

**BIOSORPTION OF SELECTED HEAVY METAL IONS FROM TEST
SOLUTIONS USING GREEN ALGAE BIOSORBENT**

BY

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DECLARATION

Declaration by the Candidate

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DEDICATION

To the Almighty God for everything, and my parents, Francis Maraga Ogwora and Christine Obonyo Ogwora for their unwavering support.

ABSTRACT

Human activities such as agricultural and industrial operations have led to the accumulation of pollutants for example, heavy metals in the environment since they do not break down. When this occurs over a long period of time, it can cause both pollution and health risks. Consequently, there is need to remove these heavy metals from environmental matrices. The different conventional ways of removing heavy metals from the environment are expensive and mostly ineffective. Among these methods, biosorption has been proved to be efficient and cheap in heavy metal removal. Most common marine weeds have been found to be potent biosorbents. This study aimed at examining the capability of green algae in the biosorption of heavy metals from waste waters. The green algae were sampled from the University of Eldoret fish ponds, washed, dried and ground using pestle and mortar. Four selected heavy metal test solutions of Ni^{2+} , Zn^{2+} , Cu^{2+} and Cd^{2+} were used in the study. A 5 gram mass of the algae sample powder was transferred into 250 ml beaker containing a 100 mL solution of specific heavy metal at pH values 4, 7 and 9, and stirred for ten minutes. Ten ml samples were then taken from the reacting vessel at time intervals of 10, 20, 30, 40, 50 and 60 minutes and metal levels measured by the atomic absorption spectrophotometer. The results showed that for each of the four selected metals, the levels significantly increased in the biosorbent faster in the first 10 minutes, while the equilibrium reached after 50 minutes. For Cu^{2+} the maximum biosorption was found to occur at pH 9, Zn^{2+} pH 7, Ni^{2+} pH 7, and Cd^{2+} pH 7, while the least biosorption occurred at pH 4 for Cu^{2+} , Zn^{2+} pH 4, Cd^{2+} pH 9 and Ni^{2+} pH 9. The isotherm data fitted Langmuir better than Freundlich from the values of R^2 . The R^2 values for pH 4, 7 and 9 ranges, were found to be tending to one, indicating a strong positive relationship between the equilibrium heavy metal ions concentration (C_e) and the metal uptake (Q_e). Freundlich, the correlation coefficients, R^2 , were found to be less than 0.900, indicating a weak positive relationship between the equilibrium heavy metal ions concentration (C_e) and the metal uptake (Q_e). Pseudo second order kinetics agreed most strongly with the four metals, the correlation coefficient of the metals were calculated and the second order kinetics, R^2 , equaled to almost 1.000, the accepted parameter in biosorption. Thus green algae are effective biosorbents for the removal of Ni^{2+} , Zn^{2+} , Cu^{2+} and Cd^{2+} from aqueous solutions. Further studies should be done for other heavy metals by the different algae species at different pH ranges.

TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii
ABSTRACT	iv
TABLE OF CONTENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF APPENDICES	xi
LIST OF ABBREVIATIONS	xii
ACKNOWLEDGEMENTS	xiii
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background of the Study	1
1.2 Statement of the Problem	3
1.3 Justification of the Study	4
1.4 Objectives	5
1.4.1 General Objective	5
1.4.2 Specific Objectives	5
1.5 Research Questions	6
CHAPTER TWO	7
LITERATURE REVIEW	7
2.1 Introduction	7
2.2 Copper	7

2.2.1 Occurrence	7
2.2.2 Applications	8
2.2.3 Copper in Alloys	8
2.2.4 Effects of Copper	9
2.2.4.1 Poisoning.....	9
2.2.4.2 Biological Role of copper	9
2.3 Zinc	10
2.3.1 Occurrence	10
2.3.2 Application Zinc Metal	11
2.3.3 Negative Effects.....	12
2.3.4 Toxicity	13
2.3.5 Poisoning.....	14
2.3.6 Biological Role of Zinc.....	15
2.4 Cadmium.....	16
2.4.1 Occurrence	16
2.4.2 Applications	16
2.4.3 Poisoning.....	17
2.4.4 Biological Role of cadmium	18
2.5 Nickel.....	18
2.5.1 Occurrence	18
2.5.2 Application.....	19
2.5.3 Biological Role of Nickel	21
2.5.4 Toxicity	21

2.6 Background Information on Green Algae.....	22
2.6.1 Cellular Structure.....	22
2.6.2 Origin of chloroplasts in Green Algae.....	22
2.7 Biosorption.....	23
2.7.1 Environmental Uses.....	24
2.7.2 Recovery of Metals.....	25
2.8 Equilibrium Isotherm Models.....	25
2.9 Order of Reaction.....	27
2.10 Atomic Absorption Spectrophotometer (AAS).....	29
2.11 Related Studies.....	30
CHAPTER THREE.....	36
MATERIALS AND METHODS.....	36
3.1 Sample and Sampling Preparation.....	36
3.1.1 Sample Preparation.....	36
3.1.2 Materials (Equipment).....	36
3.1.3 Reagents Required.....	36
3.2 Methods.....	37
3.2.1 Preparation of Heavy Metals Solutions.....	37
3.3 Optimizing Operating Conditions of AAS.....	38
Table 3.1: Optimized conditions for AAS (Varian Spectra AA).....	38
3.4 Biosorption Experiments.....	38
3.5 Data Analysis.....	39

CHAPTER FOUR.....	40
RESULTS AND DISCUSSION	40
4.1 Effect of pH and Contact Time on Metal Uptake by Green Algae.....	40
4.1.1 Effect of pH.....	40
4.1.2 Effect of Contact Time.....	42
4.1.3 Biosorption at Specific pH Values for Metal Ions.....	42
4.1.4 Optimum pH Value.....	45
4.2 Biosorption Isotherms.....	47
4.2.1 Langmuir Isotherm.....	47
4.2.2 Freundlich Isotherm.....	48
4.3 Adsorption Kinetics	53
CHAPTER FIVE	57
CONCLUSION AND RECOMMENDATIONS	57
5.1 Conclusion	57
5.2 General Recommendations	57
5.2.1 Recommendations for Further Studies.....	57
REFERENCES	58
APPENDICES	70

LIST OF TABLES

Table 3.1: Optimized conditions for AAS (Varian Spectra AA).....	38
Table 4.1: Freundlich and Langmuir parameters at pH 4.....	50
Table 4.2: Freundlich and Langmuir parameters at pH 7.....	50
Table 4. 3: Freundlich and Langmuir parameters at pH 9.....	50
Table 4.4: Pseudo 1 st and 2 nd order rate data for the metal ions at pH 4 for low initial concentration.....	55
Table 4.5: Pseudo 1 st and 2 nd order rate data for the metal ions at pH 4 for high initial concentration.....	55

LIST OF FIGURES

Figure 2.1: A Sketch Diagram of AAS (<i>Varian Spectra AA, USA</i>).....	30
Figure 4.1: Zn ²⁺ biosorbed with time at different fixed pHs	41
Figure 4.2: Cu ²⁺ biosorbed with time at different fixed pHs	41
Figure 4.3: Ni ²⁺ biosorbed with time at different fixed pHs.....	41
Figure 4.4: Cd ²⁺ biosorbed with time at different fixed pHs	41
Figure 4.5: Variation of biosorption of metal ions with time at pH 4	43
Figure 4.6: Variation of biosorption of metal ions with time at pH 7	43
Figure 4.7: Variation of biosorption of metal ions with time at pH 9	44
Figure 4.8: Green algae biosorption for metal ions at different pH values.....	45
Figure 4.9: Langmuir plot for Cu ²⁺ at pH 4	48
Figure 4.10: Freundlich plot for Ni ²⁺ at pH 4	49
Figure 4.11: Pseudo-first order sorption plot for Cu ²⁺ at pH 4.....	54
Figure 4.12: Pseudo-second order sorption plot for Cu ²⁺ at pH 4	54

LIST OF APPENDICES

Appendix I: Langmuir Isotherm	70
Appendix II: Freundlich Isotherm	78
Appendix III: Pseudo first order reaction data.....	87
Appendix IV: Pseudo second order reaction data.....	88
Appendix V: WHO, FAO and KEBS heavy metal maximum permissible limits	89
Appendix VI: Physical properties of elements that were analyzed	90
Appendix VII: Formula for calculation of stock solution (1000 ppm) metal ion concentration.....	91
Appendix VIII: Dilution formula of stock solution	92

LIST OF ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
CdCA	Cd-carbonic anhydrase
DNA	Deoxyribonucleic Acid
FAAS	Flame Atomic Absorption Spectroscopy
FAO	Food and Agriculture Organization
FDA	Food and Drugs Agency
IUPAC	International Union of Pure and Applied Chemistry
KEBS	Kenya Bureau of Standards
PARP	Pol ADP Ribose polymerase
PGE	Platinum Group Elements
RDA	Recommended Dietary Allowance
RNA	Ribonucleic Acid
SEP	Standard Electrode Potential
USDA	United States Department of Agriculture
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Metals are notable for their wide environmental dispersion from industrial waste. Heavy metals have a tendency to accumulate in selected tissues of human body and animals in general. These heavy metals have a high potential being toxic even at relatively minor levels of exposure. The accumulation of heavy metals in the environment over a long period of time can cause both pollution and health risks. Metal poisoning occurs through inhaling the fumes and through ingestion of food contaminated with toxic metals. The heavy metals in soils can be absorbed by plants up to certain levels which are toxic and through ingestion by herbivores or omnivores, metal poisoning takes place. Children are also highly vulnerable to metal poisoning since they interact with toys contaminated with toxic metals (Alkorta *et al.*, 2004). According to the agency for toxic substances and diseases registry based in Atlanta, Georgia, the top four most toxic metals in the environment are lead, mercury, arsenic, and cadmium. Other toxic heavy metals at elevated levels are copper, nickel; zinc, antimony, and selenium. Methods of heavy metal removal from the environment include biosorption, activated sludge process, bio-filter, and anaerobic digestion. Among these methods, biosorption has been proved to be very efficient in heavy metal removal (Pagnanelli *et al.*, 2001).

Biosorption is the process of removing metal ions by means of passive binding to non-living biomass from an aqueous solution. Most common marine weeds have been found to be potent biosorbents (Lewis & McCourt, 2004). This research examined the capability of green algae in the biosorption of heavy metals from waste waters. The green algae are

the large group of algae from which the embryophytes (higher plants) emerged. As such, they form a paraphyletic group, although the group including both green algae and embryophytes is monophyletic.

The green algae include unicellular and colonial flagellates, most with two flagella per cell, as well as various colonial, coccoid, and filamentous forms, and macroscopic seaweeds (Gupta *et al.*, 2001). In the Charales, the closest relatives of higher plants, full differentiation of tissues occurs. There are about 6,000 species of green algae. Many species live most of their lives as single cells, while other species form colonies, coenobia, long filaments, or highly differentiated macroscopic seaweeds (Jeffrey *et al.*, 2004)

A few other organisms rely on green algae to conduct photosynthesis for them. The chloroplasts in euglenids and chlorarachniophytes were acquired from ingested green algae, and in the latter retain a vestigial nucleus (nucleomorph *Valoniopsis pachynema* (Deng *et al.*, 2007). Some species of green algae, particularly of genera *trebouxia* and *pseudotrebouxia* (*trebouxiophyceae*), can be found in symbiotic associations with fungi to form lichens. In general the fungal species that partner in lichens cannot live on their own, while the algal species is often found living in nature without the fungus. *Trentepohlia* is a green alga parasitic on the bark of some trees (Jeffrey *et al.*, 2004).

Microorganisms, including algae, bacteria, yeast and fungi can be used as biosorbents for detoxification and recovery of toxic or valuable metals from industrial discharges. One of the most promising biosorbents is “algae” (Aksu, 2005). In fact, the uses of algae in the sorption of heavy metals were dated back to 1986 when Mehta & Gaur (2005) reported

that there was biosorption of cadmium on the cell of the green microalga *stichococcus bacillaris*. Following this work, there were a number of reports on the sorption of heavy metals by other microorganisms (Lewis & McCourt, 2004).

1.2 Statement of the Problem

Domestic and industrial waters are increasingly choking with heavy metal waste; human waste from informal settlements; industrial wastes in the form of gaseous emissions, liquid effluents and solid waste; agro-chemicals in fish ponds, and other wastes especially petro-chemicals and metals from micro-enterprises – the “Jua-kali”; and over-flowing sewers (Golub, 2005). This situation has occasioned spread of water-borne diseases, loss of sustainable livelihoods, loss of biodiversity, reduced availability and access to safe portable water, and the insidious effects of toxic substances and heavy metal poisoning which affects human productivity (Hayes, 2007). A major concern is the level of heavy metals in these waters. Heavy metals enter these waters when industrial and consumer waste, or even from water run – off from neighbouring mining sites drain directly to the water surfaces. These heavy metals bio - accumulate in the sediments and in the water to dangerous levels which pose precarious effects to human health, aquatic life and even the environment (Golub, 2005). The common polluting heavy metals are Pb, Cd, Cu, Zn, Cr and Hg. This has been hypothesized as the core trigger to the numerous heavy metal poisoning effects noted from the continuous use of boreholes and industrial water (Emsley, 2003). Such effects include memory loss, increased allergic reactions, high blood pressure, depression, mood swings, irritability, poor concentration, aggressive behaviour, sleep disabilities, fatigue, speech disorders, cholesterol, triglycerides, vascular

occlusion, neuropathy, autoimmune diseases, and chronic fatigue are just some of the many conditions resulting from exposure to such toxins (John, 2002).

As endeavours to clean these waters and thus reduce the disparaging effects, various strategies have been put in place to lower the concentration of heavy metals in these waters, sediments and in the aquatic animals which live in these water bodies. This research explored the use of green algae as a possible way of removing these heavy metals from water bodies by biosorption. Biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions. Biomass exhibits this property, acting just as a chemical substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria which is responsible for this phenomenon. This implies that removal of heavy metals from industrial effluents will be done by biological means which are not harmful to the environment. This was in line with the core objective of this study which was to contribute to the search for less expensive adsorbents and their utilization possibilities for various agricultural waste by-products such as sugarcane bagasse, rice husks, oil palm shells, coconut shell, and coconut husks among the many biosorbents for the elimination of heavy metals from wastewater (Romano & Matteucci, 2007).

1.3 Justification of the Study

Wastewaters from industrial effluent burden the water systems with high levels of heavy metals. Hence there is need to remove these heavy metals so that the waste water can be recycled for use for household or irrigation. Almost all industries discharge at least one heavy metal into the environment that is, soil or water. The effluents have been found to

contribute significantly towards the acidification, mineralization and metal contamination of the water bodies. The removal of dissolved minerals from wastewater has been given relatively little attention, because minerals have been considered to be less of a pollution hazard than other constituents, such as organic matter and suspended solids (Das *et al.*, 2008). The injurious effects of heavy metals to humans are well established, for example, excessive amounts of Pb in the human body cause hypertension and brain damage (Jnr & Spiff, 2005). Toxic metals Cd, Zn, Ni and Cu find their way to the water bodies through waste waters (Ahluwalia & Goyal, 2007). Due to their non- biodegradability and persistence, heavy metals can accumulate in the environment such as in food chains and thus pose a significant danger to human health (Cheng, 2003). There is need to develop an efficient and cheap method of removing heavy metals from waste water in-order to alleviate environmental pollution. In this study, green algae were used with the possibility of obtaining a reliable, cheap and efficient way of biosorbing Cu^{2+} , Zn^{2+} , Ni^{2+} and Cd^{2+} from wastewater effluents.

1.4 Objectives

1.4.1 General Objective

The main objective of this research was to investigate the efficiency of green algae in the biosorption of heavy metals from aqueous solution.

1.4.2 Specific Objectives

- i) To evaluate the concentration of Cu^{2+} , Zn^{2+} , Ni^{2+} and Cd^{2+} biosorbed by the green algae using atomic absorption spectroscopy.
- ii) To evaluate the efficiency of green algae in the removal of heavy metals from polluted waters.

- iii) To determine the best isotherm model that fits the data hence the order of reaction.
- iv) To determine factors that affects the rate of biosorption for a given metal ion.

1.5 Research Questions

- i. To what extent can green algae considerably biosorb heavy metals from wastewater?
- ii. To what level can green algae be effective as biosorbent of Cu^{2+} , Zn^{2+} , Ni^{2+} and Cd^{2+} ions from polluted water?
- iii. Which is the best isotherm model fitting the data and what is the order of reaction between heavy metals and green algae?
- iv. To what extent can pH, initial metal ion concentration and contact time affect the rate of biosorption for a given metal ion?

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Biosorption involves a solid phase (sorber, biological material) and a liquid phase (solvent, water) containing a dissolved species to be adsorbed, metal ions. The adsorbate is attracted and bound to the adsorbent by different mechanisms. The biosorption process continues until equilibrium is attained between the amount of solid bound; adsorbate species and its portion remaining in the solution. The extent of the adsorbent affinity for the adsorbate determines its distribution between the solid and the liquid phases. The major advantages of this process over conventional waste treatment methods include; low cost, high efficiency, minimal chemical use and possibility of metal recovery (Voleskey *et al.*, 2003).

2.2 Copper

2.2.1 Occurrence

Copper can be found as either native Cu or as part of minerals. Native Cu is a polycrystal, with the largest single crystals found to date measuring 4.4×3.2×3.2 cm (Platzman *et al.*, 2008). The largest mass of elemental Cu weighed 420 tonnes and was found in 1857 on the Keweenaw Peninsula in Michigan, US. There are many examples of Cu-containing minerals: chalcopyrite and chalcocite are copper (II) sulphides, azurite and malachite are copper (II) Cates and cuprite is a copper (II) oxide. Copper is present in the Earth's crust at a concentration of about 50 ppm, and is also synthesized in massive stars (Romano & Matteucci, 2007).

2.2.2 Applications

The major functions of Cu include electronic devices, architecture that is industrial roofing and plumbing (20%), industrial machinery (15%). Copper is mostly used as a metal, but when a higher hardness is required it is combined with other elements to make an alloy (5% of total use) such as brass and bronze (Davis, 2000a).

A small part of Cu supply is used in production of compounds for nutritional supplements and fungicides in agriculture. Machining of Cu is possible, although it is usually necessary to use an alloy for intricate parts to get good machinability characteristics. An anti-bio fouling application that is Cu has long been used as a biostatic surface to line parts of ships to protect against barnacles and mussels (Davis, 2000b).

2.2.3 Copper in Alloys

Numerous Cu alloys exist, many with important uses. Brass is an alloy of Cu and Zn and bronze usually refers to Cu-Sn alloys, but can refer to any alloy of Cu such as Al-bronze (Davis, 2001). Copper is one of the most important constituents of carat silver and gold alloys and carat solders used in the jewelry industry, modifying the colour, hardness and melting point of the resulting alloys (Süss *et al.*, 2004).

The alloy of Cu and Ni, called cuproni, is used in low-denomination statutory coins, often for the outer cladding. The US 5-cent coin called *Ni* consists of 75% Cu and 25% Ni and has a homogeneous composition. The 90% Cu/10% Ni alloy is remarkable by its resistance to corrosion and is used in various parts being exposed to seawater. Alloys of Cu with Al (about 7%) have a pleasant golden colour and are used in decorations (Ahmed *et al.*, 2006).

2.2.4 Effects of Copper

2.2.4.1 Poisoning

Gram quantities of various Cu salts have been taken in suicide attempts and produced acute Cu toxicity in humans, possibly due to redox cycling and the generation of reactive oxygen species that damage DNA. Corresponding amounts of Cu salts (30 mg/kg) are toxic in animals. A minimum dietary value for healthy growth in rabbits has been reported to be at least 3 ppm in the diet. However, higher concentrations (greater than 3ppm) of Cu in the diet of rabbits have been shown to favourably influence feed conversion efficiency, growth rates, and carcass dressing percentages. Chronic Cu toxicity does not normally occur in humans because of transport systems that regulate absorption and excretion. Autosomal recessive mutations in Cu transport proteins can disable these systems, leading to Wilson's disease with Cu accumulation and cirrhosis of the liver in persons who have inherited two defective genes (Mercer, 2001).

2.2.4.2 Biological Role of copper

Copper is required for the normal functioning of plants, animals and most microorganisms. It is incorporated into a variety of organic materials such as enzyme-cofactors, coenzymes and prosthetic group in which specific metabolic functions are performed. Because it is an essential metal, daily dietary requirements have been recommended by a number of agencies. The American Medical Association has recommended 1.2 – 1.3 mg/day (fortified in foods) as the dietary requirements for Cu (Norbert & Wiley, 2002).

The chemical nature of Cu is very important in determining its biological availability, both in the environment and in food (Wilson, 2011). Although evidence of this continues

to accumulate, the impact of excess Cu is still far too frequently inferred from levels of “total Cu” or even the “presence” of Cu (Gaetke & Chow, 2003). Some of the uses of Cu come from its ability to impare the growth of organisms. This occurs when Cu is biologically available and at concentrations that are detrimental. As a result, Cu is used in a range of cidal agents. For example, Cu has been demonstrated to be an effective antibacterial, antiplaque agent in mouthwashes and toothpastes. Cu also continues to be widely used for the control of unwanted organisms in fish farming (Conway & Pretty, 2013). Evidence in both fresh water and salt water indicates no hazardous effect to consumers of the fish. Cu antifouling agents used on fish net pens have been considered a source of metal to the sediments but there is little evidence that they provide an important source of dissolved Cu when there is adequate water exchange for fish farming (Ahmed *et al.*, 2006).

2.3 Zinc

2.3.1 Occurrence

Zinc makes up about 75 ppm (0.0075%) of the Earth's crust, making it the 24th most abundant element. Soil contains 5–770 ppm of Zn with an average of 64 ppm. Seawater has only 30 ppb Zn and the atmosphere contains 0.1–4 $\mu\text{g}/\text{m}^3$ (Bothwell *et al.*, 2003). The element is normally found in association with other base metals such as Cu and Pb in ores. Zinc is a chalcophile, meaning the element has a low affinity for oxides and prefers to bond with sulphides. Chalcophiles formed as the crust solidified under the reducing conditions of the early Earth's atmosphere (Soetan *et al.*, 2010). Sphalerite, which is a form of zinc (II) sulphide, is the most heavily mined Zn-containing ore because its concentrate contains 60–62% Zn. Other minerals, from which Zn is extracted, include

smithsonite (zinc (II) carbonate), hemimorphite (zinc (II) silicate) and wurtzite (another zinc (II) sulphide). With the exception of wurtzite, all these other minerals were formed as a result of weathering processes on the primordial zinc (II) sulphides (Iqbal & Edyvean, 2004).

Identified world Zn resources total about 1.9 billion tonnes, large deposits are in Australia, Canada and the United States with the largest reserves in Iran. At the current rate of consumption, these reserves are estimated to be depleted sometime between 2027 and 2055. About 346 million tonnes have been extracted throughout history to 2002, and one estimate found that about 109 million tonnes of that remains in use (Bothwell *et al.*, 2003).

2.3.2 Application Zinc Metal

The metal is most commonly used as an anti-corrosion agent. Galvanization, which is the coating of iron or steel to protect the metals against corrosion, is the most familiar form of using Zn in this way. In 2009 in the United States, 55% or 893 thousand tonnes of the Zn metal were used for galvanization according to United States Geological survey (Bounoughaz *et al.*, 2003).

Zinc is more reactive than iron or steel and thus will attract almost all local oxidation until it completely corrodes away (Marder, 2000). A protective surface layer of oxide and carbonate ($Zn_5(OH)_6(CO_3)_2$) forms as the Zn corrodes. This protection lasts even after the Zn layer is scratched but degrades through time as the Zn corrodes away. The Zn is applied electrochemically or as molten Zn by hot-dip galvanizing or spraying. Galvanization is

used on chain-link fencing, guard rails, suspension bridges, light posts, metal roofs, heat exchangers and car bodies (Emsley, 2003).

The relative reactivity of Zn and its ability to attract oxidation to itself makes it an efficient sacrificial anode in cathodic protection. For example, cathodic protection of a buried pipeline can be achieved by connecting anodes made from Zn to the pipe. Zn acts as the anode (negative terminus) by slowly corroding away as it passes electric current to the steel pipeline (Imlay, 2003). Zinc is also used to cathodically protect metals that are exposed to sea water from corrosion. A Zn disc attached to a ship's iron rudder will slowly corrode while the rudder stays unattacked. Other similar uses include a plug of Zn attached to a propeller or the metal protective guard for the keel of the ship (Bounoughaz *et al.*, 2003).

With a standard electrode potential (SEP) of -0.76 volts, Zn is used as an anode material for batteries while reactive lithium (SEP -3.04 V) is used for anodes in lithium batteries. Powdered Zn is used in this way in alkaline batteries and sheets of Zn metal form the cases and act as anodes in Zn-C batteries. Zn is used as the anode or fuel of the Zn-air battery/fuel cell (Cooper *et al.*, 2008).

2.3.3 Negative Effects

Soils contaminated with Zn through the mining of Zn-containing ores, refining, or where Zn-containing sludge is used as fertilizer, can contain several grams of Zn per kilogram of dry soil (Manceau *et al.*, 2008). Levels of Zn in excess of 500 ppm in soil interfere with the ability of plants to absorb other essential metals, such as Fe and Mn. Zn levels of

2000 ppm to 180,000 ppm (18%) have been recorded in some soil samples (Bounoughaz *et al.*, 2003).

2.3.4 Toxicity

Although Zn is an essential requirement for good health, excess Zn can be harmful. Excessive absorption of Zn suppresses Cu and Fe absorption. The free Zn ion in solution is highly toxic to plants, invertebrates, and even vertebrate fish. The free ion activity model is well-established in the literature, and shows that just micromolar amounts of the free ion kills some organisms. A recent example showed 6 micromolar killing 93% of all *Daphnia* in water (Muysen *et al.*, 2006). The free Zn ion is a powerful Lewis acid up to the point of being corrosive. Stomach acid contains hydrochloric acid, in which metallic Zn dissolves readily to give corrosive zinc (II) chloride. Swallowing a post-1982 American one cent piece (97.5% Zn) can cause damage to the stomach lining due to the high solubility of the Zn ion in the acidic stomach (Bothwell *et al.*, 2003).

There is evidence of induced Cu deficiency at low intakes of 100–300 mg Zn/day; a recent trial had higher hospitalizations for urinary complications compared to placebo among elderly men taking 80 mg/day. The USDA RDA is 11 and 8 mg Zn/day for men and women, respectively. Even lower levels, closer to the RDA, may interfere with the utilization of Cu and Fe or adversely affect cholesterol metabolism. Levels of Zn in excess of 500 ppm in soil interfere with the ability of plants to absorb other essential metals, such as Fe and Mn. There is also a condition called the Zn shakes or "Zn chills" that can be induced by the inhalation of freshly formed Zn oxide formed during the welding of galvanized materials (Connie & Christine, 2009).

The U.S. Food and Drug Administration (FDA) reported that Zn damages nerve receptors in the nose, which can cause anosmia. Reports of anosmia were also observed in the 1930s when Zn preparations were used in a failed attempt to prevent polio infections. On June 16, 2009, the FDA reported that consumers should stop using Zn-based intranasal cold products and ordered their removal from store shelves. The FDA reported the loss of smell can be life-threatening because people with impaired smell cannot detect leaking gas or smoke and cannot tell if food has gone bad before they eat it. Recent research suggests that the topical antimicrobial Zn pyrithione ($C_{10}H_8N_2O_2S_2Zn$) is a potent heat shock response inducer that may impair genomic integrity with induction of PARP-dependent energy crisis in cultured human keratinocytes and melanocytes (Lamore *et al.*, 2010).

2.3.5 Poisoning

In 1982, the United States Mint began minting pennies coated in Cu but made primarily of Zn. With the new Zn pennies, there is the potential for Zn toxicosis, which can be fatal. One reported case of chronic ingestion of 425 pennies (over 1 kg of Zn) resulted in death due to gastrointestinal bacterial and fungal sepsis, while another patient, who ingested 12 grams of Zn, only showed lethargy and ataxia (gross lack of coordination of muscle movements). Several other cases have been reported of humans suffering from Zn intoxication by the ingestion of Zn coins. Pennies and other small coins are sometimes ingested by dogs, resulting in the need for medical treatment to remove the foreign body (Bothwell *et al.*, 2003). The Zn content of some coins can cause Zn toxicity, which is commonly fatal in dogs, where it causes a severe hemolytic anaemia, and also liver or kidney damage; vomiting and diarrhoea are possible symptoms. Zn is highly toxic in

parrots and poisoning can often be fatal. The consumption of fruit juices stored in galvanized cans has resulted in mass parrot poisonings with Zn (Connie & Christine, 2009).

2.3.6 Biological Role of Zinc

Zinc is an essential trace element, necessary for plants, animals, and microorganisms. Zinc is found in nearly 100 specific enzymes, serves as structural ions in transcription factors and is stored and transferred in metallothioneins. It is "typically the second most abundant transition metal in organisms" after iron and it is the only metal which appears in all enzyme classes (Broadley *et al.*, 2007). In proteins, Zn ions are often coordinated to the amino acid side chains of aspartic acid, glutamic acid, cysteine and histidine (Brandt *et al.*, 2009).

There are 2–4 grams of Zn distributed throughout the human body. Most Zn is in the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye. Semen is particularly rich in Zn, which is a key factor in prostate gland function and reproductive organ growth (Berdanier *et al.*, 2007). In humans, Zn plays "ubiquitous biological roles". It interacts with "a wide range of organic ligands", and has roles in the metabolism of RNA and DNA, signal transduction, and gene expression. It also regulates apoptosis (Brandt *et al.*, 2009).

In the brain, Zn is stored in specific synaptic vesicles by glutamatergic neurons and can "modulate brain excitability". It plays a key role in synaptic plasticity and so in learning. However, it has been called "the brain's dark horse" since it also can be a neurotoxin,

suggesting Zn homeostasis which plays a critical role in normal functioning of the brain and central nervous system (Berdanier *et al.*, 2007).

2.4 Cadmium

2.4.1 Occurrence

Cadmium makes up about 0.1 ppm of the Earth's crust. Compared with the more abundant Zn with 65 ppm Cd is rare. No significant deposits of Cd-containing ores are known. Greenockite (CdS), the only Cd mineral of importance, is nearly always associated with sphalerite (ZnS). This association is caused by the geochemical similarity between Zn and Cd which makes geological separation unlikely. As a consequence, Cd is produced mainly as a byproduct from mining, smelting, and refining sulphidic ores of Zn and to a lesser degree, Pb and Cu. Small amounts of Cd, about 10% of consumption, are produced from secondary sources, mainly from dust generated by recycling Fe and steel scrap. Production in the United States began in 1907, but it was not until after World War I that Cd came into wide use (Fthenakis, 2004).

Rocks mined to produce phosphate fertilizers contain varying amounts of Cd to a concentration of up to 300 mg/kg in the produced phosphate fertilizers and thus in the high Cd content in agricultural soils. Coal can contain significant amounts of Cd, which ends up mostly in the flue dust (Hayes, 2007).

2.4.2 Applications

Cadmium has got many common industrial uses, it is a key component in battery production plants, and other common uses include electroplating and nuclear fission.

2.4.3 Poisoning

The most dangerous form of occupational exposure to Cd is inhalation of fine dust and fumes, or ingestion of highly soluble Cd compounds. Inhalation of Cd-containing fumes can result initially in metal fume fever but may progress to chemical pneumonitis, pulmonary edema and death (Hayes, 2007). Consumption of Cd contaminated food is associated with developed itai-itai disease and renal abnormalities, including proteinuria and glucosuria (Nogawa *et al.*, 2004).

Although some studies linked exposure to Cd with lung and prostate cancer, there is still a substantial controversy about its carcinogenicity. More recent studies suggest that As rather than Cd may lead to the increased lung cancer mortality rates. Furthermore, most data regarding the carcinogenicity of Cd rely on research confounded by the presence of other carcinogenic substances. Tobacco smoking is the most important single source of Cd exposure in the general population. It has been estimated that about 10% of the Cd content of a cigarette is inhaled through smoking. The absorption of Cd from the lungs is much more effective than that from the gut and as much as 50% of the Cd inhaled via cigarette smoke may be absorbed (Manzoori & Bavili-Tabrizi, 2002).

On average, smokers have 4–5 times higher blood Cd concentrations and 2–3 times higher kidney Cd concentrations than non-smokers. Despite the high Cd content in cigarette smoke, there seems to be little exposure to Cd from passive smoking. No significant effect on blood Cd concentrations has been detected in children exposed to environmental tobacco smoke (Hayes, 2007). Cd exposure is a risk factor associated with early arteriosclerosis and hypertension, which can both lead to cardiovascular disease.

2.4.4 Biological Role of cadmium

Because of its excellent correlation with phosphate, Cd is used as a paleotracer for nutrients. Over the past several years, it has been demonstrated that cadmium is an important micronutrient for marine phytoplankton (Migon, 2005). In particular, it has been discovered and characterized as a Cd-carbonic anhydrase, CdCA, the first and only known Cd metalloenzyme. Carbonic anhydrases are extremely fast enzymes that catalyze the reversible conversion of CO_2 to HCO_3^- . CdCA, along with other novel carbonic anhydrases have been discovered in marine phytoplankton, plays a critical role in the acquisition of inorganic carbon for photosynthesis (Marchitto, 2000). Some organisms that use Cd for growth do not possess CdCA, implying the existence of other Cd metalloenzyme(s). The discovery of (CdCA) establishes a remarkable link between the global cycles of cadmium and carbon in the oceans (Cutter, 2005).

2.5 Nickel

2.5.1 Occurrence

On Earth, Ni occurs most often in combination with S and Fe in pentlandite, with S in millerite, with As in the mineral niine and with As and S in Ni galena. Ni is commonly found in Fe meteorites as the alloys kamacite and taenite. The bulk of the Ni mined comes from two types of ore deposits. The first are laterites where the principal ore minerals are niiferous limonite: $(\text{Fe,Ni})\text{O}(\text{OH})$ and garnierite (a hydrous Ni silicate): $(\text{Ni, Mg})_3\text{Si}_2\text{O}_5(\text{OH})_4$. The second are magmatic sulphide deposits where the principal ore mineral is pentlandite: $(\text{Ni, Fe})_9\text{S}_8$. In terms of supply, the Sudbury region of Ontario, Canada, produces about 30% of the world's supply of Ni (Nestle *et al.*, 2002).

The Sudbury Basin deposit is theorized to have been created by a meteorite impact event early in the geologic history of the Earth. Russia contains about 40% of the world's known resources at the Norilsk deposit in Siberia. The Russian mining company MMC Norilsk Ni obtains the Ni and the associated palladium for world distribution. Other major deposits of Ni are found in New Caledonia, France, Australia, Cuba, and Indonesia. Deposits found in tropical areas typically consist of laterites which are produced by the intense weathering of ultramafic igneous rocks and the resulting secondary concentration of Ni bearing oxide and silicate minerals (Butt & Cluzel, 2013).

Based on geophysical evidence, most of the Ni on Earth is postulated to be concentrated in the Earth's core. Kamacite and taenite are naturally occurring alloys of Fe and Ni. For kamacite the alloy is usually in the proportion of 90:10 to 95:5 although impurities such as Co or C may be present, while for taenite the Ni content is between 20% and 65%. Kamacite and taenite occur in Ni,Fe meteorites (Palmieri, 2006).

2.5.2 Application

The fraction of global Ni production presently used for various applications is as follows: 60% is used for making Ni steels, 14% used in Ni-Cu alloys and Ni-Ag, 9% used to make malleable Ni, Ni clad and other super alloys 6%, (withstanding very high temperatures) used in plating, 3% used for Ni cast Fe, 3% in heat and electric resistance alloys, such as nichrome, 2% used for Ni brasses and bronzes with the remaining 3% of the Ni consumption in all other applications combined (Kuck, 2006). Ni is used in many specific and recognizable industrial and consumer products, including stainless steel, alnico magnets, coinage, rechargeable batteries, electric guitar strings, microphone capsules, and special alloys. It is also used for plating and as a green tint in glass. Ni is pre-eminently

an alloy metal and its chief uses are in the Ni steels and Ni cast irons, of which there are many varieties. It is also widely used in many other alloys, such as Ni brasses and bronzes, and alloys with Cu, Cr, Al, Pb, Co, Ag, and Au (Davis, 2000). A "horseshoe magnet" is made up of alnico Ni alloy. The composition of alnico alloys is typically 8–12% Al, 15–26% Ni, 5–24% Co, up to 6% Cu, up to 1% Ti, and the balance is Fe. The development of alnico began in 1931 when it was discovered that an alloy of Fe, Ni and Al had a coercivity of double that of the best magnet steels of the time. Alnico magnets are now being replaced by rare earth magnets in many applications. Because of its resistance to corrosion, Ni has been occasionally used historically as a substitute for decorative Ag.

Nickel was also occasionally used in some countries after 1859 as a cheap coinage metal (see above) but beginning the later years of the 20th century has largely been replaced by cheaper stainless steel that is Fe alloys, except notably in the United States. Ni is an excellent alloying agent for certain other precious metals and so used in the so-called fire assay, as a collector of Pt group elements (PGE). As such, Ni is capable of full collection of all 6 PGE elements from ores, in addition to partial collection of gold. Ni mines may also engage in PGE recovery (primarily Pt and Pd); examples are Norilsk in Russia and the Sudbury Basin in Canada. Ni foam or Ni mesh is used in gas diffusion electrodes for alkaline fuel cells (Bidault *et al.*, 2008). Ni and its alloys are frequently used as catalysts for hydrogenation reactions. Raney Ni, a finely-divided Ni-Al alloy, is one common form; however, related catalysts are also often used, including related 'Raney-type' catalysts. Ni is a naturally magneto-strictive material, meaning that in the presence of a magnetic field, the material undergoes a small change in length. In the case of Ni, this

change in length is negative (contraction of the material), which is known as negative magneto-striction and is in the order of 50 ppm. Ni is used as a binder in the cemented tungsten carbide or hard metal industry and used in proportions of six to 12% by weight. Ni can make the tungsten carbide magnetic and adds corrosion-resistant properties to the cemented tungsten carbide parts, although the hardness is lower than parts made of the binder Co (Davis, 2000).

2.5.3 Biological Role of Nickel

Although not recognized until the 1970s, Ni plays important roles in the biology of microorganisms and plants. In fact urease (an enzyme which assists in the hydrolysis of urea) contains Ni. The Ni, Fe-hydrogenases contain Ni in addition to Fe-S clusters. Such [Ni-Fe]-hydrogenases characteristically oxidise H₂. A Ni-tetrapyrrole coenzyme, F430, is present in the methyl coenzyme M reductase which powers methanogenicarchaea. One of the CO dehydrogenase enzymes consists of a Fe-Ni-S cluster. Other Ni-containing enzymes include a class of superoxide dismutase and a glyoxalase (Thornalley, 2003).

2.5.4 Toxicity

Exposure to Ni metal and soluble compounds should not exceed 0.05 mg/cm³ in Ni equivalents per 40-hour work week. NiS fume and dust is believed to be carcinogenic, and various other Ni compounds may be as well. Ni cyl, [Ni (CO)₄], is an extremely toxic gas (Van Den Hende *et al.*, 2012). The toxicity of metal cyls is a function of both the toxicity of the metal as well as the cyl's ability to give off highly toxic CO gas and this one is no exception; Ni Cyl is also explosive in air. Sensitized individuals may show an allergy to Ni affecting their skin, also known as dermatitis. Sensitivity to Ni may also be present in patients with pompholyx. Ni is an important cause of contact allergy, partly

due to its use in jewellery intended for pierced ears (Bidault *et al.*, 2008). Nickel allergies affecting pierced ears are often marked by itchy, red skin. Many earrings are now made Ni-free due to this problem. The amount of Ni which is allowed in products which come into contact with human skin is regulated by the European Union. In 2002 researchers found amounts of Ni being emitted by 1 and 2 Euro coins far in excess of those standards. This is believed to be due to a galvanic reaction (Nestle *et al.*, 2002).

2.6 Background Information on Green Algae

2.6.1 Cellular Structure

Almost all forms of green algae have chloroplasts. These contain chlorophylls a and b, giving them a bright green colour (as well as the accessory pigments beta carotene and xanthophylls), and have stacked thylakoids (Spolaore *et al.*, 2006). All green algae have mitochondria with flat cristae. When present, flagella are typically anchored by a cross-shaped system of microtubules and fibrous strands, but these are absent among the higher plants and charophytes, which instead have a 'raft' of microtubules, the spline. Flagella are used to move the organism. Green algae usually have cell walls containing cellulose, and undergo open mitosis without centrioles (Nestle *et al.*, 2002).

2.6.2 Origin of chloroplasts in Green Algae

The chloroplasts of green algae are bound by a double membrane, so presumably they were acquired by direct endosymbiosis of cyanobacteria. A number of cyanobacteria show similar pigmentation for instance prochloron and cyanobacterial endosymbiosis appears to have arisen more than once, as in the glaucophyta (cyanophora) and red algae. Indeed, the green algae probably obtained their chloroplasts from a *prochloron*-type prokaryotic ancestor, and evolved separately from the red algae (Nestle *et al.*, 2002).

Some green seaweed, such as *Enteromorpha* and *Ulva*, are quick to utilize inorganic nutrients from land runoff, and thus can be indicators of nutrient pollution (Jeffrey *et al.*, 2004). Green algae are often classified with their embryophyte descendants in the green plant clade *Viridiplantae* (or *Chlorobionta*). *Viridiplantae*, together with red algae and *glaucophyte* algae, form the super group *primoplantae*, also known as *Archaeplastida* or *Plantae sensulato*. Classification systems which have a kingdom of Protista may include green algae in the Protista or in the Plantae (Simpson & Roger, 2004).

2.7 Biosorption

Biosorption is the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Volesky, 2003). Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents (Aksu, 2005). The major advantages of biosorption over conventional treatment methods include, low cost, high efficiency, minimization of chemicals and or biological sludge, and possibility of metal recovery. It is a physiochemical process that occurs naturally in certain biomass which allows it to passively concentrate and bind contaminants onto its cellular structure. Biomass exhibits this property, acting just as a chemical substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria which is responsible for this phenomenon. The opposite of biosorption is metabolically driven active bioaccumulation by living cells (Bhatti *et al.*, 2007). Some of the biomass types which are extremely effective in concentrating metals come as a waste by-product of large-scale industrial fermentations. Other metal-binding biomass types, certain abundant seaweeds (particularly brown algae such as *Sargassum*, *Ecklonia*), can be readily collected from the

oceans. These biomass types, serving as a basis for metal biosorption processes, can accumulate in excess of 25% of their dry weight in deposited heavy metals: Pb, Cd, Cu, Zn, even Cr and others (Lesmana *et al.*, 2009; Ahmady *et al.*, 2013).

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases (Velásquez & Dussan, 2009).

2.7.1 Environmental Uses

Biosorption may be used as an environmentally friendly filtering technique. An extensive body of research has found that a wide variety of commonly discarded waste including eggshells, bones, peat, fungi, seaweed, yeast and carrot peels can efficiently remove toxic heavy metal ions from contaminated water via biosorption (Aksu, 2005; Vijayaraghavan & Yun, 2008; Basci *et al.*, 2004; Aksu, 2001 and Cordero *et al.*, 2004). In addition, adsorbing biomass, or biosorbents, can also remove other harmful metals like: arsenic, lead, cadmium, cobalt, chromium and uranium. There is no doubt that the world could benefit from more rigorous filtering of harmful pollutants created by industrial processes and all-around human activities.

The idea of using biomass as a tool in environmental cleanup has been around since the early 1900s when Arden and Lockett discovered certain types of living bacteria cultures were capable of recovering nitrogen and phosphorus from raw sewage when it was mixed in an aeration tank (Sameera *et al.*, 2011). This discovery became known as the activated sludge process which is structured around the concept of bioaccumulation and is still widely used in wastewater treatment plants today. It wasn't until the late 1970s when scientists noticed the sequestering characteristic in dead biomass which resulted in a shift in research from bioaccumulation to biosorption (Jeevitha & Sumathy, 2012).

2.7.2 Recovery of Metals

Recovery of the deposited metals from saturated biosorbent can be accomplished because they can often be easily released from the biosorbent in a concentrated wash solution which also regenerates the biosorbent for subsequent multiple reuse (Volesky, 2001). This extremely low cost of biosorbents makes the process highly economic and competitive particularly for environmental applications in detoxifying effluents of for example, metal-plating and metal-finishing operations, mining and ore processing operations, metal processing, battery and accumulator manufacturing operations, and thermal power generation (coal-fired plants in particular) nuclear power generation.

2.8 Equilibrium Isotherm Models

Langmuir and Freundlich models were used to determine the sorption equilibrium between the biosorbent and metal ions.

The Langmuir model assumes that a monomolecular layer is formed when biosorption takes place without any interaction between the adsorbed molecules (Yao, 2000).

Freundlich isotherm is an empirical equation based on a heterogeneous adsorption due to

the diversity of adsorption sites or diverse nature of the adsorbed metal ions, free or hydrolyzed species (Umpleby *et al.*, 2001). The Langmuir isotherm equation has a hyperbolic form:

$$Q_e = \frac{Q_{\max} k C_e}{1 + k C_e} \dots\dots\dots 2.1$$

where Q_e = concentration (mg/g) adsorbed at equilibrium,

Q_{\max} = maximum adsorption capacity (mg/g) corresponding to the monolayer adsorption capacity,

C_e = concentration (mg/L) of the heavy metal ions solution at equilibrium and

k is related to the strength of adsorbent-adsorbate affinity.

A linear expression of the Langmuir isotherm is expressed as:

$$\frac{C_e}{Q_e} = \frac{1}{k Q_{\max}} + \frac{C_e}{Q_{\max}} \dots\dots\dots 2.2$$

The empirical Freundlich isotherm equation is:

$$Q_e = k_f [C_e]^{\frac{1}{n}} \dots\dots\dots 2.3$$

in logarithmic linear form;

$$\log Q_e = \log k_f + \frac{1}{n} \log C_e \dots\dots\dots 2.4$$

where k_f is related to adsorption capacity and n is related to intensity of adsorption.

These models were be applied at a constant pH, and used for modelling of biosorption equilibrium in the presence of one metal.

Recently, some biosorbents have emerged as an eco-friendly, effective and low cost material options. These biosorbents include some agricultural wastes, fungi, bacteria and

yeast. Studies using biosorbents have shown that both living and dead microbial cells are able to uptake metal ions and offer potential inexpensive alternative to conventional absorbents (Brandt *et al.*, 2009).

2.9 Order of Reaction

The order of reaction explains the functional relationship between the concentration of reactants and the rate of reaction. It determines how the amount of compound speeds up or retards the reaction. It is given by the sum of the exponents of the concentration terms of the rate law;

$$Rate = k[A]^a[B]^b \dots\dots\dots 2.5$$

The reaction overall order of reaction for this equation is given by ‘a’ plus ‘b’. In a reaction where A and B react 1st order with respect to A and zero order with respect to B, the reaction rate is indicated as, $Rate = k[A]^1[B]^0 = k[A]$, the overall rate reaction is equal to one plus zero.

The data were subjected to reaction kinetics to deduce whether the process followed pseudo first order or pseudo second order. The rate equation gives an expression of the variation in concentration of metal ions with time. The order of reaction was deduced from the integrated rate equation given as;

$$\frac{dQ_t}{dt} = k(Q_e - Q_t) \dots\dots\dots 2.6$$

The symbols Q_t and Q_e are the masses of metal which are biosorbed by the biosorbent in (mg/g) at any time t and at equilibrium, respectively. The value k is the rate constant for the biosorption. On integration, equation 2.6 gives;

$$kt = \ln Q_e - \ln(Q_e - Q_t) \dots\dots\dots 2.7$$

A plot of time t against $\ln(Q_e - Q_t)$ gives a straight line with a slope of $-1/k$

If a graph of t against $\ln(Q_e - Q_t)$ is plotted, it gives a straight line with a correlation coefficient (R^2) value tending to unity, then the experimental data agrees with first order kinetics and the reaction is first order. The second order rate equation can be represented as;

$$\frac{dQ_t}{dt} = k_2(Q_e - Q_t)^2 \dots\dots\dots 2.8$$

where k_2 is the second order rate constant, Q_e and Q_t have the same meaning as defined above. Upon integration, equation 2.8 gives the equation 2.9;

$$\frac{t}{Q_t} = \frac{t}{Q_e} + \frac{1}{k_2 Q_e^2} \dots\dots\dots 2.9$$

Equation 2.9 predicts that for a second order process, plotting t/Q_t against t should give a straight line from which the rate constant k_2 and the metal uptake at equilibrium Q_e , can be calculated. From the R^2 values for the first and second order plots, it is possible to deduce the order of reaction. The curve with the higher value of linear correlation coefficient corresponds to the order of the reaction.

If the concentration of one of a reactants remains constant because it is supplied in great excess, its concentration can be absorbed within the rate constant, obtaining a pseudo first-order reaction constant, because in fact, it depends on the concentration of only one reactant . A second-order reaction depends on the concentrations of one second-order reactant, or two first-order reactants (Ho & Ofomaja, 2006).

2.10 Atomic Absorption Spectrophotometer (AAS)

Atomic absorption is the absorption of light by free atoms. An atomic absorption spectrophotometer is an instrument that uses this principle to analyze the concentration of metals in solution. The substances in solution form are suctioned into an excited phase where they undergo vaporization, and are broken down into small fragmented atoms by discharge. The sample is atomized in the flame, through which radiation of a chosen wavelength (using a hollow cathode lamp) is sent. The amount of absorbed radiation is a quantitative measure for the concentration of the element to be analyzed. The most current gas mixtures used are air/acetylene and nitrous-oxide/acetylene. The latter resulting in higher atomization efficiencies and thus better detection limits for elements like Si, Al, Sc, Ti, V and Zr. The air/acetylene flame can be used for easy atomizable elements (for instance As and Se). Background correction can be achieved with a deuterium lamp. By exposing these atoms to high temperatures they are able to “jump” to high energy levels and in return, emit light with a characteristic wavelength which can then be recorded (Welz & Sperling, 2008).

The characteristic wavelengths are element specific and accurate to 0.01-0.1nm. To provide element specific wavelengths, a light beam from a lamp whose cathode is made of the element being determined is passed through the flame. A device such as photon multiplier can detect the amount of reduction of the light intensity due to absorption by the analyte, and this can be directly related to the amount of the element in the sample.

Figure 2.1 is a sketch diagram of an AAS instrument (*Varian Spectra A*).

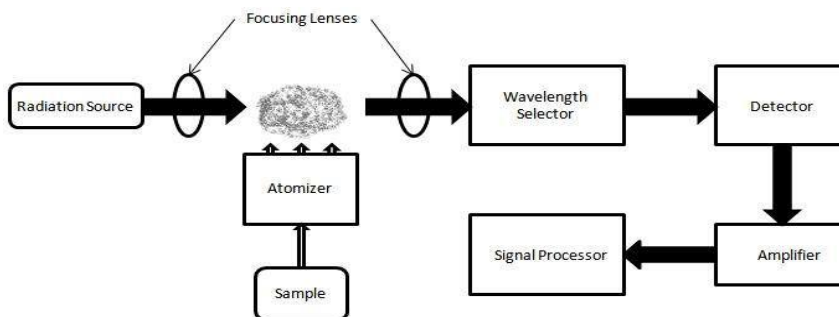


Figure 2.1: A Sketch Diagram of AAS (Varian Spectra AA, USA)

2.11 Related Studies

Much research has been done on the biosorption of heavy metals using different species of algae as biosorbents. Ayabei & Kituyi (2013) did a comparative study of rates of biosorption for selected single and mixed metal ions using a brown alga *Ascophyllum nodosum*. The analysis revealed that the order of preference to the active sites present in the algae was $Pb^{2+} > Cu^{2+} > Zn^{2+}$ the order was the same even for single metal uptake.

In another study done in Okha, India, Kumar *et al.* (2009) studied the biosorption of heavy metals from aqueous solution by green marine macro-algae. The equilibrium sorption data proved that the process conforms to Langmuir better than Freundlich isotherm model as depicted with high correlation coefficients.

To evaluate the capability of marine brown alga *Fucus vesiculosus* to absorb Cu^{2+} , Salman & Medhi (2011) did a study using test solutions. Their results suggested that *Fucus vesiculosus* biomass could be an interesting low-cost biosorbent for Cu^{2+} removal from aqueous solutions. The experimental data in deionized water was well described by the Langmuir model. Brown algae among the three groups of algae (red, green and

brown) received the most attention. Higher uptake capacity has been found for brown algae than for red and green algae (Fraile *et al.*, 2005).

A different study by Romera *et al.* (2007) on comparative study of biosorption of heavy metals using different types of algae showed that sorption capacity of the different alga depended on the pH and the biomass concentration. The optimum pH value for recovery of Cd^{2+} , Ni^{2+} and Zn^{2+} was 6 for the algae studied. The sorption pH for Cu^{2+} ranged from 4 to 9, which agrees well with the current study. Reducing the biomass concentration increased the sorption capacity, and in many cases values were higher at the lowest concentration (0.5 g/L). Experimental data fitted a Langmuir equation very well. All algae showed similar affinities for each metal tested, the sequence of affinities between each biomass and the different metal ions was as follows: $\text{Cd}^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+}$.

In another study, Volesky *et al.* (2003) did a review of the biochemistry of heavy metal biosorption by brown algae and found out that the main components responsible for biosorption in all algae are polysaccharides, lipids and proteins. Fucoidan is a branched polysaccharide sulphate ester with l-fucose building blocks as the major component with predominantly α (1-2) linkages. According to them, the sorption of heavy metals on the biosorbents could be described as a two-step process where the metal was initially taken onto the surface of the cell followed by the bioaccumulation inside the cell. Their results showed that studies on biosorption of Pb^{2+} by the algal biomass of *Spirogyra* species fitted the Langmuir isotherm accurately. Biosorption of cadmium on *Spirulina platensis* immobilized on alginate and silica gel also fitted well with the Langmuir isotherm.

The studies on Cu^{2+} removal by *Ascophyllum nodosum* done by Rangasayatorn *et al.* (2004) and Fraile *et al.* (2005) showed that the adsorptive capacity of the biomass

depended on pH. Biosorption was also found to be affected by biomass concentration. *Chlorella vulgaris* showed best sorption at lower biomass concentrations for uptake of metals. pH was found to be the most important factor affecting rate of biosorption since increase in pH showed higher metal uptake capacities. Biosorbent size did not affect the biosorption capacity and rate. The biosorption of heavy metal ions on the cell surface occurs by ion exchange process. The technique of immobilization of algal biomass increased the kinetic uptake rates of the metals.

Another biosorbent green algae species (*Spirogyra species*) was studied by Gupta & Rastogi (2008) who performed a study of the kinetics of biosorption of Pb^{2+} from aqueous solutions. Their results indicated that the biomass of *Spirogyra sp.* is an efficient biosorbent for the removal of Pb^{2+} from aqueous solutions. In addition, they showed that the uptake kinetics followed the pseudo-second-order model and equilibrium is well described by Langmuir isotherm. They also performed batch experiments to determine the biosorption properties of the biomass and it was observed that the maximum adsorption capacity of Pb^{2+} ion was around 140 mg metal/g of biomass at pH 5.0 in 100 min with 200 mg/L of initial concentration.

The biosorption capability of the green macroalga *Caulerpa lentillifera* was tested by Pavasant *et al.* (2006) who performed a batch experiment to test the sorption of Cu^{2+} , Cd^{2+} , Pb^{2+} , and Zn^{2+} by the species. Their findings indicated that the sorption of all the test metal ions rapidly reached equilibrium within 20 min. The sorption kinetics of these metals was governed by external mass transfer and intra-particle diffusion processes. The sorption isotherm followed the Langmuir isotherm where the maximum sorption capacities was $Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+}$.

In another similar study, Bulgariu & Bulgariu (2012) investigated the biosorption of Pb^{2+} , Cd^{2+} , and Co^{2+} from aqueous solution on green algae waste biomass. The results indicate that Langmuir model provide best correlation of experimental data, and the pseudo-second order kinetic equation could best describe the biosorption kinetics of the considered heavy metals. The same researcher Bulgariu *et al.* (2009) evaluated the adsorption potential of Romanian peat moss for the removal of Hg^{2+} from aqueous solutions, biosorption followed the Langmuir model.

Hamdy (2000) studied the ability of four different algae (three brown and one red) that have not been previously studied to adsorb Cr^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Cd^{2+} ions. His findings revealed that the metal uptake was dependent on the type of biosorbent, with different accumulation affinities towards the tested elements. The HCl-treated biomass decreased the metal biosorptive capacity particularly in the case of Cr^{3+} adsorption with *Laurencia obtusa*. The extent of uptake of the different metals with the tested algae was assessed under different conditions such as pH, time of algal residence in solution with the metal, and concentration of algal biomass.

Feng & Aldrich (2004) studied the adsorption of heavy metals onto biomaterial derived from the marine alga *Ecklonia maxima* via batch experiments. The rate of adsorption onto the marine alga was high. The alga particle size played an important role in the adsorption behaviour. The coarse alga particles had a higher adsorption capacity and slower adsorption kinetics and could be regenerated without significant loss of capacity. In contrast, the fine alga particles had a lower adsorption capacity and faster adsorption kinetics and could not be regenerated without significant loss of capacity. Comparison with a commercial resin indicated that the activated biomass derived from *E. maxima*

could be used as an efficient biosorbent for the treatment of waste waters containing heavy metals.

Kaewsarn (2002) studied biosorption of Cu^{2+} from aqueous solutions using marine algae *Padina* sp. Equilibrium isotherms and kinetics were obtained from batch adsorption experiments. The findings showed that biosorption capacities were solution pH dependent and the maximum capacity obtained was 0.80 mmol/g at a solution pH of about 5.

In another study, Chojnacka *et al.* (2005) studied the process of biosorption of heavy metal ions (Cr^{3+} , Cd^{2+} , Cu^{2+}) by blue-green algae *Spirulina* sp. This study investigated quantitatively the potential binding sites present at the surface of *Spirulina* sp., using both potentiometric titrations and adsorption isotherms. The kinetic experiments showed that the process equilibrium was reached quickly, in less than 5–10 min. It was found that the equilibrium dependence between biosorption capacity and bulk metal ion concentration could be described with Langmuir equation. This suggests that the mechanism of biosorption is rather chemisorption than physical adsorption and was further confirmed by the low surface area associated with physical adsorption and by the presence of cations that appeared in the solution after biosorption. Three functional groups capable of cation exchange were identified on the cell surface. The biomass was described as weakly acidic ion exchanger. Since deprotonation of each functional group depends on pH, the process of biosorption is strongly pH-dependent. This was confirmed in the biosorption experiments carried out at different pH.

Wang & Chen (2006) did a review of heavy metal biosorption using *Saccharomyces cerevisiae*. It was found that yeast biosorption largely depends on parameters such as pH, the ratio of the initial metal ion and initial biomass concentration, culture conditions, presence of various ligands and competitive metal ions in solution and to a limited extent on temperature.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample and Sampling Preparation

The green algae (*spirogyra sp.*) samples were obtained from the University of Eldoret fish ponds where they were found floating. They were collected and dried using direct sunlight for three hours after which they were put into polythene bags and transferred to the laboratory.

3.1.1 Sample Preparation

The collected green algae were washed with distilled water for several times to remove all the dirt particles. The cleaned algae were then dried in a hot air oven (Memmert UNB 300, *Germany*) at 60 °C for 24 hrs. The dried green algae were then ground using a pestle and mortar and then sieved using +60 mesh sieve to a constant particle size. The powdered green algae were then stored in plastic bottles.

3.1.2 Materials (Equipment)

Hot plate, Plastic bottles(100 mL), pH meter, Flame atomic Spectrophotometer, Oven, Stop watch, Sieves(+60 mesh), Pestle and mortar, polythene bags, 1000 mL volumetric flask, 500 mL volumetric flask, 100 mL volumetric flask, 50 mL volumetric flask, 10 volumetric flask, 100 mL beakers, and Test tubes.

3.1.3 Reagents Required

Concentrated nitric acid (HNO₃), concentrated Sulphuric acid (H₂SO₄), concentrated hydrochloric acid (HCl), deionized water (H₂O), copper(II)chloride dihydrate

(CuCl₂·2H₂O), zinc(II)nitrate hexahydrate (Zn(NO₃)₂·6H₂O), nickel(II)chloride hexahydrate(NiCl₂·6H₂O) and cadmium(II)nitrate dihydrate (Cd(NO₃)₂·2H₂O) salts.

3.2 Methods

3.2.1 Preparation of Heavy Metals Solutions

The reagents for preparing standard solutions of Ni²⁺, Zn²⁺, Cu²⁺ and Cd²⁺ were of analar grade, procured from Sigma Aldrich Company in form of CuCl₂·2H₂O, Zn(NO₃)₂·6H₂O, NiCl₂·6H₂O and Cd(NO₃)₂·2H₂O salts, respectively.

1000ppm stock solution of the heavy metals were prepared by dissolving 2.117g, 4.549g, 4.049g and 1.791 g of CuCl₂·2H₂O, Zn(NO₃)₂·6H₂O, NiCl₂·6H₂O and Cd(NO₃)₂·2H₂O, respectively in 100mL aqueous solution containing 5% concentrated nitric acid solution and then transferred to 1000mL volumetric flasks. The solutions were then diluted to the mark by using deionized water.

The volume of the required aliquot was calculated using the formular, $C_1V_1 = C_2V_2$

where: C₁ is the concentration of the stock solution,

V₁ is the aliquot volume,

C₂ is the concentration of the working heavy metal solution

V₂ is the volume of the working solution.

To minimize errors dilutions were done in series, for Cu²⁺, 1000 ppm was diluted to 100 ppm which was further diluted to 10 ppm. 10 ppm was then further reduced to 1ppm and finally diluted to the required concentration of 0.05 ppm; this was repeated for the other metal ions (Ni²⁺, Zn²⁺, and Cd²⁺).

3.3 Optimizing Operating Conditions of AAS

The instrument's operating conditions were optimized before the actual analysis. The fixed parameters were automatically set upon selection of the element to be analyzed. These parameters included; wavelength, lamp current, slit width and extra heat tension (EHT) and detection limits. The other parameters were set according to the manufacturers specifications in the manual. The acetylene and oxygen flow rate was set to attain maximum transparency of the flame. The optimized conditions for the AAS instrument during analysis are shown in table 3.1 below.

Table 3.1: Optimized conditions for AAS (Varian Spectra AA)

Meta l Ion	Wavelength λ (nm)	Lamp Current (mA)	Slit Width (nm)	Acetylene flow rate (L/min)	Oxidant flow rate (L/min)	BH (mm)	EH T (v)	Detection Limit (ppm)
Zn ²⁺	213.9	4.0	1	2	13.5	13.5	800	0.0 – 8.0
Cu ²⁺	324.8	4.0	0.5	2	13.5	13.5	494	0.0 – 8.8
Cd ²⁺	228.8	4.0	1	2	13.5	13.5	485	0.0 – 3.3
Ni ²⁺	232.0	4.0	0.5	2	13.5	13.5	486	0.0 – 16.5

3.4 Biosorption Experiments

A 5 gram mass of the green algae sample powder were measured accurately and transferred into 250 mL beaker containing a 100 mL of specific heavy metal at pH 4 and then stirred for about ten minutes. In order to determine heavy metal ion concentration biosorbed from the solution, 10 mL samples were taken out from the reacting vessel, filtered through whatman cellulose filter paper at specific time intervals of 10, 20, 30, 40,

50 and 60 minutes before measurement by the AAS machine, and the results were then tabulated. The same procedure was repeated for pH 7 and 9.

The difference between the initial and remaining metal concentration was assumed to be taken up by the biosorbent.

3.5 Data Analysis

Data collected was processed using Ms-Excel program. To test the adsorption kinetics, the data was subjected to Langmuir and Freundlich isotherms to determine which model fitted best to the adsorption data.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of pH and Contact Time on Metal Uptake by Green Algae

4.1.1 Effect of pH

Effects of pH on metal uptake by immobilized green algae cells involved biosorption experiments at pH values of 4, 7 and 9 with the heavy metal ions Cu^{2+} , Zn^{2+} , Ni^{2+} and Cd^{2+} at respective initial concentrations in ppm of 0.05, 7.00, 0.20 and 0.90 due to variation of the metallic detective limits.

Figures 4.1- 4.4 showed that the levels of the metal biosorbed initially increased steadily and rapidly reaching equilibrium after sometime. The increase of biosorption at initial stages could be due to the enormous initial binding sites on the surface of the algae. With time, these active sites were filled up, which led to eventual reduction in the uptake of the metal ions from the aqueous solution. These observations agree with those reported by Chojnacka *et al.* (2005). The amount of metal ions remaining in solution, therefore decreased, but afterwards, remained constant because the number of binding sites became saturated with the metal ions, resulting in almost zero rate of adsorption and desorption. It was also clearly seen that for Cu^{2+} , pH 9 favoured its biosorption followed by pH 7 while pH 4 gave the lowest. Biosorption for the other metal ions was similar except the suitability of pH was not consistent for all the metal ions. For instance biosorption for Ni^{2+} , pH 7 > pH 4 > pH 9, Zn^{2+} , pH 7 > pH 9 > pH 4 and Cd^{2+} , pH 7 > pH 4 > pH 9. Generally high pH values favoured the biosorption of the metal ions. This is due to surface of the algae which at a higher pH become negatively charged, while at a low pH value, it is positively charged (Hajar, 2009).

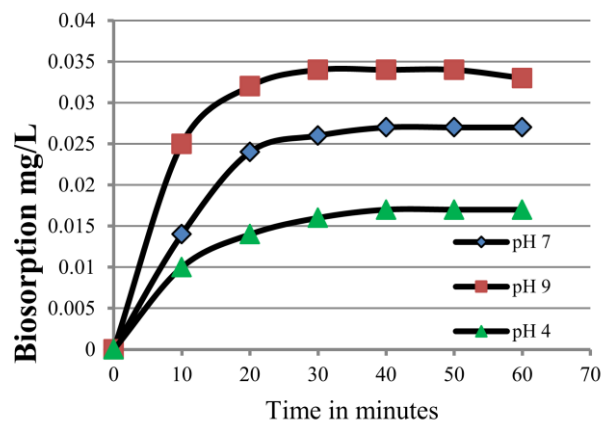


Figure 4.2: Cu²⁺ biosorbed with time at different fixed pHs

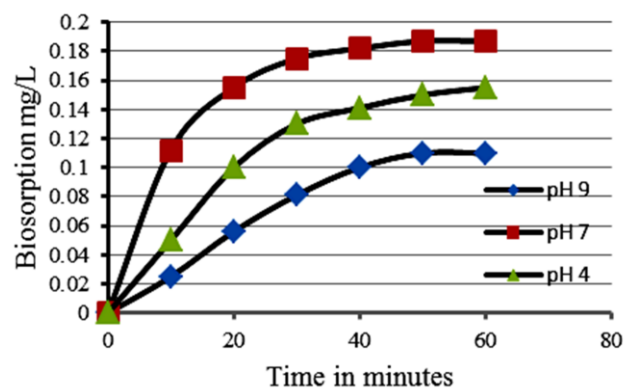


Figure 4.3: Ni²⁺ biosorbed with time at different fixed pHs

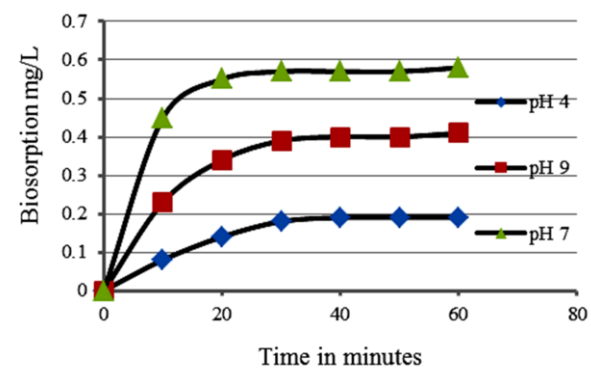


Figure 4.1: Zn²⁺ biosorbed with time at different fixed pHs

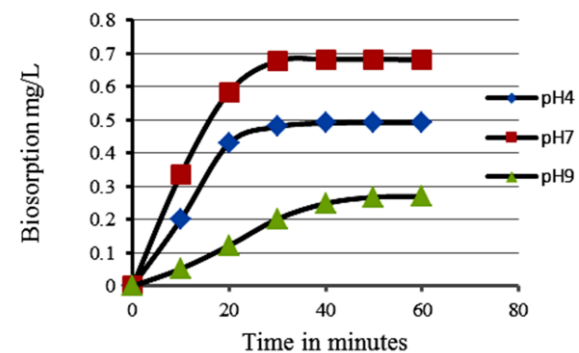


Figure 4.4: Cd²⁺ biosorbed with time at different fixed pHs

4.1.2 Effect of Contact Time

Figures 4.1 - 4.4 showed that the biosorption efficiency of the four heavy metal ions, Cu^{2+} , Zn^{2+} , Ni^{2+} and Cd^{2+} by green algae as a function of contact time increased with the rise in contact time till equilibrium was reached. The fast initial metal biosorption rate could be attributed to the surface binding while the slower sorption that followed could be as a result of the interior penetration (Bishnoi & Pant, 2004).

Different kinds of functional groups, with different affinities to the metal ions, are usually present on the biomass surface. The active binding groups with higher affinities are firstly occupied (Deng *et al.*, 2007). According to these results, a contact time of 60 min was set in order to ensure attainment of equilibrium conditions. These findings agree with those reported by Maheswari *et al.* (2008) while Karthikeyan *et al.* (2007) found a significant fraction of the total Cu^{2+} uptake was achieved within 30 minutes.

4.1.3 Biosorption at Specific pH Values for Metal Ions

Figures 4.5 – 4.7 show the comparison of metal ions at pH values of 4, 7 and 9. It was observed that, Cd^{2+} were biosorbed mostly while Cu^{2+} were the least giving the order $\text{Cd}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Cu}^{2+}$.

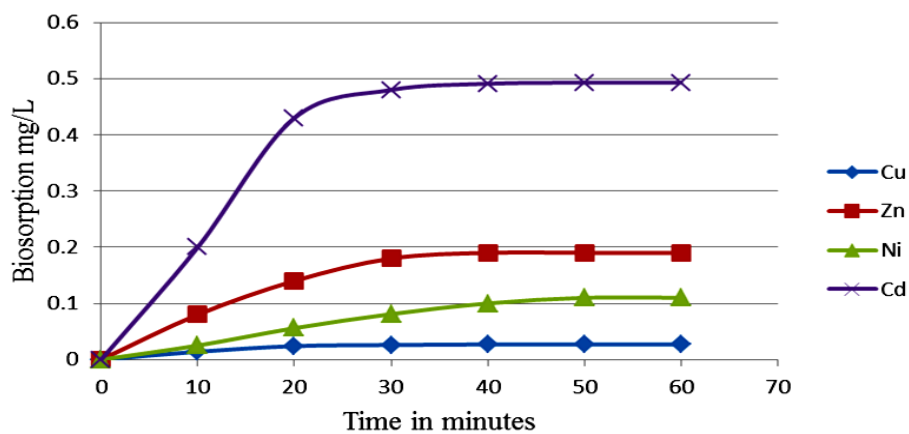


Figure 4.5: Variation of biosorption of metal ions with time at pH 4

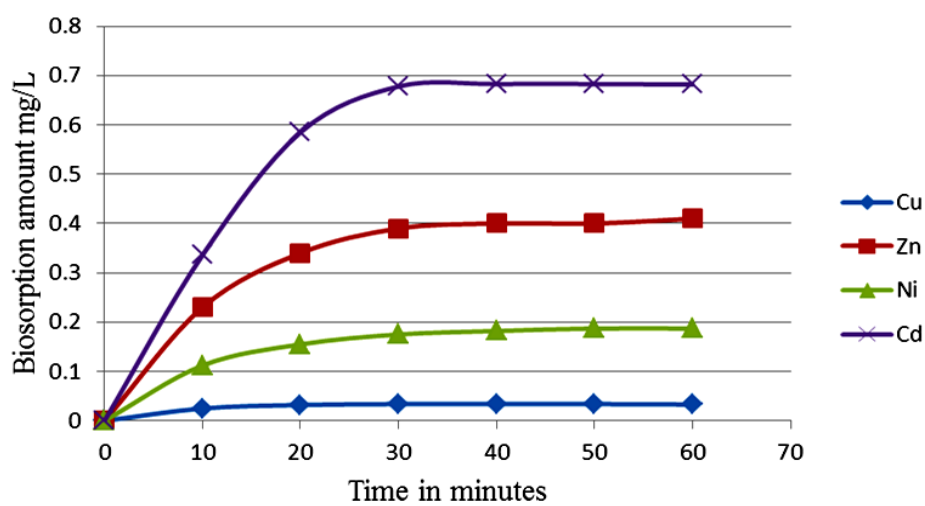


Figure 4.6: Variation of biosorption of metal ions with time at pH 7

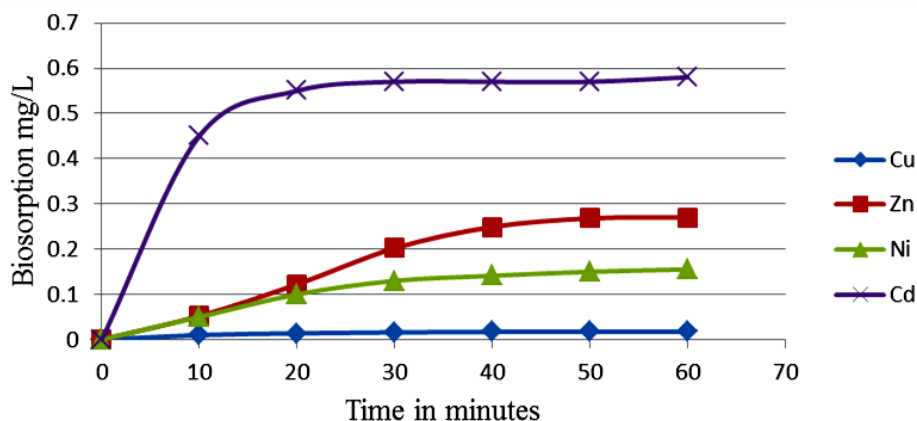


Figure 4.7: Variation of biosorption of metal ions with time at pH 9

At the optimum pH of around 6.5, the anion population reached saturation point hence no more metal uptake took place. These findings agreed with those of Salman and Mehdi (2011). These figures showed consistent biosorption of the metal ions at all the pH values of 4, 7 and 9, without changing the order of biosorption.

There was a strong link between the ionic radii of the metals and their uptake by the algae. Since Cd^{2+} has the largest ionic radius, it was least hydrated and so more biosorbed at any given pH value leading to it being the most biosorbed while Cu^{2+} was the least. The initial metal ion biosorption increased during the first few minutes and then leveled off after sometime. This is in agreement with the results reported by Sheng *et al.* (2004) illustrating the biosorption mechanism takes place in two stages. In the first stage pseudo-equilibrium was reached, while in the second stage, a slow decrease of metal uptake took place. This decrease was, however, as a result of the metal ion crossing through the cell wall, causing intracellular accumulation since the number of binding sites of the ligands on the algae decreased (Bothwell *et al.*, 2003).

4.1.4 Optimum pH Value

The result of the effect of pH on biosorption capacity, involving the uptake of Cu^{2+} , Zn^{2+} , Ni^{2+} and Cd^{2+} onto green algae as a function of pH is shown in Figure 4.8. Most biosorption generally occurred within 7 ± 2 pH values for the four metal ions giving the optimum pH value of 6.5. It can be seen that Cu^{2+} was least biosorbed while Cd^{2+} was the highest. Below and above this optimum pH value, biosorption generally decreased for all the heavy metal ions under study.

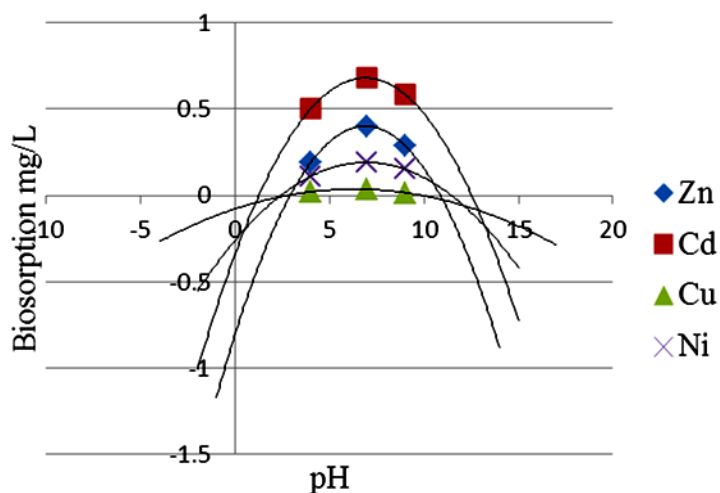
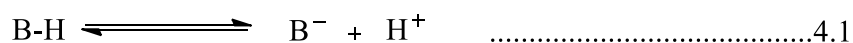


Figure 4.8: Green algae biosorption for metal ions at different pH values

It has been reported that biosorption of heavy metal ions is dependent on the pH of the solution as it affects the degree of ionization of the biosorbent (Davis *et al.*, 2003). The pH of the solution influences both metal binding sites on the cell surface and the chemistry of metal in solution. It was evident from Figure 4.8 that as the pH of the heavy metal solution increased from 4 to 6.5, the biosorption capacity of the green algae also increased rapidly reaching an optimum value before registering a decline with one exception of Zn^{2+} . The decreasing biosorption level at a lower pH was due to competition between protons and metal ions for the same sites of the alginate polymer.

Increased proton charge (H^+) density on the sites of biomass surface at low pH values (pH 4) restricted the approach of metal cations as a result of repulsive force. In contrast, when the pH value increased, biomass surface was more negatively charged, ($R-COO^-$), and the biosorption of the metal ions with positive charge reached optimum. The decrease in biosorption at higher pH values, that is more than pH 9, may be attributed to the formation of anionic hydroxide complexes of the metal ions and their competition with the active sites. These results were in agreement with those reported by Davis *et al.* (2003).

The order of biosorption was, $Cd^{2+} > Zn^{2+} > Ni^{2+} > Cu^{2+}$ at all the pH values investigated. These findings were similar to those reported by Hajar (2009). In the biosorption phenomenon, pH value affects two aspects; metal ion solubility and biosorbent total charge, since protons can be adsorbed or released (Romera *et al.*, 2007). This behaviour will depend on the functional groups present on the alga cell wall, which in turn determine the state according to the following equations, 4.1 and 4.2;



Therefore,

$$K_a = \frac{[B^-][H^+]}{[B-H]} \quad \text{and}$$

$$pK_a - pH = \frac{\log[B^-]}{[B-H]} \quad \dots\dots\dots 4.2$$

For pH values lower than pKa, equilibrium 4.1 shifts to the left, implying that more protons are consumed and pH increases until its value equals pKa. When the pH of the medium is higher than pKa, the opposite will happen, equation 4.2.

At higher pH, the number of negatively charged active sites increased, facilitating a higher electrical attraction to positively charged metal ions. However, most metal ions were highly biosorbed at pH values > 4, these findings are supported by Romera *et al.* (2007) and Gupta & Rastogi (2008).

4.2 Biosorption Isotherms

4.2.1 Langmuir Isotherm

The Langmuir model is given by the equation;

$$Q_e = \frac{Q_{\max} k C_e}{1 + k C_e} \dots\dots\dots 4.3$$

where;

Q_e = amount (mg/g) of metal biosorbed per gram of the biosorbent material;

C_e = the metal concentration (mg/L) in solution (equilibrium) concentration;

Q_{\max} = the maximum specific uptake of the metal (mg/g) by biosorbents;

k = ratio of adsorption and desorption rates.

Rewriting Langmuir equation;

$$\frac{C_e}{Q_e} = \frac{1}{k Q_{\max}} + \frac{C_e}{Q_{\max}} \dots\dots\dots 4.4$$

A plot of $\frac{C_e}{Q_e}$ versus C_e gave a straight line with y-intercept equal to $\frac{1}{k Q_{\max}}$ and the

slope is $\frac{1}{Q_{\max}}$. Using an example of Cu^{2+} , the resulting plot gave rise to Figure 4.9.

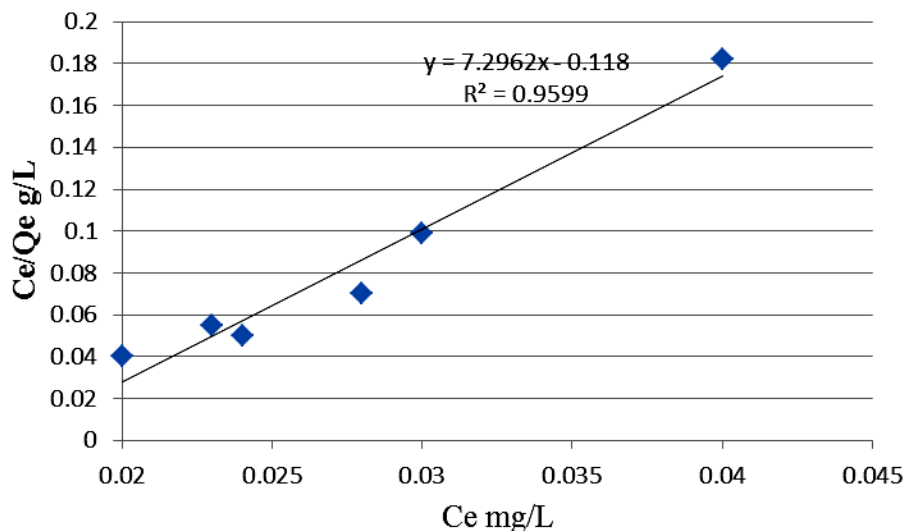


Figure 4.9: Langmuir plot for Cu^{2+} at pH 4

From the metal ions experimental data of C_e and Q_e , a linearized expression of Langmuir

isotherm $\frac{C_e}{Q_e} = \frac{1}{k Q_{\max}} + \frac{C_e}{Q_{\max}}$ was used as a reference point in making plots and from

the plots the constants k , Q_{\max} and R^2 were achieved and tabulated.

4.2.2 Freundlich Isotherm

Freundlich equation is written in linear form as;

$$\log Q_e = \log k_f + \frac{1}{n} \log C_e \quad \dots\dots\dots 4.5$$

k_f and n are constants relating to respective biosorption capacity and biosorption intensity. The corresponding values of k_f and n indicate high biosorption capacity and intensity, respectively. Using Ni^{2+} data at pH 4 Figure 4.10 was drawn.

Equation 4.5 was used to plot $\log Q_e$ (y-axis) against $\log C_e$ and a straight line was obtained with “ n ” equal to the reciprocal of the gradient and “ k_f ” the anti-logarithm of the y - intercept. From the plot the constants n , k_f and R^2 were obtained and tabulated.

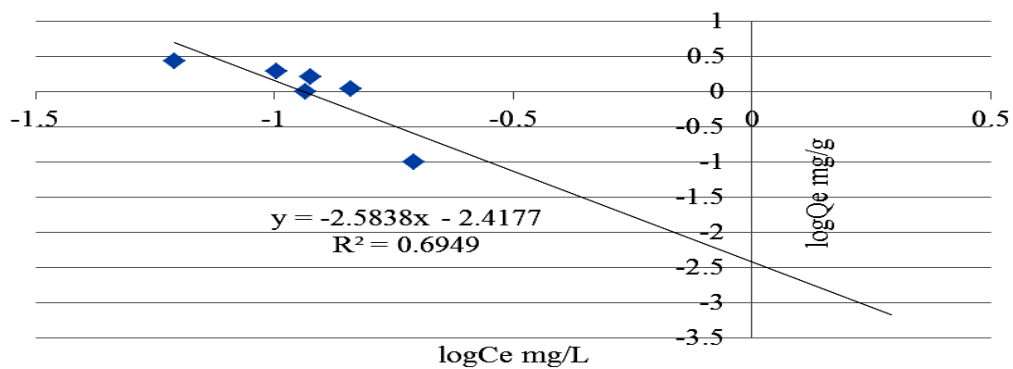


Figure 4.10: Freundlich plot for Ni²⁺ at pH 4

The same procedure was used to generate data for all the metal ions at pH values studied and plots for both the Langmuir and Freundlich isotherms that are presented in Appendices A and B, respectively. The summary of the results of both isotherm parameters were tabulated in Tables 4.3 – 4.5.

Table 4.1: Freundlich and Langmuir parameters at pH 4

Metal ion	Freundlich pH 4			Langmuir pH 4		
	n	k_f	R^2	Q_{max}	k	R^2
Cd^{2+}	0.6670	2.85	0.9916	2.07	33.761	0.9968
Cu^{2+}	0.1440	39.44	0.9335	2.441	31.3	0.9599
Ni^{2+}	0.3892	8.222	0.6733	1.233	2.57	0.9930
Zn^{2+}	0.2310	76.73	0.9864	2.443	4.406	0.9907

Table 4.2: Freundlich and Langmuir parameters at pH 7

Metal ion	Freundlich pH 7			Langmuir pH 7		
	n	k_f	R^2	Q_{max}	k	R^2
Cd^{2+}	0.5690	56.05	0.8816	2.832	6.761	0.9978
Cu^{2+}	0.744	39.44	0.8951	1.923	128	0.9376
Ni^{2+}	0.3892	8.222	0.8788	2.816	28.57	0.9684
Zn^{2+}	0.733	76.73	0.8914	1.684	892	0.9895

Table 4.3: Freundlich and Langmuir parameters at pH 9

Metal ion	Freundlich pH 9			Langmuir pH 9		
	n	k_f	R^2	Q_{max}	k	R^2
Cd^{2+}	0.6721	38.05	0.8511	8.678	4.390	0.9932
Cu^{2+}	0.2089	3.302	0.8217	9.333	4.301	0.9536
Ni^{2+}	0.8824	8.2330	0.7804	1.667	3.320	0.9321
Zn^{2+}	0.3770	6.5670	0.8442	2.857	7.292	0.9198

The R^2 values, for Ni^{2+} , Freundlich isotherm at pH 4 were slightly lower compared to other metal ions in the study at the same pH value implying it was least biosorbed. There was competition for H^+ and the other metal cations, hence the low intake by the green algae (Romera *et al.*, 2007). Ions with larger ionic radii have a higher likelihood of being biosorbed compared to those with smaller ionic radii. Larger ions are least hydrated while the smaller ions are highly hydrated in solution making them more difficult to be biosorbed as compared to the bigger ones. Although Zn^{2+} is smaller than Cu^{2+} hence more hydrated, it was unexpectedly found that it was biosorbed more than Cu^{2+} . This could be due to the fact that some green algae species are highly specific for the uptake of certain metals irrespective of their sizes and also the differences in their electronic configuration of the d orbital (Hamdy, 2000). Therefore the order of biosorption was $\text{Cd}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Cu}^{2+}$, based on their sizes and the specificity of the algae uptake for some metal ions, Zn^{2+} was preferred to Cu^{2+} by the algae.

The data fitted best the Langmuir isotherm than the Freundlich since the correlation coefficients, R^2 , at pHs 4 and 7 for the Langmuir were greater than 0.900. This shows that the sorption of these metal ions had a monolayer coverage on the surface of the algae. The values k are related to the strength of adsorbent-sorbate affinity, and at both pH values, k values were above unity which showed strong adsorbent-sorbate equilibrium. This implied that the sorption of heavy metal ions on the algae took place at the functional group binding sites strongly as is supported by Alkorta *et al.* (2004).

The extent of biosorption and the mechanisms involved are also known to be influenced by pH, initial metal concentration, and contact time with the green algae biomass. Metal

ions are important, as the associated properties of ionic charge, size and solubility determine how effectively a particular metal is absorbed and how likely it will be displaced by a more preferred competitor (Salman & Mehdi, 2011). The data was fitted to the Langmuir model at pH 9 too and the correlation coefficient, R^2 , was greater than 9.2 for all the 4 metal ions as shown in Table 4.5. This agrees with the work reported by Bulgariu *et al.* (2009). At low pH, the cell binding sites are closely linked with H^+ ions making them unavailable for metal cations. However, with an increase in pH, there is an increase in the number of ligands with negative charges which results in increased binding for cations. pH is an important parameter for adsorption of metal ions from aqueous solution because it affects the adsorption of the metal bindings to the ligands on the algae (Hamdy, 2000). The maximum monolayer coverage capacities (Q_{max}) from the Langmuir isotherm model were generally greater than one, increasing with pH. This indicated that biosorption was favourable and the data fitted well to Langmuir isotherm model. The n values between 1 and 10 represent a greater biosorption capacity; demonstrated by the three pH values of 4, 7 and 9. The magnitude of k_f and n shows easy separation of heavy metal ion from wastewater and high adsorption capacity, respectively. The k_f values at pH 7 were high compared to pHs of 4 and 9 indicating that biosorption occurred optimally around this pH value of 6.5 also supported by Davis *et al.* (2003).

The value of n, which is related to the distribution of bonded ions on the sorbent surface, is found to be almost unity for the algae, indicating that a greater adsorption of the four heavy metals was quite favourable (Fraile *et al.*, 2005). It was noted that the R^2 values for the Langmuir were much greater than those for the Freundlich at pH values of 7 and 9.

Thus biosorption followed a surface monolayer adsorption mechanism. However at pH 4, the two mechanisms seemed to have played a role in the metal ion biosorption, and their R^2 values were close for both isotherms.

4.3 Adsorption Kinetics

A pseudo-first-order kinetic equation is given as;

$$\log(Q_e - Q_t) = \log Q_e - k_1(t/2.303) \dots \dots \dots 4.6$$

where

Q_t = amount (mg/g) of heavy metal ions biosorbed at time t (minutes)

Q_e = biosorption capacity at equilibrium (mg/g)

k_1 = the pseudo-first-order rate constant (1/min), and

t = contact time (min).

Plotting $\log(Q_e - Q_t)$ versus t , the pseudo-first-order rate constant (k_1) was determined from the model presented by calculating the gradient of the graphs drawn, and the second order rate equation;

$$\frac{dQ_t}{dt} = k_2(Q_e - Q_t)^2 \dots \dots \dots 4.7$$

Where k_2 is the second order rate constant, Q_e and Q_t have the same meaning as defined earlier.

Upon integration it assumes the form,

$$\frac{t}{Q_t} = \frac{t}{Q_e} + \frac{1}{k_2 Q_e^2} \dots \dots \dots 4.8$$

Plotting t/Q_t against t gave a straight line from which the rate constant k_2 and the metal uptake at equilibrium Q_e , calculated. From the R^2 values for the first and second order plots, the order of reaction was deduced.

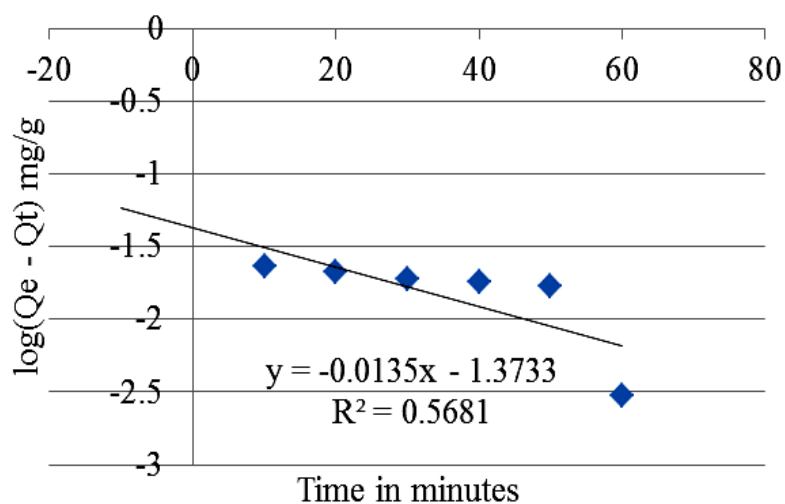


Figure 4.11: Pseudo-first order sorption plot for Cu^{2+} at pH 4

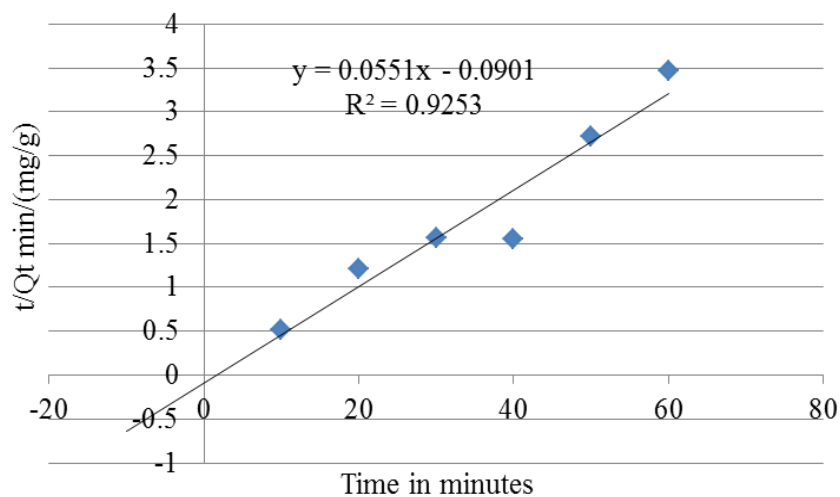


Figure 4.12: Pseudo-second order sorption plot for Cu^{2+} at pH 4

The same procedure was used for biosorption of Cu^{2+} ions by green algae biosorbent at pH values of 7 and 9 and the remaining metal ions at all the pH values.

From the plots for Cu^{2+} R^2 for pseudo 1st order were lower than those for the pseudo 2nd order reaction kinetics implying biosorption by the green algae proceeded by this pseudo second order rate kinetics. The different adsorption parameters for each metal ion at low and high initial concentrations were obtained and used to evaluate the pseudo 1st and 2nd order rate data as presented in Tables 4.6 and 4.7.

Table 4.4: Pseudo 1st and 2nd order rate data for the metal ions at pH 4 for low initial concentration

Metal ion	Pseudo 1 st order rate			Pseudo 2 nd order rate		
	k	Q_e	R^2	k_2	Q_e	R^2
Cu^{2+}	0.012	34.051	0.5681	6.163	0.033	0.9253
Ni^{2+}	0.007	17.270	0.861	2.412	0.109	0.9850
Cd^{2+}	0.062	1.223	0.896	0.1554	4.125	0.9971
Zn^{2+}	0.004	5.904	0.577	4.650	0.137	0.9757

Table 4.5: Pseudo 1st and 2nd order rate data for the metal ions at pH 4 for high initial concentration

Metal ion	Pseudo 1 st order rate			Pseudo 2 nd order rate		
	k	Q_e	R^2	k_2	Q_e	R^2
Cu^{2+}	0.036	7.478	0.541	0.126	2.09	0.9967
Ni^{2+}	0.052	1.23	0.856	0.156	3.14	0.9915
Cd^{2+}	0.003	1.627	0.917	0.309	2.43	0.9640
Zn^{2+}	0.020	2.638	0.878	0.081	2.87	0.95843

From Tables 4.6 and 4.7 the pseudo second order kinetics gave R^2 values that were closer to unity for all the four metals implying that biosorption took place by this mechanism.. This showed that the biosorbent and sorbate (reactants) were both involved in the

biosorption reaction. The values obtained closely correlate with those reported by Bulgariu and Bulgariu (2012). A comparison of R^2 between first and second order at both low and high initial concentrations showed that the second order values were higher than those for first order. Therefore the biosorption of the metal ions by green algae followed pseudo second order kinetics. The order of biosorption from R^2 values was: $\text{Cu}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+} > \text{Zn}^{2+}$ at high initial concentration and at low initial concentration; $\text{Cd}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Cu}^{2+}$, implying that initial concentration affects the order of biosorption of these metal ions and this is supported by Salman & Mehdi (2011).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

- ❖ Contact time influenced the biosorption of Cu^{2+} , Cd^{2+} , Zn^{2+} and Ni^{2+} ions
- ❖ From the results, maximum biosorption of Cu^{2+} , Cd^{2+} , Zn^{2+} and Ni^{2+} was generally observed near pH 7 (optimum pH value recorded at pH 6.5).
- ❖ The order of biosorption was $\text{Cd}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Cu}^{2+}$
- ❖ The equilibrium data for Cu^{2+} , Cd^{2+} , Zn^{2+} and Ni^{2+} ions followed the Langmuir isotherm.
- ❖ Kinetic data was better described by the pseudo-second order mechanism.
- ❖ Green algae biomass effectively biosorbed metal ions
- ❖ The initial metal ion concentration influenced the order of biosorption.

5.2 General Recommendations

Green algae powder which can be obtained cheaply and is easily available should be used as a biosorbent for the removal of heavy metals from contaminated waste waters.

5.2.1 Recommendations for Further Studies

A further study on other pH values at different contact times on different metal pollutants is recommended. It is also prudent that studies be carried out on other alga species to establish the best biosorbent.

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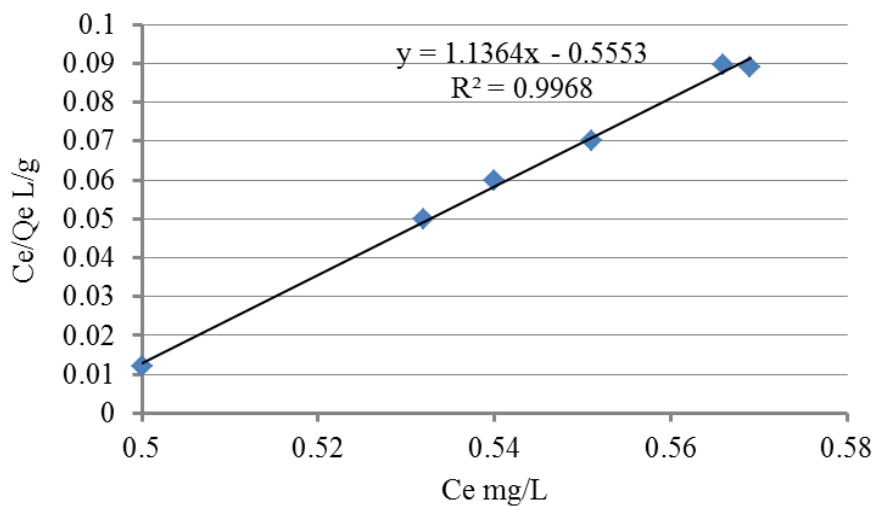
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APPENDICES

Appendix I: Langmuir Isotherm

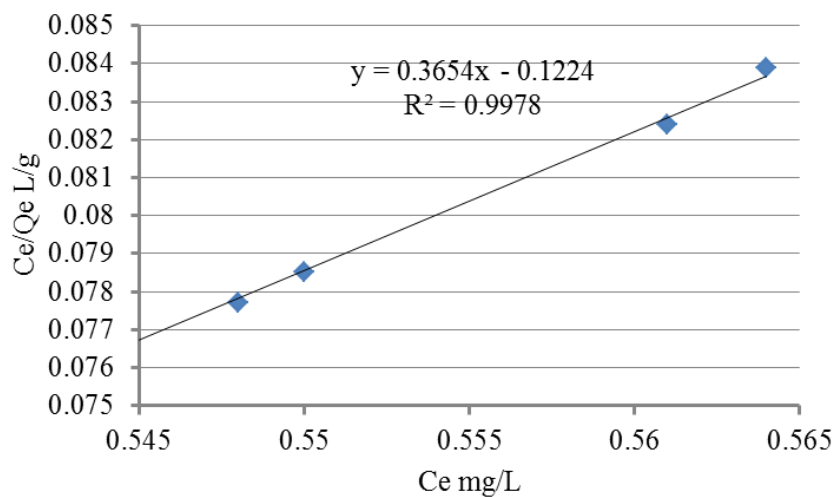
I) Langmuir Isotherm data for Cd^{2+} at pH 4

Ce	Qe	Ce/Qe
0.569	6.72	0.089
0.566	6.8	0.0897
0.551	7	0.07
0.54	7.04	0.06
0.532	7.14	0.05
0.5	7.14	0.012

II) Langmuir plot for Cd^{2+} at pH 4III) Langmuir Isotherm data for Cd^{2+} at pH 7

Ce	Qe	Ce/Qe
0.564	6.72	0.0839
0.561	6.81	0.0824
0.55	7.01	0.0785
0.548	7.05	0.0777
0.543	7.14	0.0761
0.543	7.14	0.0761

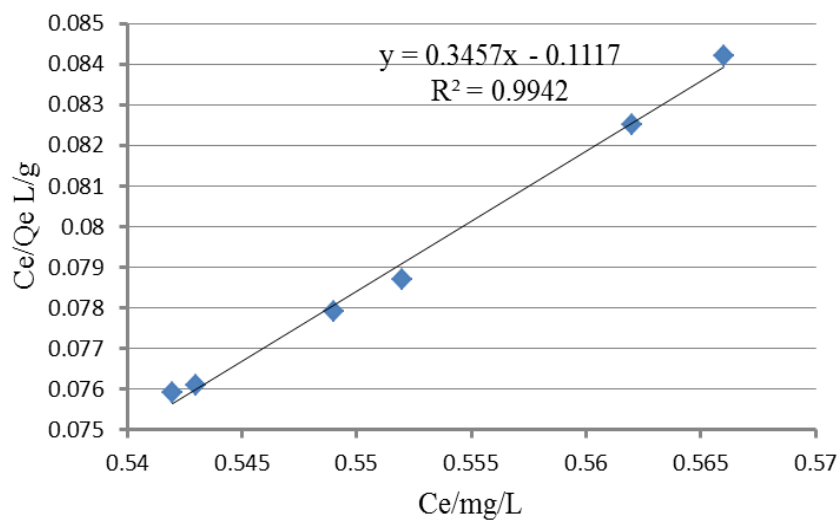
IV) Langmuir plot for Cd²⁺ at pH 7



V) Langmuir Isotherm data for Cd²⁺ at pH 9

Ce	Qe	Ce/Qe
0.566	6.72	0.0842
0.562	6.81	0.0825
0.552	7.01	0.0787
0.549	7.05	0.0779
0.542	7.14	0.0759
0.543	7.14	0.0761

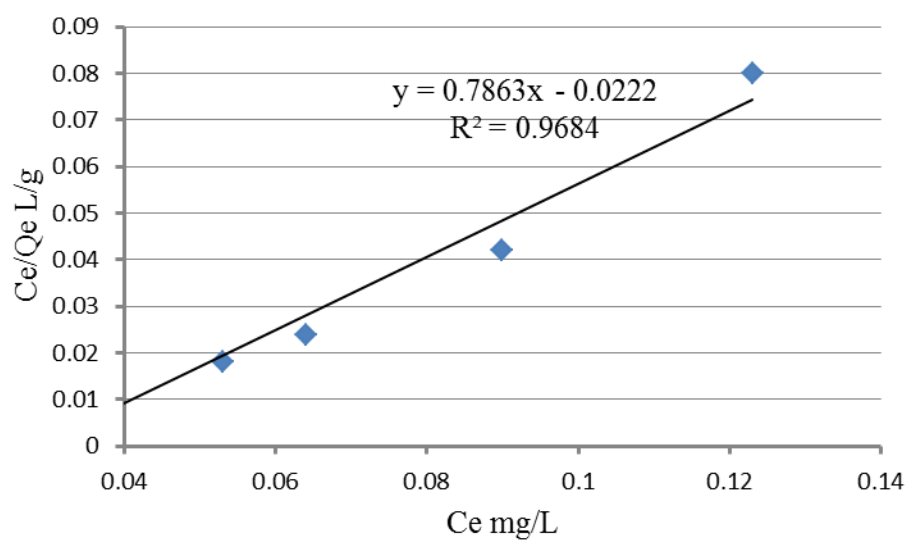
VI) Langmuir plot for Cd²⁺ at pH 9



VII) Langmuir Isotherm data for Ni²⁺ at pH 7

Ce	Qe	Ce/Qe
0.123	1.54	0.08
0.09	2.12	0.042
0.064	2.7	0.024
0.053	3	0.018
0.038	3.38	0.011
0.037	3.39	0.010

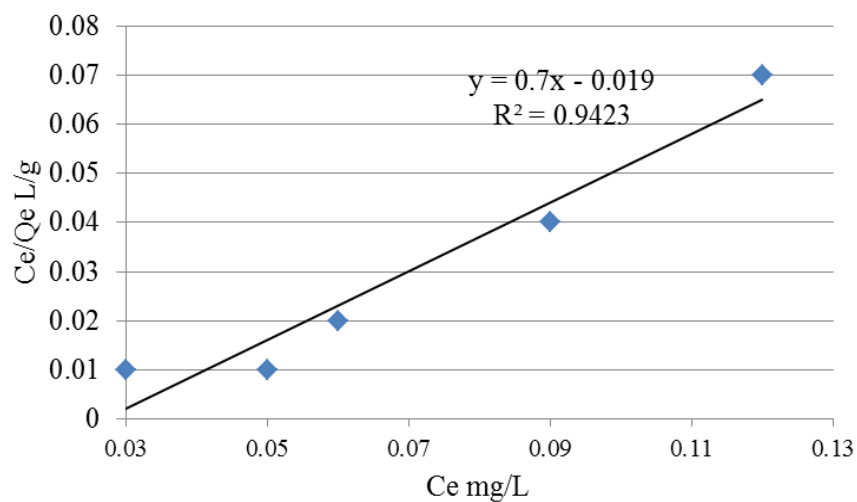
VIII) Langmuir plot for Ni²⁺ at pH 7



IX) Langmuir Isotherm data for Ni²⁺ at pH 9

Ce	Qe	Ce/Qe
0.12	1.54	0.07
0.09	2.12	0.04
0.06	2.7	0.02
0.05	3	0.01
0.03	3.38	0.01
0.03	3.37	0.01

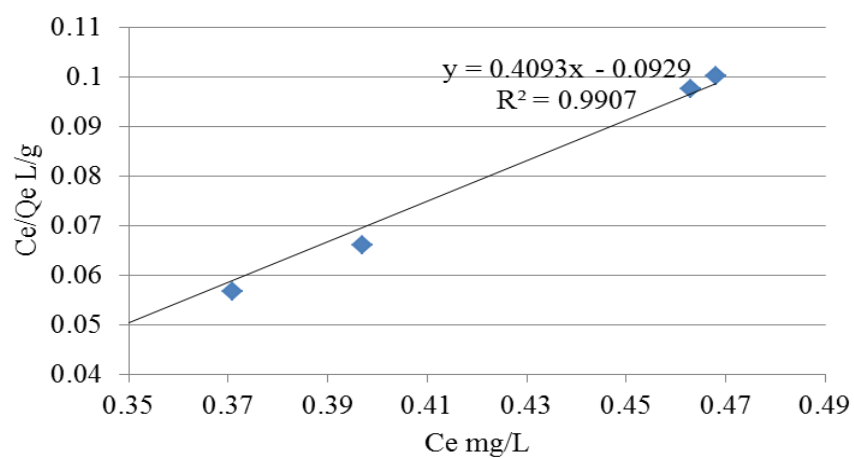
X) Langmuir plot for Ni²⁺ at pH 9



XI) Langmuir Isotherm data for Zn²⁺ at pH 4

Ce	Qe	Ce/Qe
0.468	4.673	0.1001
0.463	4.744	0.0976
0.397	6.011	0.066
0.371	6.541	0.0567
0.335	7.271	0.0461
0.335	7.289	0.046

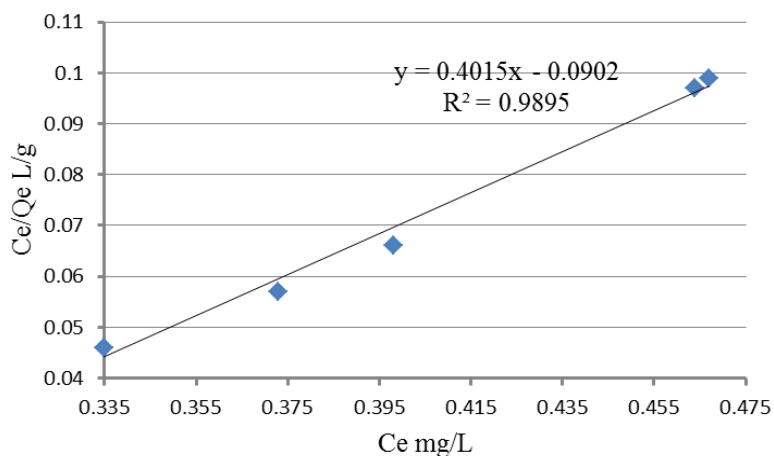
XII) Langmuir plot for Zn²⁺ at pH 4



XIII) Langmuir Isotherm data for Zn²⁺ pH 7

Ce	Qe	Ce/Qe
0.467	4.672	0.099
0.464	4.744	0.097
0.398	6.01	0.066
0.373	6.542	0.057
0.334	7.272	0.046
0.335	7.288	0.046

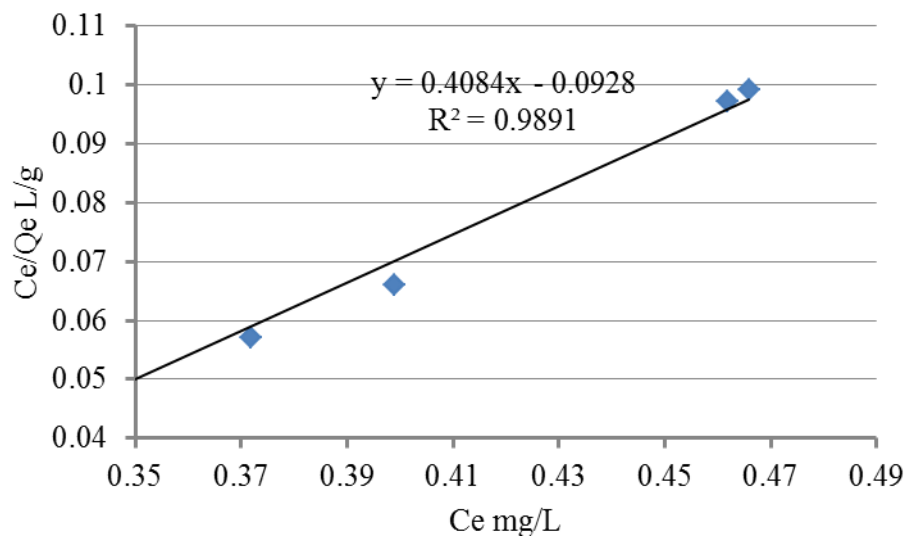
XIV) Langmuir plot for Zn²⁺ at pH 7



XV) Langmuir Isotherm data for Zn²⁺ at pH 9

Ce	Qe	Ce/Qe
0.466	4.672	0.099
0.462	4.744	0.097
0.399	6.01	0.066
0.372	6.542	0.057
0.336	7.272	0.046
0.335	7.288	0.046

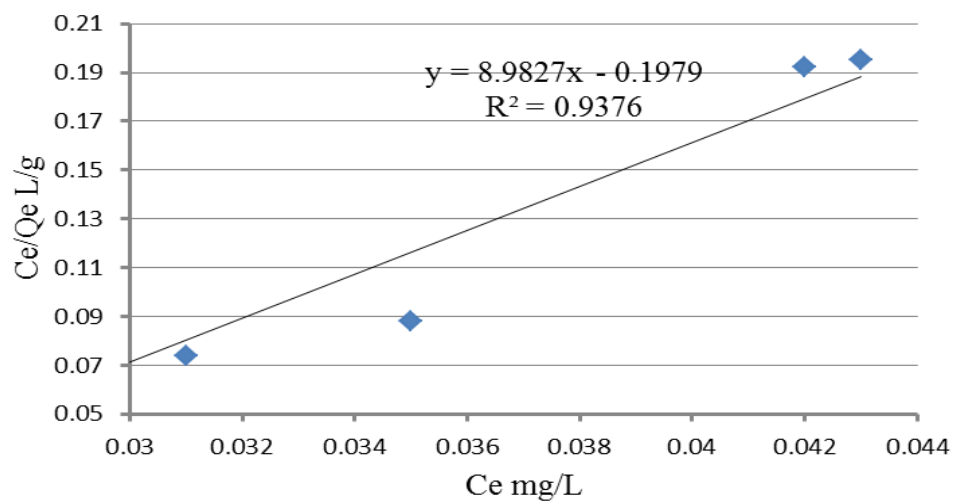
XVI) Langmuir plot for Zn²⁺ at pH 9



XVII) Langmuir Isotherm data for Cu²⁺ at pH 7

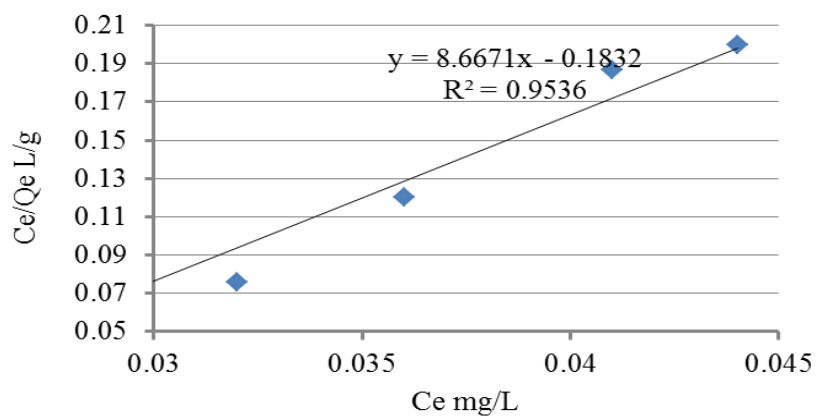
Ce	Qe	Ce/Qe
0.043	0.22	0.195
0.042	0.219	0.192
0.035	0.4	0.088
0.031	0.42	0.074
0.029	0.48	0.06
0.025	0.56	0.045

XVIII) Langmuir plot for Cu²⁺ at pH 7

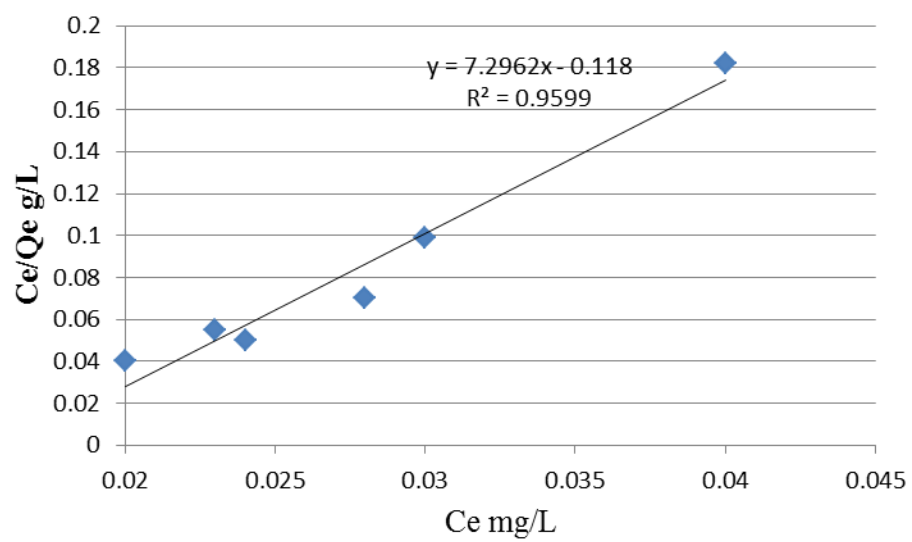


XIX) Langmuir Isotherm data for Cu²⁺ at pH 9

Ce	Qe	Ce/Qe
0.044	0.22	0.2
0.041	0.219	0.187
0.036	0.3	0.12
0.032	0.42	0.076
0.029	0.48	0.06
0.024	0.56	0.043

XX) Langmuir plot for Cu²⁺ at pH 9**XXI) Langmuir Isotherm data for Cu²⁺ at pH 4**

Ce	Qe	Ce/Qe
0.04	0.22	0.182
0.03	0.38	0.099
0.028	0.4	0.07
0.023	0.42	0.055
0.024	0.48	0.05
0.02	0.56	0.04

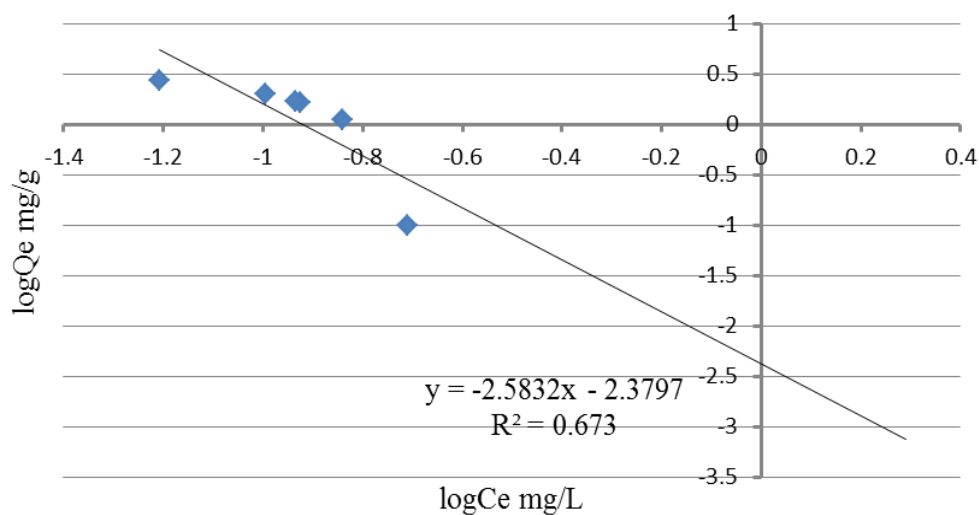
XXII) Langmuir plot for Cu^{2+} at pH 4

Appendix II: Freundlich Isotherm

I) Freundlich data for Ni²⁺ at pH 4

TIME	Ce	logCe	Qe	logQe
10	0.195	-0.709	0.1	-1
20	0.144	-0.841	1.12	0.049
30	0.119	-0.924	1.62	0.209
40	0.116	-0.935	1.68	0.225
50	0.101	-0.995	1.98	0.296
60	0.062	-1.208	2.76	0.44

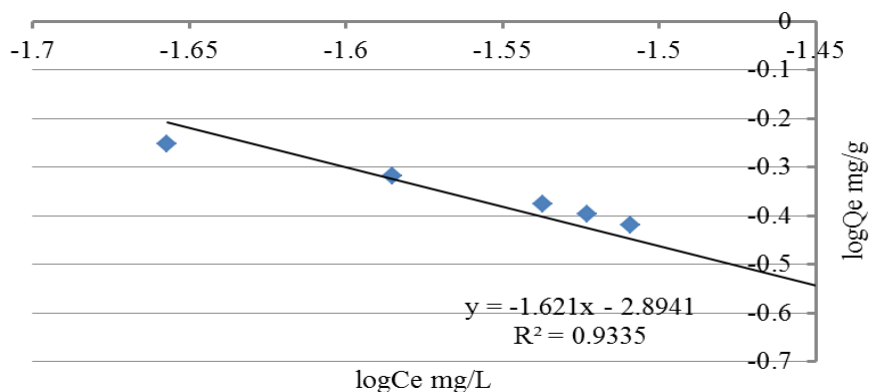
II) Freundlich plot for Ni²⁺ at pH 4



III) Freundlich data for Cu²⁺ at pH4

TIME	Ce	Qe	logCe	logQe
10	0.039	0.22	-1.409	-0.657
20	0.031	0.38	-1.509	-0.42
30	0.03	0.4	-1.523	-0.397
40	0.029	0.42	-1.537	-0.376
50	0.026	0.48	-1.585	-0.318
60	0.022	0.56	-1.657	-0.251

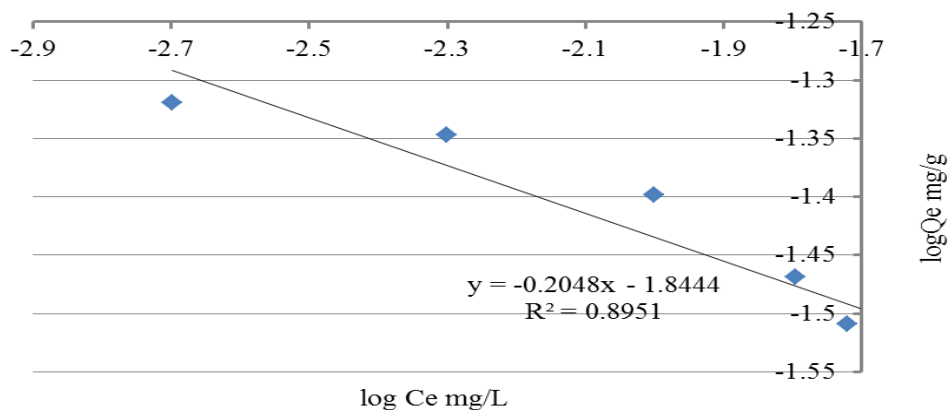
IV) Freundlich plot for Cu^{2+} at pH 4



V) Freundlich data for Cu^{2+} at pH 7

TIME	Ce	log Ce	Qe	logQe
10	0.02	-1.699	0.03	-1.523
20	0.019	-1.721	0.031	-1.509
30	0.016	-1.796	0.034	-1.469
40	0.01	-2	0.04	-1.398
50	0.005	-2.301	0.045	-1.347
60	0.002	-2.699	0.048	-1.319

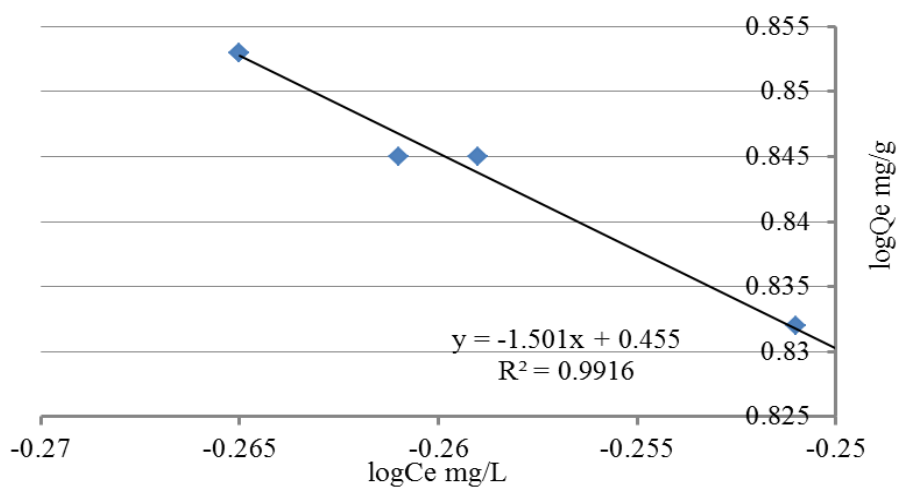
VI) Freundlich plot for Cu^{2+} at pH 7



VII) Freundlich data for Cd^{2+} at pH 4

TIME	Ce	logCe	Qe	logQe
10	0.564	-0.248	6.72	0.827
20	0.56	-0.251	6.8	0.832
30	0.55	-0.259	7	0.845
40	0.548	-0.261	7.04	0.845
50	0.543	-0.265	7.14	0.853
60	0.543	-0.265	7.14	0.853

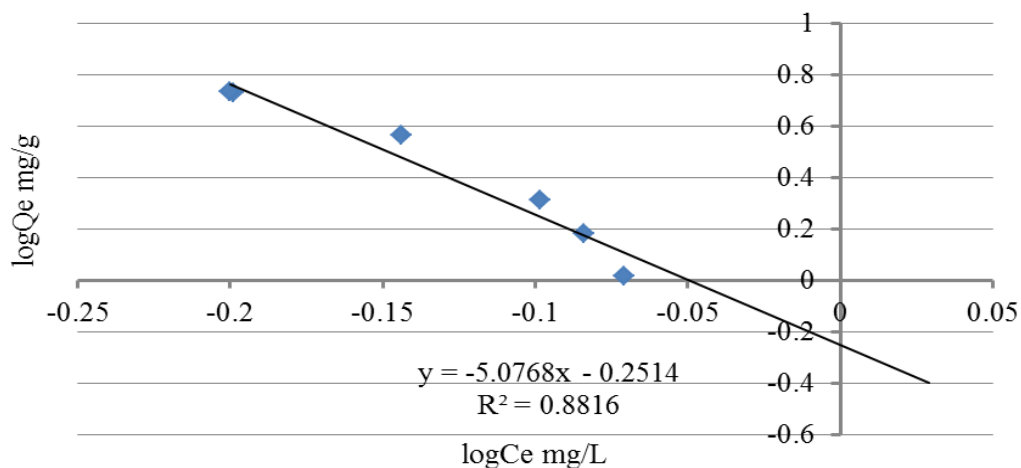
VIII) Freundlich plot for Cd^{2+} at pH 4



IX) Freundlich data for Cd^{2+} at pH 7

Time	Ce	logCe	Qe	logQe
10	0.848	-0.071	1.04	0.017
20	0.824	-0.084	1.52	0.181
30	0.797	-0.0985	2.06	0.313
40	0.717	-0.144	3.66	0.563
50	0.632	-0.199	5.36	0.729
60	0.63	-0.2	5.4	0.732

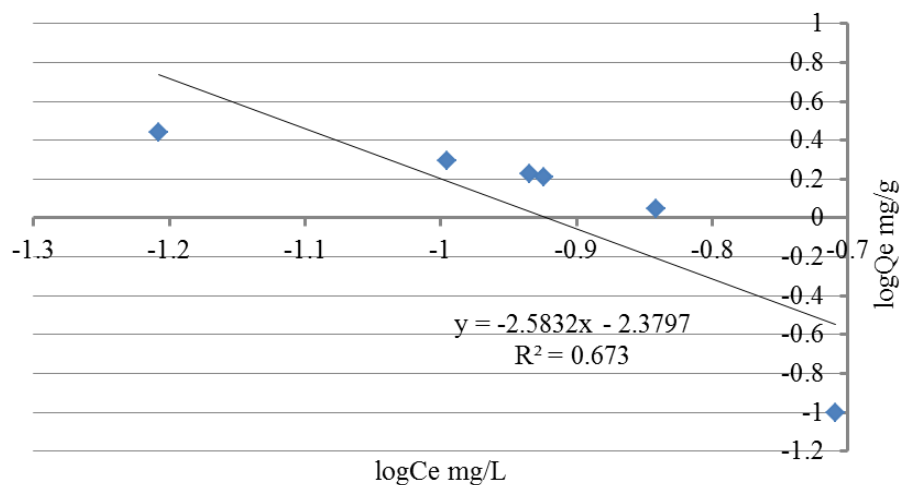
X) Freundlich plot for Cd²⁺ at pH 7



XI) Freundlich data for Ni²⁺ at pH 4

TIME	Ce	logCe	Qe	logQe
10	0.195	-0.709	0.1	-1
20	0.144	-0.841	1.12	0.049
30	0.119	-0.924	1.62	0.209
40	0.116	-0.935	1.68	0.225
50	0.101	-0.995	1.98	0.296
60	0.062	-1.208	2.76	0.44

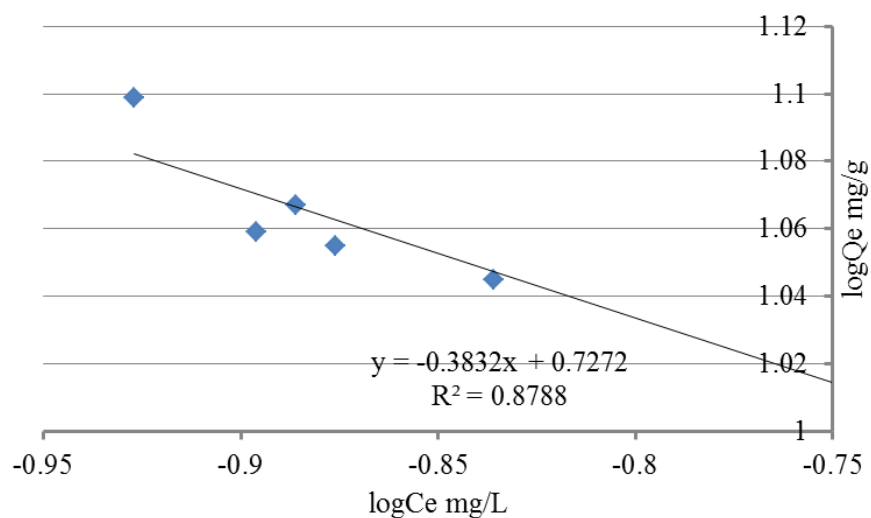
XII) Freundlich plot for Ni²⁺ at pH 4



XIII) Freundlich data for Ni²⁺ at pH 7

TIME	Ce	logCe	Qe	logQe
10	0.186	-0.728	10.26	1.011
20	0.145	-0.836	11.08	1.045
30	0.132	-0.876	11.34	1.055
40	0.129	-0.886	11.4	1.067
50	0.126	-0.896	11.46	1.059
60	0.118	-0.927	11.64	1.099

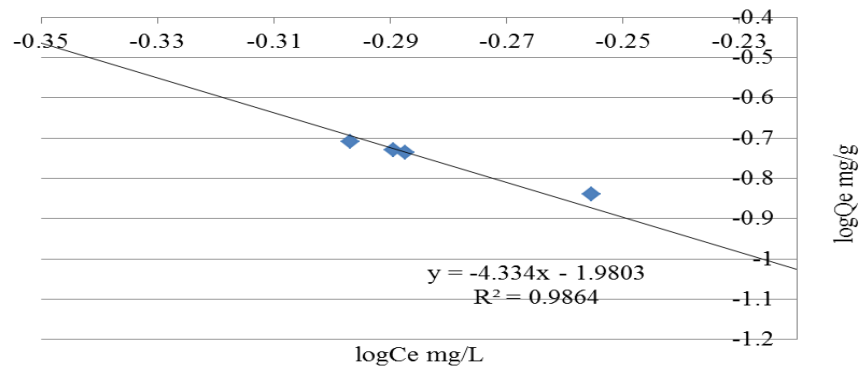
XIV) Freundlich plot for Ni²⁺ at pH 7



XV) Freundlich data for Zn²⁺ at pH 4

TIME	Ce	logCe	Qe	logQe
10	0.6225	-0.2059	0.0775	-1.111
20	0.6072	-0.2167	0.0928	-1.032
30	0.5554	-0.2554	0.1446	-0.8398
40	0.516	-0.2874	0.184	-0.7352
50	0.5135	-0.2894	0.1865	-0.7293
60	0.5048	-0.2969	0.1952	-0.7095

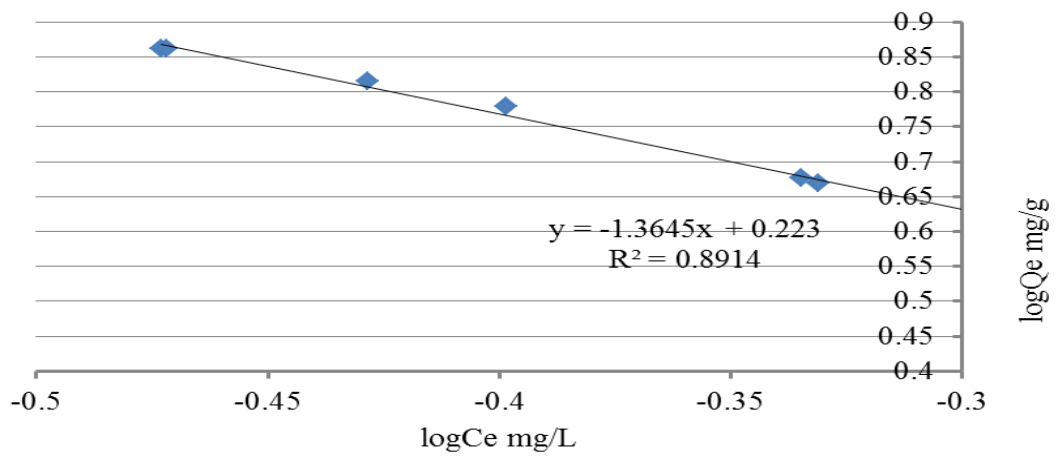
XVI) Freundlich plot for Zn²⁺ at pH 4



XVII) Freundlich data for Zn²⁺ at pH 7

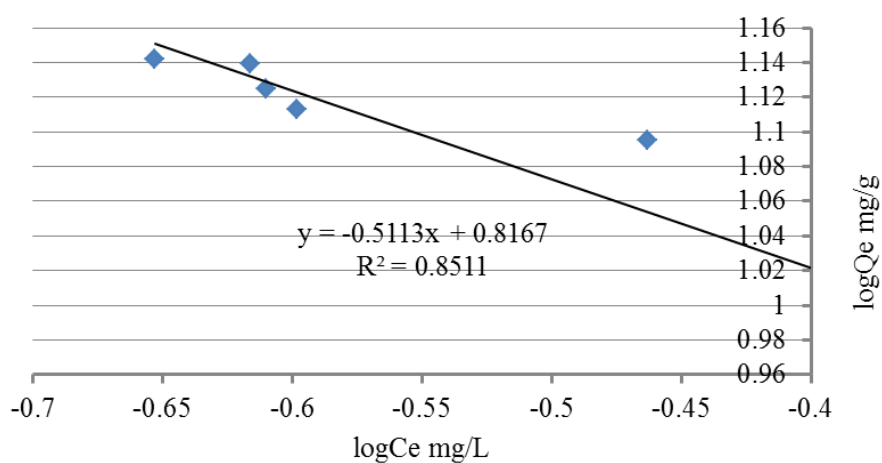
TIME	Ce	logCe	Qe	logQe
10	0.4664	-0.3312	4.672	0.6695
20	0.4628	-0.3347	4.744	0.6761
30	0.3995	-0.3985	6.01	0.7789
40	0.3729	-0.4284	6.542	0.8157
50	0.3364	-0.4731	7.272	0.8617
60	0.3356	-0.472	7.288	0.8626

XVIII) Freundlich plot for Zn²⁺ at pH 7



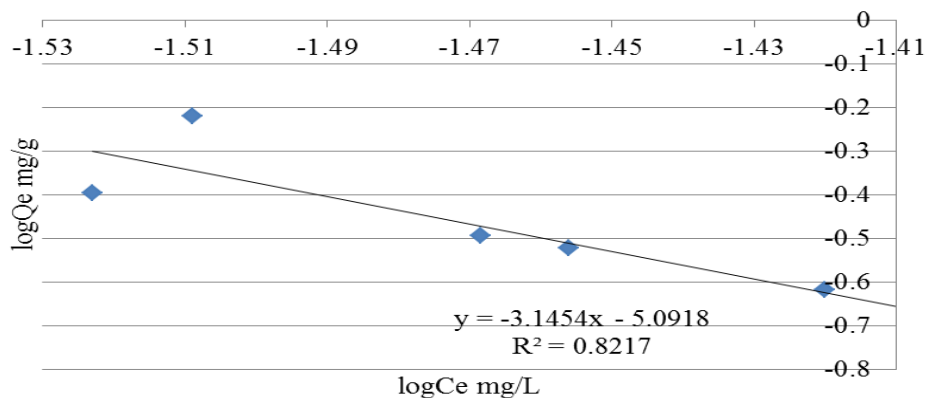
XIX) Freundlich data for Cd²⁺ at pH 9

TIME	Ce	logCe	Qe	logQe
10	0.42	-0.376	9.6	0.982
20	0.252	-0.598	12.96	1.113
30	0.222	-0.653	13.56	1.142
40	0.217	-0.463	13.66	1.095
50	0.218	-0.6101	13.64	1.125
60	0.202	-0.616	13.16	1.139

XX) Freundlich plot for Cd²⁺ at pH 9**XXI) Freundlich data for Cu²⁺ at pH 9**

TIME	Ce	logCe	Qe	logQe
10	0.42	-0.376	9.6	0.982
20	0.252	-0.598	12.96	1.113
30	0.222	-0.653	13.56	1.142
40	0.217	-0.463	13.66	1.095
50	0.218	-0.6101	13.64	1.125
60	0.202	-0.616	13.16	1.139

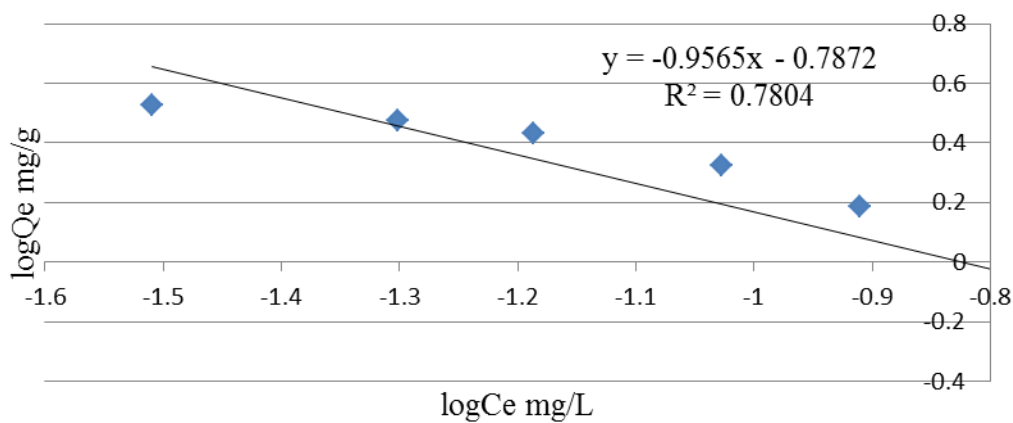
XXII) Freundlich plot for Cu^{2+} at pH 9



XXIII) Freundlich data for Ni^{2+} at pH 9

TIME	Ce	$\log C_e$	Qe	$\log Q_e$
10	0.173	-0.762	0.54	-0.267
20	0.123	-0.91	1.54	0.187
30	0.094	-1.027	2.12	0.326
40	0.065	-1.187	2.7	0.431
50	0.05	-1.301	3	0.477
60	0.031	-1.509	3.38	0.528

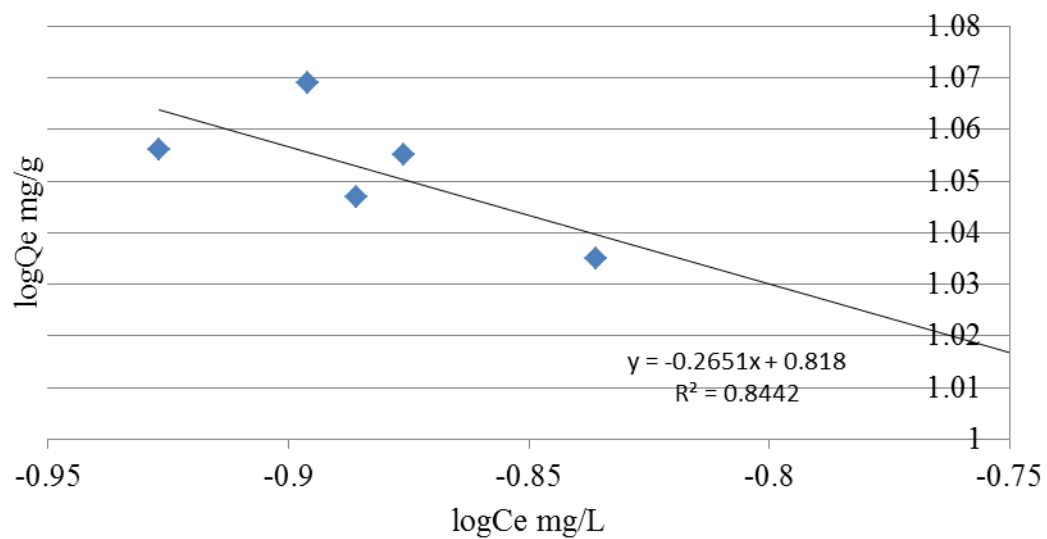
XXIV) Freundlich plot for Ni^{2+} at pH 9



XXV) Freundlich data for Zn²⁺ at pH 9

TIME	Ce	LogCe	Qe	logQe
10	0.186	-0.728	10.26	1.011
20	0.145	-0.836	11.08	1.035
30	0.132	-0.876	11.34	1.055
40	0.129	-0.886	11.4	1.047
50	0.126	-0.896	11.46	1.069
60	0.118	-0.927	11.64	1.056

XXVI) Freundlich plot for Zn²⁺ at pH 9



Appendix III: Pseudo first order reaction data for Cu^{2+} at pH 4

Time in minutes	10	20	30	40	50	60
$\log(Q_e - Q_t)$ mg/g	-1.638	-1.678	-1.721	-1.745	-1.770	-2.523

Appendix IV: Pseudo second order reaction data for Cu^{2+} at pH 4

Time in minutes	10	20	30	40	50	60
t/Qt Min/(mg/g)	0.516	1.212	1.56	1.55	2.715	3.471

Appendix V: WHO, FAO and KEBS heavy metal maximum permissible limits

Element	WHO and FAO maximum permissible limits (ppm)	KEBS maximum permissible limits (ppm)
Ni	0.01	0.1
Cd	0.003	N/A
Cu	1	0.1
Zn	5	5

Appendix VI: Physical properties of elements that were analyzed

Element	Atomic number	Mass number	M.P. °C	B.P. °C	Density (g/cm ³)
Ni	28	58.693	1453	2730	8.9
Cd	48	112.4	321	765	8.65
Zn	30	65.38	420	907	7.13
Cu	29	64	1083	2595	8.96

Appendix VII: Formula for calculation of stock solution (1000 ppm) metal ion concentration

$$\frac{1 \text{ gram of element dissolved in } 12\% \text{ Nitric acid}}{\text{R.A.M of element to be analysed}} \times \text{RFM of the salt used} = \text{Mass of salt.}$$

This gives 1.000 µg/ml or 1000 ppm at density range of 1.01 – 1.02 g/ml at 20⁰ C-25⁰C

Appendix VIII: Dilution formula of stock solution

$$C_1V_1 = C_2V_2,$$

Where,

C_1 = Initial concentration of the stock solution

V_1 = Initial volume of the stock

C_2 = Final concentration required of analyte

V_2 = Final volume required to prepare the required standard solution