.

3.4 Sample Preparation

3.4.1 Preparation of biosorbent (*Allium cepa*)

The *Allium cepa* bulbs was carefully washed with tap water to remove adhering materials from their surface and then rewashed with deionised water. They were sliced and air dried for a period of 10 days. The dried Allium cepa was washed with deionised water to remove dust and dried in an oven at a temperature of $60^{\circ}C$, at interval of 2hr the dried sample was weighed until constant weight was attained at 48 hr (Gupta and Rastogi, 2007). The dried sample was pulverized with agate mortar and passed through 0.25 to 0.50 mm micro sieve. This was to allow for shorter diffusion path allowing the adsorbate to penetrate deeper into the biosorbent more quickly, resulting in a higher rate of biosorption (Adeyinka *et al.,* 2007). The powdered sample was stored in polyethylene bottles prior to analysis and was then used as biosorbent.

3.4.2 Preparation of human blood plasma

Blood sample was drawn into blood collection tubes containing 1.80 mg citrate phosphate dextrose adenine (CPDA) per blood cm^3. The actual volume was drawn to ensure the correct blood to anticoagulant ratio. The blood collection tubes were inverted carefully 10 times to mix blood and anticoagulant. Samples were centrifugated at 175 rpm immediately for a minimum of 10 min at room temperature. This gave three layers: (from top to bottom) plasma, leucocytes (buffy coat) and erythrocytes, after which the supernatant (Plasma) was carefully aspirated into centrifuge tubes at room temperature. Care was taken not to disrupt the cell layer or transfer any cell. The plasma was inspected for turbidity. Turbid samples were centrifuged and aspirated again to remove remaining insoluble matter. The plasma was poured into a blood collection tube. It was ensure that the blood collection tubes were adequately labelled with the relevant information, including details of additives present in the human blood (Berthold *et al*., 2001). This was carried out at the Haematology Department, Ahmadu Bello University Teaching Hospital, (ABUTH) Zaria.

3.5 Preparation of stock solutions

3.5.1 Preparation of stock solution of copper (II) ions (1000 mgL^{-1})

The stock solution was prepared by dissolving 3.9291 g of $CuSO_4.5H_2O$ in 50.00 cm^3 of deionised water in a 250 cm^3 beaker. The solution in the beaker was then quantitatively transferred into a 1000 cm^3 volumetric flask and made up to 1000.00 cm^3 with deionised water in a volumetric flask giving 1000 mgL^{-1} Cu (II) ions solution. The working solution for analysis were then prepared from the standard solution via serial dilution (Eaton *et al*., 2005).

3.5.2 Preparation of Stock solution of lead (II) ion (1000 mgL^{-1})

The stock solution was prepared by dissolving 1.5986 g $Pb(NO_3)_2$ salt in 7.50 cm^3 of concentrated Nitric acid (HNO_3). The solution was made up to 1000.00 cm^3 with deionised water in a volumetric flask giving 1000 mgL^{-1} Lead solution. The working solution for analysis were then prepared from the standard solution via serial dilution (Eaton *et al*., 2005).

3.5.3 Preparation of Stock solution of zinc (II) ions (1000 mgL^{-1})

The stock solution was prepared by dissolving 4.3980 g $Zn(NO_3)_2.6H_2O$ salt in 50.00 cm^{3}of deionised water. The solution was made up to 1000.00 cm^3 with deionised water in a volumetric flask giving 1000 mgL^{-1} Zinc ion solution. The working solution for analysis were then prepared from the standard solution via serial dilution (Eaton *et al*., 2005).

3.5.4 Preparation of 0.1M HNO_3

Concentrated HNO_3(aq) (4.44 cm ^{3}) with specific gravity 1.42g/cm^3, was measured into a 1000.00 cm^3 volumetric flask and made up to mark using deionised water.

3.5.5 Preparation of 0.1M NaOH

Sodium hydroxide pellets (4.00 g) were dissolved in 50.00 cm^3 deionise water in a volumetric flask and made up to 1000.00 cm^3 mark with the deionised water.

3.6 Characterization of Samples

3.6.1 Screening of Cu^{2+} in the human blood plasma

Human blood plasma samples were digested by the Conventional Wet Acid Method by adopting the method of (Memon *et al*., 2007). Accurately 1.00 cm^3 of whole blood was taken into Pyrex flask separately. To this was added 6.00 cm^3 of freshly prepared mixture of concentrated nitric acid and hydrogen peroxide (2:1v/v) and was made to stand for 10 min. The flasks were covered with watch glass and then digested at 100^{0}C for 2 hr. The digests were then treated with 4.00 cm^3 nitric acid and 8 drops of H_2O_2, while heating continued on hot plate at 80^{0}C until a clear digested solution was obtained. The excess acid mixture was evaporated to semi - dry mass, cooled and diluted with 2.00 cm^3 of 5% nitric acid. The mixture was separated using Whatman No 11 filter paper. The filtrate was transferred into 100 cm^3 volumetric flask and diluted to mark using deionised water prior to analysis. The same procedure was repeated for Pb (II) and Zn ions.

3.6.2 Screening of Cu^{2+} in the biosorbent

The prepared biosorbent samples were digested by the Conventional Wet Acid Method by adopting the method of (Memon *et al*., 2007). Accurately 1.00 g of biosorbent was placed into Pyrex flask separately. To this was added 6.00 cm^3 of freshly prepared mixture of concentrated nitric acid and hydrogen peroxide (2:1V/V) and was made to stand for 10 min. The flasks were covered with watch glass and then digested at 100^0C for 2 hr. The digests were then treated with 4.00 cm^3 nitric acid and 8 drops of H_2O_2, while heating continued on hot plate at about 100^0C until a clear digested solution was obtained. The excess acid mixture was evaporated to semi - dry mass, cooled and diluted with 2.00 cm^3 of 5% nitric acid. The mixture was separated using Whatman No 11 filter paper and was transferred into 100 cm^3 volumetric flask and diluted to mark using deionised water prior to analysis (Screening). The same procedure was repeated for Pb (II) and Zn ions.

3.6.3 Fourier Transform Infrared Spectroscopy (FTIR) analysis of biosorbent.

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups present in the biosorbent. Biosorbent (10.00 mg) was mixed with 100.00 mg of KBr and then ground to a homogeneous blend and compressed to give a small translucent disc of 13 mm diameter. A background spectrum of pure KBr was run before the spectrum of the samples to account for the absorption due to impurities in the KBr. The disc containing the sample was then placed in the infrared spectrometer and the spectrum of the samples was collected over a range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}. With this technique, functional group(s) responsible for the metal sorption was determined. This analysis was conducted at National Research Institute for Chemical Technology (NARICT) Zaria.

3.6.4 Fourier Transform Infrared Spectroscopy (FTIR) analysis of Cu^{2+} treated biosorbent

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups responsible for binding in the biosorbent. This was done by accurately weighing 0.60 g of the biosorbent into 50.00 cm^3 conical flask, to this was added 20.00 cm^3 prepared human plasma solution spiked with 50 mgL^{-1} of Cu (II) ion solution. The mixture was agitated for a period of 100 min. After the preset contact time was reached, the biosorbent was separated from the mixture via filtration using Whatman No 11 filter paper. The residue was placed in an oven and dried at a temperature of 60^0C for a period of 72 hr. The dried residue (10 mg) was mixed with 100.00 mg of KBr and then ground to a homogeneous blend and compressed to give a small translucent disc of 13 mm diameter. A background spectrum of pure KBr was run before the spectrum of the samples to account for the absorption due to impurities in the KBr. The disc containing the sample was then placed in the infrared spectrometer and the spectrum of the samples was collected over a range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}. This analysis was conducted at National Research Institute for Chemical Technology (NARICT) Zaria. This same procedure will be repeated for Pb (II) and Zn (II) ions.

3.6.5 Scanning Electron Microscope (SEM) analysis of biosorbent

The effect of metal biosorption on the surface of the *Allium cepa* biomass was analyzed using SEM. Surface micrographs of the Native and heavy metals (Cu^{2+}, Pb^{2+} and

Zn^{2+}) treated *allium cepa* biomass powder were recorded using SEM apparatus linked with ISIS Computer Software, U = 10kv and 20kv for SEM Scans were collected. This analysis was conducted at the Department of Chemical Engineering, Ahmadu Bello University, Zaria.

3.7 Spiking of the human blood plasma using various concentration Cu (II) ions solutions

The sample (Human plasma) was spiked with 20 mgL^{-1}, 30 mgL^{-1}, 40 mgL^{-1}, 50 mgL^{-1}, and 60 mgL^{-1} of Cu (II) ions solution respectively, thereafter the biosorbent was introduced into the spiked sample and agitated for 100 min. This was carried out to ascertain the detoxification capacity of the biosorbent under study (EPA 2006). The same procedure was repeated for Pb (II) and Zn (II) ions respectively.

The amount of spiking solution to be added to the sample was determined as expressed in equation 3.1;

$$V_{sol} = \frac{S_{conc.} * V_{sam.}}{C_{spik.}} \quad (3.1)$$

Where:

V_{sol} = Volume of spiking solution (Solution of metal (II) ions)

S_{conc} = Spike concentration desired

V_{sam} = Volume of sample to which spike is added

C_{spik}= Concentration of spiking solution

3.8 Optimisation of biosorption parameters for Cu (II) ions adsorption

3.8.1 Optimization of pH for Cu (II) ions adsorption

The optimisation of pH was studied by introducing 0.60 g of biosorbent into 250.00 cm^3 conical flasks containing 20.00 cm^3 of human blood plasma solution spiked with 50

mgL^{-1} Cu (II) ion solution. The pH of the mixture was adjusted to pH 2 with 0.1M HNO_3 or 0.1M NaOH and the mixture was agitated for a contact time of 100 min (Gochev *et al*., 2010; Elhadi *et al.,* 2013). After the preset contact time was reached, the biomass was separated from the mixture using Whatman No 11 filter paper. The filtrate was then digested and analysed using AAS for residual Cu (II) ions. This procedure was repeated for pH values of 4, 6, 8 and 10 respectively. An Optimum pH 6 was obtained for further experiment.

3.8.2 Digestion of filtrate

Filtrate (3.00 cm^3) was measured into 50.00 cm^3 Pyrex flask separately and 10.00 cm^3 of freshly prepared mixture of concentrated HNO_3 and 5.00 cm^3 of H_2O_2 (2:1) added. The mixture was allowed to stand for 10 min and flasks were covered with watch glass and digested at 70^0C for 2 hr while heating continued on hot plate until a clear digested solution was obtained. The excess acid mixture was evaporated to semi dry mass and cooled. These were transferred into 100.00 cm^3 volumetric flask and diluted to mark using deionised water. The amount of Cu (II) ions in the filtrate was determined by Atomic Adsorption spectroscopy (AAS) method (AA240FS Varian) (Memon *et al.,* 2007).

3.8.3 Optimisation of Cu (II) ions concentration

Biosorbent (0.60 g) was transferred into 250 cm^3 conical flask containing 20 cm^3 human plasma solution spiked with 20 mgL^{-1} Cu (II) ions solution. The mixture was adjusted to pH 6 (Optimum pH) using 0.1M HNO_3 or 0.1M NaOH (Elhadi *et al.*, 2013) and the mixture was agitated for contact time of 100 min after which the biomass was separated from the mixture using Whatman No 11 filter paper. The filtrate was then digested (as presented in 3.8.2) and analysed using AAS for residual Cu (II) ions. This procedure was repeated for Cu (II) ion solution concentrations of 30 mgL^{-1}, 40 mgL^{-1}, 50 mgL^{-1} and 60 mgL^{-1} respectively. An optimum value (50 mgL^{-1}) was obtained for further experiment.

3.8.4 Optimisation of biosorbent dosage for Cu (II) ions adsorption

The optimisation of biosorbent dosage was investigated by accurately weighing biosorbent (0.20 g) and transferred into 250 cm^3 conical flask containing 20 cm^3 human plasma solution spiked with 50 mgL^{-1} Cu (II) ions (Optimum concentration) solution, the mixture was maintained at pH 6 (Optimum pH) using 0.1M HNO_3 and 0.1M NaOH (Elhadi *et al.,* 2013) and the mixture was agitated for contact time of 100 min. The biomass was separated from mixture using Whatman No 11 filter paper. The filtrate was digested (as presented in 3.8.2) and the Cu (II) ions concentration of the filtrate was determined using AAS. This procedure was repeated using 0.40 g, 0.60 g, 0.80 g and 1.00 g biosorbent dosage respectively (Adelaja *et al.,* 2011). An Optimum (0.60 g) was obtained for further experiment.

3.8.5 Study of the rate of biosorption of Cu (II) ions

Optimum mass (0.60 g) of the biosorbent was transferred into 250 cm^3 conical flask containing 20 cm^3 human plasma solution spiked with 50 mgL^{-1} Cu (II) ions (Optimum concentration) (Gochev *et al*., 2010), the mixture was maintained at pH 6 (Optimum pH) using 0.1M HNO_3 and 0.1M NaOH (Elhadi *et al.,* 2013). The mixture was agitated for various contact time of 10 min, 20 min, 30 min, 40 min, 50 min, 60 min, 70 min, 80 min, 90 min, 100 min, 110 min and 120 min respectively. After the pre-set contact time was reached, the solutions were separated from the biomass by filtration using Whatman No 11 filter paper. The filtrate was then digested (as presented in 3.8.2) and residual Cu (II) ions in the solution were determined using Atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200) respectively. The highest rate of biosorption was observed at 80 min.

3.9 Optimisation of biosorption parameters for Pb (II) ions adsorption

3.9.1 Optimization of pH for Pb (II) ions adsorption

The optimisation of pH was studied by introducing the biosorbent (0.60 g) into 250 cm^3 conical flask containing 20 cm^3 of human plasma solution spiked with 50 mgL^{-1} Pb (II) ion solution. The pH of the mixture was adjusted to pH 2 with 0.1M HNO_3 or 0.1M NaOH and the mixture was agitated for a contact time of 100 min (Elhadi *et al.*, 2013). After the preset contact time was reached, the biomass was separated from the mixture using Whatman No 11 filter paper. The filtrate was then digested and determined using AAS for residual Pb (II) ions. This procedure was repeated for pH values of 4, 6, 8 and 10 respectively. An optimum pH 4 was obtained for further experiment.

3.9.2 Digestion of filtrate

Filtrate (3.00 cm^3) was measured into 5.00 Pyrex flask separately and 10.00 cm^3 of freshly prepared mixture of concentrated HNO_3 and 5.00 cm^3 of H_2O_2 (2:1) added. The mixture was allowed to stand for 10.00 min and flasks were covered with watch glass and digested at 70^0C for 2.00 hr while heating continued on hot plate until a clear digested solution was obtained. The excess acid mixture was evaporated to semi dry mass and cooled. These were transferred into 100.00 cm^3 volumetric flask and diluted to mark using deionised water. The amount of Pb (II) ions in the filtrate was determined by Atomic Adsorption spectroscopy (AAS) method (AA240FS Varian) (Memon *et al.*, 2007).

3.9.3 Optimization of Pb (II) ions concentration

Biosorbent (0.60 g) was transferred into 250 cm^3 conical flask containing 20 cm^3 human plasma solution spiked with 20 mgL^{-1} Pb (II) ions solution, (Gochev *et al*., 2010). The mixture was adjusted to pH 4 (Optimum pH) using 0.1M HNO_3 or 0.1M NaOH (Elhadi *et al.,* 2013) and the mixture was agitated for contact time of 100 min after which the biomass was separated from the mixture using Whatman No 11 filter paper. The filtrate was then digested (as presented in 3.9.2) and determined using AAS for residual Pb (II) ions. This procedure was repeated for Pb (II) ion solution concentrations of 30 mgL^{-1}, 40 mgL^{-1}, 50 mgL^{-1} and 60 mgL^{-1} respectively. An optimum concentration (50 mgL^{-1}) was obtained for further experiment.

3.9.4 Optimization of biosorbent dosage for Pb (II) ions adsorption

The optimisation of biosorbent dosage was investigated by accurately weighing biosorbent (0.20 g) and transferred into 250 cm^3 conical flask containing 20 cm^3 human plasma solution spiked with 50 mgL^{-1} Pb (II) ions (Optimum concentration) solution, the mixture was maintained at pH 4 (Optimum pH) using 0.1M HNO_3 and 0.1M NaOH (Elhadi *et al.,* 2013) and the mixture was agitated for contact time of 100 min. The biomass was separated from mixture using Whatman No 11 filter paper. The filtrate was digested (as presented in 3.9.2) and the Pb (II) ions concentration of the filtrate was determined using AAS. This procedure was repeated using 0.40 g, 0.60 g, 0.80 g and 1.00 g biosorbent dosage respectively (Adelaja *et al.,* 2011). Optimum biosorbent dose (0.40 g) was obtained for further experiment.

3.9.5 Study of the rate of biosorption of Pb (II) ions

Optimum mass (0.40 g) of the biosorbent was transferred into 250 cm^3 conical flask containing 20 cm^3 human plasma solution spiked with of 50 mgL^{-1} Pb (II) ions (Optimum concentration) solution (Gochev *et al*., 2010), the mixture was maintained at pH 4 (Optimum pH) using 0.1M HNO_3 and 0.1M NaOH (Elhadi *et al.,* 2013). The mixture was agitated for contact time of 10 min, 20 min, 30 min, 40 min, 50 min, 60 min, 70 min, 80 min, 90 min, 100 min, 110 min and 120 min respectively. After the pre-set contact time was reached, the solutions were separated from the biomass by filtration using Whatman No 11 filter paper. The filtrate was then digested (as presented in 3.9.2) and residual Pb (II) ions in the solution were determined using Atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200) respectively. The highest rate of biosorption was observed at 90 min.

3.10 Optimisation of biosorption parameters for Zn (II) ions adsorption

3.10.1 Optimization of pH for Zn (II) ions adsorption

The optimisation of pH was studied by introducing 0.60 g of biosorbent into 250 cm^3 conical flasks containing 20 cm^3 of human plasma solution spiked with 50 mgL^{-1} Zn (II) ion solution. The pH of the mixture was adjusted to pH 2 with 0.1M HNO_3 or 0.1M NaOH and the mixture was agitated for a contact time of 100 min (Gochev *et al*., 2010) and (Elhadi *et al.,* 2013). After the preset contact time was reached, the biomass was separated from the mixture using Whatman No 11 filter paper. The filtrate was then digested and determined using AAS for residual Zn (II) ions. This procedure was repeated for pH values of 4, 6, 8 and 10 respectively. An optimum pH 6 was obtained for further experiment.

3.10.2 Digestion of filtrate

Filtrate (3.00 cm^3) was measured into 5.00 Pyrex flask separately and 10.00 cm^3 of freshly prepared mixture of concentrated HNO_3 and 5.00 cm^3 of H_2O_2 (2:1) added. The mixture was allowed to stand for 10.00 min and flasks were covered with watch glass and digested at 70^0C for 2.00 hr while heating continued on hot plate until a clear digested solution was obtained. The excess acid mixture was evaporated to semi dry mass and cooled. These were transferred into 100.00 cm^3 volumetric flask and diluted to mark using deionised water. The amount of Zn (II) ions in the filtrate was determined by Atomic Adsorption spectroscopy (AAS) method (AA240FS Varian) (Memon *et al.,* 2007).

3.10.3 Optimization of Zn (II) ions Concentration

Biosorbent (0.60 g) was transferred into 250 cm^3 conical flask containing 20 cm^3 human plasma solution spiked with 20 mgL^{-1} Zn (II) ions solution, (Gochev *et al*., 2010). The mixture was adjusted to pH 6 (Optimum pH) using 0.1M HNO_3 or 0.1M NaOH (Elhadi *et al.,* 2013) and the mixture was agitated for contact time of 100 min after which the biomass was separated from the mixture using Whatman No 11 filter paper. The filtrate was then digested (as presented in 3.10.2) and determined using AAS for residual Zn (II) ions. This procedure was repeated for Zn (II) ion solution concentrations of 30 mgL^{-1}, 40 mgL^{-1}, 50 mgL^{-1} and 60 mgL^{-1} respectively. An optimum concentration (50 mgL^{-1}) was obtained for further experiment.

3.10.4 Optimization of biosorbent dosage for Zn (II) ions adsorption

The optimisation biosorbent dosage was investigated by accurately weighing biosorbent (0.20 g) and transferred into 250 cm^3 conical flask containing 20 cm^3 human plasma solution spiked with 50 mgL^{-1} Zn (II) ions (Optimum concentration) solution, the mixture was maintained at pH 6 (Optimum pH) using 0.1M HNO_3 and 0.1M NaOH (Elhadi *et al.,* 2013) and the mixture was agitated for contact time of 100 min. The biomass was separated from mixture using Whatman No 11 filter paper. The filtrate was digested (as presented in 3.10.2) and the Zn (II) ions concentration of the filtrate was determined using

AAS. This procedure was repeated using 0.40 g, 0.60 g, 0.80 g and 1.00 g biosorbent dosage respectively (Adelaja *et al.,* 2011). An optimum mass (0.80 g) was obtained for further experiment.

3.10.5 Study of the rate of biosorption of Zn (II) ions

Optimum mass (0.80 g) of the biosorbent was transferred into 250 cm^3 conical flask containing 20 cm^3 human plasma solution spiked with 50 mgL^{-1} Zn (II) ions (Optimum concentration), the mixture was maintained at an pH 6 (Optimum pH) using 0.1M HNO_3 and 0.1M NaOH (Elhadi *et al.,* 2013). The mixture was agitated for various contact time of 10 min, 20 min, 30 min, 40 min, 50 min, 60 min, 70 min, 80 min, 90 min, 100 min, 110 min and 120 min respectively. After the pre-set contact time was reached, the solutions were separated from the biomass by filtration using Whatman No 11 filter paper. The filtrate was then digested (as presented in 3.10.2) and residual Zn (II) ions in the solution were determined using Atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200) respectively. The highest rate of biosorption was observed at 70 min.

3.11 Data Evaluations

The quality of biosorbent is judged by the metal uptake capacity. Amount of metal ions bond onto the biosorbent which disappeared from the solution was evaluated as presented in equation 3.2:

$$q = \frac{V(C_i - C_f)}{S} \qquad (3.2)$$

where;

q = Metal ion uptake capacity (mgg^{-1})

C_i = Initial concentration of metal in solution, before the sorption analysis (mgL^{-1}).

C_f = Final concentration of metal in solution, after the sorption analysis (mgL^{-1}).

S = Weight of biosorbent (gm)

V = Solution volume (cm^3)

The difference between the initial metal ion concentration and final metal ion concentration was assumed to be bound to the biosorbent.

The biosorption efficiency of the metal ion was calculated using equation 3.3:

$$A\% = \frac{C_o - C_e}{C_0} * 100 \quad (3.3)$$

where;

A% is biosorption efficiency, C_0 and Ce are the initial and equilibrium metal ion concentrations (mgL^{-1}), respectively (Ding *et al.*, 2012).

3.12 Data Analysis

Equilibrium isotherms describe how adsorbate interacts with biosorbent and equilibrium is established between adsorbed metal ions on the biosorbent (Reddy *et al.*, 2013). The experimental data was fitted into Langmuir isotherm, Freundlich isotherm and Tempkin isotherm. To study the kinetics of the adsorbent-adsorbate interaction, pseudo first and second order kinetics model were used to study the experimental data, (Nasution *et al*., 2005).

CHAPTER FOUR

RESULTS

4.1 Fourier Transform Infrared (FTIR) of Biosorbent (*Allium cepa*).

Fourier Transform Infrared (FTIR) spectra of the native and metal ions (Cu^{2+}, Pb^{2+} and Zn^{2+}) treated biosorbent (*Allium cepa*) are presented in Appendices III, IV, V and VI respectively while Table 4.1, 4.2, 4.3, and 4.4 represents the assignment of IR bands of functional groups present in the native and metal ions (Cu^{2+}, Pb^{2+} and Zn^{2+}) treated biosorbent respectively.

Table 4.1: Assignment of IR bands of Functional groups in *Allium cepa* biomass

S/N	Frequency (cm^{-1})	Absorption, cm^{-1}	Assignment	Class of Compounds
1.	810-840	816.3	C-H bend	Aromatic compound
2.	650-1000	920.7	=C-H bend	Alkenes
3.	1025-1075	1025.0	=C-O-C Symmetric	Ethers
4.	1200-1275	1252.4	=C-O-O asym. Stretch	Ethers
5.	1300-1375	1364.2	S=O	Sulphoxides
6.	1630-1680	1636.3	C=O stretch	Amides
7.	2850-3000	2922.2	C-H stretch	Alkanes and Alkyls

8.	2500-3500	3272.6	O-H stretch	Carboxylic Acids

Table 4.2: Assignment of IR bands of Functional groups in Cu (II) ions treated *Allium cepa* biomass

S/N	Frequency (cm^{-1})	Absorption, cm^{-1}	Assignment	Class of Compounds
1.	810-840	816.7	C-H bend	Aromatic compound
2.	650-1000	920.7	=C-H bend	Alkenes
3.	1020-1075	1016.8	=C-O-C Symmetric	Ethers
4.	1200-1275	1242.4	=C-O-O asym. Stretch	Ethers
5.	1300-1375	1364.2	S=O	Sulphoxides
6.	1630-1680	1634.8	C=O stretch	Amides
7.	2850-3000	2922.2	C-H stretch	Alkanes and Alkyls
8.	2500-3500	3269.4	O-H stretch	Carboxylic Acids

Table 4.3: Assignment of IR bands of Functional groups in Pb (II) ions treated *Allium cepa* biomass

S/N	Frequency (cm^{-1})	Absorption, cm^{-1}	Assignment	Class of Compounds
1.	1020-1075	1013.8	=C-O-C Symmetric	Ethers
2.	1200-1275	1237.5	=C-O-O asym. Stretch	Ethers
3.	1000-1350	1319.5	S=O	Sulphoxides
4.	1300- 1375	1367.9	S=O	Sulphoxides
5.	1630-1680	1632.6	C=O stretch	Amides
6.	1715-1730	1733.2	C=O stretch	Esters
7.	2850-3000	2855.2	C-H stretch	Alkanes and Alkyls
8.	2500-3500	3272.6	O-H stretch	Carboxylic Acids

Table 4.4: Assignment of IR bands of Functional groups in Zn (II) ions treated *Allium cepa* biomass

S/N	Frequency (cm^{-1})	Absorption, cm^{-1}	Assignment	Class of Compounds
1.	885-895	890.8	=C-H bend	Alkenes
2.	1020-1075	1010.1	=C-O-C Symmetric	Ethers
3.	1200-1275	1237.5	=C-O-O asym. Stretch	Ethers
4.	1000-1350	1319.5	C-F stretch	Alkyl halides
5.	1300- 1375	1364.2	S=O	Sulphoxides
6.	1580-1650	1625.1	N-H bend	Amines
7.	1735-1750	1736.9	C=O stretch	Esters
8.	2850-3000	2922.2	C-H stretch	Alkanes and Alkyls
9.	2500-3500	3268.9	O-H stretch	Carboxylic Acids

4.2 Scanning Electron Microscope (SEM) of Biosorbent (*Allium cepa* biomass)

Scanning Electron Microscope (SEM) micrograph of the native and metal ions (Cu (II), Pb (II) and Zn (II) ions) treated biosorbent are presented in Figures 4.1, 4.2, 4.3 and 4.4 respectively.

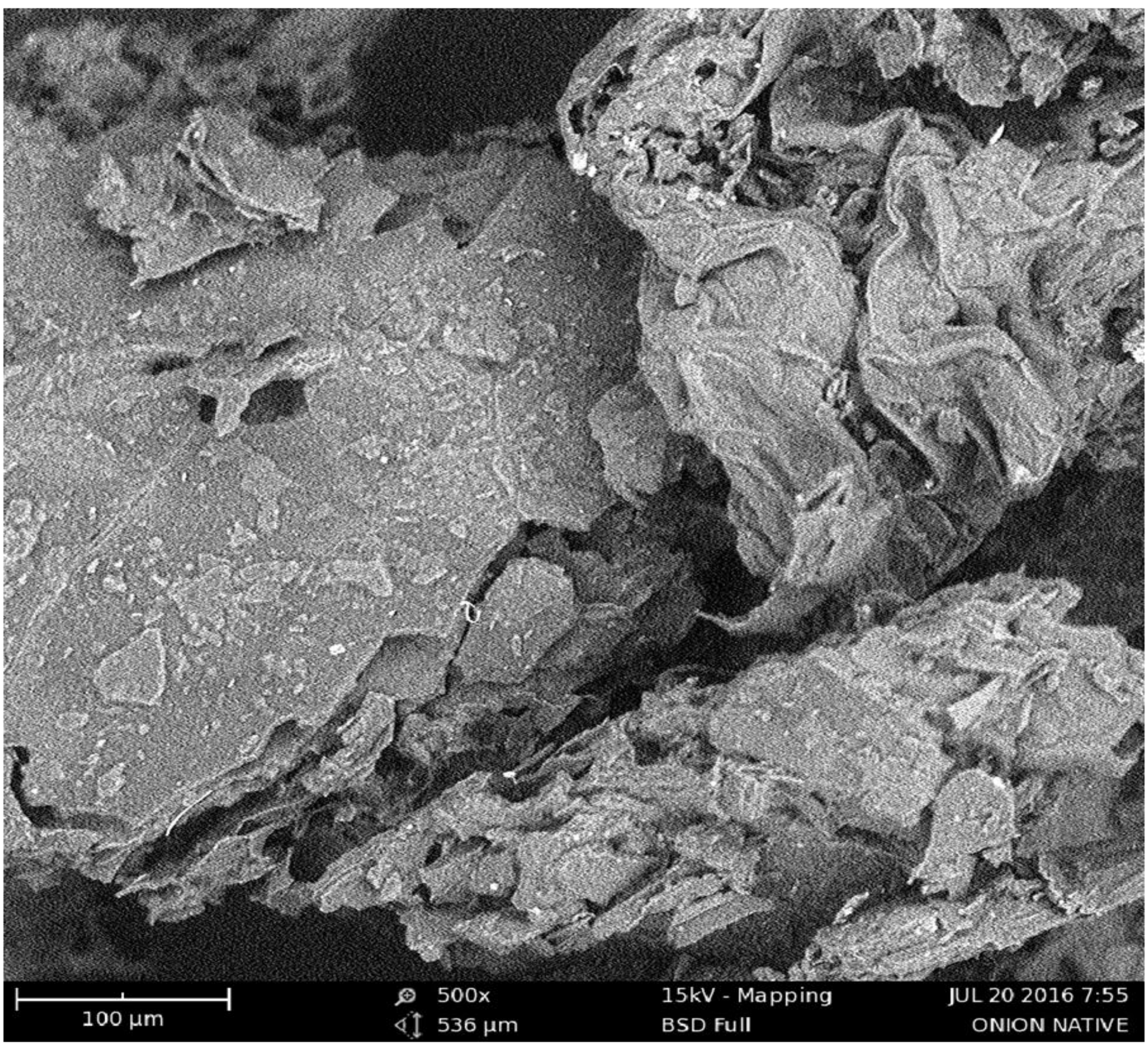


Figure 4.1: SEM micrograph of biosorbent (*Allium cepa*)

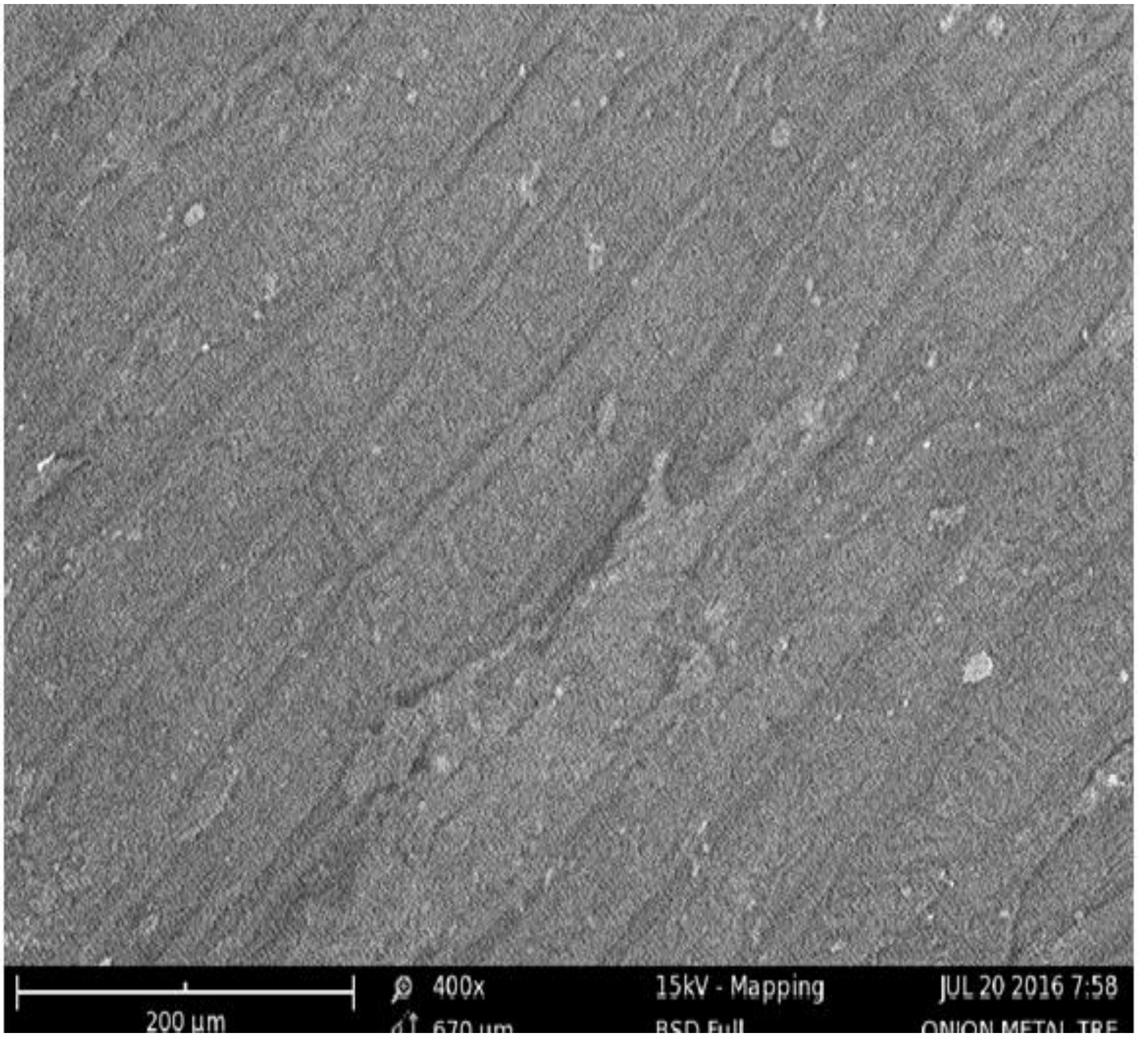


Figure 4.2: SEM micrograph of biosorbent treated with Cu (II) ions

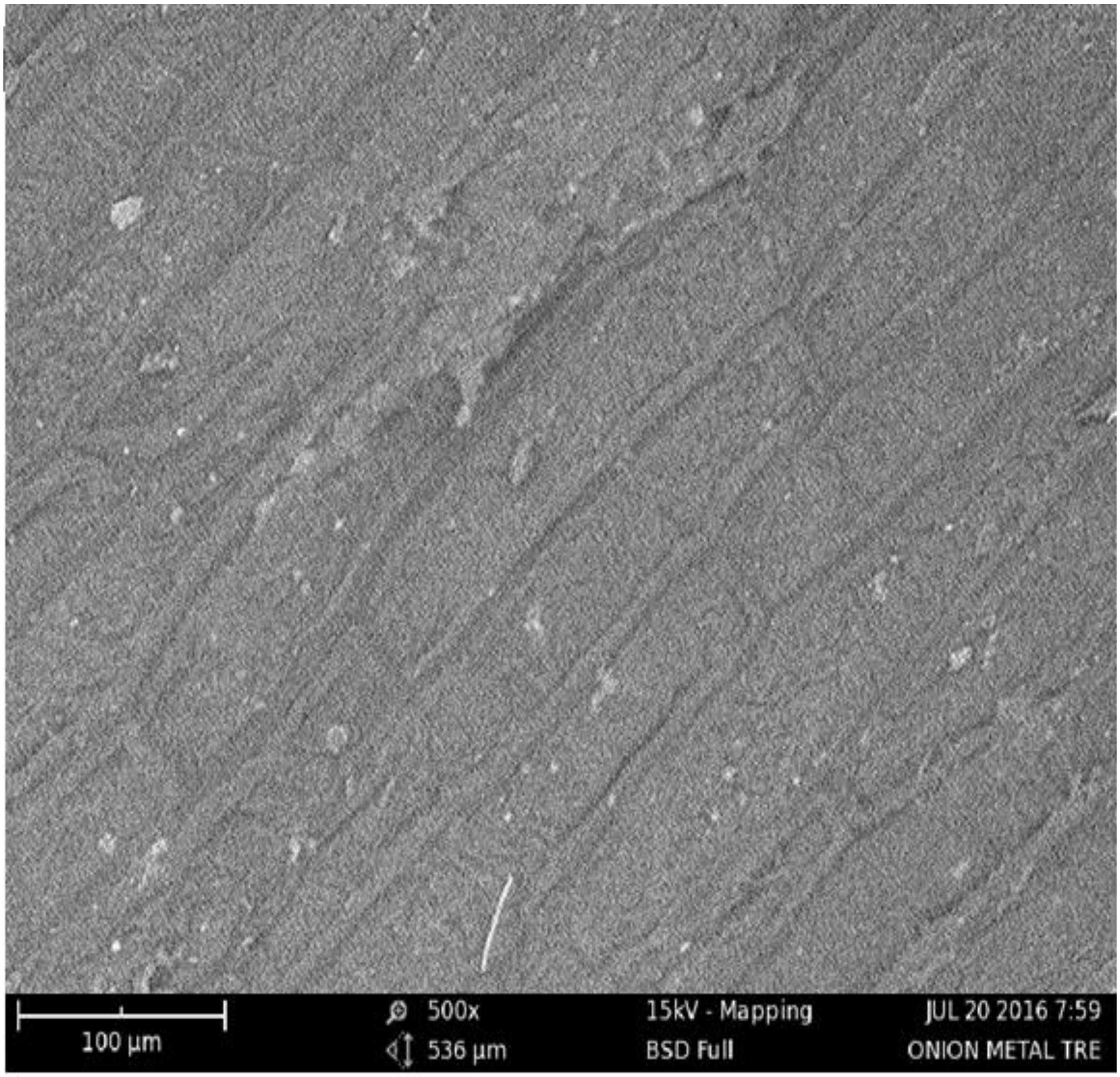


Figure 4.3: SEM micrograph of biosorbent treated with Pb (II) ion

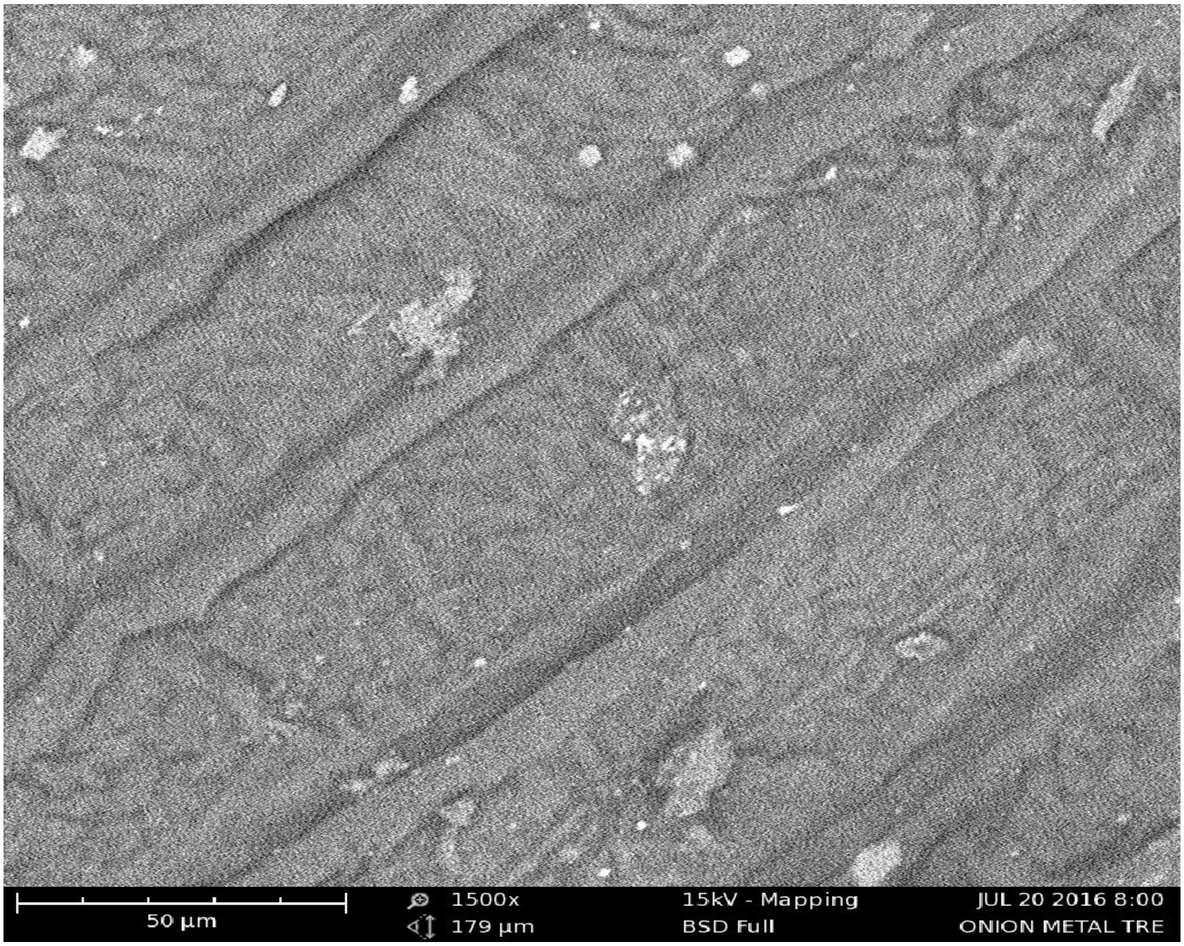


Figure 4.4: SEM micrograph of biosorbent treated with Zn (II) ion

4.3 Optimum Parameters

4.3.1 Optimum parameters for biosorption of Cu (II) ions

Figures 4.5, 4.6 and 4.7 represent the optimised pH, concentration of Cu (II) ions and biosorbent dose respectively for biosorption of Cu (II) ions. The values for the plot are presented in Appendices VII, VIII and IX respectively.

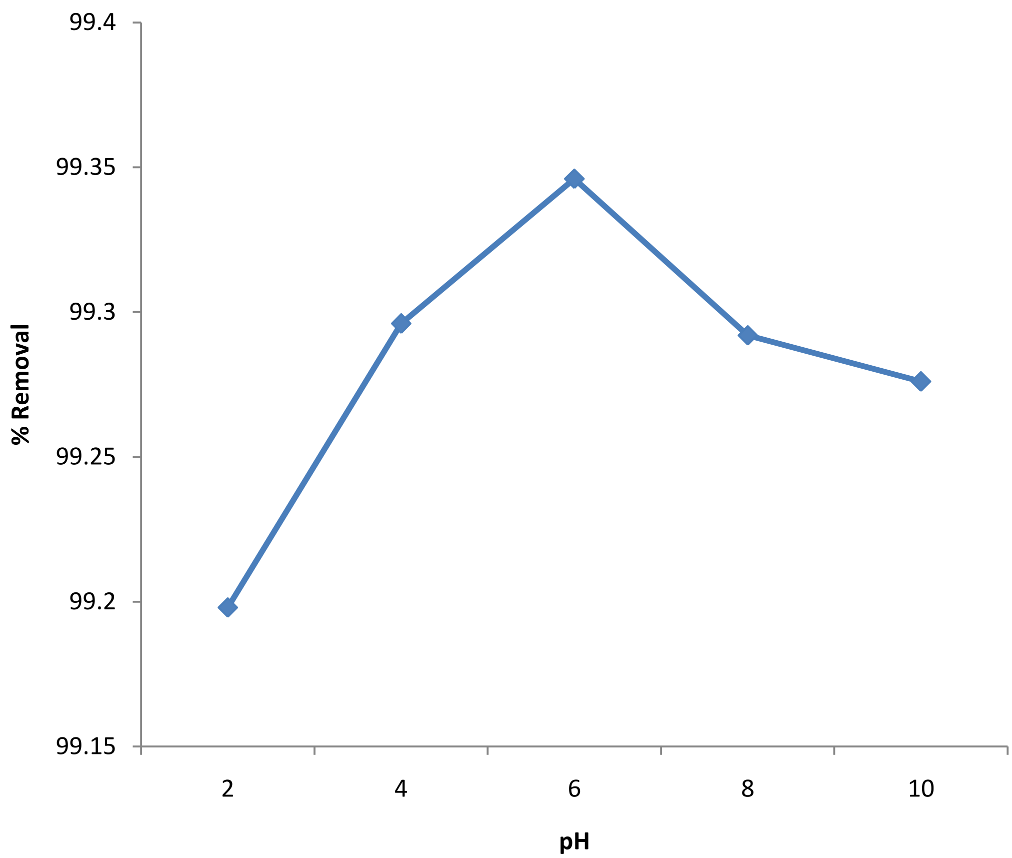


Figure 4.5: Percentage removal of Cu^{2+} as a function of pH

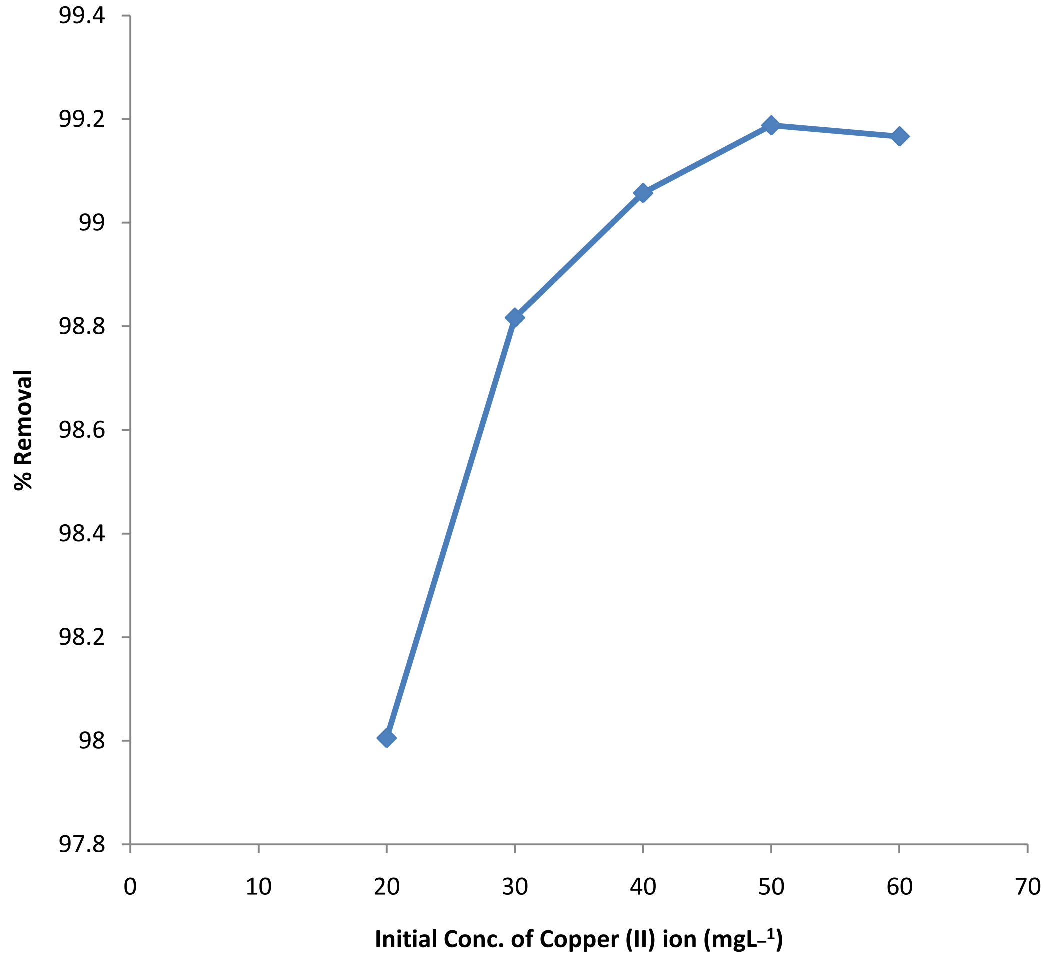


Figure 4.6: Percentage removal of Cu (II) ions as a function of Concentration

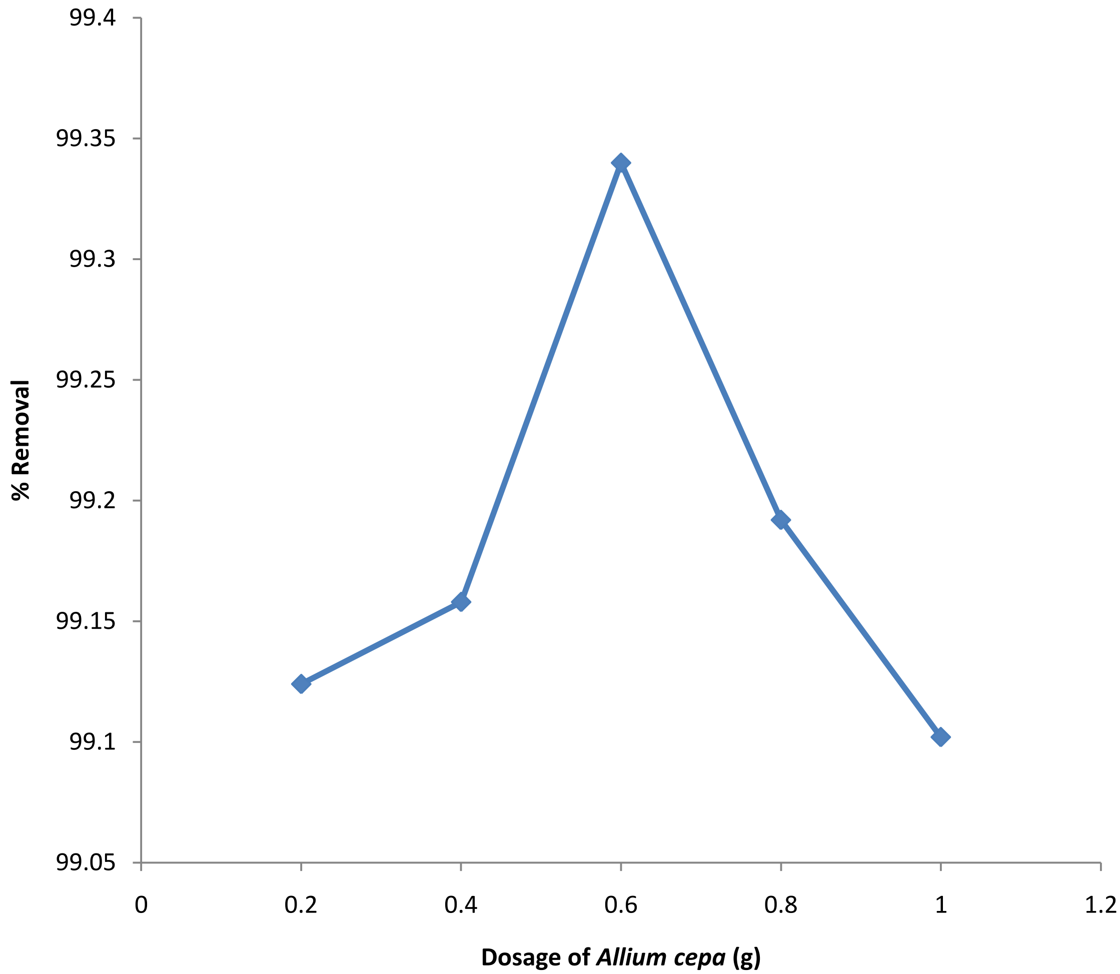


Figure 4.7: Percentage removal of Cu^{2+} as a function of *Allium cepa* Dosage

4.3.2 Rate of removal of Cu (II) ions

Figure 4.8 represents the percentage removal of Cu (II) ions by *allium cepa* biomass at different time intervals. The values for the plot are presented in Appendix X.

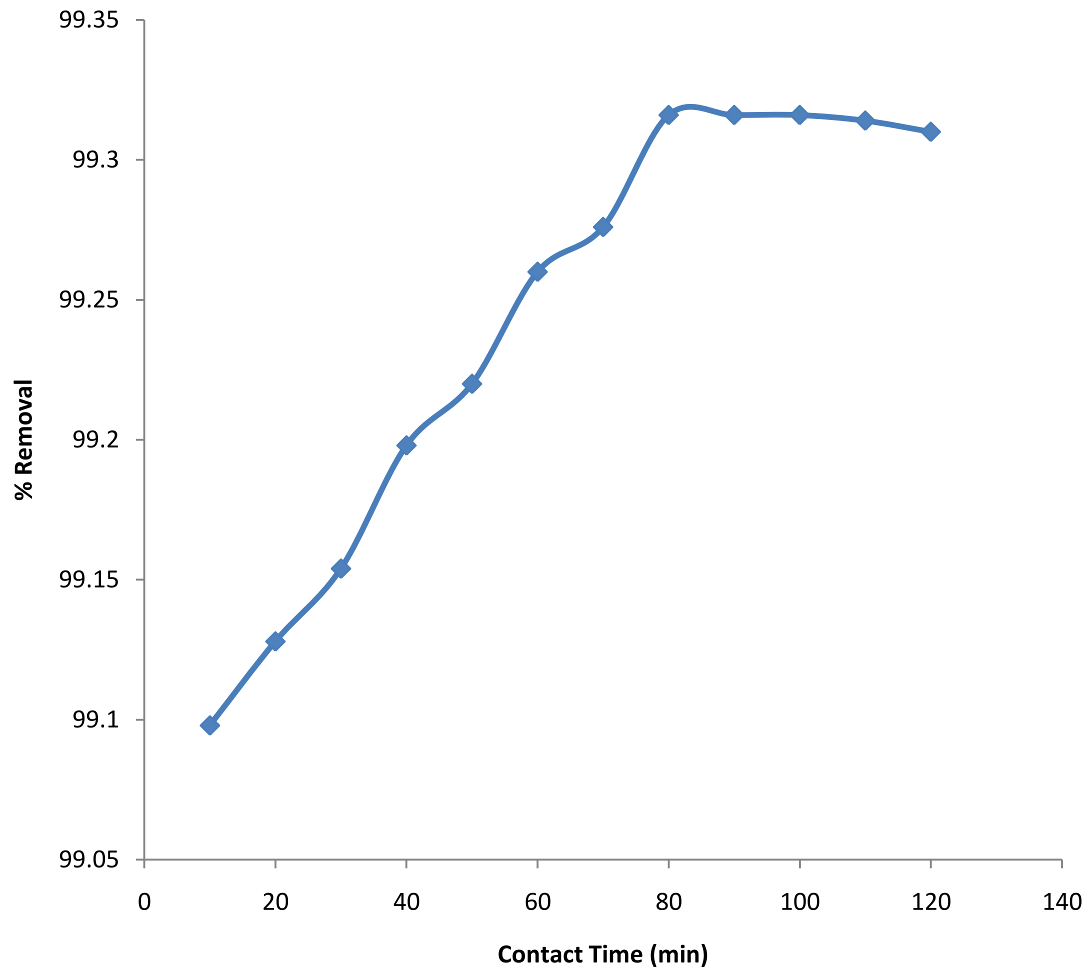


Figure 4.8: Rate of removal of Cu^{2+}

4.3.3 Optimum parameters for biosorption of Pb (II) ions

Figures 4.9, 4.10 and 4.11 represent the optimised pH, concentration of Pb (II) ions and biosorbent dose respectively for the biosorption of Pb (II) ions. The values for the plot are presented in Appendices XI, XII and XIII respectively.

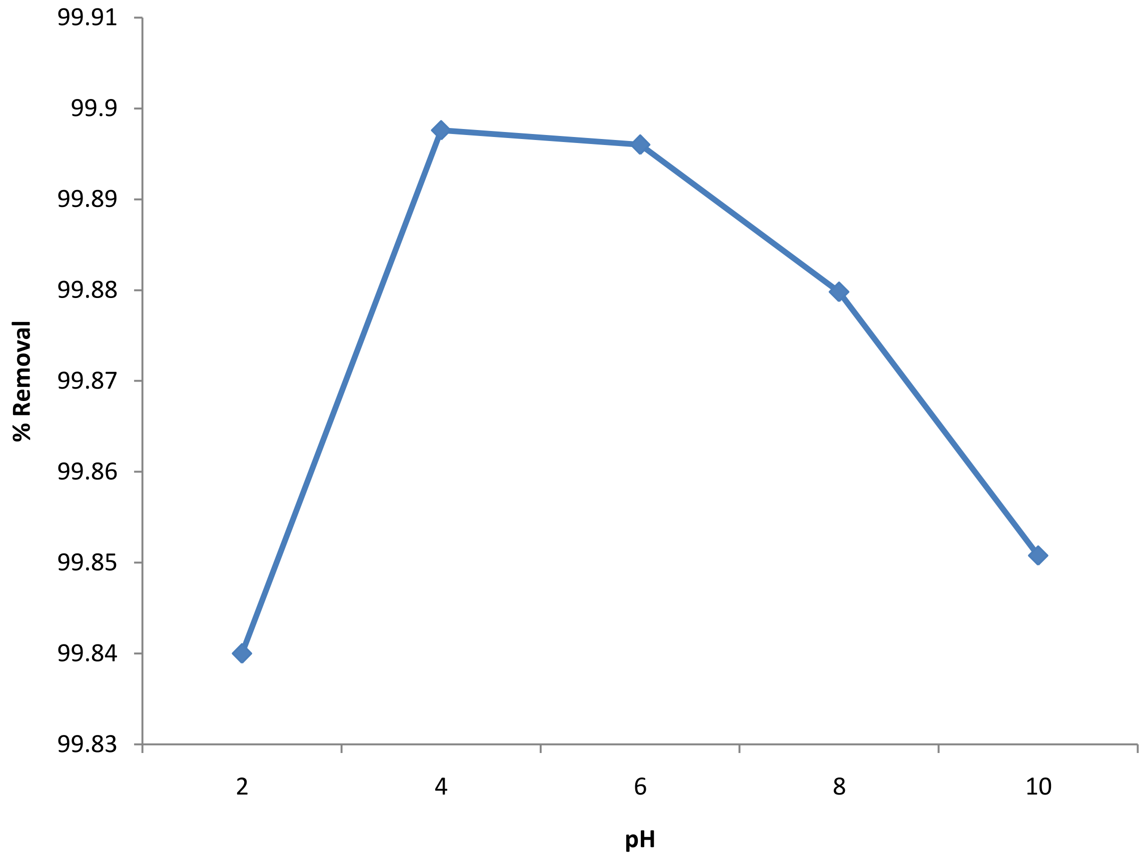


Figure 4.9: Percentage removal of Pb^{2+} as a function of pH

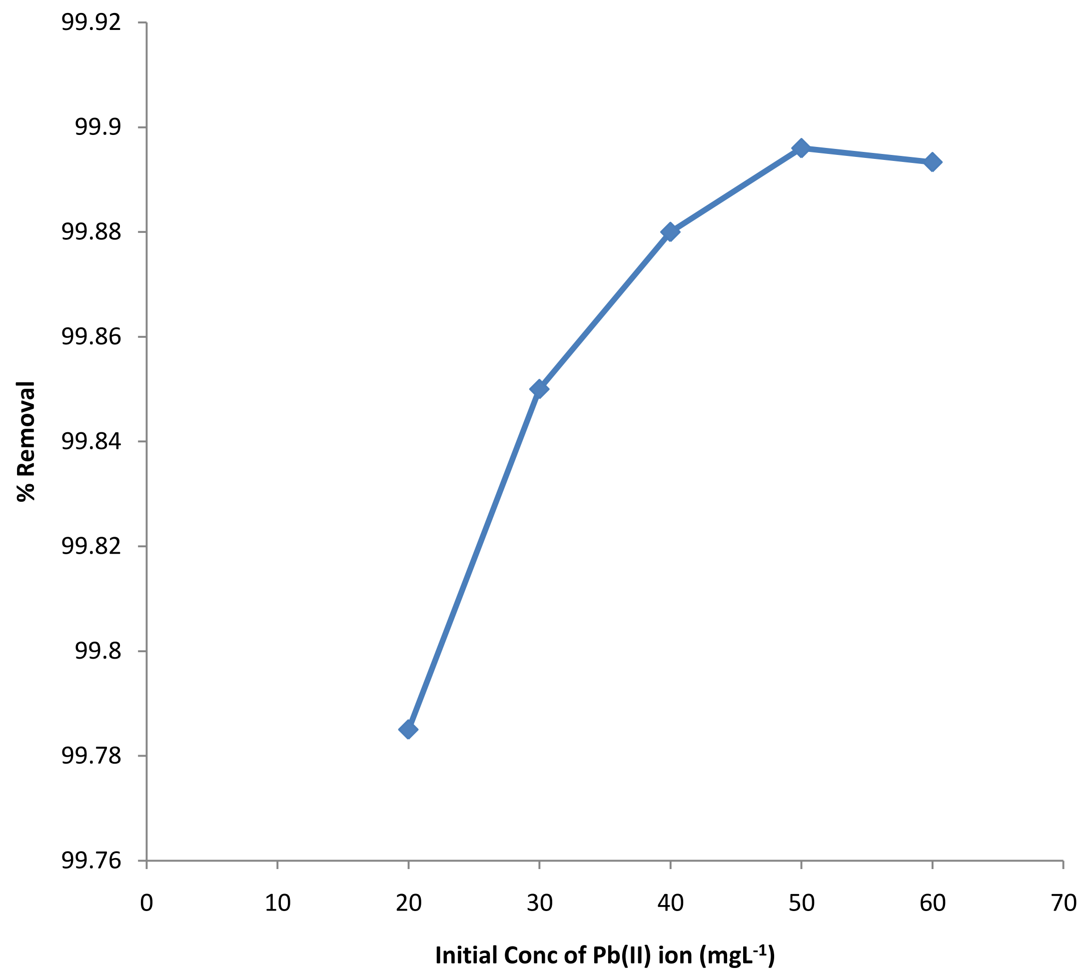


Figure 4.10: Percentage removal of Pb^{2+} as a function of Concentration

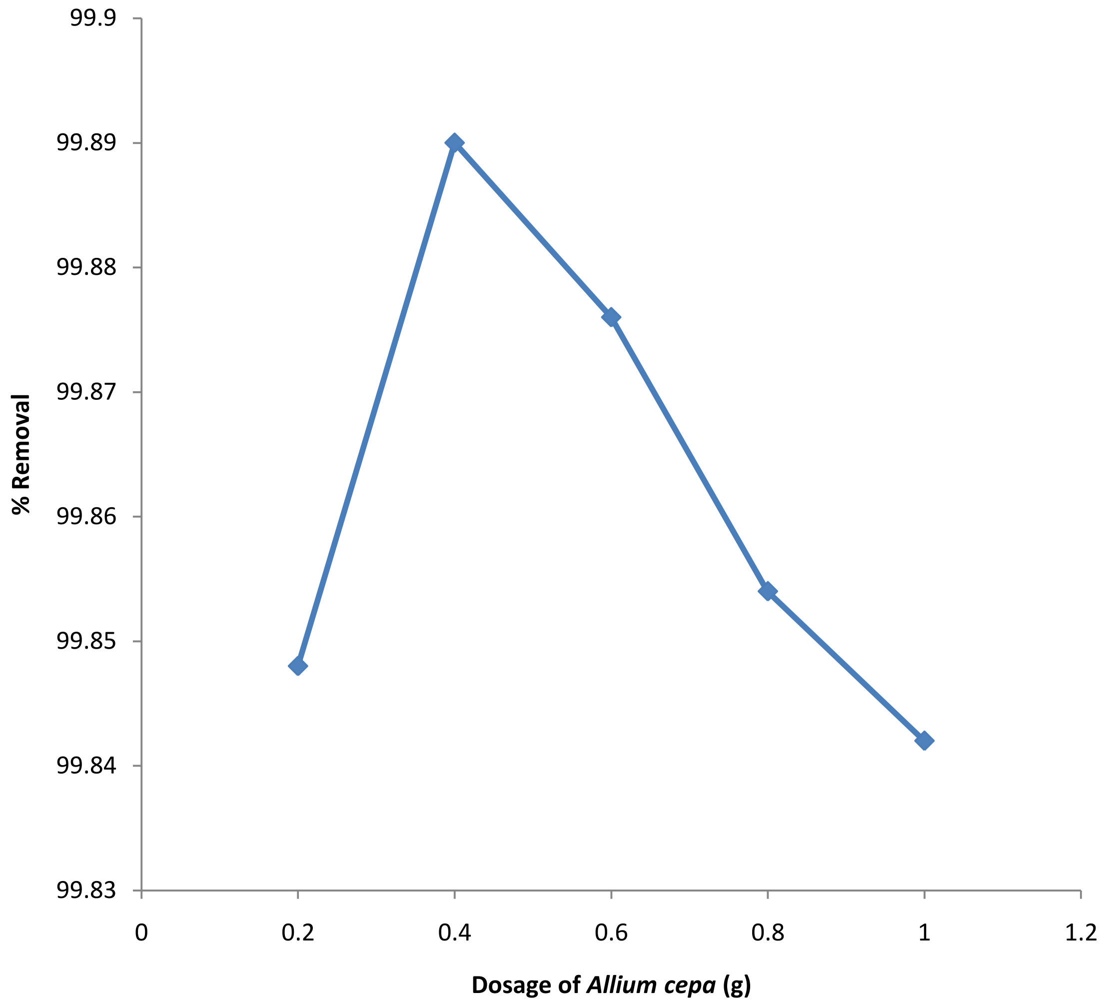


Figure 4.11: Percentage removal of Pb^{2+} as a function of *Allium cepa* Dosage

4.3.4 Rate of removal of Pb (II) ions

Figure 4.12 shows the percentage removal of Pb (II) ions at different time intervals. The values for the plot are presented in appendix XIV.

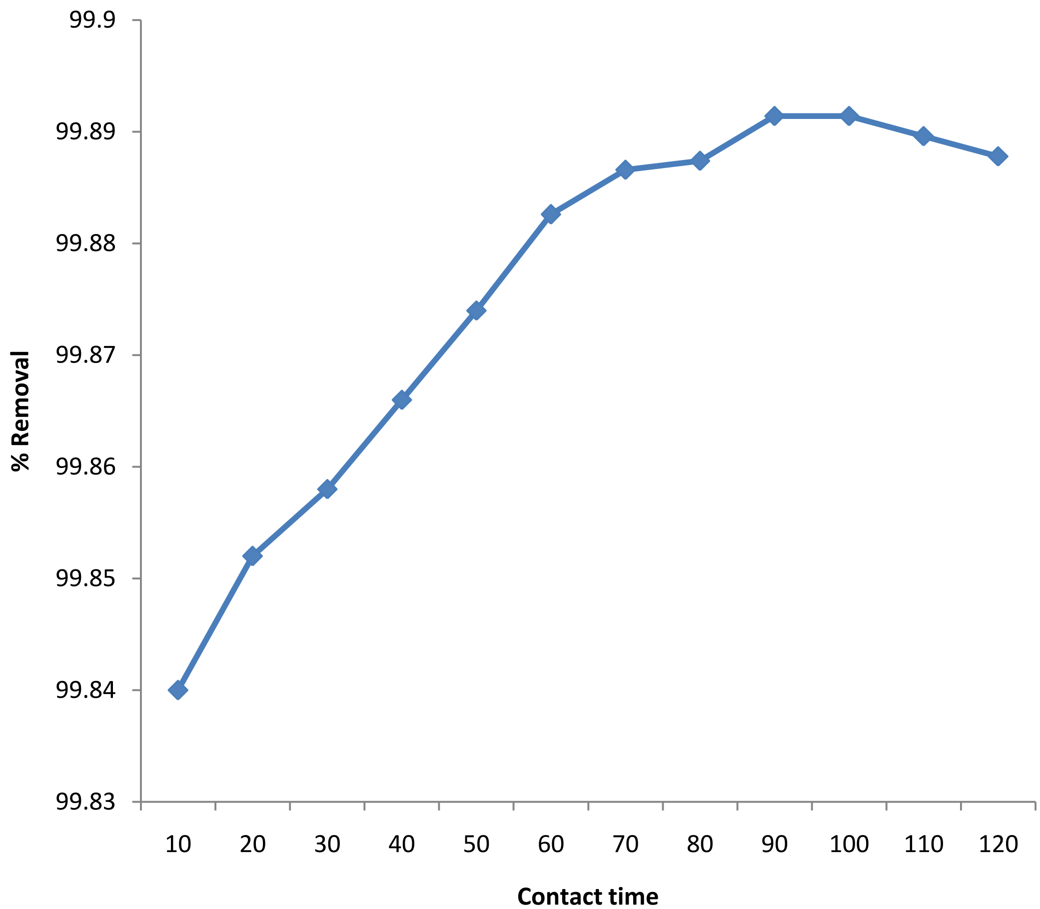


Figure 4.12: Rate of removal of Pb^{2+}

4.3.5 Optimum parameters for biosorption of Zn (II) ions

Figures 4.13, 4.14 and 4.15 represent the optimised pH, concentration of Zn (II) ions and biosorbent dose respectively for biosorption of Zn (II) ions. The values for the plot are presented in appendices XV, XVI and XVII respectively.

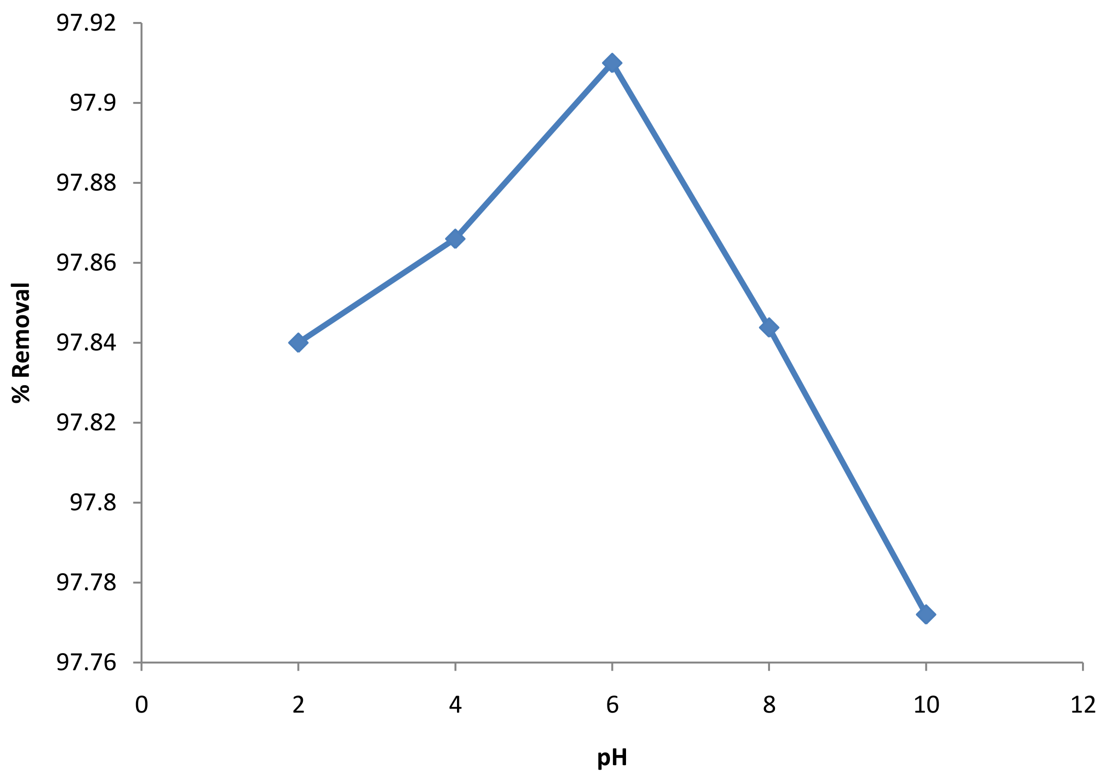
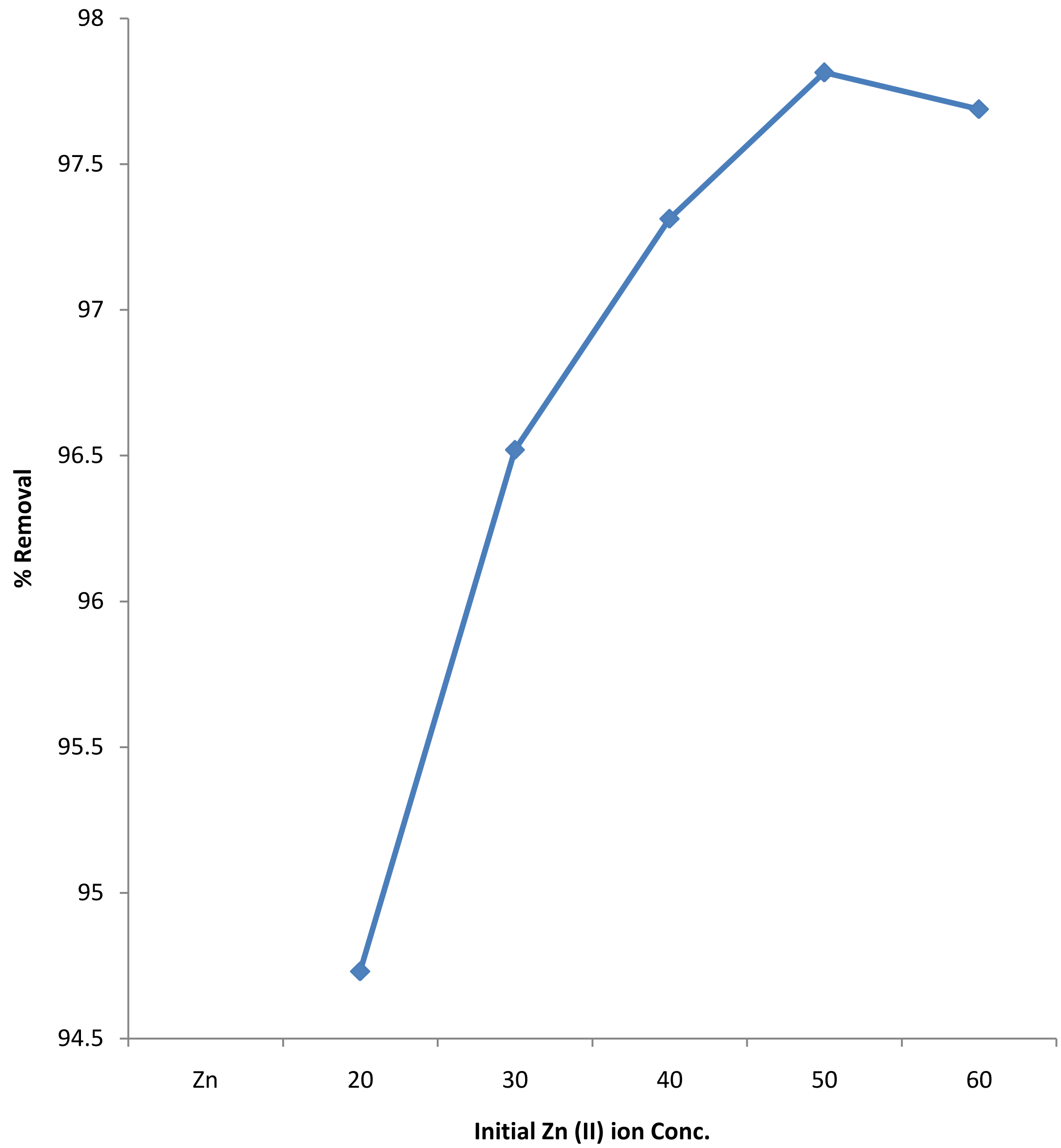


Figure 4.13: Percentage removal of Zn^{2+} as a function of pH


98
97.5
97
96.5
96
95.5
95
94.5
% Removal
Zn
20
30
40
50
60
Initial Zn (II) ion Conc.

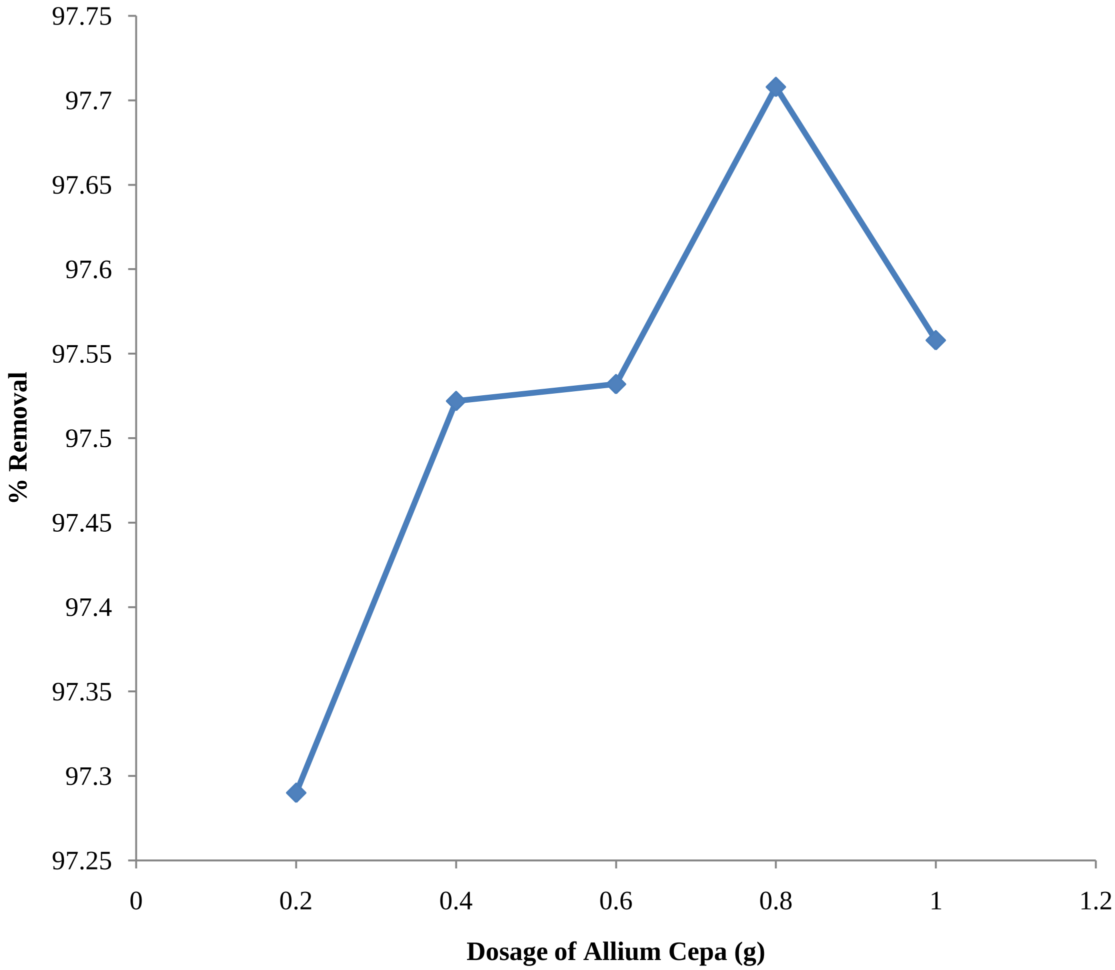


Figure 4.15: Percentage removal of Zn^{2+} as a function of *Allium cepa* dosage

4.3.6 Rate of removal of Zn (II) ions

Figure 4.16 represents the percentage removal of Zn (II) ions at different time intervals. The values for the plot are presented in appendix XVIII.

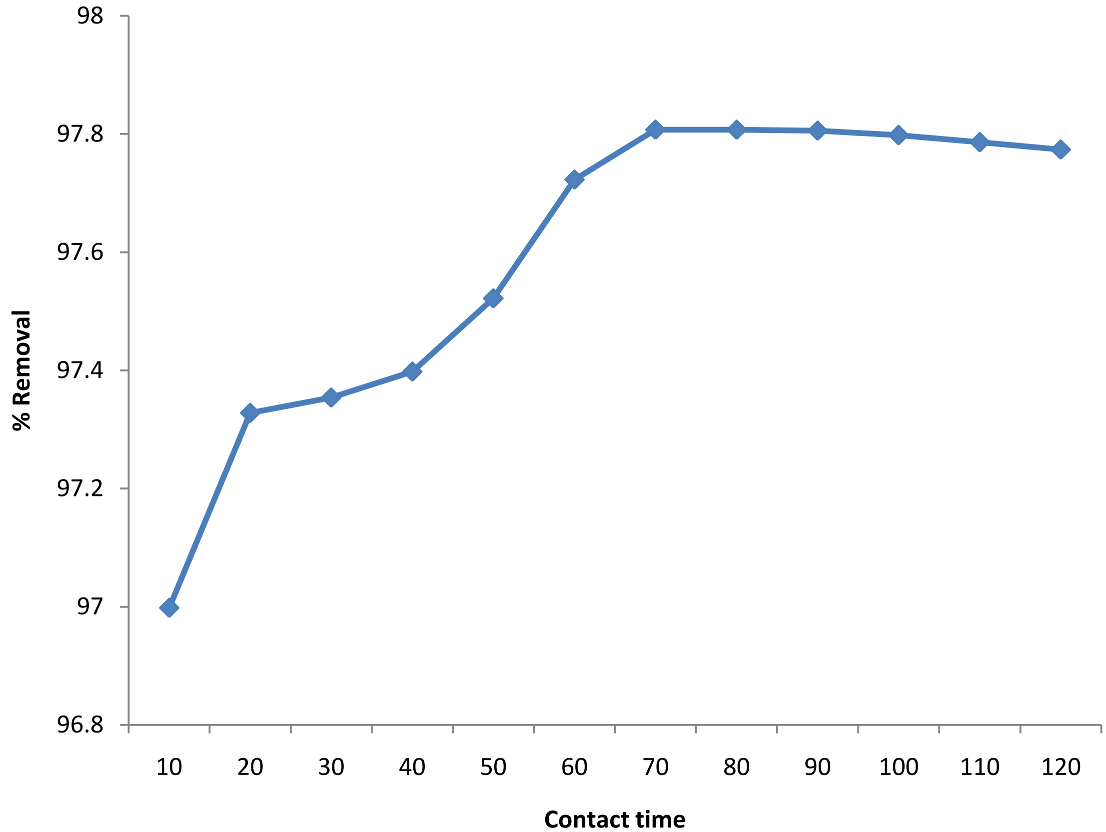


Figure 4.16: Rate of removal of Zn^{2+}

4.4 Equilibrium Isotherms

4.4.1 The Langmuir, Freundlich and Tempkin Isotherms Modelling for Cu (II) ions uptake

The biosorption capacity of *allium cepa* was studied using Langmuir, Freundlich and Tempkin Isotherms Modelling as shown in Figures 4.17, 4.18 and 4.19 respectively. The values for the plot are presented appendices XIX, XX and XXI respectively. The correlation parameters of Langmuir, Freundlich and Tempkin Isotherms for the adsorption of Cu (II) ions onto the biosorbent are presented in Table 4.5.

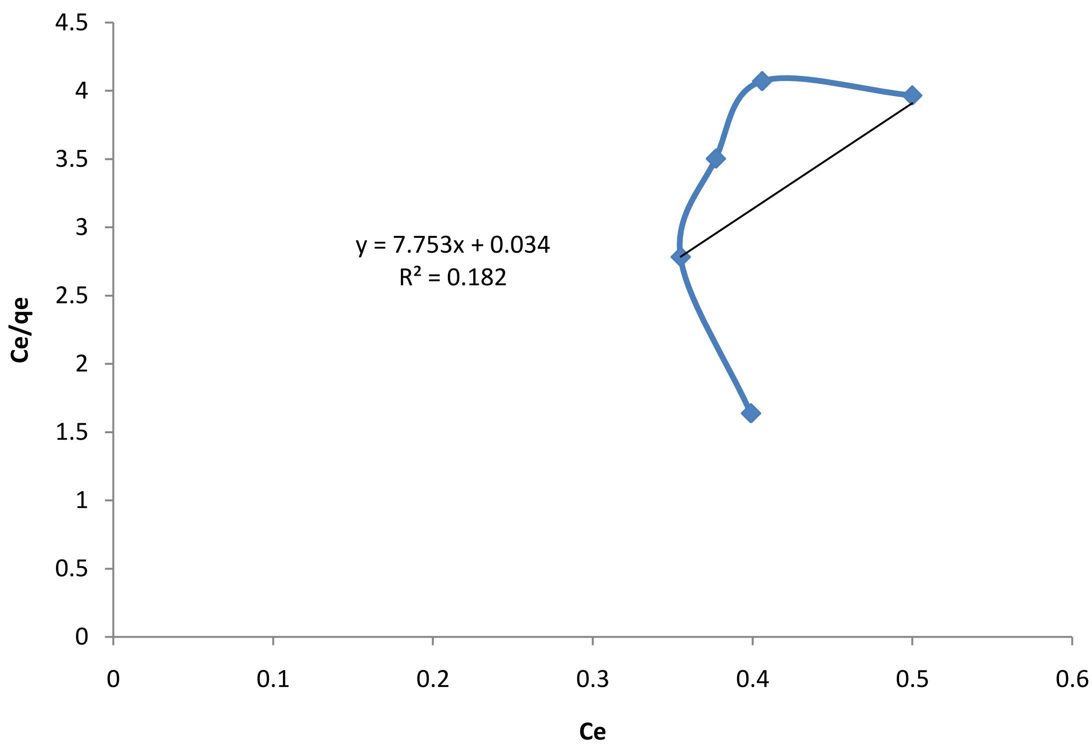


Figure 4.17: Langmuir isotherm for biosorption of Cu^{2+}

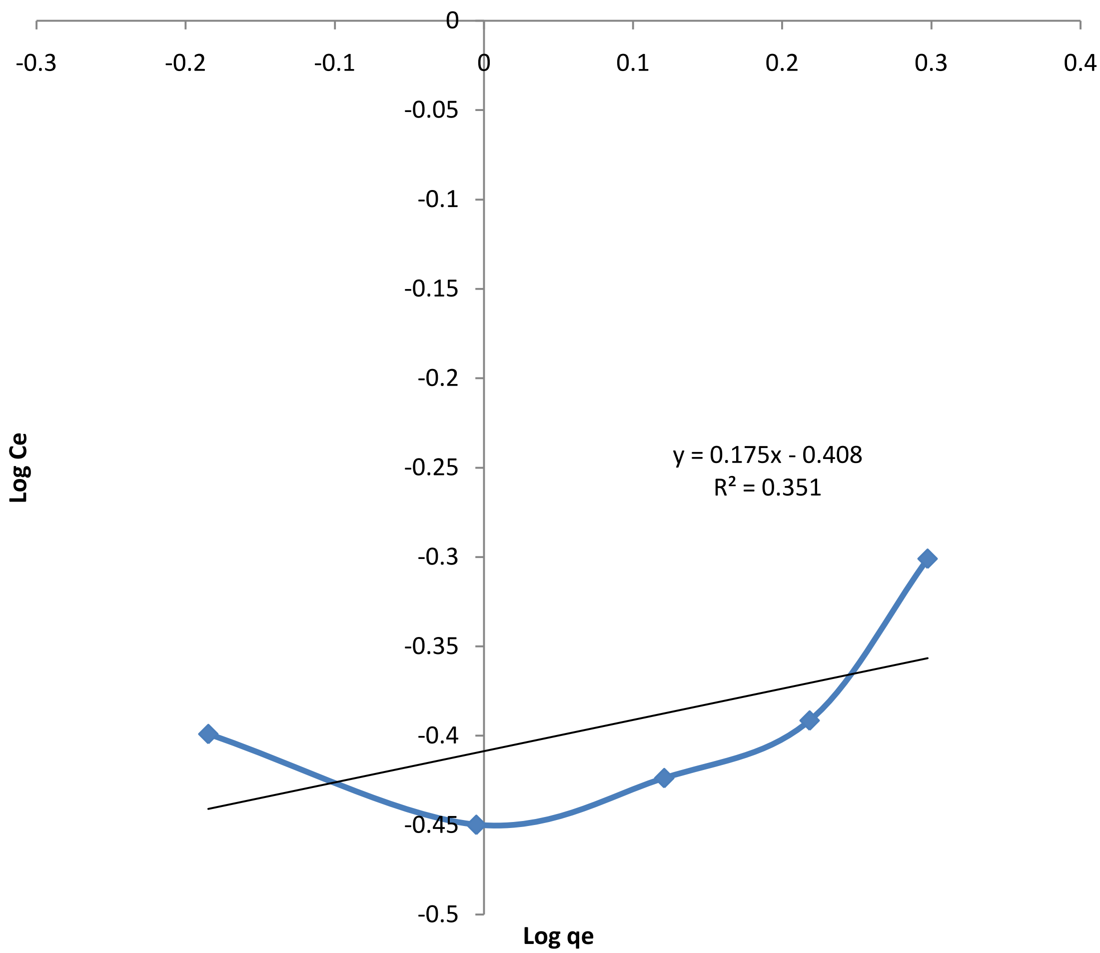


Figure 4.18 Freundlich Isotherm for biosorption of Cu^{2+}

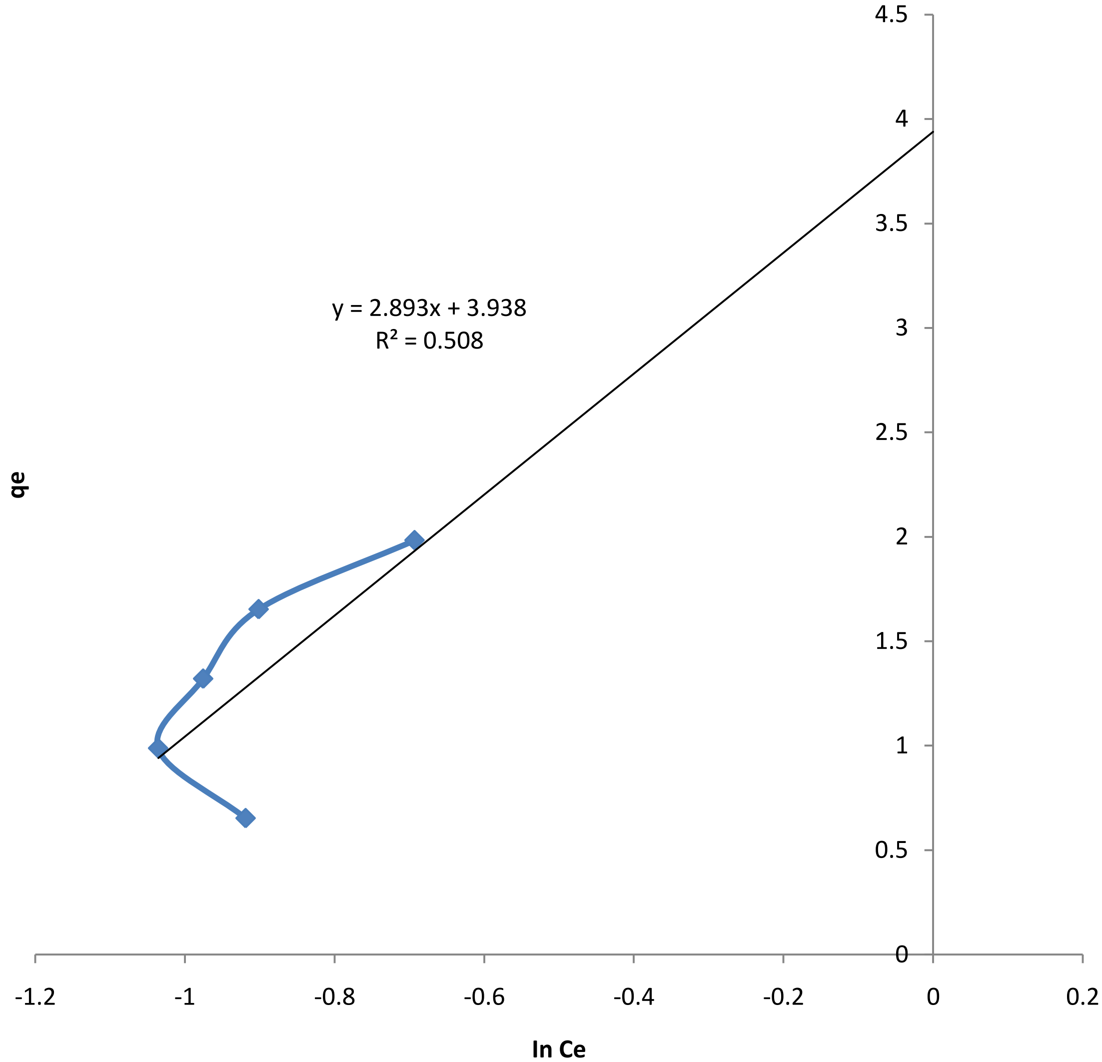


Figure 4.19: Tempkin Isotherm for biosorption of Cu^{2+}

Table 4.5: Correlation parameters of Langmuir, Freundlich and Temkins Isotherms for Cu (II) ion adsorption onto biosorbent (*Allium cepa*)

.

Metal	Langmuir Model				Freundlich Model			Temkin		
	Qmax (mg/g)	K_L	**R^2**	**R^L**	K_f	**N**	**R^2**	b_T	A_T	**R^2**
Cu	**0.129**	**227.99**	**0.182**	**8.77×10^{-5}**	**0.3908**	**5.714**	**0.351**	**86.220**	**3.900**	**0.508**

Key: R^2 is the correlation coefficient, Qmax is the maximum metal uptake (mg/g), K_L is the Langmuir constant (L/mg), R_L is the separation factor, K_f is the Freundlich constant measuring biosorption capacity, A_T is the Tempkin equilibrium binding constant (L/g) and b_T is the Tempkin isotherm constant.

4.4.2 The Langmuir, Freundlich and Tempkin Isotherms Modeling for Pb (II) ions Uptake

The biosorption capacity of *allium cepa* was studied using Langmuir, Freundlich and Tempkin isotherms modeling as shown in Figures 4.20, 4.21 and 4.22 respectively. The values for the plot are presented in appendices XXII, XXIII and XXIV respectively. The correlation parameters of Langmuir, Freundlich and Tempkin Isotherms for the adsorption of Pb (II) ion onto the biosorbent are presented in Table 4.6.

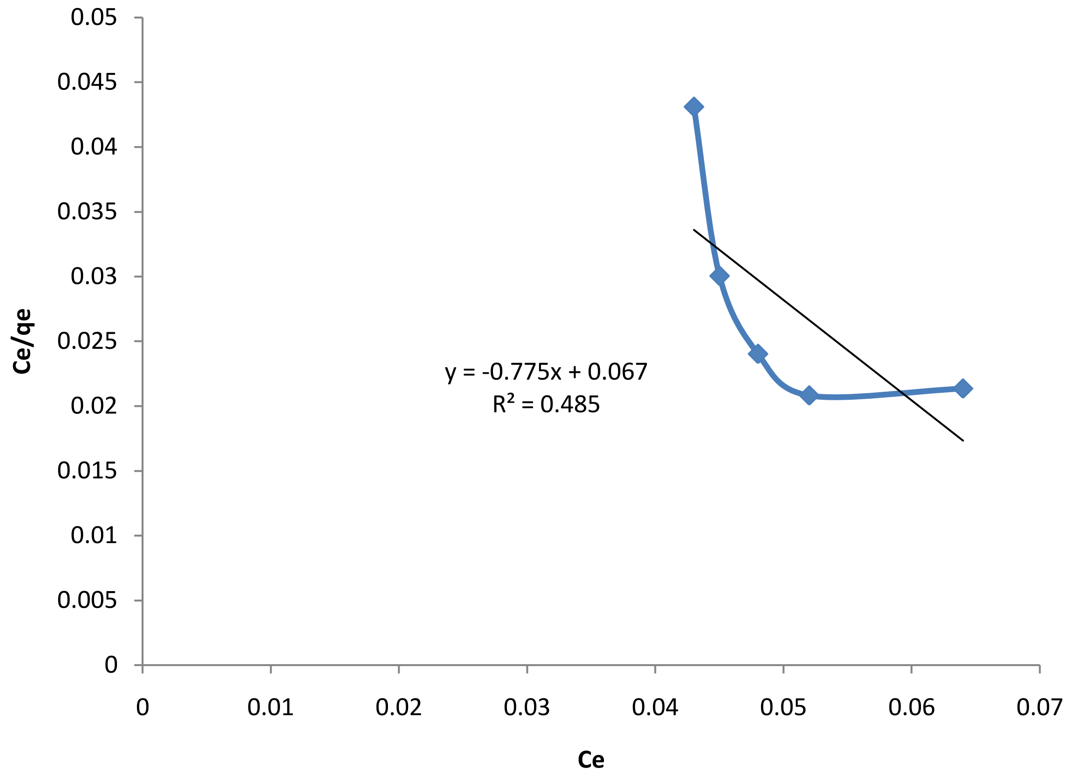


Figure 4.20: Langmuir isotherm for biosorption of Pb^{2+}

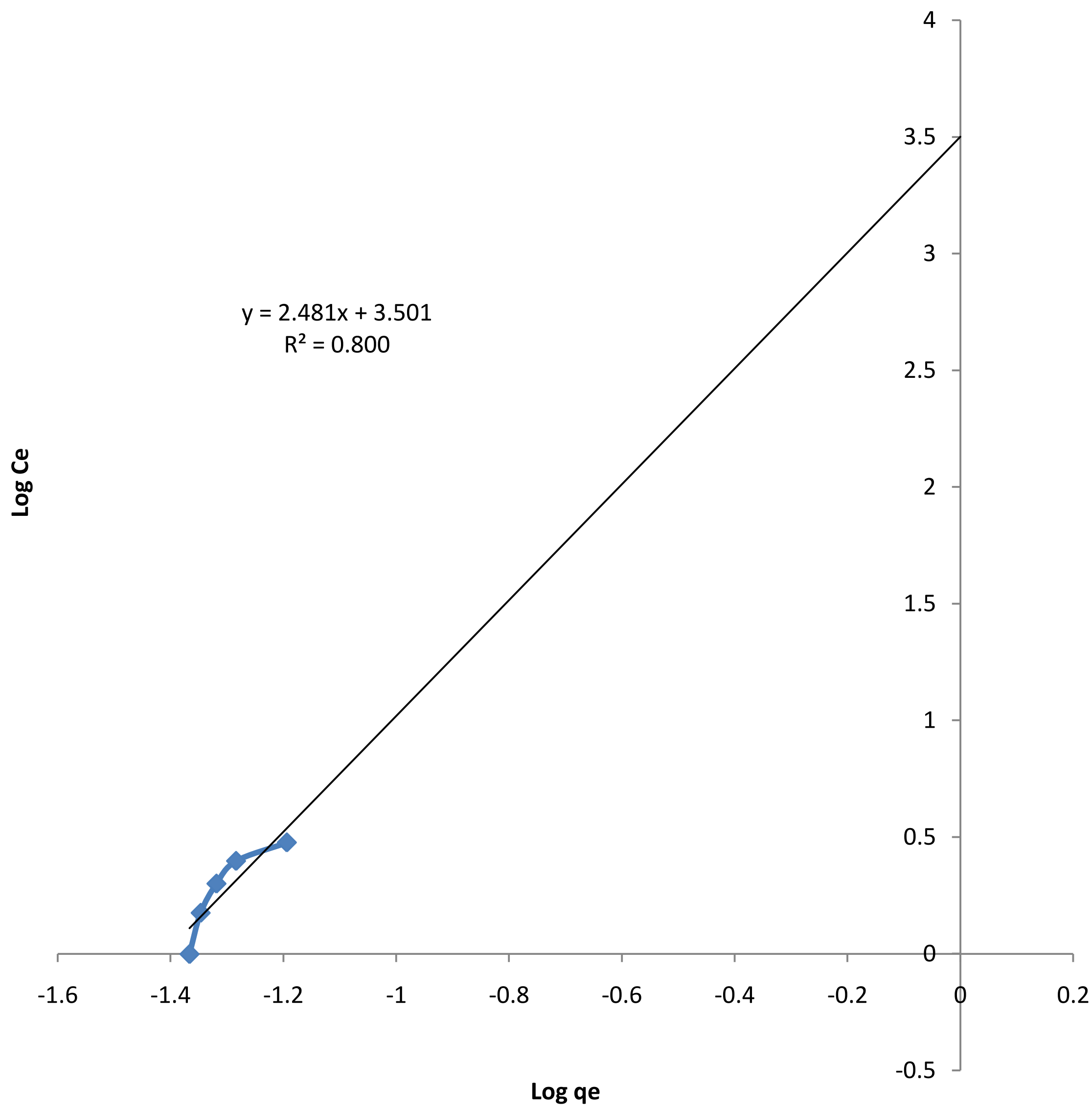


Figure 4.21: Freundlich Isotherm for biosorption of Pb^{2+}

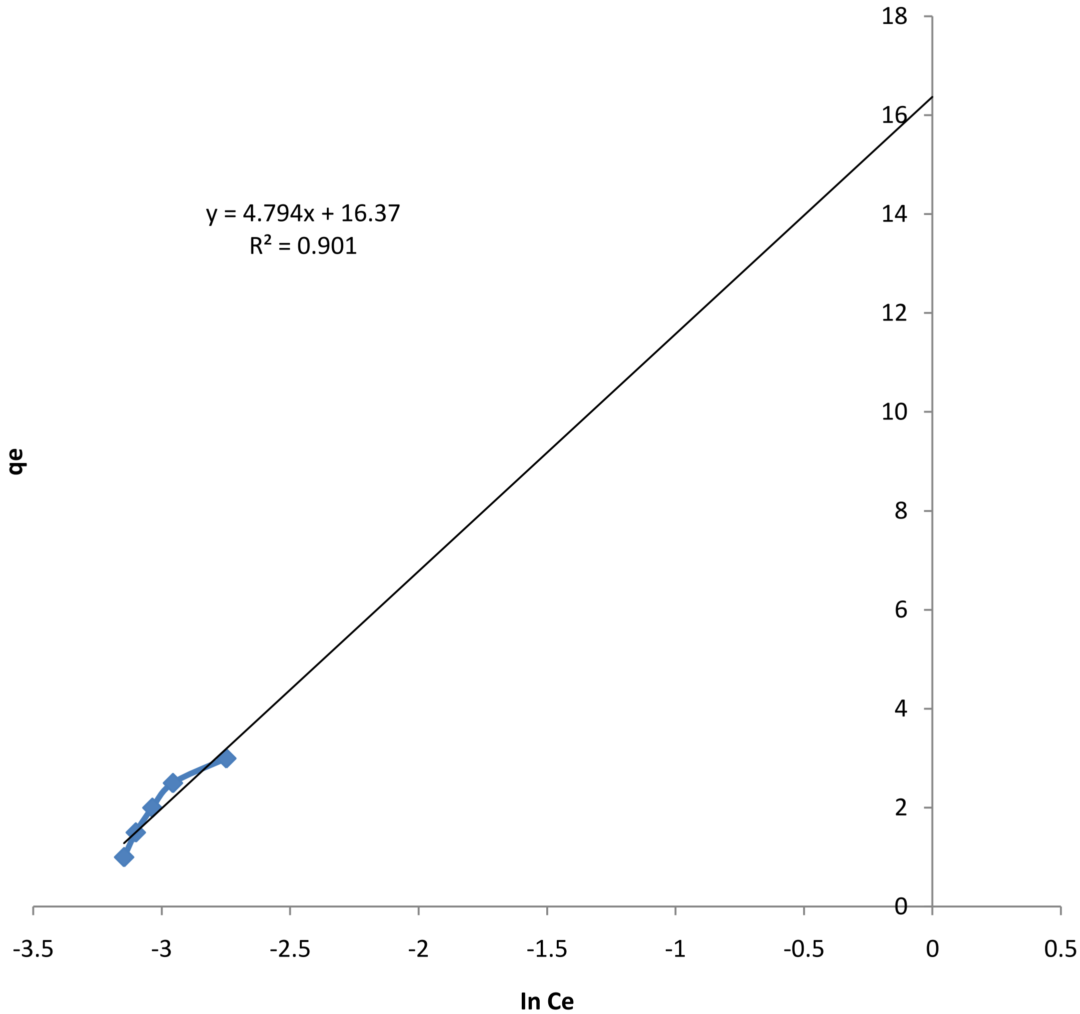


Figure 4.22: Tempkin Isotherm for biosorption of Pb^{2+}

Table 4.6 Correlation parameters of Langmuir, Freundlich and Tempkin Isotherms for Pb (II) ion adsorption onto biosorbent (*Allium cepa*)

Metal	***Langmuir Model***				***Freundlich Model***			***Tempkin***		
	Qmax (gm/g)	K_L	R^2	R_L	K_f	**n**	R^2	b_T	A_T	R^2
Pb	**-1.290**	**-11.570**	**0.485**	**-1.73×10^{-3}**	**3,169.57**	**0.403**	**0.800**	**52.0307**	**1.340**	**0.901**

Key: R^2 is the correlation coefficient, Qmax is the maximum metal uptake (mg/g), K_L is the Langmuir constant (L/mg), R_L is the separation factor, K_f is the Freundlich constant measuring biosorption capacity, A_T is the Tempkin equilibrium binding constant (L/g) and b_T is the Tempkin isotherm constant.

4.4.3 The Langmuir, Freundlich and Tempkin Isotherms Modeling for Zn (II) ions Uptake

The biosorption capacity of *allium cepa* was studied using Langmuir, Freundlich and Tempkin isotherms modeling as shown in Figures 4.23, 4.24 and 4.25 respectively. The values for the plot are presented in appendices XXV, XXVI and XXVII respectively. The correlation parameters of Langmuir, Freundlich and Tempkin Isotherms for the adsorption of Zn (II) ion onto the biosorbent are presented in Table 4.7.

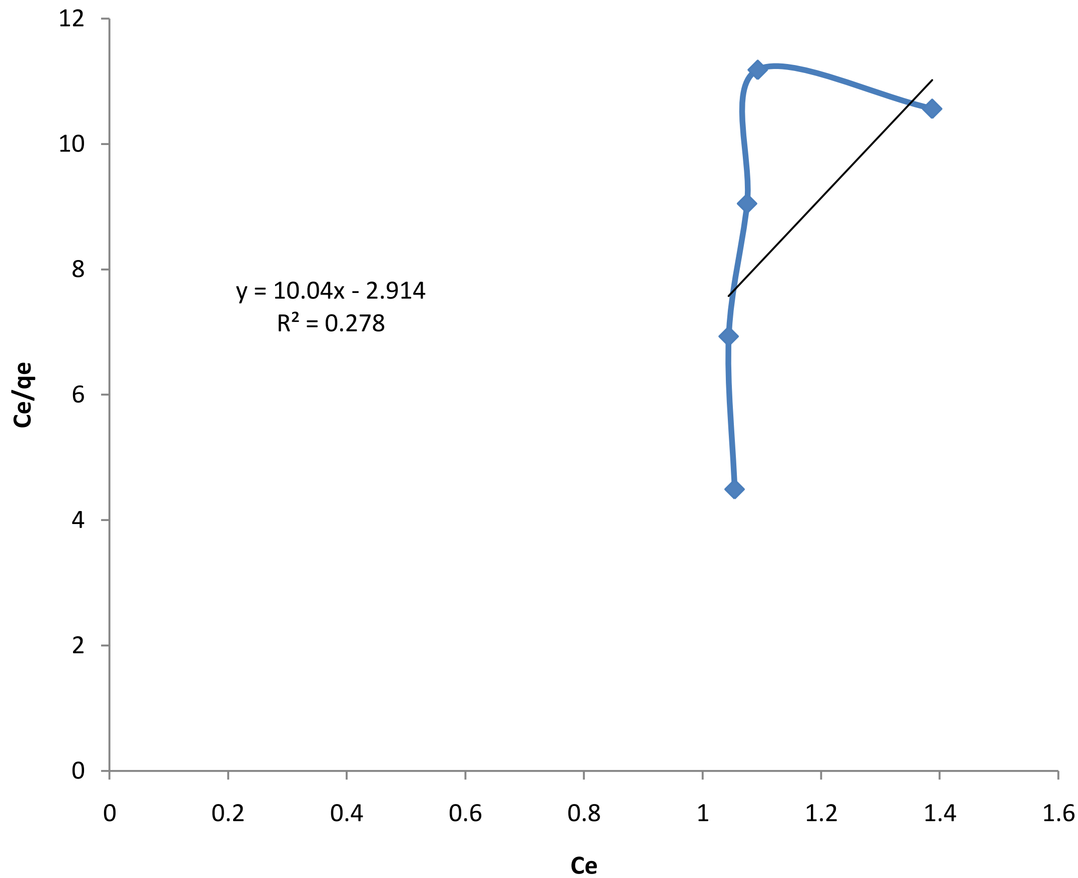


Figure 4.23: Langmuir isotherm for biosorption of Zn^{2+}

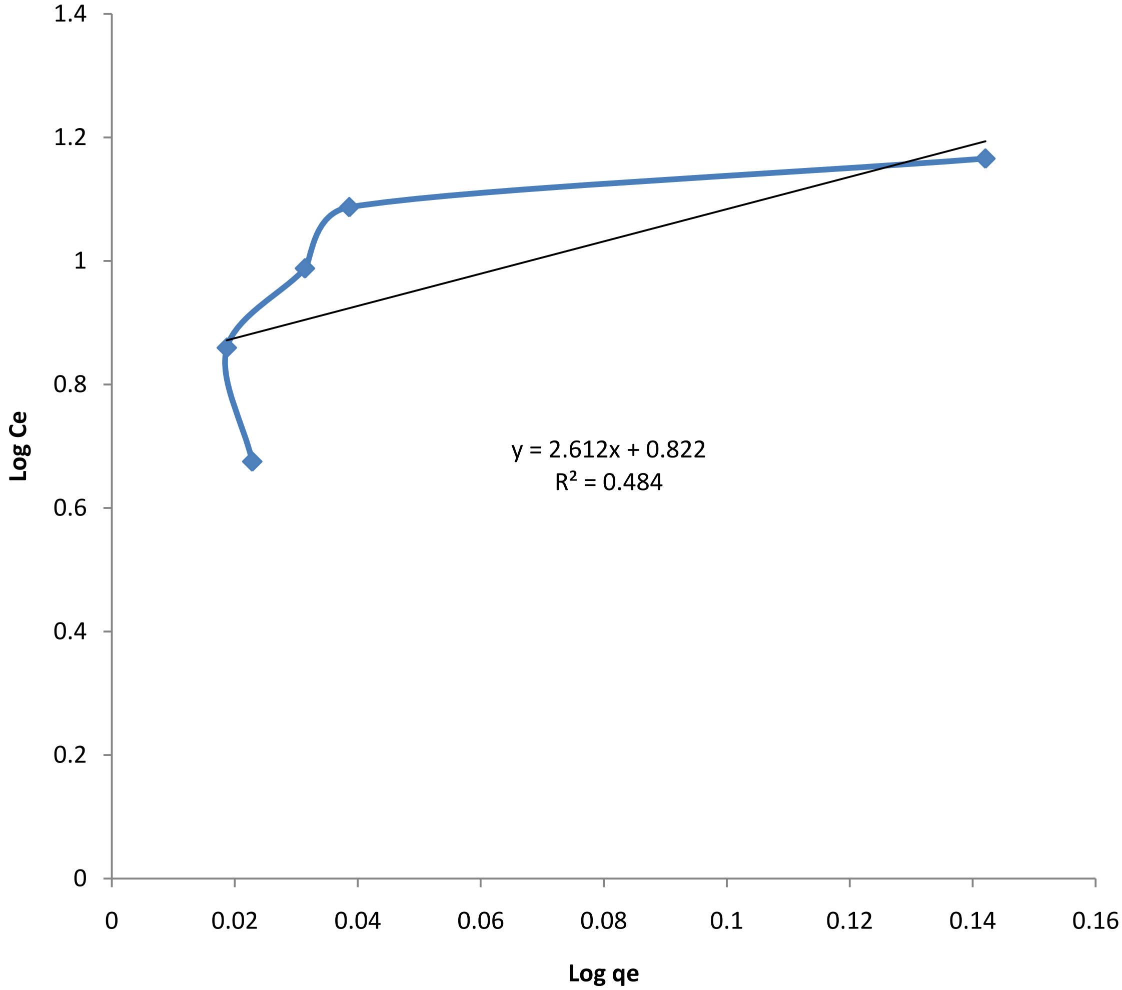


Figure 4.24: Freundlich Isotherm for biosorption of Zn^{2+}

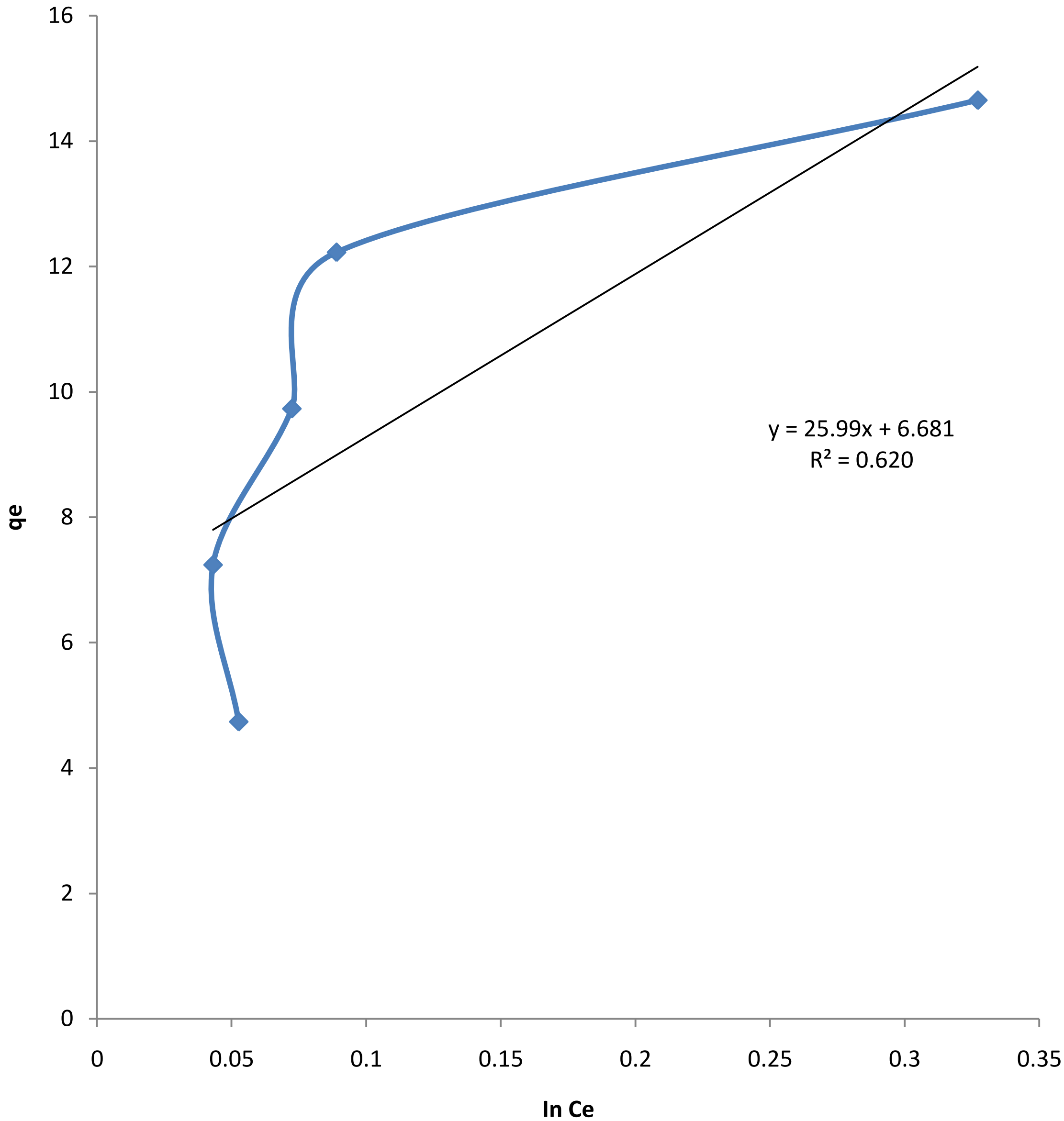


Figure 4.25: Tempkin Isotherm for biosorption of Zn^{2+}

Table 4.7 Correlation parameters of Langmuir, Freundlich and Tempkins Isotherms for Zn (II) ion adsorption onto biosorbent (*Allium cepa)*

Metal	***Langmuir Model***				***Freundlich Model***			***Tempkin***		
	Qmax (gm/g)	K_L	R^2	R_L	K_f	**n**	R^2	b_T	A_T	R^2
Zn	**0.0996**	**-34.621**	**0.278**	**-5.77×10^{-4}**	**6.6374**	**0.383**	**0.484**	**9.5974**	**48.910**	**0.620**

Key: R^2 is the correlation coefficient, Qmax is the maximum metal uptake (mg/g), K_L is the Langmuir constant (L/mg), R_L is the separation factor, K_f is the Freundlich constant measuring biosorption capacity, A_T is the Tempkin equilibrium binding constant (L/g) and b_T is the Tempkin isotherm constant.

4.5 Biosorption kinetics

4.5.1 Lagergren Pseudo first order, Pseudo second order and Intra-particle diffusion kinetic models for biosorption of Cu (II) ions

Figures 4.26, 4.27 and 4.28 represent the Pseudo first order, Pseudo second order and Intra-particle diffusion models for Cu (II) ions biosorption respectively. Values for the plot are presented in appendices XXVIII, XXIX and XXX respectively. The correlation parameters of the Pseudo first order and Pseudo second order kinetics model for the adsorption of Cu (II) ion onto biosorbent are presented in Table 4.8.

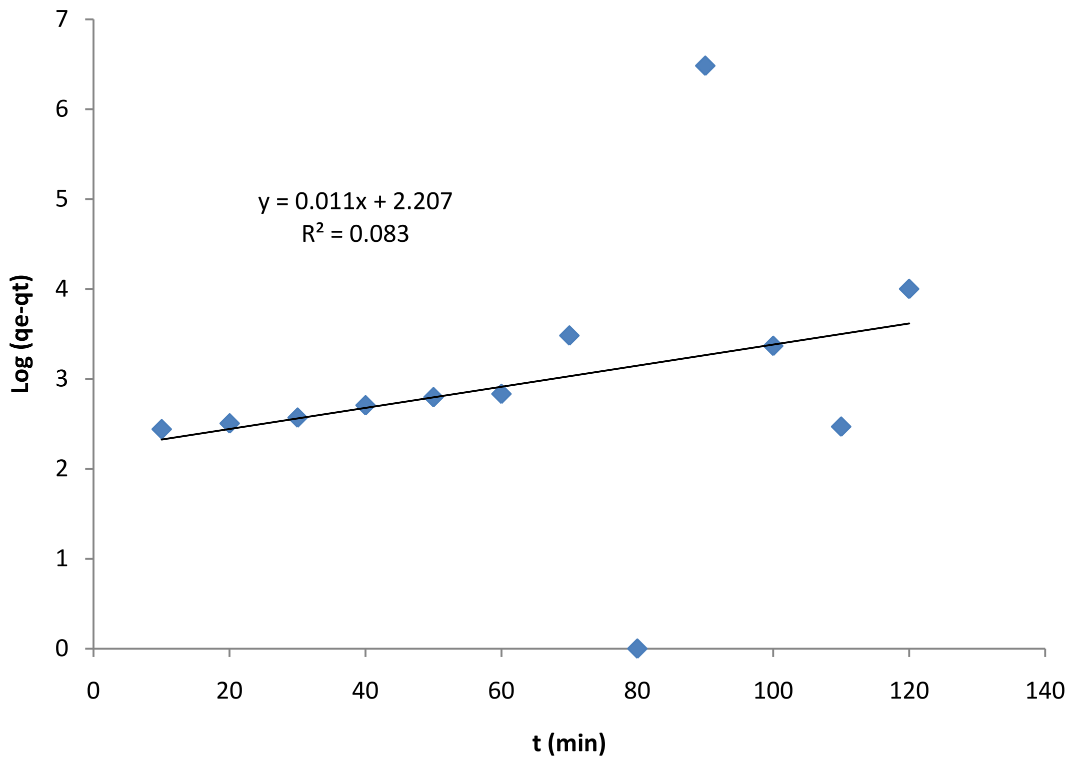


Figure 4.26: Lagergren Pseudo first order kinetics for biosorption of Cu^{2+}

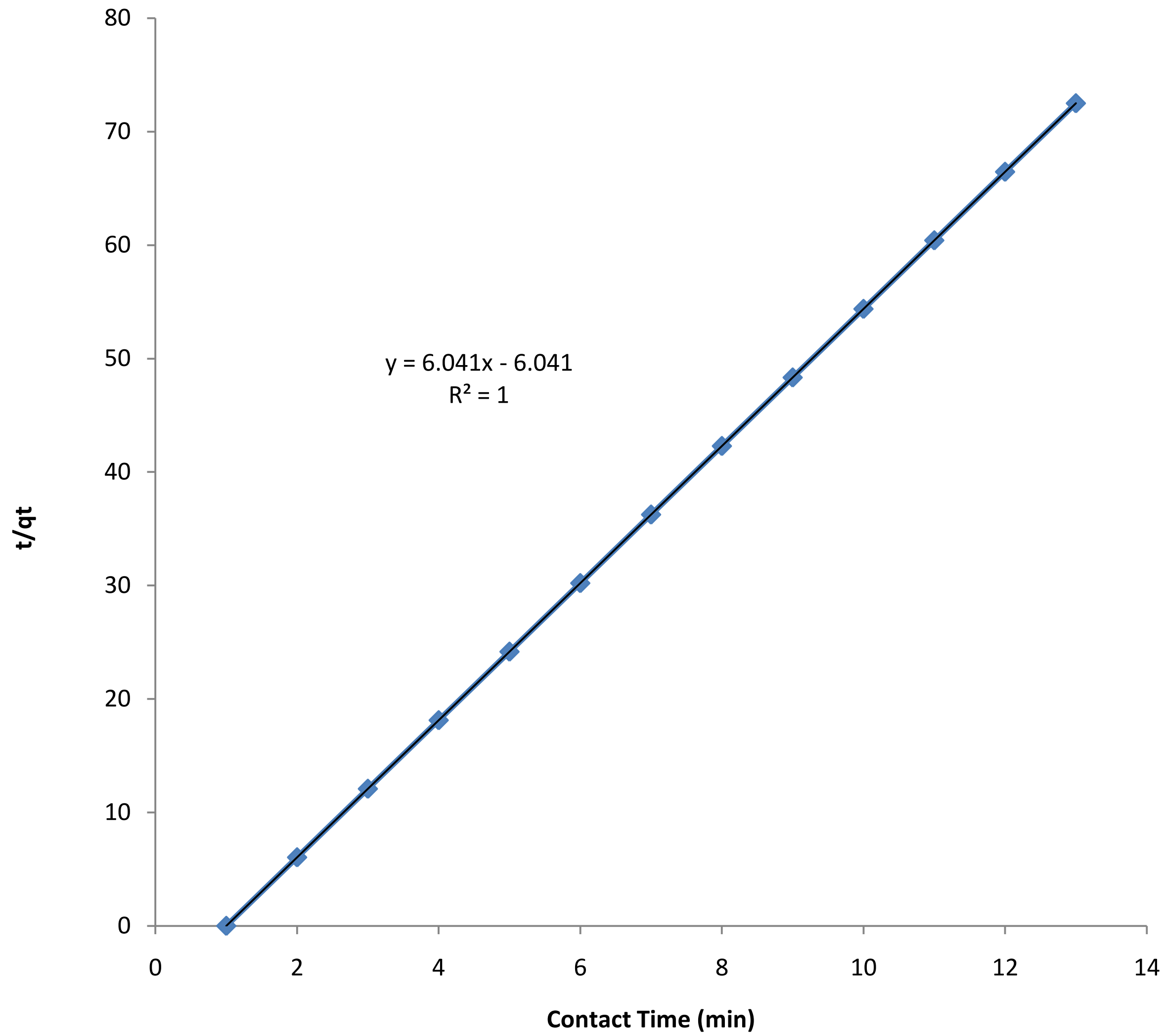


Figure 4.27: Lagergren Pseudo second order kinetics for biosorption of Cu^{2+}

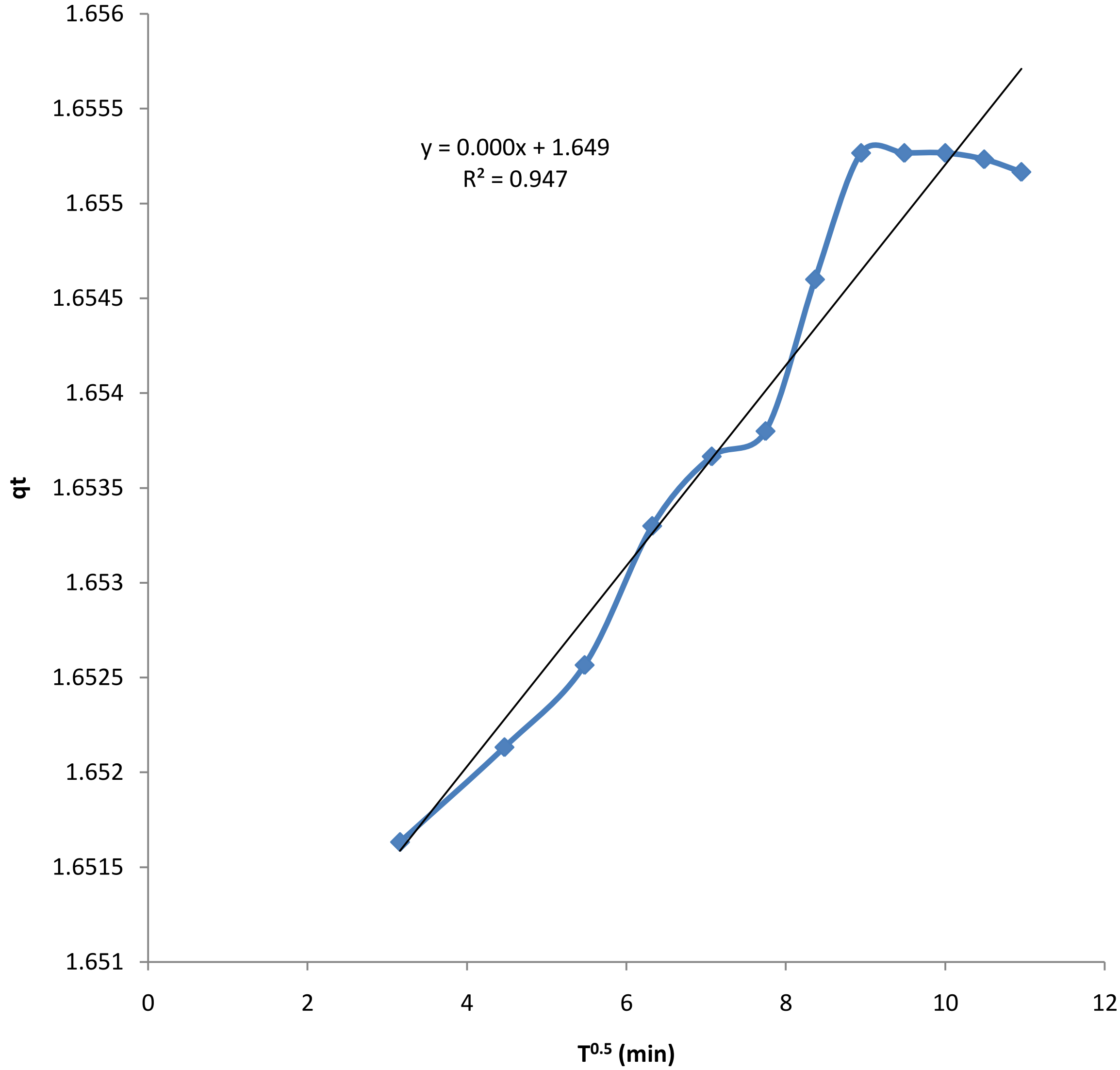


Figure 4.28: Intraparticle diffusion model for Cu (II) ion biosorption

Table 4.8 Rate constants and correlation coefficient values of Pseudo first order, Pseudo second order and Intra-particle diffusion model for Cu (II) ion adsorption onto *Allium cepa*

	Isotherms	Parameters	Values
Cu^{2+}	Pseudo first order	q_e (mg/g)	161.0646
		K_1 (min^{-1})	-0.02533
		R_1^2	0.012
	Pseudo Second Order	q_e(mg/g)	0.1655
		K_2 (g/mg/min)	-6.0436
		R_2^2	1
	Intra-particle diffusion	K_{id}	0.000
		C_{id}	1.649
		R^2	0.947

Key: R^2 is the correlation coefficient, q_e is the maximum metal uptake (mg/g), K_1 is the Pseudo first order rate constant ($Lmin^{-1}$), K_2 is the pseudo second order rate constant (gmg^{-1}.min), Ki is intra-particle diffusion rate constant (g/mg/min), C is the intercept.

4.4.2 Lagergren Pseudo first order, Pseudo second order and intra-particle diffusion kinetic models for biosorption of Pb (II) ions

Figures 4.29, 4.30 and 4.31 represent the Pseudo first order, Pseudo second order models and intra-particle diffusion model for Pb (II) ions biosorption respectively. Values for the plot are presented in appendices XXXI, XXXII and XXXIII respectively. The correlation parameters of the Pseudo first order and Pseudo second order kinetics model for the adsorption of Pb (II) ion onto biosorbent are presented in Table 4.9.

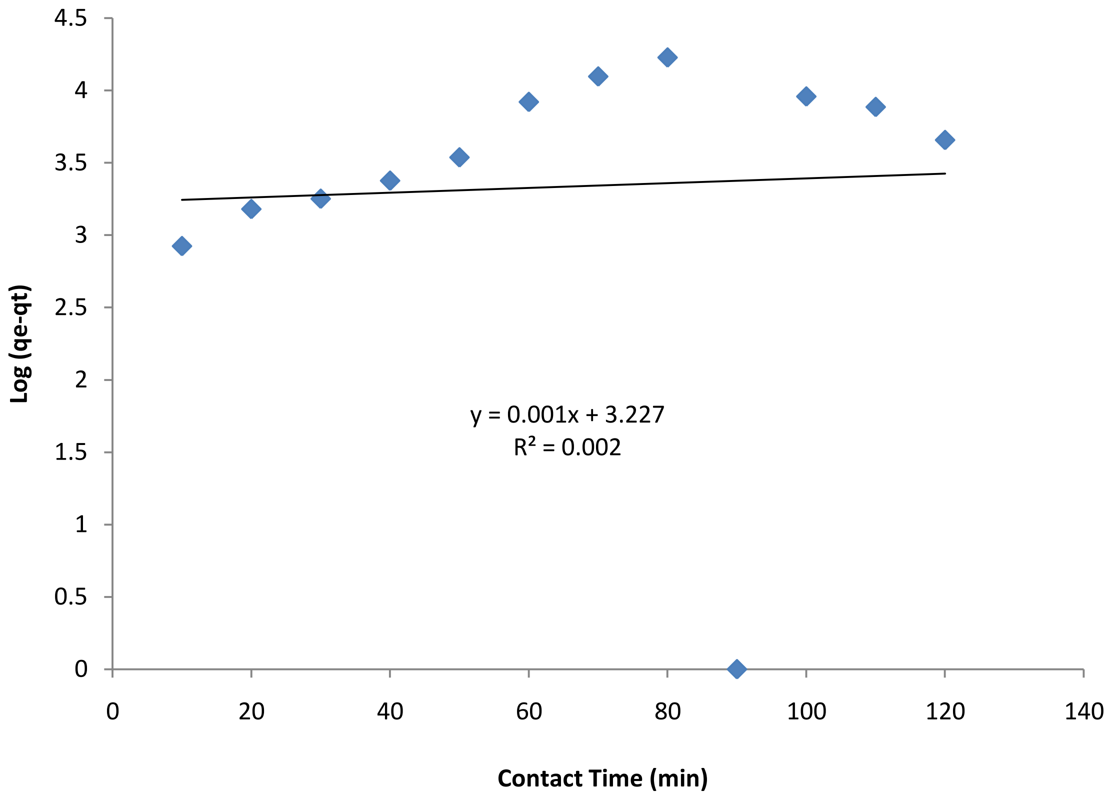


Figure 4.29: Lagergren Pseudo first order kinetics for biosorption of Pb^{2+}

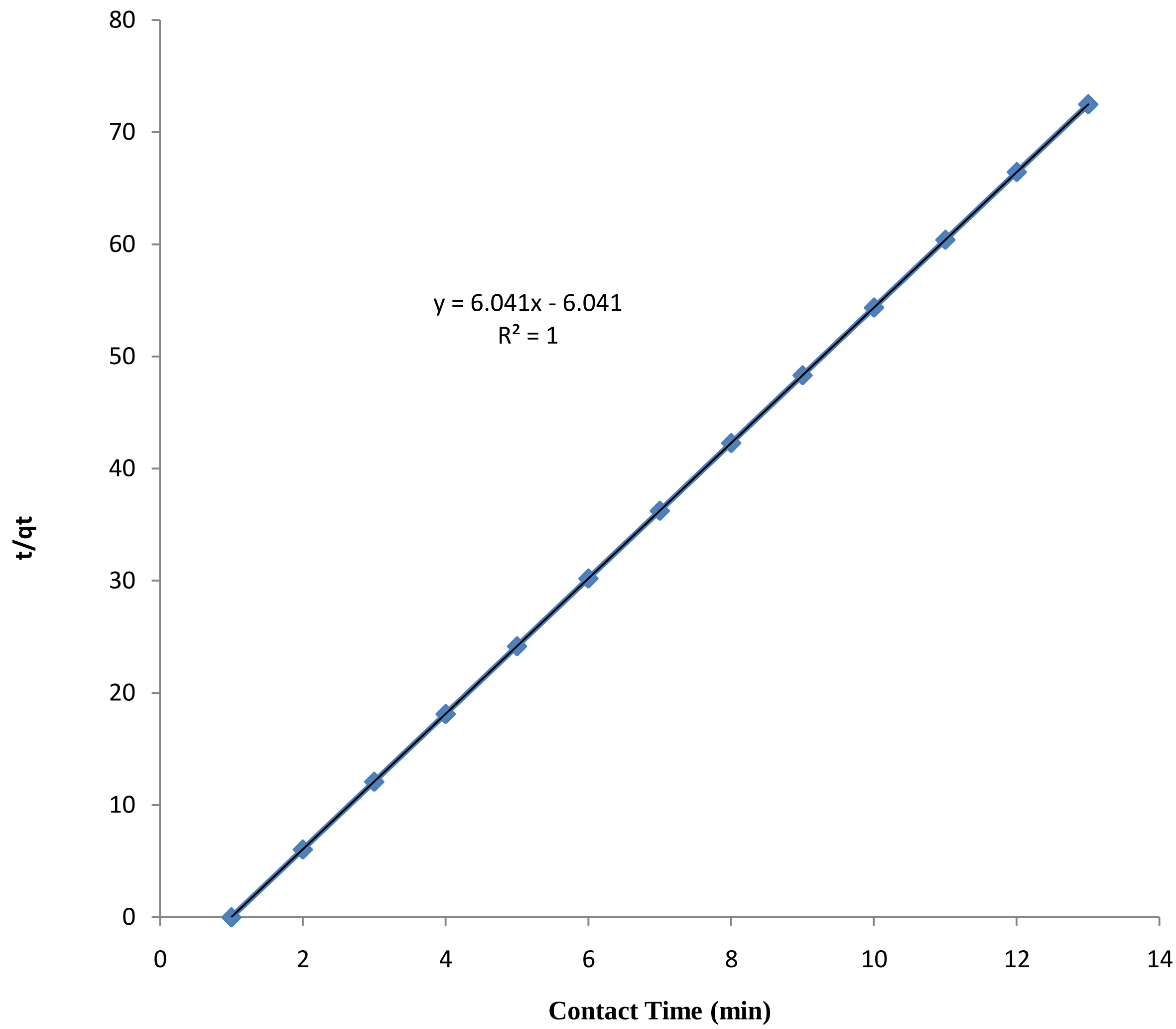


Figure 4.30 Lagergren Pseudo second order kinetics for biosorption of Pb^{2+}

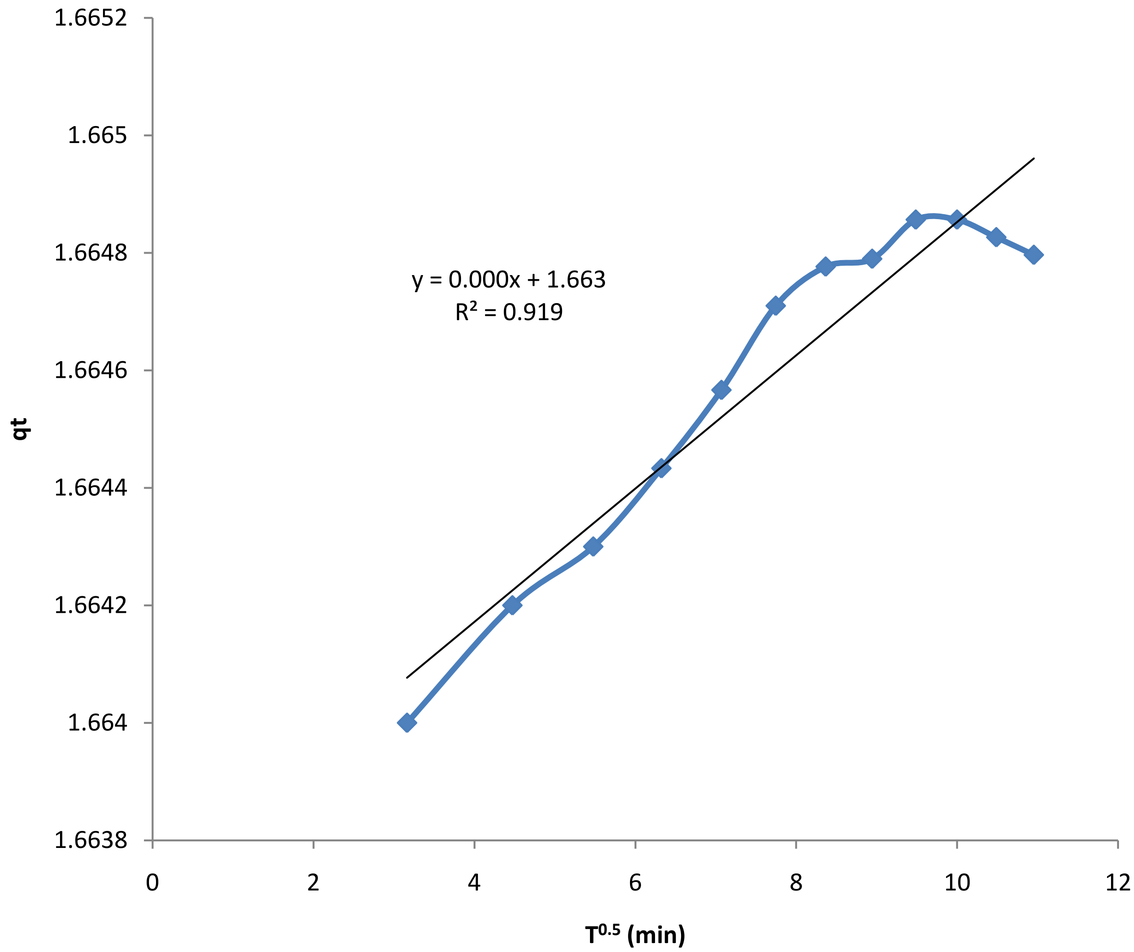


Figure 4.31 Intraparticle diffusion for Pb^{2+} biosorption

Table 4.9 Rate constants and correlation coefficient values of Pseudo first order, Pseudo second order and Intra-particle diffusion model for Pb (II) ion adsorption onto *Allium cepa*

	Isotherms	Parameters	Values
Pb^{2+}	Pseudo first order	q_e (mg/g)	1,686.55
		K_1 (min^{-1})	-0.002303
		R_1^2	0.002
	Pseudo Second Order	q_e(mg/g)	0.1665
		K_2 (g/mg/min)	-6.006
		R_2^2	1
	Intra-particle diffusion	K_{id}	0.000
		C_{id}	1.663
		R^2	0.919

Key: R^2 is the correlation coefficient, q_e is the maximum metal uptake (mg/g), K_1 is the Pseudo first order rate constant ($Lmin^{-1}$), K_2 is the pseudo second order rate constant ($gmg^{-1}.min$), K_{id} is intra-particle diffusion rate constant (g/mg/min), C is the intercept.

4.4.3 Lagergren Pseudo first order, Pseudo second order and Intra-particle diffusion kinetic models for biosorption of Zn (II) ions

Figures 4.32 and 4.33 and 4.34 represent the Pseudo first order, Pseudo second order and intra-particle diffusion kinetic models for Zn (II) ions biosorption respectively. Values for the plot are presented in appendices XXXIV, XXXV and XXXVI respectively. The correlation parameters of the Pseudo first order and Pseudo second order model for the adsorption of Zn (II) ion onto biosorbent are presented in Table 4.10.

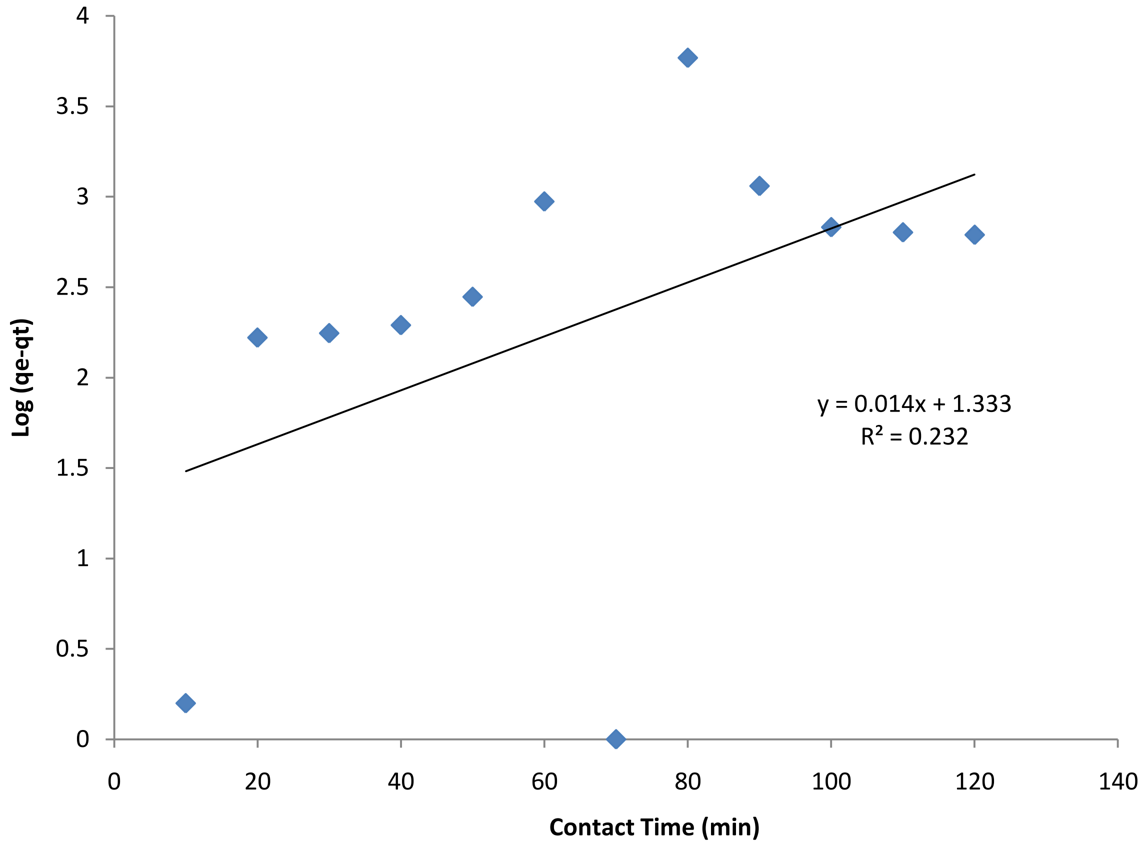


Figure 4.32: Lagergren Pseudo first order kinetics for biosorption of Zn^{2+}

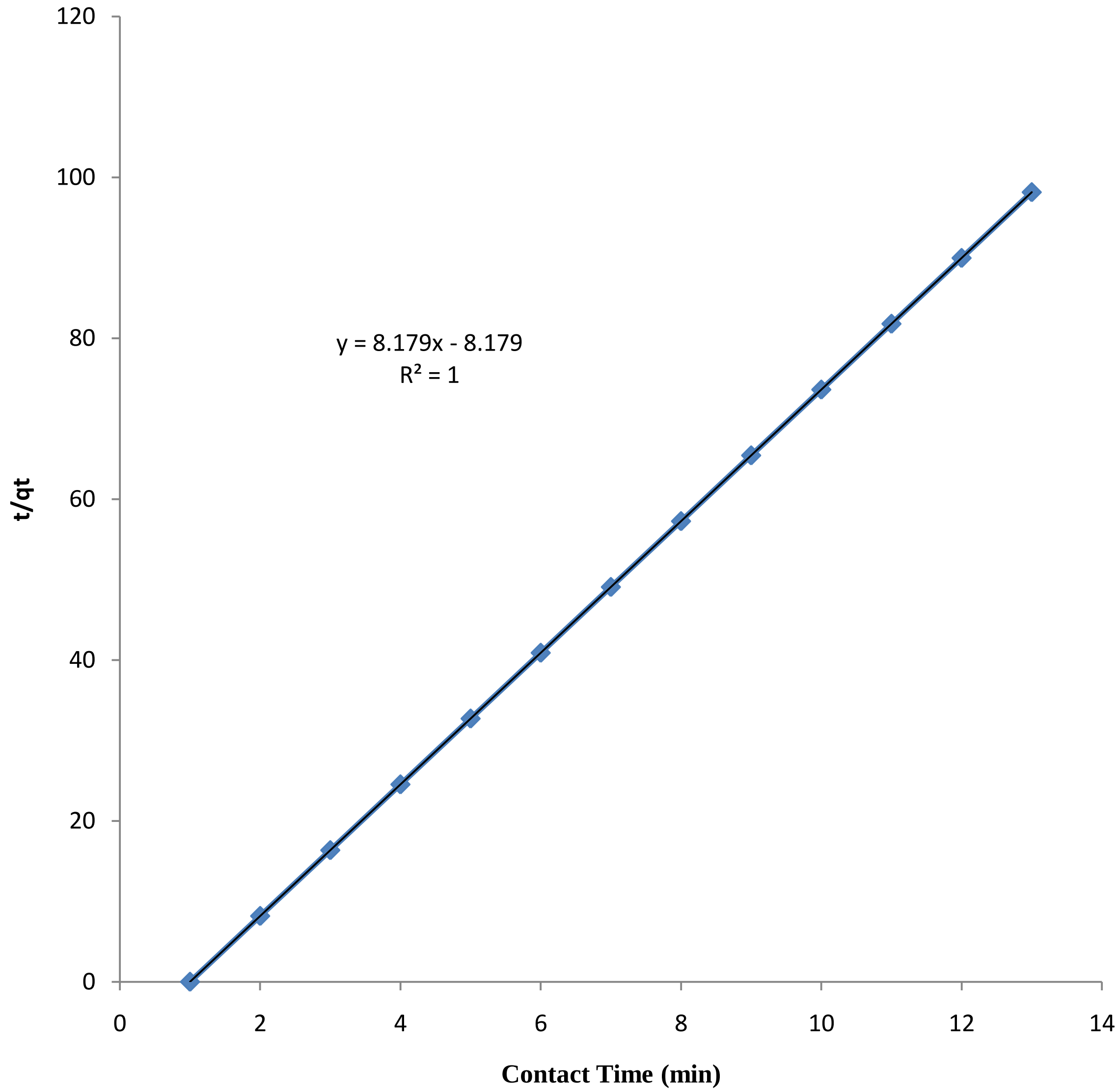


Figure 4.33: Lagergren Pseudo second order kinetics for biosorption of Zn^{2+}

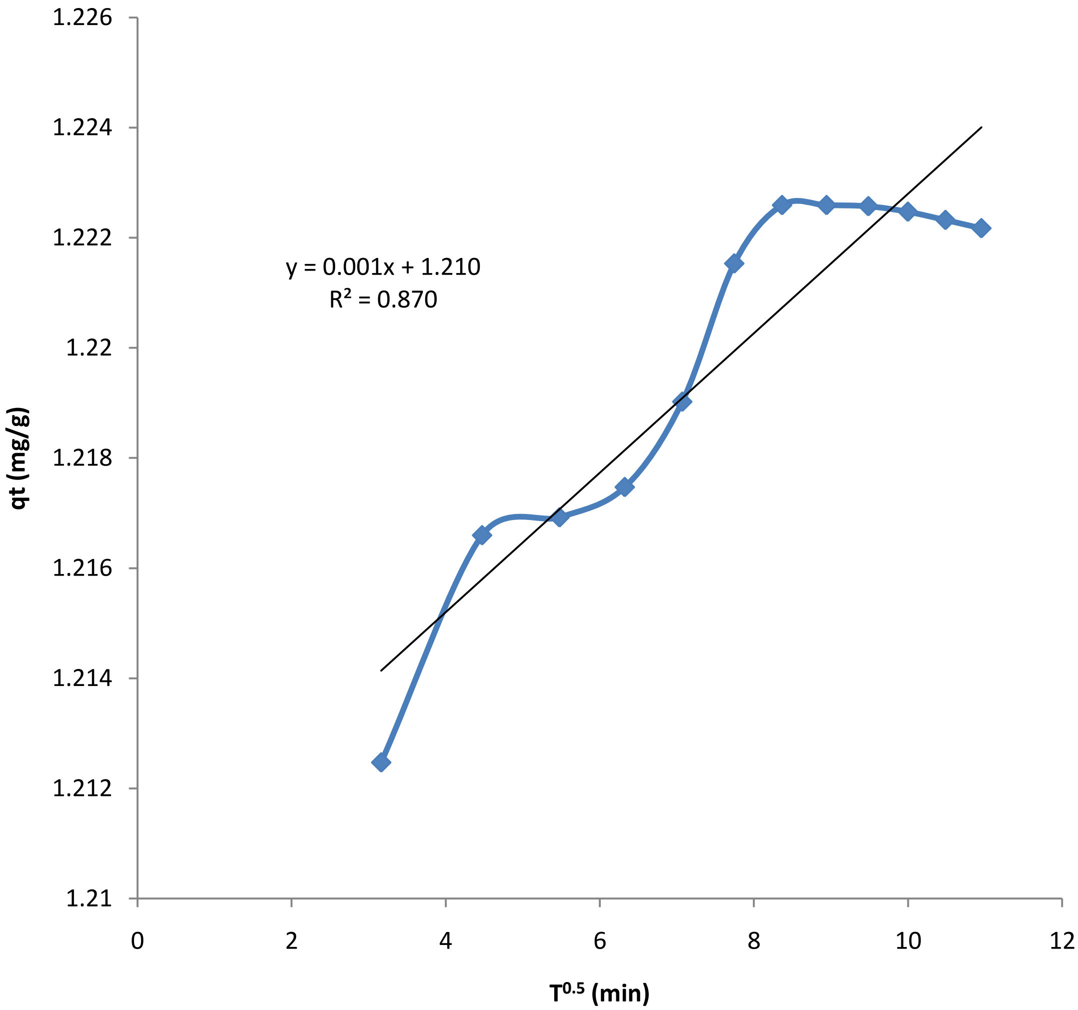


Figure 4.34 **Intraparticle diffusion for Zn^{2+} biosorption.**

Table 4.10 Rate constants and correlation coefficient values of Pseudo first order, Pseudo second order and Intra-particle diffusion model for Zn (II) ion adsorption onto *Allium cepa*

	Isotherms	Parameters	Values
Zn^{2+}	Pseudo first order	q_e (mg/g)	21.5278
		K_1 (min^{-1})	-0.03224
		R_1^2	0.232
	Pseudo Second Order	q_e(mg/g)	0.1222
		K_2 (g/mg/min)	-8.1782
		R_2^2	1
	Intra-particle diffusion	K_{id}	0.001
		C_{id}	1.21
		R^2	0.870

Key: R^2 is the correlation coefficient, q_e is the maximum metal uptake (mg/g), K_1 is the Pseudo first order rate constant ($Lmin^{-1}$), K_2 is the pseudo second order rate constant (gmg^{-1}.min), Ki is intra-particle diffusion rate constant (g/mg/min), C is the intercept.

CHAPTER FIVE

DISCUSSIONS

5.1 Characterization of Samples

5.1.1 Atomic Adsorption Spectroscopy Analysis of Samples

Appendix II represents the determination of Cu, Pb and Zn (II) ions concentration in samples. It can be seen that the concentration of Cu, Pb and Zn (II) ions present in the sample of *Allium cepa* biomass are 0.002 mgL^{-1}, 0.0000 mgL^{-1} and 0.003 mgL^{-1} respectively while in the human blood plasma, concentration of Cu, Pb and Zn (II) ions present are 0.0000 mgL^{-1} for the three metal ions. The result shows the concentration of Cu^{2+}, Pb^{2+} and Zn^{2+} are within permissible limits. The World Health Organization (WHO) and the Food and Agricultural Administration (FAA) suggested that the population mean intake of copper should not exceed 12 mg/day for adult males and 10 mg/day for adult females and there is no known level of lead exposure that is considered safe (WHO, 2015). The National Institute of Health Office of Dietary supplement in 2016 stated that, the intake of zinc is dependent on the age and safe highest level of intake for Zinc is 13 mg/day.

5.1.2 Fourier Transform Infrared Spectroscopy (FTIR) of dried *Allium Cepa.*

An FTIR spectrum of dried *Allium cepa* biomass is presented in Appendix III, while Table 4.1 presents the identified functional groups. There was presence of a medium peak at 816.3 cm^{-1} corresponding to a C-H bend confirming the presence of aromatic compound. A strong and sharp absorption peak at 920.7 cm^{-1} indicating =C-H bend, conforming the presence of alkenes, a sharp peak at 1025.0 indicating =C-O-C symmetric stretch confirming the presence of ethers, a medium peak at 1252.4 indicating =C-O-O asymmetric stretch confirming the presence of ether, a sharp and medium peak at 1364.2 indicating S=O confirming the presence of sulphoxides, a sharp peak at 1636.3 indicating C=O stretch confirming the presence of amides, a medium peak at 2922.2 indicating C-H stretch confirming the presence of alkanes and alkyl groups, a strong and broad peak at 3272.6 confirming the presence of carboxylic acid.

5.1.3 Fourier Transform Infrared Spectroscopy (FTIR) of Cu (II) ions treated *Allium Cepa*

FTIR spectra of the Cu^{2+} treated *Allium cepa* is presented in Appendix IV while Table 4.2 presents the identified functional groups. The spectra showed generally a shift in the peaks from 3272.6 cm^{-1}, 1636.3 cm^{-1}, 1252.4 cm^{-1}, 1025.0 cm^{-1} and 816.3 cm^{-1} in the native biomass to peaks 3269.4 cm^{-1}, 1634.8 cm^{-1}, 1242.4 cm^{-1}, 1016.8 cm^{-1} and 816.7 cm^{-1} respectively in the Cu^{2+} treated biosorbent. The general shift in the peaks as observed from the FTIR spectra analysis confirmed that =C-H bend of amide group, =C-O-C symmetric stretch of aromatic compounds, O-H stretch of carboxyl group and =C-O-C asymmetric stretch of ethers are the overall functional groups involved in the successful biosorption of Cu (II) ions by *Allium cepa* biomass.

5.1.4 Fourier Transform Infrared Spectroscopy (FTIR) of Pb (II) ions treated *Allium Cepa*

FTIR spectra of the Pb^{2+} treated *Allium cepa* is presented in Appendix IV, while Table 4.2 presents the identified functional groups. The spectra showed generally a shift in the peaks from 3272.6 cm^{-1}, 2922.2 cm^{-1}, 1636.3 cm^{-1}, 1364.2 cm^{-1}, 1252.4 cm^{-1} and 1025.0 cm^{-1} in the native biomass to peaks 3272.6 cm^{-1}, 2855.1 cm^{-1}, 1632.6 cm^{-1}, 1367.9 cm^{-1}, 1237.5 cm^{-1} and 1013.8 cm^{-1} respectively in the Pb^{2+} treated biosorbent. The general shift in the peaks as observed from the FTIR spectra analysis confirms O-H stretch of alkanes and alkyl group, C=O stretch of amide group, O-H stretch of carbonyl group, =C-O-C symmetric and asymmetric stretch of ether group and S=O of sulphoxide group were involved in the overall successful biosorption of Pb (II) ions by *Allium cepa* biomass.

5.1.5 Fourier Transform Infrared Spectroscopy (FTIR) of Zn (II) ions treated *Allium Cepa*

FTIR spectra of the Zn^{2+} treated *Allium cepa* is presented in Appendix IV while Table 4.2 presents the identified functional groups. The spectra showed generally a shift in the peaks from 3272.6 cm^{-1}, 1636.3 cm^{-1}, 1364.2 cm^{-1}, 1252.4 cm^{-1} and 920.7 cm^{-1} in the native biomass to peaks 3268.9 cm^{-1}, 1625.1 cm^{-1}, 1319.5 cm^{-1}, 1237.5 cm^{-1} and 890.8 cm^{-1} respectively in the Zn^{2+} treated biosorbent. The general shift in the peaks as observed from the FTIR spectra analysis confirms that O-H stretch of carbonyl group, C=O stretch of amide group, S=O of sulphoxide group, =C-O-C asymmetric stretch of ether group and =C-H bend of aromatic compounds were involved in the overall successful biosorption of Zn (II) ions by *Allium cepa* biomass.

5.1.6 Scanning Electron Microscope (SEM)

Scanning Electron Microscope clearly revealed the surface texture and morphology of the *Allium cepa* biomass before and after biosorption at different magnifications (400x, 500x, and 1500x). The SEM micrograph of native and Cu^{2+}, Pb^{2+} and Zn^{2+} treated *allium cepa* biomass are shown in Figures 4.1, 4.2, 4.3 and 4.4 respectively. At the different magnifications of the native *Allium cepa* biomass, uneven surface texture along with a lot of irregular surface format was observed. These Figures displayed evidently that over the biosorption period, the surface morphology of the native *Allium cepa* biomass had undergone remarkable physical disintegration resulting to emergence of protrusions and rough surface area which is evidence of high Cu (II), Pb (II) and Zn (II) ions uptake.

SEM micrograph for Pb (II) ions treated *Allium cepa* biomass in Figure 4.3 showed more disintegrations and rough surface than the other heavy metal ion treated biomass SEM micrographs. This is due to the high surface interactions between the binding sites on the surface of the *Allium cepa* biomass and Pb (II) ions. Similar SEM observations have been reported (Gupta and Rastogi, 2008; Sethuraman and Balasubramanian, 2010; Shakirullah *et al.*, 2006; Wilke *et al.*, 2006;).

5.2 Optimum Parameters for biosorption of Cu (II) ions

5.2.1 Optimum pH for biosorption of Cu (II) ions

Figure 4.5 is a graphical illustration of the optimised pH, by plotting % removal of Cu (II) ions against different pH of the mixture. At pH 2, a percentage sorption of 99.198 % was observed. The maximum sorption of Cu (II) by *allium cepa* biomass occurred at pH 6 (Optimum pH) with percentage sorption of 99.346%. The impact of pH on sorption of Cu (II) in the range of 2 to 10, no significant variation (99.198 % - 99.276 %) was observed in the sorption capacity. pH has no significant influence on the ionic state of the active sites and ligands involved in the sorption of Cu (II) ions by *Allium cepa* biomss. The optimum pH for Cu sorption was found to be pH 6. Similar result was reported by Mesfin, (2009).

5.2.2 Optimum concentration for biosorption of Cu (II) ions

Figure 4.6 is a graphical illustration of the optimised initial Cu (II) ions concentration, by plotting % removal of Cu (II) ion against various concentrations of Cu (II) ions**.** There was an increase in percentage removal from 98.005 % - 99.188 % when the concentration was varied from 20 mgL^{-1} – 50 mgL^{-1}. The maximum sorption of Cu (II) by

Allium cepa occurred at 50 mgL^{-1} (Optimum concentration) with percentage sorption of 99.188 %. This is due to higher interaction between metal ions and the biosorbent. Thereafter, varying the concentration from 50 mgL^{-1} – 60 mgL^{-1} resulted in a decrease in percentage removal from 99.188 % - 99.16667 % as observed.

This is because for a fixed dose of the biomass the total available binding sites are limited, thereby binding the same amount of metal ions. Therefore the increase in Cu (II) ions uptake by *Allium cepa* with an increase in Cu (II) ions concentration might be due to the increase in the ratio of initial number of moles of Cu (II) ions to the available surface area (Mataka *et.al.*, 2010).

5.2.3 Optimum biosorbent dosage for biosorption of Cu (II) ions

Figure 4.7 is a graphical illustration of the optimised biosorbent dosage for Cu (II) ions removal. The uptake of Cu (II) by *Allium cepa* from the human plasma was rapid initially and slowed down in the latter stages as it got to its optimum at 0.60 g. As the biosorbent dosage was varied from 0.20 g – 0.60 g, the removal efficiency increased to 99.34 % due to the availability of more binding cites for complexation of metal ions (Gong *et al*., 2005). However, during the interval of 0.80 g - 1.00 g, the % removal decreased. Analysis for 1.00 g residual concentration resulted to an efficiency of 99.102 %, indicating no significant uptake by the *Allium cepa*.

This may be due to the fact that some adsorption sites may remain unsaturated during the adsorption process where the number of sites available for sorption increases by increasing the biosorbent dose. This could be attributed to the fact that at high biosorbent dosages the available metal ions are insufficient to cover all the exchangeable sites on the biosorbent, usually resulting into low metal uptake (Fourest & Roux, 1992; Vijayaraghavan *et al*., 2006).

5.2.4 Rate of biosorption of Cu (II) ions

Figure 4.8 is a graphical illustration of the study of rate of biosorption of Cu (II) ions, by plotting % removal of Cu (II) ion against various contact time. The curve shows an increase in Cu (II) ions uptake from 99.098 - 99.316 % as the contact time was varied from 10 min – 80 min. The rate of uptake was initial very fast in the first 40 min and it then slowed down as contact time continues to vary; therefore it can be observed that the highest percentage sorption occurred at 80 min (99.31 %). Thereafter, increase in contact time resulted in a decrease in percentage sorption from 99.316 - 99.31 % at 120 min. The decrease in the rate of sorption can be attributed to the smaller ratio of free binding sites to free Cu (II) ions in the solution (Odokuma and Akponah, 2010).

5.2.5 Langmuir Isotherm for biosorption of Cu (II) ions

Figure 4.17 represents Langmuir isotherm for Cu (II) ions biosorption by plotting $\frac{C_e}{q_e}$ againstC_e, while Table 4.5 shows the correlation parameter of Langmuir isotherm for Cu (II) ions removal. Qmax (0.129mgg^{-1}) depicts the capacity of biosorbent to detoxify maximum amount of Cu (II) ions, R_L (8.77×10^{-5}) indicates that the removal of Cu (II) ion by *allium cepa* was favourable and correlation coefficient (R^2) 0.182 signifies that the experimental data did not fit into Langmuir adsorption isotherm (Al-Bayati, 2011).

5.2.6 Freundlich isotherm for biosorption Cu (II) ions

Figure 4.18 represents the Freundlich isotherm for Cu (II) ions biosorption by plotting Inq_e against InC_e while Table 4.5 presents the correlation parameters of Freundlich Isotherm of Cu (II) ion removal. K_f (0.3908 Lmg^{-1}) value depicts that maximum amount of Cu (II) ion was binded by *allium cepa* with high biosorption intensity, n value of 5.714 suggest a favourable Cu (II) ions sorption since $1/n<1$ and R^2 (0.351) value suggests that the experimental data for Cu (II) ions biosorption did not fit into the Freundlich isotherm (Panida *et al*., 2014).

5.2.7 Temkin Isotherm for biosorption of Cu (II) ions

Figure 4.19 represents Temkin isotherm for Cu (II) ions biosorption by plotting q_e against InC_e, while Table 4.5 presents the correlation parameter of Temkin model for Cu (II) ion biosorption. A_T (3.900) indicates low binding energy with b (29.246) corresponding to a low heat of adsorption. R^2 (0.508) depicts that the experimental data obtained for biosorption of Cu (II) ion fits more into Tempkin model (Kumar and Kirthika, 2009).

5.2.8 Lagergren Pseudo first order model for biosorption of Cu (II) ions.

Figure 4.26 represents pseudo first order model for Cu (II) ions biosorption by plotting $log\,(q_e - q_t)$ against t, while Table 4.8 presents the correlation parameters of Pseudo first order model of Cu (II) ion removal. The qe (161.0646 mgg^{-1}) value indicated that biosorbent adsorbed excess Cu (II) ion within a short contact time and the correlation coefficient, R^2 (0.01200) value indicated that the experimental data obtained for Cu (II) ions sortion did not fit into Pseudo first order (Febrianto *et al.,* 2009).

5.2.9 Lagergren Pseudo Second order model for biosorption of Cu (II) ions.

Figure 4.27 represents Pseudo second order model for Cu (II) ions sorption by plotting $\frac{t}{q_t}$ against t, while Table 4.8 presents the correlation parameters of Pseudo second order model of Cu (II) ion removal. Correlation coefficient, R^2 (1.00) value suggested that the data fits into Pseudo second order kinetics model. The qe (0.1655 mgg^{-1}) value indicates that biosorbent was capable of removing Cu (II) ions within a limited time as shown by rate constant, K_1 (-6.0436 gmg $^{-1}$.min).

5.2.10 Intra-particle diffusion model for Cu (II) ion biosorption

Figure 4.28 is an intra-particle diffusion experimental data of a plot of qt against $t^{0.5}$ for Cu (II) ion sorption. Rate constants and correlation coefficient values Intra-particle diffusion model for Cu (II) ion adsorption onto *Allium cepa* are presented in Table 4.8. For investigated Cu (II) ions sorption by *allium cepa* biomass, the plots *Qt versus* $t^{1/2}$ did not pass through the origin suggesting that even though the adsorption process involved intra-particle diffusion, it was not the only rate-controlling step (Yakout and Elsherif, 2010). The correlation coefficient R^2 (0.947) which indicates the experimental data fits into intra-particle diffusion model, and the positive value of intercept C_{id} (1.649) is indicative of some degree of boundary layer control (Ozcan and Ozcan, 2004).

5.3 Optimum Parameters for biosorption of Pb (II) ions

5.3.1 Optimum pH for biosorption of Pb (II) ions

Figure 4.9 is a graphical illustration of the optimised pH, by plotting % removal of Pb (II) ions against various pH of the mixture. At pH 2, the percentage sorption of 99.84 % was observed. Further increase in pH of the mixture, a maximum sorption of Pb (II) ions by *allium cepa* occurred at pH 4 with a percentage sorption of 99.8976 %. Thereafter increase in pH from 6 – 10, the percentage sorption showed a negligible decreased from 99.8976 %

- 99.8508 %, probably due to the degree of protonation of the adsorbent functional group decreased gradually and therefore percentage removal also slight decreased. The impact of pH on sorption of Pb (II) in the range of 2 to 10, no significant variation (99.84 % - 99.8508 %) was observed in the sorption capacity. Therefore pH has no significant influence on the ionic state of the active sites and ligands involved in the sorption of Pb (II) ions by *Allium cepa* biomass. The optimum pH for Pb sorption was found to be pH 4. Similar result was reported by Mesfin, (2009).

5.3.2 Optimum concentration for biosorption of Pb (II) ions

Figure 4.10 is a graphical illustration of the optimised initial Pb (II) ions concentration, by plotting % removal of Pb (II) ion against various concentrations of Pb (II) ions**.** As observed, the uptake capacity of Pb (II) ion increased from 99.785 % - 99.896 % when the initial Pb (II) ions concentration was varied from 20 mgL^{-1} – 50 mgL^{-1} Pb (II) ions, this is due to the availability of binding cite on the biosorbent. The maximum sorption of Pb (II) by *allium cepa* occurred at 50 mgL^{-1} Pb (II) ions (Optimum concentration) with percentage sorption of 99.896 %. Further increase in Pb (II) ions concentration from 50 mgL^{-1} – 60 mgL^{-1}, a decrease in % removal of Pb (II) ions from 99.896 % - 99.8933 % was observed.

This behaviour was attributed to the fact that, the exchangeable sites in 0.60 g of biosorbent will not be enough to cover these concentrations so the depletion in percentage removal will be obvious, i.e. the biosorption of Pb (II) ions decreased due to a few active sites available on the surface of the *allium cepa* biomass (Azza *et al.,* (2012).

5.3.3 Optimum biosorbent dose for biosorption of Pb (II) ions

Figure 4.11 represents the optimised biosorbent dosage for Pb (II) ions removal, by plotting % removal of Pb (II) ions against various biosorbent dosages**.** It can be observed that during the interval of 0.20 g - 0.40 g dosage, the efficiency increased to 99.89 %. The maximum sorption of Pb (II) by *allium cepa* occurred at 0.40 g (Optimum biosorbent dose) with percentage sorption of 99.89%. Thereafter a negligible decrease in the Pb (II) ions biosorption from 99.89 % - 99.842 while the biosorbent dosage was varied from 0.40 g – 1.00 g as observed.

This might be due to partial aggregation of biomass, which has reduced the surface area available for biosorption (Karthikeyan *et al*., 2007; Sari and Tuzen, 2008). Therefore, the optimum biomass dosage was selected as 0.40 g for further experiment.

5.3.4 Rate of biosorption of Pb (II) ions

Figure 4.12 is a graphical illustration of the study of rate of biosorption of Pb (II) ions, by plotting % removal of Pb (II) ion against various contact time. The curve shows an increase from 99.84 - 99.8914 % in Pb (II) ions sorption by the biosorbent as the contact time was varied from 10 – 90 min. Therefore, the maximum sorption of Pb (II) by *allium cepa* occurred at 90 min with percentage sorption of 99.8914%. Thereafter a decrease in percentage sorption from 99.8914 to 99.8878 % was observed as agitation continued from 90 min – 120 min indicating no significant uptake of Pb (II) ions from solution of human blood plasma.

The decrease in the rate of sorption can be attributed to the unavailability of free binding sites for Pb (II) ions uptake. Such rapid biosorption process has been correlated with the characteristics of the biomass, and its physico-chemical interactions with the metal ion. The rapid metal sorption is also highly desirable for successful deployment of the biosorbents for practical applications, (Volesky *et al.,* 1990).

5.3.5 Langmuir Isotherm for biosorption of Pb (II) ions

Figure 4.20 represents Langmuir isotherm Pb (II) ions biosorption by plotting $\frac{C_e}{q_e}$ againstC_e while Table 4.6 presents the correlation parameter of Langmuir isotherm for Pb (II) ion biosorption. Q_{max} (-1.290 mgg^{-1}) and K_L (-11.570 Lm^{-1}) are the biosorption capacity and adsorption affinity for binding Pb (II) ions in solution respectively. The result implies that this model is not suitable to explain the removal of Pb (II) ions, since these constants are indicative of the surface binding energy and monolayer coverage (Kiurski *et al.,* 2003). R_L (-1.73×10^{-3}) value indicates that removal of Pb (II) ions by biosorbent was not favourable, the correlation coefficient, R^2 (0.485) value indicates that the experimental data obtained did not fit into Langmuir adsorption isotherm (Elaigwu *et al.,* 2009).

5.3.6 Freundlich isotherm for biosorption of Pb (II) ions

Figure 4.21 represents Freundlich isotherm for Pb (II) ions biosorption by plotting Inq_e against InC_e while Table 4.6 presents the Freundlich isotherm constants and correlation coefficient of Pb (II) ion biosorption. An n value of 0.403 suggests that the removal of Pb (II) ions was not favourable since 1/n >1 with K_f (3,169.27 Lmg^{-1}) depicting large amount of Pb (II) ions adsorbed per site and correlation coefficient (R^2) value of 0.800 suggest that the experiment data obtained fit into Freundlich adsorption isotherm (Gupta and Rastogi, 2008).

5.3.7 Temkin Isotherm for biosorption of Pb (II) ions

Figure 4.22 shows the Temkin isotherm for Pb (II) ions biosorption by plotting as q_e against InC_e while Table 4.6 presents the correlation parameter of Temkin model for Pb (II) ions biosorption. A_T (1.340 Lg^{-1}) value indicated low binding energy between Pb (II) ion and the biosorbent, b (48.4625) value suggest a higher heat of biosorption for Pb (II) ions sorption and correlation coefficient, R^2 (0.901) value indicates that the experimental data obtained for of Pb (II) ion biosorption fits into Temkin isotherm (Choy *et al.*, 1999).

5.3.8 Lagergren Pseudo first order model for biosorption of Pb (II) ions.

Figure 4.29 represents Pseudo first order kinetics for Pb (II) ions biosorption by plotting $log\ (q_e - q_t)$ against t, while the Pseudo first order correlation parameters for Pb (II) ions biosorption are presented in Table 4.9. The qe (1,686.55 mg/g) value is the amount of Pb (II) ion adsorbed per biosorbent; this suggests that significant amount of Pb (II) ion could be detoxified within a short time. Correlation coefficient, R^2 (0.002) value indicates that the removal of Pb (II) ion by biosorbent did not fits into pseudo first order model (Febrianto *et al.*, 2009).

5.3.9 Lagergren Pseudo Second order model for biosorption of Pb (II) ions.

Figure 4.30 represents Pseudo second order kinetics for Pb (II) ions biosorption by plotting $\frac{t}{q_t}$ against t while the Pseudo second order correlation parameters for Pb (II) ions biosorption are presented in Table 4.9. The qe (0.1665 mgg^{-1}) value indicates that large amount of Pb (II) ion was removed by biosorbent within limited time. Correlation coefficient, R^2 (1.00) suggests that the biosorption of Pb (II) ion by *allium cepa* fits into pseudo second order model (Mahamadi *et al.*, 2007).

5.3.10 Intra-particle diffusion model for Pb (II) ion biosorption

Figure 4.31 is an intra-particle diffusion experimental data of a plot of qt against $t^{0.5}$ for Pb (II) ion sorption, while the rate constants and correlation coefficient values of intra-particle diffusion model for Pb (II) ion adsorption onto *Allium cepa* are presented in Table 4.9. For investigated Pb (II) ions sorption by *allium cepa* biomass, the plots *Qt versus* $t^{1/2}$ did not pass through the origin suggesting that even though the adsorption process involved intra-particle diffusion, it was not the only rate-controlling step (Yakout and Elsherif, 2010), therefore the K_{id} value for the plot showed that intra-particle diffusion rate constant is 0. The correlation coefficient R^2 (0.919) indicates the experimental data fits into intra-particle diffusion model, the positive value of intercept C_{id} (1.663) is indicative of some degree of boundary layer control (Ozcan and Ozcan, 2004).

5.4 Optimum Parameters for biosorption of Zn (II) ions

5.4.1 Optimum pH for biosorption of Zn (II) ions

Figure 4.13 is a graphical illustration of the optimised pH, by plotting % removal of Zn (II) ions against various pH of the mixture. At pH 2, a percentage sorption of 97.84 % was observed. Further increase in pH, the maximum percentage sorption of Zn (II) ions occurred at pH 6 (Optimum pH) at percentage sorption of 97.91 %. Thereafter increase in pH result to a decrease in percentage sorption from 97.91% - 97.772 % as the pH of the mixture was varied from pH 6 - 10. This is probably due to, higher pH significantly reduces the solubility of metals and metal hydroxides are formed, which collide and thus impede biosorption, (Pagnanelli *et al*., 2003), For most metals are absorbed as the optimum pH range 3 – 6 (Ahalya *et al*., 2003; Naja *et al*., 2007; Kratochvil *et al*., 1998). The impact of pH on sorption of Zn (II) in the range of 2 to 10, no significant variation (97.84 % - 97.772

%) was observed in the sorption capacity. Therefore pH has no significant influence on the ionic state of the active sites and ligands involved in the sorption of Cu (II) ions by *Allium cepa* biomss. The optimum pH for Zn sorption was found to be pH 6. Similar result was reported by Mesfin, (2009).

5.4.2 Optimum concentration for biosorption of Zn (II) ions

Figure 4.14 represents the optimised initial Zn (II) ions concentration, by plotting % removal of Zn (II) ion against various concentrations of Zn (II) ions**.** It was observed that the uptake capacity of Zn (II) ions increased from 94.73 % - 97.814 % when the initial metal concentration was varied from 20 mgL^{-1} – 50 mgL^{-1} Zn (II) ions, this is may be due to the availability of binding cite on the biosorbent. The maximum sorption of Zn (II) ions by *allium cepa* occurred at 50 mgL^{-1} Zn (II) ions (Optimum concentration) with percentage sorption of 97.814 %. Further increase in Zn (II) ion concentration from 50 mgL^{-1} – 60 mgL^{-1} Zn (II) ions, resulted in a decrease in percentage sorption of Zn (II) ions from 97.814 % - 97.68 %.

This behaviour was attributed to the fact that, initially, all binding sites on the biomass surface were vacant resulting in high metal biosorption at the beginning. Further increase in Zn (II) ions concentration, the biosorption of Zn (II) ions decreased due to few active sites were available on the surface of the biosorbent, (Azza *et al.*, 2012).

5.4.3 Optimum biosorbent dose for biosorption of Zn (II) ions

Figure 4.15 is a graphical illustration of the optimised biosorbent dosage for Zn (II) ions removal, by plotting % removal of Zn (II) ions against various biosorbent dosages**.** The result of biomass concentration study shows that there is an increase in the biosorption percentage from 97.29 - 97.708 % with increase in biomass concentration from 0.20 g -

0.80 g. The maximum sorption of Zn (II) by *allium cepa* occurred at 0.80 g (Optimum biosorbent dose) with percentage sorption of 97.708 %. Further increase in concentration of the biosorbent from 0.80 g – 1.00 g showed a decrease in biosorption percentage from 97.708 - 97.558 %.

This may be due to the fact that some adsorption sites may remain unsaturated during the adsorption process where as the number of sites available for sorption increases by increasing the biosorbent dose, i.e. reduction in metal uptake with increasing biomass dose can be attributed to an insufficiency of metal ions in solution with respect to available binding sites (Fourest & Roux, 1992).

5.4.4 Rate of biosorption of Zn (II) ions

Figure 4.16 represents the study of rate of biosorption of Zn (II) ions, by plotting % removal of Zn (II) ion against various contact time. The curve shows an increase in Zn (II) ions sorption by *Allium cepa* as the contact time was varied from 10 – 70 min. The rate of uptake was initial fast in the first 20 min, and it then slows down with as time increase, Therefore the maximum sorption of Zn (II) by *allium cepa* occurred at 70 min with percentage sorption of 97.8076 %. As shaking continued the percentage sorption began to decrease from 97.8076 % - 97.774 % indicating no significant uptake of Zn (II) ions from solution of plasma.

The decrease in the rate of sorption can be attributed to the unavailability of free binding sites for Zn (II) ion uptake from solution of human plasma by *allium cepa* biomass (Gardea *et al.*, 2000).

5.4.5 Langmuir Isotherm for biosorption of Zn (II) ions

Figure 4.23 represents Langmuir isotherm parameter for Zn (II) ion sorption by plotting $\frac{C_e}{q_e}$ against C_e while Table 4.7 is the correlation parameter of Langmuir isotherm of Zn (II) ion sorption. Q_{max} (0.0996 mgg^{-1}) suggests that some heterogeneity in the surface or pores of biosorbent will play a role in large amount of Zn (II) ion removal. R_L (-5.77×10^{-4}) value indicated that the removal of Zn (II) ion biosorbent was favourable and correlation coefficient (R^2) of 0.278 suggest that the experimental data did not fit into Langmuir adsorption isotherm.

5.4.6 Freundlich isotherm for biosorption Zn (II) ions

Figure 4.24 represents the Freundlich isotherm for Zn (II) ion sorption by plotting Inq_e against InC_e while Table 4.7 shows the Freundlich isotherm constants and correlation coefficient of Zn (II) ions sorption. The n (0.383) value suggests that the removal of Zn (II) ions was not favourable since $n < 1$, were $1/n = 2.611$ indicates unfavourable biosorption with K_f value of 6.6374 depicting the amount of Pb (II) ions adsorbed per site and correlation coefficient, R^2 (0.484) value indicated that sorption data didn't fit into Freundlich isotherm (Reddy *et al*. 2012).

5.4.7 Tempkin Isotherm for biosorption of Zn (II) ions

Figure 4.25 represents the Temkin isotherm for Zn (II) ion sorption by plotting q_e against InC_e while Table 4.7 shows the correlation parameter of Temkin model of Zn (II) ion sorption. A_T (48.910 L/g) value indicated high binding energy, b (262.73) value indicated high heat of biosorption and R^2 (0.620) value suggest that sorption data fit into Tempkin isotherm due to good correlation coefficient, R^2 (Wang and Qin, 2005).

5.4.8 Lagergren Pseudo first order model for biosorption of Zn (II) ions

Figure 4.30 represents Pseudo first order kinetics for Zn (II) ions biosorption by plotting $log\ (q_e - q_t)$ against t, while the Pseudo first order correlation parameters for Zn (II) ions biosorption are presented in Table 4.10. The qe (21.5278 mg/g) value indicated that significant amount of Zn (II) ions were adsorbed within a limited time K_1 (-0.03224 L/min) and the correlation of determination R^2 (0.232) suggested that removal of Zn (II) ions by biosorbent did not fits into pseudo first order model due to poor correlation coefficient (Febrianto *et al.*, 2009).

5.4.9 Lagergren Pseudo Second order model for biosorption of Zn (II) ions.

Figure 4.31 represents Pseudo second order kinetics for Zn (II) ions sorption by plotting $\frac{t}{q_t}$ against t while the Pseudo second order correlation parameters for Zn (II) ions biosorption are presented in Table 4.10. The qe (2.002 mg/g) value indicated that significant amount of Zn (II) ions was adsorbed (Igwe *et al.,* 2007). R^2 (0.9998) value signify that the removal of Zn (II) ions fits into pseudo second order model due to good correlation coefficient.

5.4.10 Intra-particle diffusion model for Zn (II) ion biosorption

Figure 4.34 is an intra-particle diffusion experimental data of a plot of qt against $t^{0.5}$ for Zn (II) ion sorption, while the rate constants and correlation coefficient values of Intra-particle diffusion model for Zn (II) ion adsorption onto *Allium cepa* are presented in Table 4.10. For investigated Zn (II) ions sorption by *allium cepa* biomass, the plots *Qt versus* $t^{1/2}$ did not pass through the origin suggesting that even though the adsorption process involved intra-particle diffusion, it was not the only rate-controlling step (Yakout and Elsherif, 2010), therefore the K_{id} value for the plot showed that intra-particle diffusion rate constant is 0.001. The correlation coefficient (R^2) for the intra-particle diffusion model is 0.870 suggests that the experimental data fit into the intra-particle diffusion kinetic model. The positive value of intercept C_{id} (1.21) is indicative of some degree of boundary layer control (Ozcan and Ozcan, 2004).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The FTIR analysis of native and Cu^{2+}, Pb^{2+} and Zn^{2+} treated *Allium cepa* biomass showed a shift in adsorption peaks of the metal ions treated biosorbent. The FTIR spectra showed absorbance peaks of amide, amine, alcohol, alkane, alkyl, carboxyl and Sulphonyl functional group. Therefore, these functional groups are responsible for the biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} from human blood plasma.

SEM analysis of Cu^{2+}, Pb^{2+} and Zn^{2+} ions treated *Allium cepa* biomass showed that the surface morphology of the native *Allium cepa* biomass had undergone remarkable physical disintegration resulting to emergence of protrusions and rough surface area. This is due to the high surface interactions between the binding sites on the surface of the *Allium cepa* biomass.

Optimisation of biosorption parameters showed uptake of Cu^{2+}, Pb^{2+} and Zn^{2+} by *Allium cepa* biomass increases with increasing initial metal ion concentration and increasing biosorbent dosage. The uptake of Cu^{2+}, Pb^{2+} and Zn^{2+} ions increased in contact time till equilibrium is achieved. The uptake of Cu^{2+}, Pb^{2+} and Zn^{2+} shows a negligible increased with increase in pH from 2 – 6. The maximum adsorption capacities of Cu^{2+}, Pb^{2+} and Zn^{2+} were determined to be 99.316 %, 99.8914 % and 97.8076 % respectively at optimum pH 4, pH 6 and pH 6 respectively, Optimum metal ion concentration of 50 mgL^{-1}, optimum *Allium cepa* dose of 0.60 g, 0.40 g and 0.80 g respectively, and highest sorption rate at 80 min, 90 min and 70 min respectively. The order of biosorption of these heavy metal ions are Pb> Cu> Zn.

Langmuir, Freundlich and Temkin isotherm models were used for the mathematical description of biosorption of the Cu^{2+}, Pb^{2+} and Zn^{2+} on to *Allium cepa* biomass and it was found that the adsorption equilibrium data of Cu^{2+}, Pb^{2+} and Zn^{2+} fitted well to the Temkin model. The biosorption processes of Cu^{2+}, Pb^{2+} and Zn^{2+} followed pseudo second order model indicating the chemisorption ccured during the overall biosorption process. The experimental data for Cu^{2+}, Pb^{2+} and Zn^{2+} sorption fitted well into Intra-particle diffusion where it proved not to be the only rate controlling step (Yakout and Elsherif, 2010).

6.2 Conclusion

As revealed by FTIR spectra analysis amide, amine, alcohol, alkane, alkene, alkyl, carboxyl and Sulphonyl functional groups are responsible for the biosorption of the Cu^{2+}, Pb^{2+} and Zn^{2+} from the human blood plasma. The SEM image of native and Cu^{2+}, Pb^{2+} and Zn^{2+} treated *Allium cepa* biomass showed remarkable physical disintegration of the surface morphology of the biosorbent which is evidence of biosorption took place. The maximum adsorption capacities of Cu^{2+}, Pb^{2+} and Zn^{2+} were determined to be 99.316 %, 99.8914 % and 97.8076 % respectively at optimum experimental conditions. The isotherm study of Cu (II), Pb (II) and Zn (II) ions onto *Allium cepa* biomass showed that the biosorption process is not monolayer sorption process. The study of the biosorption kinetics of Cu^{2+}, Pb^{2+} and Zn^{2+} sorption showed that chemisorption occurred during the overall biosorption process, whereas intra-particle diffusion proved not to be the only rate controlling step. With advantage of high metal biosorption capacity, the biomass of *Allium cepa* biomass has the potential to be used as an efficient and economic biosorbent material for the detoxification of Cu^{2+}, Pb^{2+} and Zn^{2+} from the human blood plasma.

6.3 Recommendations

It is now proven without doubt that *Allium cepa* biomass has the potential to remove heavy metal ions from human blood plasma. Bearing this in mind, the following recommendations are necessary:

- Further research should be done on plant extract of this biomass to determine its potential to remove these heavy metal ions from other blood component in the human system since it has been reported that biosorption is metabolism dependent.
- Research should be carried out on the potential of this *Allium cepa* biomass to absorb other metals not studied in this research and the ability or potential of the chemically modified form of the *Allium cepa* biomass to remove the heavy metals from human blood plasma.
- Application to column analysis of the *Allium cepa* biomass may also be explored as this will give a different dimension as compared to that of batch analysis and using

ICP-MS as the method of analysis as it may give a different result as compared to the AAS used for this study.

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Appendix Ia

List of apparatus and equipment

Conical flask- 50cm^3, 250 cm^3

Beaker -50 cm^3, 100 cm^3, 250 cm^3

Volumetric flask-1000 cm^3

Measuring cylinder- 100 cm^3, 10 cm^3, 5 cm^3

Plastic bottles for digest – 50 cm^3

Polythene bags

Wash bottle

Funnel

Filter paper- whatman no. 42 grades

Mortar and pestle

Nose guard

Eye goggle

Hot plates

Analytical balance

Centrifuge tube (50 cm^3)

Fume cupboard

Centrifuge

Class rod

Pipette- 5cm^3, 10cm^3

2mm sieve

Spatula

Appendix Ib

List of Reagents and Chemicals (Analar Grade)

- $Cu(SO)_4.7H_2O$ – Copper tetraoxosulphate (IV) heptahydrate
- $Pb(NO_3)_2$ – Lead nitrate
- $Zn(NO_3)_2$ – Zinc nitrate
- HNO_3 – Nitric acid
- H_2O_2 – Hydrogen peroxide

- NaOH – Sodium hydroxide
- HNO_3 – Nitric acid

Appendix II

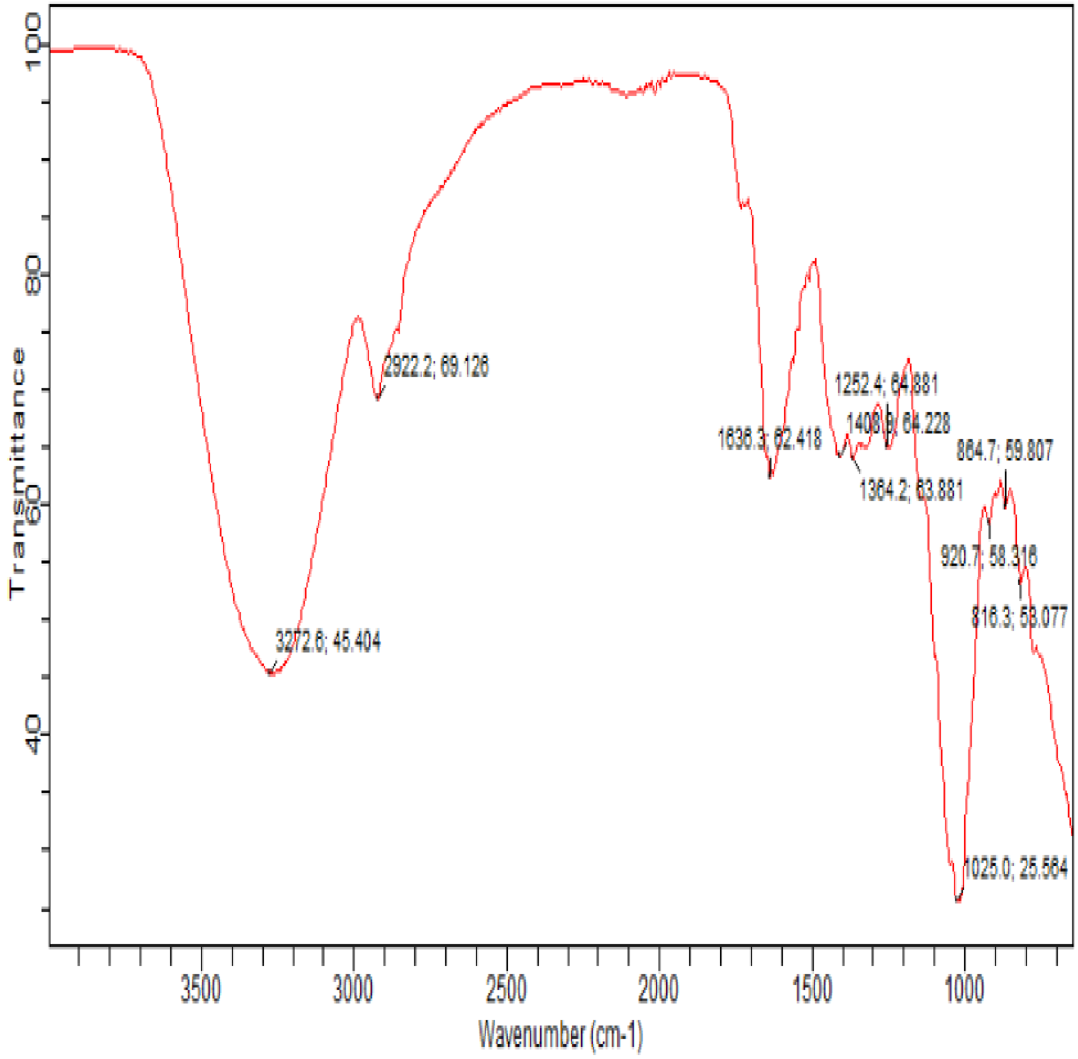
Atomic Adsorption Spectroscopy (AAS) Analysis of Samples

Metal ions	Samples	
	Allium cepa	**Human Blood Plasma**

Copper	**0.0002 mgL^{-1}**	**0.0000 mgL^{-1}**
Lead	**0.0000 mgL^{-1}**	**0.0000 mgL^{-1}**
Zinc	**0.0003 mgL^{-1}**	**0.0000 mgL^{-1}**

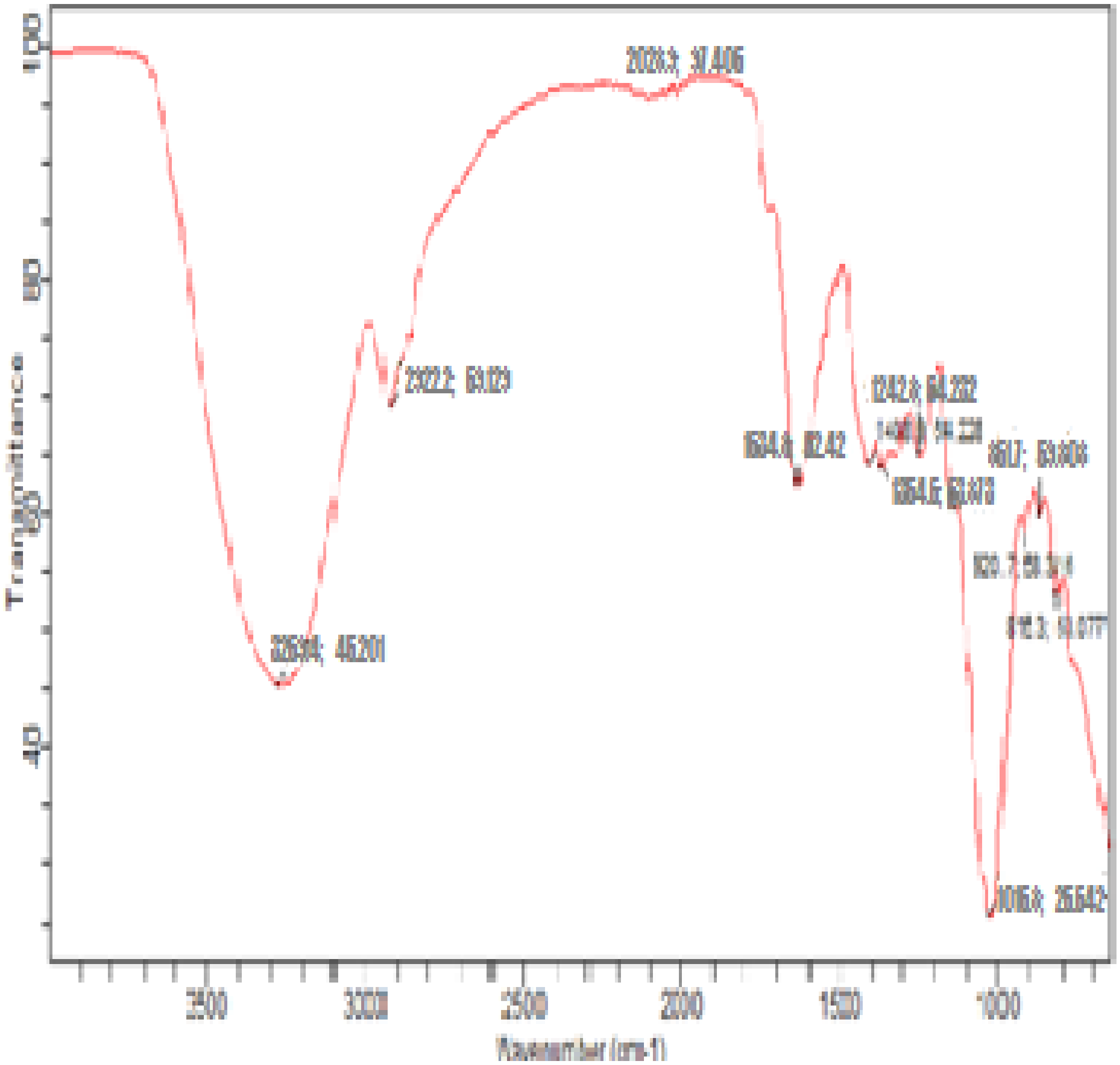
Appendix III

Fourier Transform Infrared Spectra (FTIR) analysis of dried *Allium cepa* biomass


Transmittance
100
80
60
40
2922.2; 69.126
3272.6; 45.404
1636.3; 62.418
1252.4; 64.881
1408.8; 64.228
1364.2; 63.881
864.7; 59.807
920.7; 58.316
816.3; 53.077
1025.0; 25.564
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

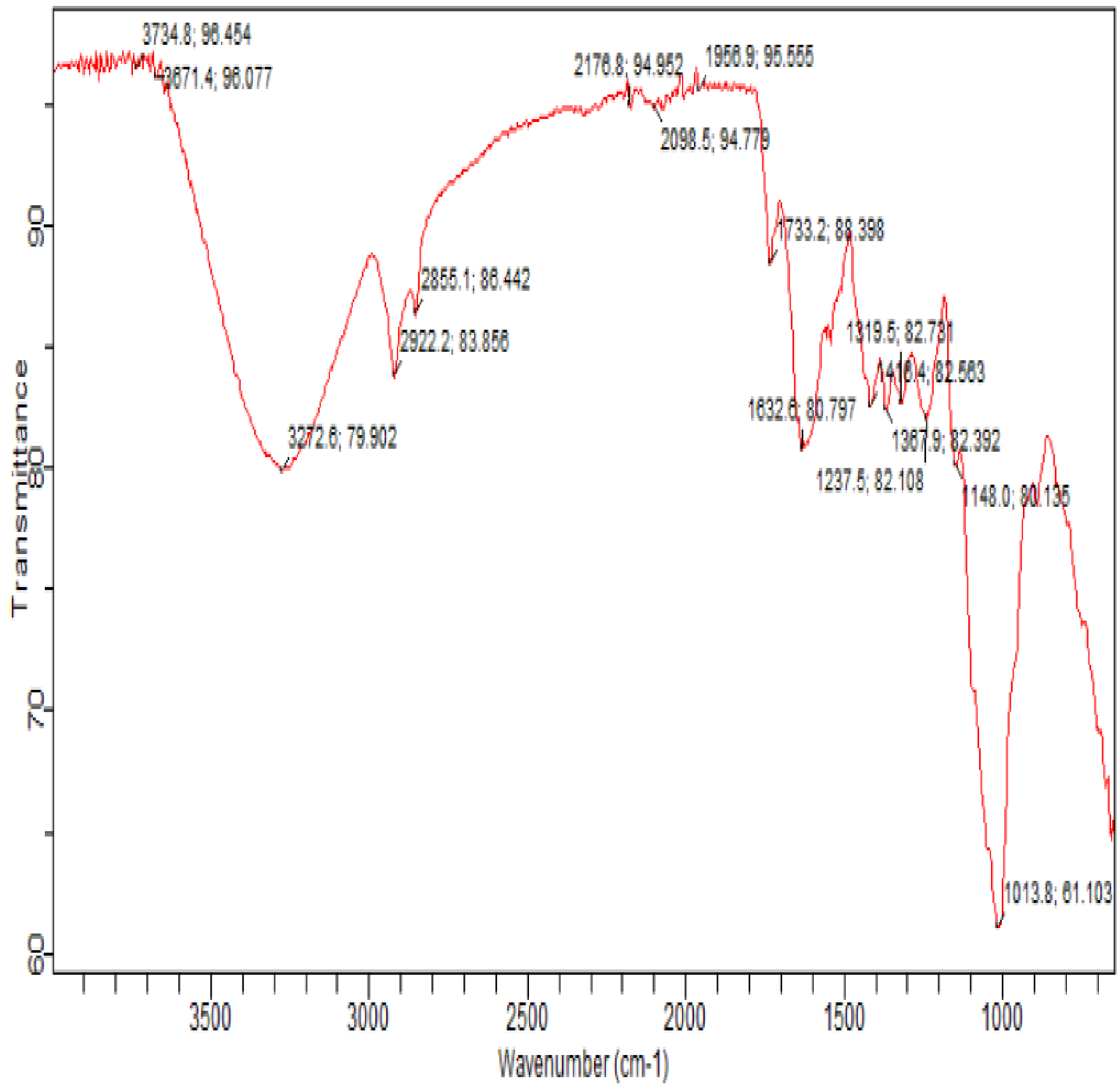
Appendix IV

Fourier Transform Infrared Spectra (FTIR) analysis of Cu^{2+} treated *Allium cepa* biomass



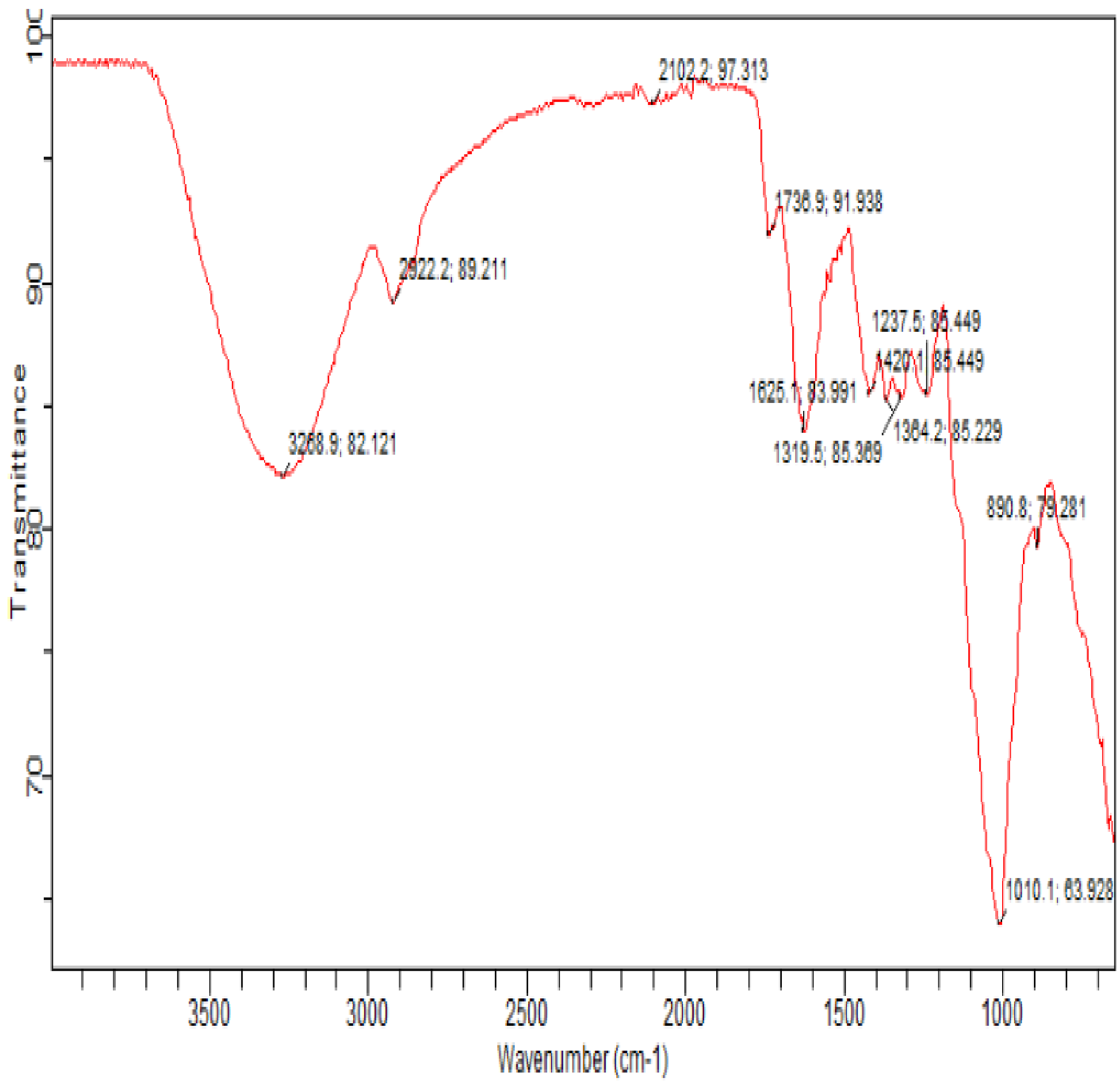
Appendix V

Fourier Transform Infrared Spectra (FTIR) analysis of Pb^{2+} treated *Allium cepa* biomass



Appendix VI

Fourier Transform Infrared Spectra (FTIR) analysis of Zn^{2+} treated *Allium cepa* biomass



Appendix VII

Optimum pH for removal of Cu (II) ions from solution of human blood plasma by *Allium cepa*

Initial conc. of Cu (mg/L)	**Residual conc of Cu (mg/L)**	**Volume of human plasma (L)**	**Mass of *Allium cepa* (g)**	**pH**	**Qe (mgg^{-1})**	**% Removal**
50	0.401	0.02	0.4	2	1.6533	99.198
50	0.352	0.02	0.4	4	1.65493	99.296
50	0.327	0.02	0.4	6	1.65576	99.346
50	0.354	0.02	0.4	8	1.65486	99.292
50	0.362	0.02	0.4	10	1.6546	99.276

Appendix VIII

Optimum Concentration for Removal of Cu (II) Ions from Aqueous Solution by *Allium Cepa*

Initial conc. of Cu (II) ion (mg/L)	Residual conc of Cu (II) ion (mg/L)	Volume of human plasma (L)	Mass of *Allium cepa.* (g)	Qe (mgg^{-1})	% Removal
20	0.399	0.02	0.40	0.653367	98.005
30	0.355	0.02	0.40	0.988167	98.81667
40	0.377	0.02	0.40	1.320767	99.0575
50	0.406	0.02	0.40	1.653133	99.188
60	0.5	0.02	0.40	1.983333	99.16667

Appendix IX

Optimum *Allium cepa* dose for removal of Cu (II) ions from solution of human blood plasma by *Allium cepa*

Initial conc. of Cu(II) ion (mg/L)	**Residual conc Cu (II) ion (mg/L)**	**Volume human plasma (L)**	**Mass of *Allium cepa* (g)**	**Qe (mgg^{-1})**	**% removal**
50	0.438	0.02	0.20	4.9562	99.124
50	0.421	0.02	0.40	2.4789	99.158
50	0.330	0.02	0.60	1.6557	99.34
50	0.404	0.02	0.80	1.2399	99.192
50	0.449	0.02	1.00	0.9910	99.102

Appendix X

Effect of time on removal of Cu (II) ions from solution of human blood plasma by *allium cepa*

Initial conc. of Cu (mg/L)	Residual conc of Cu (mg/L)	Volume of human plasma (L)	Mass of *Allium cepa* (g)	t (min)	Qe (mgg^{-1})	% Removal
50	0.451	0.02	0.6	10	1.6516	99.098
50	0.436	0.02	0.6	20	1.6521	99.128
50	0.423	0.02	0.6	30	1.6526	99.154
50	0.401	0.02	0.6	40	1.6533	99.198
50	0.39	0.02	0.6	50	1.6536	99.22
50	0.386	0.02	0.6	60	1.6538	99.26
50	0.362	0.02	0.6	70	1.6546	99.276
50	0.342	0.02	0.6	80	1.6553	99.316
50	0.342	0.02	0.6	90	1.6553	99.316
50	0.342	0.02	0.6	100	1.6553	99.316
50	0.343	0.02	0.6	110	1.6552	99.314

50	0.345	0.02	0.6	120	1.6552	99.31

Appendix XI

Optimum pH for removal of Pb (II) ions from solution of human blood plasma by *Allium cepa*

Initial conc. of Pb (mg/L)	**Residual conc of Pb (mg/L)**	**Volume of human plasma (L)**	**Mass of *Allium cepa* (g)**	**pH**	**Qe (mgg-^{1})**	**% Removal**
50	0.08	0.02	0.4	2	2.496	99.84
50	0.057	0.02	0.4	4	2.49715	99.886
50	0.0512	0.02	0.4	6	2.49744	99.8976
50	0.0601	0.02	0.4	8	2.496995	99.8798
50	0.0746	0.02	0.4	10	2.49627	99.8508

Appendix XII

Optimum concentration for removal of Pb (II) ions from aqueous solution by *Allium cepa*

Initial conc. of Zn (mg/L)	**Residual conc of Zn (mg/L)**	**Volume of human plasma (L)**	**Mass of *Allium cepa* (g)**	**Qe (mgg^{-1})**	**% Removal**
20	0.043	0.02	0.40	0.99785	99.785
30	0.045	0.02	0.40	1.49775	99.85
40	0.048	0.02	0.40	1.9976	99.88
50	0.052	0.02	0.40	2.4974	99.896
60	0.064	0.02	0.40	2.9968	99.89333

Appendix XIII

Optimum *Allium cepa* dose for removal of Pb (II) ions from solution of human blood plasma by *Allium cepa*

Initial conc. of Pb (II) ion (mg/L)	Residual conc. of Pb (II) ion (mg/L)	Volume of human plasma (L)	Mass of *Allium cepa* (g)	Qe (mgg^{-1})	% Removal
50	0.076	0.02	0.20	4.9924	99.848
50	0.055	0.02	0.40	2.49725	99.89
50	0.062	0.02	0.60	1.6646	99.876

50	0.073	0.02	0.80	1.248175	99.854
50	0.079	0.02	1.00	0.99842	99.842

Appendix XIV

Optimum *Allium cepa* dose for removal of Pb (II) ions from solution of human blood

Initial conc. of Pb (mg/L)	**Residual conc. of Pb (mg/L)**	**Volume of human plasma (L)**	**Mass of *Allium cepa* (g)**	**t (min)**	**Qe (mgg^{-1})**	**% Removal**
50	0.080	0.02	0.4	10	1.6640	99.84
50	0.074	0.02	0.4	20	1.6642	99.852
50	0.071	0.02	0.4	30	1.6643	99.858
50	0.067	0.02	0.4	40	1.6644	99.866
50	0.063	0.02	0.4	50	1.6646	99.874
50	0.0587	0.02	0.4	60	1.6647	99.8826
50	0.0567	0.02	0.4	70	1.6648	99.8866

50	0.0563	0.02	0.4	80	1.6647	99.8874
50	0.0543	0.02	0.4	90	1.6649	99.8914
50	0.0543	0.02	0.4	100	1.6649	99.8914
50	0.0552	0.02	0.4	110	1.6648	99.8896
50	0.0561	0.02	0.4	120	1.6648	99.8878

Appendix XV

Optimum pH for removal of Zinc ions from solution of human blood plasma by *allium cepa* controlled at 37 ^{o}C

Initial conc. of Pb (mg/L)	Residual conc of Pb (mg/L)	Volume of human plasma (L)	Mass of *Allium cepa.* (g)	pH	Qe (mgg-^{1})	% Removal
50	1.08	0.02	0.4	2	1.630667	97.84
50	1.067	0.02	0.4	4	1.6311	97.866

50	1.045	0.02	0.4	6	1.631833	97.91
50	1.0781	0.02	0.4	8	1.63073	97.8438
50	1.114	0.02	0.4	10	1.629533	97.772

Appendix XVI

Optimum concentration for removal of Zinc (II) ions from human blood plasma by *allium cepa*

Initial conc. of Zn (mg/L)	**Residual conc of Zn (mg/L)**	**Volume of human plasma (L)**	**Mass of *Allium cep.* (g)**	**Qe (mgg^{-1})**	**% Removal**
20	1.054	0.02	0.40	4.7365	94.73
30	1.044	0.02	0.40	7.239	96.52

40	1.075	0.02	0.40	9.73125	97.3125
50	1.093	0.02	0.40	12.22675	97.814
60	1.387	0.02	0.40	14.65325	97.68833

Appendix XVII

Optimum *Allium cepa* dose for removal of Zn (II) ions from solution of human blood plasma by *allium cepa*

Initial conc. of Zn (II) ion (mg/L)	Residual conc of Zn (II) ion (mg/L)	Volume of human plasma (L)	Mass of *Allium cepa* (g)	Qe (mgg^{-1})	% Removal
50	1.355	0.02	0.20	4.8645	97.29

50	1.239	0.02	0.40	2.43805	97.522
50	1.234	0.02	0.60	1.625533	97.532
50	1.146	0.02	0.80	1.22135	97.708
50	1.221	0.02	1.00	0.97558	97.558

Appendix XVIII

Optimum *Allium cepa* dose for removal of Zn (II) ions from solution of human blood plasma

Initial conc. of Zn (mg/L)	**Residual conc. of Zn (mg/L)**	**Volume of human plasma (L)**	**Mass of *Allium cepa* (g)**	**t (min)**	**Qe (mgg^{-1})**	**% Removal**
50	1.501	0.02	0.8	10	1.212475	96.998
50	1.336	0.02	0.8	20	1.2166	97.328

50	1.323	0.02	0.8	30	1.216925	97.354
50	1.301	0.02	0.8	40	1.217475	97.398
50	1.239	0.02	0.8	50	1.219025	97.522
50	1.1386	0.02	0.8	60	1.221535	97.7228
50	1.0962	0.02	0.8	70	1.222595	97.8076
50	1.0962	0.02	0.8	80	1.222595	97.8076
50	1.097	0.02	0.8	90	1.222575	97.806
50	1.101	0.02	0.8	100	1.222475	97.798
50	1.107	0.02	0.8	110	1.222325	97.786
50	1.113	0.02	0.8	120	1.222175	97.774

Appendix XIX

Langmuir isotherm for adsorption of Cu (II) ion unto *Allium Cepa*

Qe	**Ce**	**Ce/qe**

0.653367	0.399	1.63751
0.988167	0.355	2.783568
1.320767	0.377	3.50336
1.653133	0.406	4.071757
1.983333	0.5	3.966667

Appendix XX

Freundlich Isotherm for adsorption of Cu (II) ion onto *Allium cepa*

Ce	Qe	Log qe	Log Ce

0.399	0.653367	-0.18484	-0.39903
0.355	0.988167	-0.00517	-0.44977
0.377	1.320767	0.120826	-0.42366
0.406	1.653133	0.218308	-0.39147
0.5	1.983333	0.297396	-0.30103

Appendix XXI

Tempkin Isotherm for adsorption of Cu (II) ion onto *Allium cepa*

Qe	Ce	In Ce

0.653367	0.399	-0.91879
0.988167	0.355	-1.03564
1.320767	0.377	-0.97551
1.653133	0.406	-0.9014
1.983333	0.5	-0.69315

Appendix XXII

Langmuir isotherm for adsorption of Pb (II) ion unto *Allium Cepa*

Qe	Ce	ce/qe

0.99785	0.043	0.043093
1.49775	0.045	0.030045
1.9976	0.048	0.024029
2.4974	0.052	0.020822
2.9968	0.064	0.021356

Appendix XXIII

Freundlich Isotherm for adsorption of Pb (II) ion onto *Allium cepa*

Ce	Qe	log ce	log qe
0.043	0.99785	-1.36653	-0.00093
0.045	1.49775	-1.34679	0.175439
0.048	1.9976	-1.31876	0.300509
0.052	2.4974	-1.284	0.397488
0.064	2.9968	-1.19382	0.476658

Appendix XXIV

Langmuir isotherm for adsorption of Zn (II) ion unto *Allium Cepa*

Qe	Ce	Ce/qe
4.7365	1.054	4.493833
7.239	1.044	6.933908
9.73125	1.075	9.052326
12.22675	1.093	11.18641
14.65325	1.387	10.56471

Appendix XXV

Freundlich Isotherm for adsorption of Zn (II) ion onto *Allium cepa*

Ce	Qe	logCe	LogQe
1.054	4.7365	0.022841	0.675458
1.044	7.239	0.0187	0.859679
1.075	9.73125	0.031408	0.988169
1.093	12.22675	0.03862	1.087311
1.387	14.65325	0.142076	1.165934

Appendix XXVI

Tempkin Isotherm for adsorption of Pb (II) ion onto *Allium cepa*

Qe	Ce	In Ce
0.99785	0.043	-3.14656
1.49775	0.045	-3.10109
1.9976	0.048	-3.03655
2.4974	0.052	-2.95651
2.9968	0.064	-2.74887

Appendix XXVII

Tempkin Isotherm for adsorption of Zn (II) ion onto *Allium cepa*

Qe	Ce	In Ce
4.7365	1.054	0.052592
7.239	1.044	0.043059
9.73125	1.075	0.072321
12.22675	1.093	0.088926
14.65325	1.387	0.327143

Appendix XXVIII

Pseudo First Order Kinetics for biosorption of Cu (II) ion onto the biosorbent

Qe	Qt	qe-qt	t (min)	log(qe-qt)
1.6516	1.655267	-0.00363	10	2.440093
1.6521	1.655267	-0.00313	20	2.504456
1.6526	1.655267	-0.0027	30	2.568636
1.6533	1.655267	-0.00197	40	2.705534
1.6537	1.655267	-0.0016	50	2.79588
1.6538	1.655267	-0.00147	60	2.832683
1.6546	1.655267	-0.00067	70	3.481486
1.6553	1.655267	-3.3E-07	80	0
1.6553	1.655267	-3.3E-07	90	6.481486
1.6553	1.655267	-3.3E-07	100	3.366532
1.6552	1.655267	-3.4E-05	110	2.468521
1.6552	1.655267	-0.0001	120	4

Appendix XXIX

Pseudo Second Order Kinetics for biosorption of Cu (II) ion onto the biosorbent

Qt	T	t/qt
1.655267	10	6.041321
1.655267	20	12.08264
1.655267	30	18.12396
1.655267	40	24.16529
1.655267	50	30.20661
1.655267	60	36.24793
1.655267	70	42.28925
1.655267	80	48.33057
1.655267	90	54.37189
1.655267	100	60.41321
1.655267	110	66.45454
1.655267	120	72.49586

Appendix XXX

Intraparicle diffusion Kinetic model for biosorption of Cu (II) ion onto the biosorbent

t	√t	qt
10	**3.162278**	**1.651633**
20	**4.472136**	**1.652133**
30	**5.477226**	**1.652567**
40	**6.324555**	**1.6533**
50	**7.071068**	**1.653667**
60	**7.745967**	**1.6538**
70	**8.3666**	**1.6546**
80	**8.944272**	**1.655267**
90	**9.486833**	**1.655267**
100	**10**	**1.655267**
110	**10.48809**	**1.655233**
120	**10.95445**	**1.655167**

Appendix XXXI

Pseudo First Order Kinetics for biosorption of Pb (II) ion onto the biosorbent

Qe	Qt	qe-qt	t	log(qe-qt)
1.664	1.664857	-0.00086	10	2.9245
1.6642	1.664857	-0.00066	20	3.1805
1.6643	1.664857	-0.00056	30	3.2518
1.664433	1.664857	-0.00042	40	3.3766
1.664567	1.664857	-0.00029	50	3.5376
1.66471	1.664857	-0.00015	60	3.9208
1.664777	1.664857	-8E-05	70	4.09691
1.66479	1.664857	-6.7E-05	80	4.221849
1.664857	1.664857	-3.3E-07	90	0
1.664857	1.664857	-3.3E-07	100	0
1.664827	1.664857	-3E-05	110	4.522879
1.664797	1.664857	-6E-05	120	4.221849

Appendix XXXII

Pseudo Second Order Kinetics for biosorption of Pb (II) ion onto the biosorbent

Qt	T	t/qt
1.664857	10	6.006522
1.664857	20	12.01304
1.664857	30	18.01957
1.664857	40	24.02609
1.664857	50	30.03261
1.664857	60	36.03913
1.664857	70	42.04565
1.664857	80	48.05218
1.664857	90	54.0587
1.664857	100	60.06522
1.664857	110	66.07174
1.664857	120	72.07826

Appendix XXXIII

Intraparicle diffusion Kinetic model for biosorption of Pb (II) ion onto the biosorbent

t	√t	qt
10	**3.162278**	**1.664**
20	**4.472136**	**1.6642**
30	**5.477226**	**1.6643**
40	**6.324555**	**1.664433**
50	**7.071068**	**1.664567**
60	**7.745967**	**1.66471**
70	**8.3666**	**1.664777**
80	**8.944272**	**1.66479**
90	**9.486833**	**1.664857**
100	**10**	**1.664857**
110	**10.48809**	**1.664827**
120	**10.95445**	**1.664797**

Appendix XXXIV

Pseudo First Order Kinetics for biosorption of Zn (II) ion onto the biosorbent

Qe	Qt	qe-qt	t	log(qe-qt)
1.212475	1.222595	-0.01012	10	0.19948
1.2166	1.222595	-0.006	20	2.2218
1.216925	1.222595	-0.00567	30	2.2464
1.217475	1.222595	-0.00512	40	2.2907
1.219025	1.222595	-0.00357	50	2.4473
1.221535	1.222595	-0.00106	60	2.9747
1.222595	1.222595	0	70	0
1.222595	1.222595	0	80	0
1.222575	1.222595	-2E-05	90	3.69897
1.222475	1.222595	-0.00012	100	3.920819
1.222325	1.222595	-0.00027	110	3.568636
1.222175	1.222595	-0.00042	120	3.376751

Appendix XXXV

Pseudo Second Order Kinetics for biosorption of Zn (II) ion onto the biosorbent

Qt	T	t/qt
1	10	8.179323
1	20	16.35865
1	30	24.53797
1	40	32.71729
1	50	40.89662
1	60	49.07594
1	70	57.25526
1	80	65.43459
1	90	73.61391
1	100	81.79323
1	110	89.97256
1	120	98.15188

Appendix XXXVI

Intraparicle diffusion Kinetic model for biosorption of Zn (II) ion onto the biosorbent

t	$\sqrt{t}$	qt
10	**3.162278**	**1.212475**
20	**4.472136**	**1.2166**
30	**5.477226**	**1.216925**
40	**6.324555**	**1.217475**
50	**7.071068**	**1.219025**
60	**7.745967**	**1.221535**
70	**8.3666**	**1.222595**
80	**8.944272**	**1.222595**
90	**9.486833**	**1.222575**
100	**10**	**1.222475**
110	**10.48809**	**1.222325**
120	**10.95445**	**1.222175**