BIOSORPTION OF SELECTED HEAVY METALS FROM AQUEOUS SOLUTIONS BY DEAD BIOMASS OF FRESH WATER GREEN ALGAE *Cosmarium panamense*

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DEPARTMENT OF CHEMISTRY,

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

AUGUST, 2011

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NIGERIA

AUGUST, 2011

DECLARATION

I declare that the work in the thesis entitled "**BIOSORPTION OF SELECTED HEAVY METALS FROM AQUEOUS SOLUTIONS BY DEAD BIOMASS OF FRESH WATER GREEN ALGAE *Cosmarium panamense***" has been performed by me in the Department of Chemistry under the supervision of Prof. V. O. Ajibola and Prof. J.A. Kagbu. The informations derived from the literature have been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university or institution.

Onwuka, Jude Chinedu

| --- | --- | --- |
| Name of Student | Signature | Date |

CERTIFICATION

This project thesis entitled "**BIOSORPTION OF SELECTED HEAVY METALS FROM AQUEOUS SOLUTIONS BY DEAD BIOMASS OF FRESH WATER GREEN ALGAE *Cosmarium panamense***" by Onwuka Jude Chinedu meets the regulations governing the award of degree of M.Sc. Analytical Chemistry of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to the Almighty God.

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All glory, honour, power and majesty to Almighty God, whose mercies, favours, guidiance and protection have been my strength.

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ABSTRACT

This study investigated biosorption of Cd(II), Cr(VI), Co(II), Pb(II), and Ni(II) ions by fresh water dead algal biomass *Cosmarium panamense* from aqueous solution. Batch experiments were conducted to determine the biosorption behaviour of the biomass and it was observed that the adsorption capacity for Cd(II), Cr(VI), Co(II), Pb(II), and Ni(II) ions by the biomass are initial metal ion concentration, biosorbent dose, shaking time, pH and temperature dependence. The maximum adsorption capacities for Cd(II), Cr(VI), Co(II), Pb(II), and Ni(II) ions are 12.89mgCd/g, 109.9mgCr/g, 16.69mgCo/g, 19.72mgPb/g and 10.73mgNi/g of the biomass respectively at initial concentration of 20mg/l and pH of 5.05 for Cd(II), 2.05 for Cr(VI), 4.05 for Co(II), 4.02 for Pb(II), and 5.02 for Ni(II). The equilibrium data of the selected metals was well described by Langmuir isotherm model, which was used to determine thermodynamic parameters (free enegy change, enthalpy change and entropy change) of the metal ions sorption process. The nature of the sorption process was found to be endothermic and spontaneous. Uptake kinetics of the selected heavy metal ions follow pseudo - second order model. Effect of biosorption on various properties of the algal biomass, as adsorbent, was characterized by Neutron Activation Analysis (NAA) and Energy dispersive X – ray (EDAX), Thermal Gravimetric Analysis – Differential Scanning Calorimetry (TGA – DSC), Scanning Electron Microscope (SEM) images and Fourier Transform Infra – red (FTIR). EDAX revealed variation in the amount or number of elements available for detection before and after biosorption while TGA – DSC showed that heat flow absorbed by the heavy metal treated algal biomass, to begin decomposition at the same temperature with the native biomass, are higher than the heat absorbed by the native biomass. TGA – DSC also revealed that percent residue weight of the native algal biomass, remaining after decomposition is higher than that of the

various heavy metal treated algal biomass samples. FTIR analysis showed the presence of amino, carboxyl, hydroxyl, carbonyl, phosphonate, silica, methyl and methylene functional group which are responsible for the biosorption of these metal ions on the algal biomass. This results indicate that the biomass of *Cosmarium panamense* is an efficient biosorbent for the removal of Cd(II), Cr(VI), Co(II), Pb(II) and Ni(II) ions from aqueous solutions.

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ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
DSC	Differential Scanning Calorimetry
EDAX	Energy Dispersive X - ray
EPA	Environment Protection Agency
FTIR	Fourier Transform Infrared
NAA	Neutron Activation Energy
SEM	Scanning Electron Microscope
STA	Simultaneous Thermal Analysis
TGA	Thermal Gravimetric Analysis

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Study

The major characteristics of developed countries and primary desires of third world countries or developing countries are industrialization and urbanization. This, though brought development but economic solution to its disadvantages has become a global problem. A high degree of industrialization and urbanization have substantially enhanced the degradation of aquatic environments through the discharge of industrial wastewater and domestic wastes. This has resulted in significant amount of heavy metals being deposited into natural aquatic and terrestrial ecosystems. It has also increased the biological cycling of toxic heavy metals (Nilanjana *et al.,* 2008). Modern industry is to a large degree, responsible for contamination of the environment. Lakes, rivers and oceans are being overwhelmed with bacteria and waste matter. Among toxic substances reaching hazardous levels are heavy metals. Some inland water bodies in Europe, America, and Niger Delta Region in Nigeria are closed for fishing. Newspapers are full with reports of frequent ecological disasters in marine environments (Vieira and Volesky, 2000).

It was only in 1990's that a new scientific area, biosorption; was developed to help recover heavy metals. The first reports described how abundant biological materials could be used to remove , at very low cost, even small amounts of toxic heavy metals from industrial effluents. Metal sequestering properties of non – viable biomass provide basis for a new approach to remove heavy metals when they occur at low concentration (Volesky, 1990).

During evolution, aquatic and terrestrial organisms have develop diverse strategies to maintain an equilibrated relation with heavy metal ions present and available in the surrounding medium. Cells face two tasks, the first is to select those heavy metals essential for growth and exclude those that are not, and the second to keep essential ion at optimal intracellular concentrations (Cobbett and Goldsbrough, 2002).

Land plants, aquatic plants and algae have all attracted considerable attention for the capacity to eliminate metals. Much of the knowledge concerning algae is based in observations of higher plants. Research has shown ecological importance of algae chelating mechanisms in real environments (Ahner *et al.,*1995). Microalgae, related eukaryotic photosynthetic organisms and some fungi have preferentially developed the production of peptides capable of binding heavy metals. These molecules as organometallic complexes , are further partitioned inside vacuoles to facilitate appropriate control of the cytoplasmic concentration of heavy metal ions, thus preventing or neutralizing their potential toxic effect (Cobbett and Goldsbrough, 2002).

1.2 Algae

Algae are primitive aquatic plants with no vascular structure, contain chlorophyll, and largely dependent upon water for their sustenance and reproduction. They range in size from microscopic cells, the size of bacteria to the massive fronds of the kelps, which are the tallest marine plants known. Those that float freely on water are known as phytoplankton and are generally single-celled and microscopic, although some species are large enough to be observed by the naked eye. Multi-celled or macrophytic algae (large algae or all seed- bearing plants) are usually attached to the bottom or other solid substrate (benthic). Algae occupy

either freshwater or marine environments, sometimes both, and some species can tolerate relatively extreme conditions of temperature and salinity. In addition, some can be found in moist terrestrial habitats. Because they are immersed, algae do not require specialized tissues for internal transport, as do higher land plants, but obtain their nutrients directly from the surrounding water by diffusion. Algae also use water, much as some land plants use wind, to achieve fertilization and dispersal of the reproductive products (Nova Scotia Museum of Natural History, 1994).

In freshwater, phytoplankton mainly diatoms, green algae, and blue green algae, is the most abundant form of plant life in the open- water habitats, although less obvious than the benthic macrophytes. The numerous species differ in the depths they inhabit, the type of nutrients they require, their growth rates, and reproductive seasons. Freshwater Algae often give rise to a greenish tinge in lakes, and ponds, by multiplying rapidly to create what are known as algal blooms at the surface (Nova Scotia Museum of Natural History, 1994).

In marine environment, microscopic algae of various kinds live in the upper layers of the ocean, in a huge but inconspicuous biomass, and contribute oxygen to the surface water. Marine phytoplanktons are of basic importance to ocean ecosystems since they are primary food source for oceans and support major offshore fisheries. Marine algae contribute oxygen to the water and also provide shelter to various marine animals. Larger species protect coastlines by moderating waves and current (Darley, 1982).

1.2.1 Types of Algae

Early attempts to divide the algae into groups relied on their pigmentation (and this still applies to most groups) but modern taxonomy relies on other characteristics as well. Algae are

extremely diverse in form, colour, habit and habitat. According to Ontario Ministry of Environment (2004); Algae is generally broken into four different groups:

- Diatoms (Bacillariophyceae)
- Green Algae (Chlorophyceae)
- Blue Green Algae (Cyanophyceae)
- Pigmented Flagellates (Dinophyceae, Cryptophyceae, Chrysophyceae, Euglenophyceae)

Diatoms (Bacillariophyceae)

Diatoms are algae commonly found in both fresh and salt waters. A feature that helps distinguish them from other algae is the silicon wall that encloses them. These silicon walls are often marked with intricate patterns. The population growth rate depends on the amount of silica in the water column. Diatoms are the main food source for many aquatic microscopic animals. Diatoms are perhaps the most important group of marine algae, as they indirectly support most of the world's major fisheries. This is the group of algae most likely to cause filter- clogging problems. The rigid silica walls of diatoms are not subject to decomposition. Some diatoms produce tastes and odour in the water. When taste and odour are a problem in less eutrophic northern lakes, diatoms may be the cause. Some commonly found diatoms are *Asterionella* (which floats in the water like parachute), *Stephanodiscus* (often shaped like a disk), *Coscinodiscus, Nitzschia Fragilaria*, and *Cyclotella* (Ontario Ministry of Environment, 2004).

Green Algae (Chlorophyceae)

Green Algae are primarily of two types. One is attached and the other is free-floating. Filamentous greens (or attached algae) range from several millimeters to a meter in length. In many cases they are not found as isolated filaments but develop into large colonies of floating or attached mats. A few have been given common names such as pond silk, green felt, frog-spawn algae and stoneworts. These may produce a slime that can interfere with some industrial uses of water. They may also benefit the lake, along with other algae, to help purify the water and maintain a favorable oxygen level. Some examples of filamentous green algae are *Spirogyra, Mougeotia and Cladophora*. These can also be responsible for causing odors in water and filter clogging. Free-floating green algae are important in the aquatic food chain and help to maintain oxygen levels. Some examples of free-floating algae are *Chlorella, Pediastrum,* and the desmids (Ontario Ministry of Environment, 2004). *Spirogyra* and desmids are important to the ecology of lakes, often producing an enormous amount of biomass where levels of nitrates and phosphates are high (Nova Scotia Museum of Natural History, 1994). Green algae are also found in moist terrestrial habitats. The genus *prococcus* lives on tree trunks and is particularly conspicuous after rain (Ontario Ministry of Environment, 2004).

Blue Green Algae (Cyanophyceae)

Blue Green Algae are unicellular, colonial or filamentous. Some forms are gelatinous masses of various shapes floating in the water. When a "bloom" of blue-greens develops, the algae sometimes drift into bays or along beaches where they decompose. Soluble phyco – biliprotein pigments can be released when cells break, giving the water a bluish or pinkish. They have a colour. pleasant grassy odour while healthy, but this may change to an unpleasant

musty smell or to a rather revolting foul odour upon disintegration and decomposition. Poisonings of animals and waterfowl have been attributed to the ingestion of lethal doses of toxic blue-greens accumulating along the shores of lakes. There are three common types of blue-greens that appear to be primarily responsible for most of these incidents: *Anabaena, Microcystis and Aphanizomenon* (Ontario Ministry of Environment, 2004).

Pigmented Flagellates (Dinophyceae, Cryptophyceae, Chrysophyceae, - Euglenophyceae)

All flagellated algae possess one or more flagella per cell. A flagellum is a whip-like appendage that acts as a propeller. These algae can sometimes produce strong tastes and odours when present in water supplies. For example, *Synura*, a yellow, colonial flagellated alga can impart a perceptible cucumber odour to raw water even when present in low numbers. Some commonly found flagellates are *Synura, Dinobryon and Euglena* (Ontario Ministry of Environment, 2004).

Red Algae (Rhodophyta)

Red Algae are mostly macrophytic and are associated with the marine environment. Several single- celled genera are known, from both marine and freshwaters. Red algae form a common group of seaweeds occurring in the intertidal zones and below the low – watermark on rocky shores. Their pigment allows them to use light that greener than the brown or green algae can use, enabling their growth at greater depths. They include leafy, filamentous branched and crustose forms. Several species mostly crusts, have calcareous walls and are the so - called coralline algae, *Corallina officinalis* and *Lithothamnium spp* (Catling *et al*., 1986; Bird and McLachlan, 1992).

Brown Algae (Phaeophyta)

This are exclusively marine and are the dominant group of seaweeds found in the intertidal subtidal zones of hard- bottom shores, particularly in areas exposed to wave and surf activity but also in tidal marshes and on stones on muddy shores. They are highly variable in form, ranging from thin crusts and tiny, intricately branching species to the heavy, thick blades of kelps, which are the largest seaweeds. The rockweeds *Ascophyllum nodosum* and *Fucus* spp. are the most common intertidal algae, while the kelps (*Laminaria digitata, L. longicruris, Agarum clathratum,* and *Alaria esculenta*) are found in the subtidal zone of rocky shores (Bird, 1993).

1.2.2 Uses of Algae

Fertilizer

Algae are used by humans in many ways. They are used as fertilizers, soil conditioners and are a source of livestock feed. Because many species are aquatic and microscopic, they are cultured in clear tanks or ponds and either harvested or used to treat effluents pumped through the ponds (McHugh, 2003).

Energy source

- Algae can be used to make biodiesel (see algaculture), bioethanol and biobutanol and by some estimates can produce vastly superior amounts of vegetable oil, compared to terrestrial crops grown for the same purpose.
- Algae can be grown to produce hydrogen. In 1939 a German researcher named Hans Gaffron, while working at the University of Chicago, observed that the algae he was

studying, *Chlamydomonas reinhardtii* (a green-algae), would sometimes switch from the production of oxygen to the production of hydrogen.

- Algae can be grown to produce biomass, which can be burned to produce heat and electricity (Lewis *et al*., 1988).

Pollution control

- Algae are used in wastewater treatment facilities, reducing the need for greater amounts of toxic chemicals than are already used.
- Algae can be used to capture fertilizers in runoff from farms. When subsequently harvested, the enriched algae itself can be used as fertilizer.
- Algae Bioreactors are used by some powerplants to reduce CO_2 emissions. The CO_2 can be pumped into a pond, or some kind of tank, on which the algae feed. Alternatively, the bioreactor can be installed directly on top of a smokestack (Lewis *et al*., 1988).

Stabilizing substances

Chondrus crispus, (probably confused with *Mastocarpus stellatus*, common name: Irish moss), is also used as "carrageen". The name carrageenan comes from the Irish Gaelic for *Chondrus crispus*. It is an excellent stabilizer in milk products - it reacts with the milk protein caesin, other products include: petfoods, toothpaste, ice-creams and lotions etc. Alginates in creams and lotions are absorbable through the skin (Bigongo *et al.,* 2002).

Nutrition

Seaweeds are an important source of food, especially in Asia; They are excellent sources of many vitamins including: A, B1, B2, B6, niacin and C. They are rich in iodine, potassium, iron, magnesium and calcium (Simoons, 1991). Algae is commercially cultivated as a nutritional supplement. One of the most popular microalgal species is Spirulina *(Arthrospira platensis),* which is a Cyanobacteria (known as blue-green algae), and has been hailed by some as a superfood. Other algal species cultivated for their nutritional value include; Chlorella (a green algae),and Dunaliella (*Dunaliella salina*), which is high in beta-carotene and is used in vitamin C supplements (Morton, 2008).

1.3 Adsorption

Adsorption is the interactive force of physical attraction between the surface of a porous solid and a component molecule being removed from the bulk phase. The most fundamental property of adsorbate-adsorbent interaction is the adsorption equilibrium. The equilibrium at a given temperature is usually presented under the form of adsorption isotherms, which are useful for selecting the most appropriate adsorbent and also for predicting the performance of adsorption system (Ribeiro *et al*., 2001).

A physical-chemical process that involves the mass transfer of a solute (adsorbate) from the fluid phase to the adsorbent surface till the thermodynamic equilibrium of the adsorbate concentrations is attained with no further net-adsorption is a general expression for adsorption (Ribeiro *et al*., 2001). It involves the interphase accumulation or concentration of substances at a surface or interface between any two phases such as liquid-liquid, gas-solid, or liquid-solid

interfaces (Cheremisinoff and Ellerbusch, 1978). The material being concentrated or adsorbed is the adsorbate and the adsorbing phase is termed adsorbent.

1.3.1 Theory of adsorption

Adsorption is the formation of a layer of gas, liquid or solid on the surface of a solid or less frequently of a liquid (Daintith, 2000). Adsorption occurs at solid-solid, gas-solid, solid-liquid, liquid-solid, liquid-liquid, liquid-gas interfaces (Cheremisinoff and Ellerbusch, 1978).

1.3.2 Types of adsorption

There are two main types of adsorption, these are physisorption and chemisorptions.

Physisorption

This is an interaction between adsorbate and adsorbent in which non-specific forces of attraction such as dispersion or dipole is involved and the binding energy of the adsorbate is less than 20kJ/mol (Atkins, 2003; Adamson, 1960). These forces are non specific since they do not depend on any property of either the adsorbate or the adsorbent (Parfitt and Sing, 1976). Thus, physical adsorption takes place with the help of van der Waals' forces. It occurs in minutes and the process is reversible. Multilayers are easily formed at higher pressure/concentration in physisorption due to small heat of adsorption (Wedler, 1976; Atkins, 2003).

Chemisorption

This is a type of adsorption in which some degree of specific chemical interaction between the adsorbate and adsorbent is involved in the energy of adsorption which may be quite large

comparable to those of chemical bond formation. Typically, the enthalpy values are in the region of 200kJ/mol. The enthalpy of adsorption depends on the extent of surface coverage, mainly because the adsorbate particles interact. If the particles repel each other, the adsorption becomes less exothermic as coverage increases. If the adsorbate particles attract one another, then they tend to cluster together in islands, and growth occurs at the boarders. The distance between the surface and the closest adsorbate atom is also typically for chemisorption than for physisorption. At low temperature chemisorption may be slow that for practical purpose only physisorption is observed, where as chemisorptions predominate at high temperature (Adamson, 1960). On a general note, monolayer is formed in chemisorptions due to the short-range chemical forces between the adsorbate and adsorbent (Atkins, 2003).

1.3.3 Extent of Adsorption

The extent of surface coverage is normally expressed as the fractional coverage, θ. It represents the extent of adsorption $\boldsymbol{\theta} = \frac{\textbf{No.of adsorption sites occupied}}{\textbf{No.of adsorption sites available}}$

The fraction coverage θ can also be expressed in terms of volume of adsorbate (Atkins, 2003).

1.3.4 Rate of Adsorption

The of adsorption $d\theta/dt$ is the change of surface coverage and can be determined by observing the change of fractional coverage θ overtime (Atkins, 1999).

1.4 ADSORPTION MODELING

1.4.1 Adsorption isotherm

Sorption isotherms are plots of the sorption uptake (q) and the final equilibrium concentration of the residual sorbate remaining in the solution (C_e). A wide array of equilibrium- based

models have been used to describe sorption on solid surfaces. These include the usual isotherms equations such as those describe by Langmuir, Freundlich, Brunauer Emett and Teller or Sips (Cheremisinoff and Ellerbusch, 1978) but the most widely used models to describe metal uptake in algae for a system at equilibrium are the well-known Langmuir and Freundlich sorption isotherms.

1.4.2 Langmuir Isotherm

In 1918, Langmuir described adsorbate-adsorbent system in which the extent of adsorbate coverage is limited to monolayer at or before a relative pressure of unity is reached. Although the theory is more appropriate for the description of chemisorption, it is adopted for a number of systems where the coverage is low, Thus Langmuir adsorption isotherm is applicable to monolayer sorption onto a surface having homogeneously distributed identical binding sites over the surface sorbent. Langmuir adsorption made the following assumptions:

- **I.** The adsorption is an equilibrium process.
- **II.** Adsorption cannot proceed beyond monolayer coverage.
- **III.** Each adsorption site is equivalent in terms of energy of adsorption and uniform i.e. the surface is perfectly flat on a microscopic scale.
- **IV.** There are no interactions between adjacent adsorbate molecules.

The Langmuir isotherm is represented mathematically as:

$$q_e = \frac{Q^o b C_e}{1 + bC_e} \quad \ldots\ldots (1.1)$$

The Langmuir isotherm proposed by Gupta and Rastogi (2007) for mathematical description of equilibrium data is stated below

$$\frac{1}{q_e} = \frac{1}{Q^o} + \frac{1}{bQ^o C_e} \quad \ldots\ldots (1.2)$$

Where;

q_e = the amount of adsorbate adsorbed per gram of dried adsorbent at equilibrium (mg adsorbate/g of dried adsorbent)

Q^o = Constant relating to the maximum amount of adsorbate ion bound per g of adsorbent for a monolayer (mg/g).

b = Langmuir constant or adsorption coefficient or the adsorption affinity (l/mg) for binding of adsorbate on the adsorbent sites.

C_e = Equilibrium (residual) adsorbate concentration in solution after sorption (mg/l)

The values of Q^o and b can be calculated from the intercept $\left(\frac{1}{Q^o}\right)$ and slope $\left(\frac{1}{Q^o b}\right)$ of the plot $\frac{1}{q_e}$ against $\frac{1}{C_e}$ (Gupta and Rastogi, 2007).

1.4.3 Separation Factor, R_L

The shape of Langmuir isotherm can be used to predict whether the sorption system is favorable or unfavorable in batch process. The essential characteristics of Langmuir isotherm can be expressed in terms of a dimensionless constant called equilibrium parameter or separation constant, R_L, which is defined as

$$R_L = \frac{1}{1+bC_o} \quad \text{.....(1.3)}$$

Where:

b = the Langmuir constant (l/mg).

C_o = the initial metal ion concentration of the selected metal (mg/l).

It is known that R_L values between 0 and 1 indicate favorable adsorption (Mckay and Roberts, 1982; Raghavarao *et al.,* 2009).

1.4.4 Freundlich Isotherm

The Freundlich adsorption isotherm which provide the equilibrium data required for the designing of the adsorption system, is a special case for heterogeneous surface and is usually used to model adsorption from solution. The Freundlich equation has the general form:

$$q_e = K_F C_e^{1/n} \quad \text{.....(1.4)}$$

The Freundlich isotherm proposed by Gupta and Rastogi (2007) for mathematical description of equilibrium data is stated below

$$lnq_e = lnK_F + \frac{1}{n} ln\, C_e \quad \text{..... (1.5)}$$

Where:

q_e = Amount of adsorbate adsorbed per unit weight of biosorbent.

K_F= Freundlich Constant measuring adsorption capacity.

C_e= Equilibrium concentration of the adsorbent in solution.

n= Constant related to adsorption efficiency and energy of adsorption or adsorption intensity of the adsorbent.

The Freundlich equation is basically empirical but is often useful as a means for data description. This is because the equation is adequate to describe non-linear adsorption in a narrow range of adsorbate concentration**.** The mathematical simplicity of the equation enables it to be used easily. It describes the adsorption processes on surface adsorption sites that are energetically heterogeneous. However, theoretically, the amount of adsorbed solute may become infinite as bulk solute concentration increases and this is a disadvantage. Taking the logarithm of equation (1.4), we obtained equation (1.5).

A plot of lnq_e against lnC_e gives a straight line with a slope 1/n and an intercept of lnK_F. The K_F value increases with the total adsorption capacity of the adsorbent to bind the adsorbate. The numerical value of n is a useful index of the adsorption capacity or efficiency and is related to the energy of adsorption intensity. The adsorption isotherm is useful for predicting the capacity that an adsorbent has for adsorbing adsorbate. The steeper the isotherm, the more effective is the adsorbent. As the concentration increases, the higher will be the effective capacity at the concentration level desired for adsorption. (Weber, 1973).

1.5 Sorption Kinetics

Kinetic tests show the time – concentration profile for adsorption. The sorption reaction itself is inherently an extremely fast one. It is mainly the particle mass transfer which controls the overall adsorption kinetics (sorbent particle size, porosity, sorbate size and mixing in the sorption system). Monitoring a kinetic experiment enables us to see how the system is affected by these factors and to understand the stages which limit adsorption. The sorption kinetics thus constitutes a criterion in the determination of the interest sorption processes (Volesky *et al.,* 1999).

Kinetic studies are carried out by mixing an amount of sorbent with a given volume of metal solution, with various metal concentrations. The different tank reactors are agitated at the same speed. Alternatively, we can keep the same metal concentration and vary the amount of adsorbent (or other factors). In both cases the samples were regularly withdrawn, filtered and analyzed. The values of different concentrations are represented on timescale. We can also represent the evolution of sorption capacity over time. It is important to analyze kinetics with a prior knowledge of the isotherms. Isotherms enable us to know at all times the deviations from equilibrium which drives the transfer. Isotherm also enable us to know if the kinetics has lead

to the saturation of sorbent. We must bear in mind that the last point of the kinetics refers to the equilibrium concentration; by applying the material balance we can obtain the sorption capacity in equilibrium; this should coincide with the curve of the sorption isotherm earlier obtained (Bhatti *et al.*, 2006).

1.5.1 Pseudo - First Order

The pseudo first-order equation (Lagergren, 1898) is generally expressed as (McKay and Ho, 1999):

$$\frac{dq}{dt} = k_1(q_e - q_t) \quad \text{.....(1.6)}$$

Where q_e and q_t are the amount of sorbate adsorbed on adsorbent at equilibrium and time *t*, respectively (mg/g), and k_1 is the rate constant of first order adsorption (min^{-1}). Integrating equation (1.6) for the boundary conditions t = 0 to t = t is the following:

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \quad \text{.....(1.7)}$$

The plot of $\log(q_e - q_t)$ versus t will give a straight line and the value of k_1 can be obtained from the slope of the graph while $q_{e(\text{calculated})}$ is obtained from the intercept of the graph (Gupta and Rastogi, 2007).

1.5.2 Pseudo - Second Order

The second-order kinetic model is expressed as:

$$\frac{dq}{dt} = k_2(q_e - q_t)^2 \quad \text{.....(1.8)}$$

Where, k_2 is the pseudo-second-order rate constant of adsorption (g $mg^{-1}min^{-1}$). The linearised integrated form of (1.8) is given as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad \text{.....(1.9)}$$

If the pseudo second-order kinetics is applicable to the system, then the plot of $\frac{t}{q_t}$ versus t will give a linear relationship with $\frac{1}{q_e}$ and $\frac{1}{k_2 q_e^2}$ as slope and intercept, respectively. The values of q_e and k_2 can be determined from the slope and intercept and there is no need to know any parameters beforehand. The pseudo second-order kinetics model has been successfully applied to several biosorption systems as reported by McKay and Ho (1999).

1.6 Thermodynamic Modeling

The thermodynamic parameters such as change in standard free change energy (ΔG^o), enthalpy change (ΔH^o) and entropy change (ΔS^o) for adsorption process can be determined using the following equations proposed by Gupta and Rastogi (2007):

$$\Delta G^o = -RT\, ln(b) \quad \text{.....(1.10)}$$

$$\Delta G^\circ_{ads} = \Delta H^\circ_{ads} - T\Delta S^\circ_{ads} \quad \text{.....(1.11)}$$

Where:

R (8.314 J/mol K) is the gas constant, T (K) the absolute temperature and b (L/mol) is the Langmuir constant related to free energy or net enthalpy of adsorption. By plotting a graph of ΔG°_{ads} versus *T,* the values ΔH^o and ΔS^o can be estimated from the intercept and slope respectively.

1.7 Justification for the Research

Most cities in Nigeria are industrialized and densely populated resulting in indiscriminate use of our natural resources consequently causing serious contamination on aquatic ecosystems, principally due to chemical pollution of organic and inorganic origins especially by organic chlorinated compounds and heavy metals. Among pollutants, the contribution of heavy metals

to environment is of major concern because of its toxicity, bioaccumulation, persistence and non-biodegradable nature (Pattabhi *et al.,* 2008).

The removal of heavy metals from industrial wastewater and naturally occurring waters remains a problem both technically and economically. Hence in recent years, there has been a considerable interest in the use of cost effective adsorbents (biosorbents) which studies have shown to exhibit high biosorption capacity (Soltmann *et al.,* 2010).

The search for new technologies for removing heavy metals from wastewaters directed attention to biosorption, based on the metal binding capacities of various biological materials like bacteria, algae, fungi and yeast.

There are many reports and reviews on biosorption of heavy metals ion on marine algae, green seaweeds, and freshwater green algae species with varying removal efficiencies, maximum adsorption capacities and binding constants. Among the algal biomass used for biosorption, *Cosmarium panamense*, is a green abundantly available algal biomass found in most ponds in Zaria. Its capability and capacity to remove heavy metals from aqueous solutions has not been studied, hence, prompting the need for this study.

1.8 Aims and Objectives

The aim of this research is to study biosorption of heavy metals (Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+}) from aqueous solutions by dead biomass of fresh water green algae *Cosmarium panamense*. This aim will be achieved with the following objectives;

1. Studying the effects of contact time, initial metal ion concentration, solution pH, biosorbent dose and temperature in order to ascertain the optimum conditions, kinetics,

as well as thermodynamic nature of the biosorption process of these heavy metals (Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+}) on the algal biomass *Cosmarium panamense.*

2. Examining the biosorption capacity of *Cosmarium panamense* for these heavy metals by evaluating equilibrium adsorption isotherms. Langmuir and Freundlich Isotherms specifically.
3. Studying and characterization of the properties of native and heavy metals (Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+}) treated algal biomass by gravimetric, energy dispersive X – ray (EDAX) and simultaneous thermal analysis (STA) method .
4. Studying the surface morphology of the native and heavy metals (Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+}) treated algal biomass using scanning electron microscope (SEM).
5. Determining FTIR Spectra of native and selected heavy metals (Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+}) treated algal biomass in order to ascertain the functional groups responsible for each heavy metal ion biosorption on the algal biomass *Cosmarium panamense.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Water Pollution with Heavy Metals

A heavy metal is defined as one whose density is above $5gcm^{-3}$ or 50 $gmol^{-1}$.The water pollution is mainly due to organics, inorganics, sediments, radioactive materials and heavy metals. Among these pollutants, the contribution of heavy metals to the environment is of major concern because of its toxicity, bioaccumulation, persistence and non-biodegradable nature (Pattabhi *et al.,* 2008). The presence of heavy metals in aqueous water streams has become a problem due to their harmful effects on the human health and on the flora and fauna of receiving water bodies (Gupta and Rastogi, 2007). Heavy metals even at low concentration can cause toxicity to human and other forms of life (Hima *et al.,* 2007). Heavy metals of concern include Lead, Chromium, Mercury, Uranium, Selenium, Zinc, Arsenic, Cadmium, Silver, Gold, and Nickel (Ahalya *et al*., 2003). The toxicity of metal ion is due to their ability to bind with protein molecules and prevent replication of DNA and thus, subsequent cell division. Main sources of heavy metal contamination include urban industrial aerosols, solid waste from animals, mining activities, industrial and agricultural chemicals. To avoid health hazards, it is essential to remove these toxic heavy metals from wastewater before its disposal(Kar *et al*., 1992; Hima *et al*., 2007). Therefore it is recognized that finding methods for removal of heavy metals from aqueous water is of great importance (Gupta and Rastogi, 2007).

2.2 Sources and Toxicity of Selected Heavy Metals

2.2.1 Cadmium

Cadmium is produced as an inevitable by-product of zinc (or occasionally) refining, since these metals may occur naturally within the raw ore, the most significant use of cadmium is in nickel/cadmium batteries, as rechargeable of secondary power source exhibiting high output, long life, low maintenance and high tolerance to physical and electrical stress. Cadmium coatings provide good corrosion resistance, particularly in high stress environment such as marine and aerospace applications where high safety and reliability is required. Other uses of cadmium are as pigments, stabilizers for polyvinyl chloride, in alloys and electronic compounds; cadmium is also present as an impurity in several products and refined petroleum products (Csuros and Csuros, 2002).

Cadmium derives its toxicological properties from its chemical similarity with zinc, an essential micronutrient for plants, animals and humans. Cadmium is biopersistent and once absorbed by an organism, remains resident for many years, although it is eventually excreted. Uptake of cadmium by plants is greatest from soils but is decreased by raising the pH through liming. Uptake varies with soil properties especially those that control adsorption and also with the plant species and the source of cadmium. The average daily intake for humans is estimated as 0.15ug from air and 1ug from water. Smoking a packet of cigarette can lead to the inhalation of around 2-4ug of cadmium. Airborne cadmium comes primarily from steel industry and waste incineration, followed by volcanic activity and zinc production. There is no evidence of dermal absorption and oral absorption is very low. The effects of exposure to cadmium include vomiting, increased salivation, abdominal pain, and diarrhoea. Acute inhalation is

characterized by coughing and tightness in the chest. Chronic exposure produces a variety of effects on kidneys, lungs, heart, bones, and gonads. Cadmium fumes can damage the olfactory organs. Cadmium toxicity is decreased by the presence of other metals (Csuros and Csuros, 2002).

2.2.2 Chromium

Hexavalent chromium is present in the effluents produced during the electroplating, leather tanning, cement, mining, dyeing and fertilizer and photography industries and causes severe environmental and public health problems. Hexavalent chromium has been reported to be toxic to animals and humans and it is known to be carcinogenic (Raji and Anirudhan, 1998).Its concentrations in industrial wastewaters range from 0.5 to 270.000 mg·l-1 (Patterson, 1985). The tolerance limit for Cr(VI) for discharge into inland surface waters is 0.1 mg·l-1 and in potable water is 0.05 mg·l-1 (EPA, 1990). Chromium is necessary for the proper utilization of sugars and other carbohydrates by optimizing the production and effects of insulin. It is widely distributed in the body. The RDA is 0.05 to 2mg. sources include liver, meat, cheese, whole grain, yeast and wine (Csuros and Csuros, 2002).

Chromium deficiency causes impaired insulin function, hence increased insulin secretion and the risk of adult onset diabetes mellitus (Csuros and Csuros, 2002). The human body will need trace amounts of chromium III (Cr^{3+})in order to help metabolize fats and carbohydrates. But if the body ingests more or if the body is exposed to the more dangerous chromium VI, it will most likely suffer from lung cancer, weak immune system, ulcer, and liver damage (Mayflor, 2009) .

2.2.3 Cobalt

Cobalt is a hard, bluish – white metal that is used mainly in alloys, such as stainless and satellite (an alloy of iron, copper, and tungsten), which is used in surgical instruments. Cobalt is used to prepare the alloy alnico, which forms powerful magnets (Csuros and Csuros, 2002). Cobalt is used for manufacturing glasses and ceramics and in various metallurgical processes. It enters the air and then eventually settles down to the ground and contribute to water pollution (Mayflor, 2009). Aqueous solutions of cobalt (II) salts are characteristically rose colored. Cobalt salts, usually oxidation states II and III, are used to give a brilliant blue color to glass, tiles and pottery. Anhydrous cobalt(II) chloride is used in water quality testing and as a heat sensitive ink. Artificially produced cobalt – 60 is used as a radioactive tracer and cancer treatment agent. Cobalt is a part of vitamin B_{12} and is considered an essential nutrient, but concentrations higher than 1 mg/kg of body weight are regarded as a health hazard (Csuros and Csuros, 2002).

Cobalt exhibits toxic effects on the heart, kidneys, and thyroid gland. Consumption of large quantities of coffee or beer may lead to high concentrations of cobalt,. Cobalt toxicity resulting in heart failure (about 40% mortality) has been reported among heavy drinkers who had consumed products containing cobalt additives used as a foam stabilizer (Csuros and Csuros, 2002). Overexposure to cobalt leads to vomiting, nausea, and coma or death (Mayflor, 2009).

2.2.4 Lead

Lead is found in the earth crust as a constituent of the minerals galena (PbS), angelside ($PbSO_4$) and cerusite ($PbCO_3$) (US EPA Consumer fact-sheet). The most important natural emission of lead to the atmosphere is from wind blow dust (Elder, 1988). Combustion of fossil

fuels accounts for the largest anthropogenic emissions into the environment. Other major inputs are the production of non ferrous metals, iron and steel production (Pacyna *et al.,* 2001). Corrosion of lead based paints and metal recycling releases lead into surface water (Holt, 2000), while lead in groundwater comes from scrap metal recycling and industrial discharges (Jensen *et al*., 2000; Pacyna and Pacyna, 2000). Dissolved lead is mainly present in the inorganic form (Pb^{2+}) but can also be in tetravalent forms such as alkyl leads (Besser *et al.,* 2001). Until recently a major source of lead in drinking water was from the corrosion of the solder used in copper plumbing systems. Also, sporting activities such as fishing and wild fowling can deposit significant quantities of lead in aquatic and terrestrial environment (Goyer, 1993).

Lead is highly toxic to humans, flora and fauna with cumulative and non - degradative characteristics (Tong *et al*., 2000). Lead has been used extensively by humans since prehistoric times and has become widely distributed within the environment (Gilfillan, 1965). Consequently, the exposure to and uptake of lead has increased bringing significant health and environmental implications (Guinee *et al.,* 1999). High levels of lead ingested into the body causes damage to the brain, kidneys, nervous system and the red blood cells. The US EPA considers lead to be highly toxic and a major health and environmental threat (US EPA metal toxicity profiles). Chronic lead exposure is a particular concern for infant children exposed to low levels via contaminated drinking water or passed over in their mother's milk (Goyer, 1993). The MCL for lead in drinking water is 0.005 and 0.0015 mg/L for the EU 2006 and US EPA 2006 respectively.

2.2.5 Nickel

The major sources of nickel contamination of water comes from industrial processes such as electroplating, battery and accumulator manufacturing and porcelain enamelling. Nickel is an important element in the steel industry. Nickel is used in making batteries, welding rods, adding pigments to paints, and producing dental and surgical prostheses (Mayflor, 2009). Because it is quite resistant to corrosion, nickel is often used in plating more active metals. Nickel and chromium are the chief additives to iron in making stainless steel. In combination with copper, it produces a hard, strong, corrosion resistant alloy called monel (Csuros and Csuros, 2002).

With nickel in practically everything that people use, exposure is unavoidable. Fortunately, there are no lethal effects due to minimal exposures. Overexposures, however, lead to lung cancer, asthma-like symptoms, and various organ problems (Mayflor, 2009). Nickel itch or contact dermatitis is the most commonly seen reaction to nickel compounds especially in women, resulting from use of nickel in costume jewelry, especially earrings. Chronic exposure to nickel causes cancer in the respiratory tract and the lungs. Acute poisoning of nickel causes headache, nausea and vomiting, dizziness, chest pain, tightness of the chest, dry cough, and shortness of breath and rapid respiration (Csuros and Csuros, 2002).

2.3 Biosorption Process

Biosorption is an innovative technology using living or dead biomass to remove toxic heavy metals from aqueous solutions (Vieira and Volesky, 2000). It can be defined as the removal of metallic ions by means of passive adsorption or complexation by live biomass or organic waste

(Eccles, 1995; Saradhi *et al.,* 2010) or as "a non directed physicochemical interaction that may occur between metal/radionuclide species of microbial cells" (Shumate and Stranberg, 1985).

Compared with conventional methods for removal of toxic metals from wastewater, biosorption offers potential advantages such as low operating cost, minimization of chemical and biological sludge, high efficiency of heavy metal removal from diluted solutions, regeneration of biosorbents, possibility of metal recovery and environmental friendly (Ahluwalia and Goyal, 2007). Recent biosorption experiments have focused attention on waste materials, which are by-products or the waste material from large scale industrial operations for example the waste mycelia available from fermentation processes, olive mill solid residues (Pagnanelli *et al*., 2002), activated sludge from sewage treatment plants (Hammaini *et al*., 2003), biosolids (Norton, 2003), aquatic macrophytes (Keskinan *et al*., 2003) etc.

Biosorption process involves a solid phase (sorbent or biosorbent usually a biological material) and a liquid phase (solvent, normally water) containing dissolved species to be sorbed (sorbate, a metal ion). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound with different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. While there is a preponderance of solute (sorbate) molecules in the solution, there are more in the sorbent particle to start with. This imbalance between the two environments creates a driving force for the solute species. The heavy metals adsorb on the surface of the biomass thus, the biosorbent becomes enriched with metal ions in the sorbate (Hima *et al.,* 2007).

2.4 Biosorption Mechanisms

The complex structure of microorganisms implies that there are many ways for metal to be taken up by the microbial cell. Biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms are divided into:

1. Metabolism dependent and
2. Non –metabolism dependent.

Metabolism dependent is a slow process and includes transport across cell membrane and precipitation while Non – metabolism dependent is a rapid process and includes precipitation, physical adsorption, ion – exchange and complexation (Sannasi *et al.,* 2006).

According to the location where the metal removed from solution is found, biosorption can be classified as

1. Extra cellular accumulation/precipitation
2. Cell surface sorption/precipitation
3. Intracellular accumulation.

Transport of metal across the cell membrane yield intracellular accumulation, which is dependent on the cell metabolism. This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal. During non-metabolism dependent biosorption, metal uptake is by physico – chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal

binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible (Kuyucak and Volesky, 1988). In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface (Ercole, *et al.,* 1994). Further, it may be dependent on the cell's' metabolism if, in the presence of toxic metals, the microorganism produces compounds that favours the precipitation process. Precipitation may not be dependent on the cells' metabolism, if it occurs after a chemical interaction between the metal and cell surface (Ahalya *et al.*, 2003).

2.4.1 Transport across Cell Membrane

Heavy metal transport across microbial cell membranes may be mediated by the same mechanism used to convey metabolically important ions such as potassium, magnesium and sodium. The metal transport systems may become confused by the presence of heavy metal ions of the same charge and ionic radius associated with essential ions. This kind of mechanism is not associated with metabolic activity. Basically biosorption by living organisms comprises of two steps. First, a metabolism independent binding where the metals are bound to the cell walls and second, metabolism dependent intracellular uptake, whereby metal ions are transported across the cell membrane. (Gadd *et.al.,* 1988; Nourbaksh *et.al.,* 1994). Thus, Biosorption of metals is not based on only one mechanism. It may consist of several mechanisms that quantitatively and qualitatively differ according to the species used, the origin of the biomass and its processing. A number of different metal-binding mechanisms have been postulated to occur in biosorption.

2.4.2 Physical Adsorption

In this category, physical adsorption takes place with the help of van der Waals' forces. Kuyucak and Volesky (1988), hypothesized that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomasses of algae, fungi and yeasts takes place through electrostatic interactions between the metal ions in solutions and cell walls of microbial cells. Electrostatic interactions have been demonstrated to be responsible for copper biosorption by bacterium *Zoogloea ramigera* and alga *Chiarella vulgaris* (Aksu *et al.,* 1992), for chromium biosorption by fungi *Ganoderma lucidum* and *Aspergillus niger* (Ahalya *et al.*, 2003).

2.4.3 Ion Exchange

Cell walls of microorganisms contain polysaccharides and bivalent metal ions exchange with the counter ions of the polysaccharides. For example, the alginates of marine algae occur as salts of K^+, Na^+, Ca^{2+}, and Mg^{2+}. These ions can exchange with counter ions such as CO^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+} resulting in the biosorptive uptake of heavy metals (Kuyucak and Volesky 1988). The biosorption of copper by fungi *Ganoderma lucidium* (Muraleedharan and Venkobachr, 1990) and *Aspergillus niger* was also up taken by ion exchange mechanism (Ahalya *et al.,* 2003).

2.4.4 Complexation

The metal removal from solution may also take place by complex formation on the cell surface after the interaction between the metal and the active groups. Aksu *et al* (1992) reported that biosorption of copper by C. *vulgaris* and Z. *ramigera* takes place through both adsorption and formation of coordination bonds between metals and amino and carboxyl groups of cell wall

polysaccharides. Complexation was found to be the only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury accumulation by *Pseudomonas syringae*. Micro organisms may also produce organic acids (e.g., citric, oxalic, gluonic, fumaric, lactic and malic acids), which may chelate toxic metals resulting in the formation of metallo-organic molecules. These organic acids help in the solubilisation of metal compounds and their leaching from their surfaces. Metals may be biosorbed or complexed by carboxyl groups found in microbial polysaccharides and other polymers (Ahalya *et al.,* 2003).

2.4.5 Precipitation

Precipitation may be either dependent on the cellular metabolism or independent of it. In the former case, the metal removal from solution is often associated with active defense system of the microorganisms. They react in the presence of a toxic metal producing compounds, which favours the precipitation process. In the case of precipitation not dependent on the cellular metabolism, it may be a consequence of the chemical interaction between the metal and the cell surface (Ahalya *et al.,* 2003). The various biosorption mechanisms mentioned above can take place simultaneously. The active groups responsible in the metals biosorption were listed in Table 2.1

Table 2.1: Functional groups that are responsible in metal ion biosorption (Birch and Bachofen, 1990; Le Cloirec *et al.*, 2003)

Formula	Basic Groups	Formula	Acidic Groups
$-NH_2$	Amino	-COOH	Carboxylic
=NH	Immino	$-SO_3H$	Sulphonic
-N=	Cyclic or Non – Cyclic	$-PO(OH)_2$	Phosphonic
=CO	Carbonyl	--OH	Enolic, Phenolic
-O-	Ether	=N-OH	Oxime
-OH	Alcohol	-SH	Mercaptan
-S-	Thio Ether		

2.4.6 Factors affecting Biosorption

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

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

Roux, 1992; Gadd *et al*., 1988). Gadd *et al* (1988) suggested that an increase in biomass concentration leads to interference between the binding sites. Fourest and Roux (1992) invalidated this hypothesis attributing the responsibility of the specific uptake decrease to metal concentration shortage in solution. Hence this factor needs to be taken into consideration in any application of microbial biomass as biosorbent.

4. Biosorption is mainly used to treat wastewater where more than one type of metal ions would be present; the removal of one metal ion may be influenced by the presence of other metal ions. For example: Uranium uptake by biomass of bacteria, fungi and yeasts was not affected by the presence of manganese, cobalt, copper, cadmium, mercury and lead in solution (Sakaguchi and Nakajima, 1991). In contrast, the presence of Fe^{2+} and Zn^{2+} was found to influence uranium uptake by *Rhizopus arrhizus* (Tsezos and Volesky, 1982) and cobalt uptake by different microorganisms seemed to be completely inhibited by the presence of uranium, lead, mercury and copper (Sakaguchi and Nakajima, 1991).

2.5 Selection and Types of Biosorbents

Both living and nonliving microorganisms such as algae, bacteria, fungal and yeast are used as biosorbent materials for heavy metals biosorption (Wang and Chen, 2006). Some biomass types are very effective in accumulating heavy metals, some can bind and collect a wide range of heavy metals with no specific priority while others are specific for certain types of metals. In the literature, some microorganisms are capable to remove heavy metals even at low concentration, in the range 1-100 mg/L (Chong and Volesky, 1995).

Availability is a major factor to be taken into account to select biomass for clean-up purposes. The economy of environmental remediation dictates that the biomass must come from nature or even has to be a waste material. While choosing the biomass for metal biosorption, its origin is a major factor to be taken into account. Biomass can come from, (I) activated sludge or fermentation waste from industries like those of food, diary and starch, which should be obtained free of charge . (II) organisms (eg., bacteria, yeast, fungi, and algae) coming from their natural habitats are good sources of biomass and are easily available in large amounts in nature. (III) fast growing organisms that are specifically cultivated for biosorption purposes (eg., crab shells, seaweeds) can be used as biosorbents (Vieira and Volesky, 2000).

Apart from the microbial sources even agricultural products such as wool, rice, straw, coconut husks, peat moss, exhausted coffee (Dakiky *et al.*, 2002), waste tea (Ahluwalia and Goyal, 2007), walnut skin, coconut fibre, cork biomass (Chubar *et al*., 2003), seeds of *Ocimum basilicum* (Melo and D'souza, 1994), defatted rice bran, rice hulls, soybean hulls and cotton seed hulls (Teixeria *et al.*, 2004), wheat bran, hardwood (*Dalbergia sissoo*), saw dust, pea pod, cotton and mustard seed cakes (Saeed *et al*., 2002) are also proven as good biomass sources. However, seaweeds, molds, yeasts, bacteria have been tested for metal biosorption with encouraging results (Vieira and Volesky, 2000).

2.5.1 Seaweeds

Seaweeds are large groups of marine benthic algae. They offer several advantages for biosorption because of their large surface area which gives a convenient basis for the production of biosorbent particles suitable for sorption process. They contain many polyfunctional metal-binding sites for both cationic and anionic metal complexes. Potential

metal cation-binding sites of algal cell components include carboxyl, amine, imidazole, phosphate, sulphate, sulfhydryl, hydroxyl and chemical functional groups contained in cell proteins and sugars (Crist *et al.*, 1981). Brown algae stands out as very good biosorbent of heavy metals (Gonzalez *et al.*, 2006). Their cell walls contain fucoidin and alginic acid. The alginic acid offers carboxylate and sulfate ions at neutral pH.

Volesky *et al* (1999) worked extensively with one of the best metal-sorbing biomass types, ubiquitous *Sargassum* seaweed. They compared three different species of non living *Sargassum* biomass for their equilibrium Cd and Cu uptakes from aqueous solutions by using experimental sorption isotherms. Uptakes of Cd at the optimum pH 4.5 were *qmax* = 87 mg Cd/g, for *Sargassum vulgare*, 80 mg Cd/g for *S. fluitans*, and 74 mg Cd/g for *S. filipendula*. Uptakes of Cu at pH 4.5 were *qmax* = 59 mg Cu/g for *S. vulgare*, 56 mg Cu/g for *S. filipendula*, and 51 mg Cu/g for *S. fluitans.*

2.5.2 Fungi and Yeast

The majority of fungi show filamentous or hyphal growth. Cell walls of fungi present a multi-laminate architecture where up to 90% of their dry mass consist of amino or non-amino polysaccharides. The fungal cell walls can be considered as a two phase system consisting of chitin framework embedded on an amorphous polysaccharide matrix (Yan and Viraraghavan, 2000). The cell walls are rich in polysaccharides and glycoproteins such as glycans (β- 1 -6 and β- 1 -3 linked D-glucose residues), chitin (β- 1 -4 linked N-acetyl-D- glucosamine), chitosan (β- 1 -4 linked D-glucosamine), mannans (β- 1 -4 linked mannose), and phosphormannans (phosphorylated mannans). Various metal binding groups, viz amine, imidazole, phosphate, sulphate, sulfhydryl, and hydroxyl are present in polymers (Crist *et al.,* 1981).

The most common yeast biomass (*Saccharomyces cerevisiae*) is not usually a waste, but a commercial commodity (feed-lot uses). Some chemical compounds of yeast cells can also act as ion exchangers with rapid reversible binding of cations (Vieira and Volesky, 2000).

Volesky *et al* (1993) working on cadmium biosorption by *Saccharomyces cerevisiae* demonstrated that this yeast is a reasonably potent biosorbent material for cadmium.

2.5.3 Bacteria

The evaluation of bacterial metal-sorbing properties has aroused some controversy. Most of the experiments done with metals and bacteria have really concerned metabolically mediated bioaccumulation**,** while the basic principle of biosorption is the use of dead biomass (Vieira and Volesky, 2000). According to Volesky and Holan (1995), who presented an extensive review of biosorption results, the strong biosorbent behavior of certain types of microbial biomass toward metallic ions is a function of the chemical makeup of microbial cells. In fact, the biomass is dead and all cells are metabolically inactive. There is also much confusion in the methodology used and in the evaluation and expression of experimental data which shows an inadequate understanding of sorption principles (Volesky and Schiewer, 1999).

The most relevant work on true bacterial biosorption has been done by the Brierley (1990), who took the metal biosorption concept all the way to the commercial stage (Brierley *et al.,* 1986). Fein *et al* (1997) used *Bacillus subtilis* to examine further the bacterium interaction with Cd, Cu, Pb and Al. Their results quantified not only the deprotonation constants for the important organic functional groups on the bacterial cell wall, but also the stability constants for adsorption of environmentally important metal species onto the individual sites. Bacterial

cell walls are negatively charged under acidic pH conditions and the cell wall chemically functional groups display a high affinity for metal ions in solution (Collins and Stotzky, 1992).

According to Beveridge (1989), bacteria make excellent biosorbents because of their high surface-to-volume ratios and a high content of potentially active chemisorption sites such as on teichoic acid in their cell walls. Churchill *et al* (1995) used two Gram-negative strains *Escherichia coli* K-12 and *Pseudomonas aeruginosa* and a Gram-positive strain *Micrococcus luteus* to demonstrate biosorption of Cu^{2+}, Cr^{3+}, Co^{2+} and Ni^{2+}. Their sorption binding constants suggested that *E. coli* cells were the most efficient at binding copper, chromium and nickel and *M. luteus* sorbed cobalt most efficiently. Hu *et al* (1996) worked with *Pseudomonas aeruginosa* strain CSU, a genetically not altered bacterial strain known to bind dissolved hexavalent uranium. *P. aeruginosa* CSU biomass was sorbing significantly more uranium than certain novel, patented biosorbents derived from algal or fungal biomass sources. A good basis for ongoing work in the field has been provided by Mann (1990) in one of the most relevant reviews of bacterial metal biosorption, which remains very attractive and exciting for study and contribution.

2.6 Overview of Metal-Binding Biosorbents

One of the major challenges in the field of biosorption is to select the most promising types of biomass from an extremely large pool of readily available and inexpensive biomaterials. The meaningful search for metal-sorbent biomaterials can be greatly expedited, if the phenomenon of metal biosorption was better understood. Such knowledge could serve as a guide in looking for potentially high-sorbing materials in the natural domain. However, the-state-of-the-art being in its infancy, tedious experimental screening of selected biomass types

readily available still is the basis for discovering new biosorbents. Considering the number of candidate biomass types and the number of metallic species of interest, as well as the number of variable experimental or process parameters, the scope of the task of prospecting for new and potentially feasible metal biosorbents is rather large.

Table 2.2 summarize the results of research attempted to identify metal – sorbing algae and biomass types in the biosorption world. The results of these studies vary widely due to the different criteria used by the authors in searching for suitable materials.

Table 2.2:
Biosorption of Cd(II), Cr(VI), Co(II), Pb(II) and Ni(II) by Algae

	q_{max}(mg/g)	pH	Biomass Conc.(g/l)	
Cd(II)				
Ascophyllum. nodosum	115.36	5	21	Yu *et al.,* 1999
Sargassum filipendula	73.9	4.5	22	Davis *et al.,* 2000
Codium vermilara	21.8	5	27	Gonzalez *et a.l,* 2006
Spirogyra insignis	22.9	5	27	Gonzalez *et a.l,* 2006
Fucus spiralis	114.9	5	27	Gonzalez *et a.l,* 2006
Sargassum Sp.	84.7	5	25	Hajar M, 2009
Cladophora fasicularis	8.26	5	RT	Kumar *et al*., 2009
Ulva lactuca	20.4	5	RT	Kumar *et al.*,2009
Cr(VI)				
Sargassum seaweed	60	3.5	22	Barkhordar and Ghaiseddin, 2004
Spirogyra Sp.	22.5	2	35	Gupta *et al.,* 2001
Co(II)				
Ascophyllum. nodosum	100.3	4	RT	Davis *et al.,* 2003
Pb(II)				
Spirogyra Sp.	140	5	25	Gupta and Rastogi, 2006
Cladophora glomerata	72.5	4.5	30	Jalali *et al*., 2002
Codium vermilara	63.3	5	27	Gonzalez *et al.,* 2006
Spirogira insignis	51.5	5	27	Gonzalez *et a.l,* 2006
Asparapsis armata	63.7	4	27	Gonzalez *et a.l,* 2006
Chondrus crispus	204.1	4	27	Gonzalez *et a.l,* 2006
Ascophyllum nodosum	178.6	3	27	Gonzalez *et a.l,* 2006
Sargassum sp.	351.9	4.5	20	Ramin *et al.,* 2006
Ni(II)				
Sargassum fluitans	70.2	5	RT	Davis *et al.,* 2000
Ascophyllum nodosum	1.43	5	RT	Davis *et al.,* 2000
Codium vermilara	13.2	6	27	Gonzalez *et al.,* 2006
Spirogira insignis	17.5	6	27	Gonzalez *et a.l,* 2006
Asparapsis armata	17.1	6	27	Gonzalez *et a.l,* 2006
Chondrus crispus	37.2	6	27	Gonzalez *et a.l,* 2006
Ascophyllum nodosum	43.3	6	27	Gonzalez *et a.l,* 2006
Fucus Spiralis	50	6	27	Gonzalez *et a.l,* 2006

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Collection, Identification and Treatment.

The sample was collected from a swimming pool located at Ahmadu Bello University, Zaria. After collection, it was taken to Biological Sciences Department of the University where it was identified as *Cosmarium panamense* using the method described by Prescott, 1977. Before use, it was washed several times with tap water and then with de-ionized water to remove impurities and salts. The biomass was sun –dried and then dried in an oven at 60^{o}C for 48hours. The dried algae biomass was cut, ground in a mortar, sieved and particles with an average size 0.5mm was used for biosorption experiments.

3.2 Characterization of the Biosorbent

3.2.1 Preliminary Study

3.2.2 Moisture and Dry Matter Content (of Dry Sample) Determination.

Clean silica crucibles were dried in a desiccator and weighed. 1g of the dry sample was weighed. It was dried in an air – circulated oven at 105^{0}C for 3hours after which it was cooled in a dessicator and then weighed (ASTMA, 1994).

Calculation

$$\text{Moisture (\%)} = \frac{Loss\ \ in\ Weight\ on\ Drying(g)}{Initial\ Sample\ Weight(g)} \times 100 \quad \ldots\ldots(3.1)$$

$$\text{Dry Matter (\%)} = \frac{Oven\ Dry\ Weight\ (g)}{Initial\ Sample\ Weight\ (g)} \times 100 \quad \ldots\ldots(3.2)$$

3.2.3 Ash Content Determination

Copper crucible was heated in a furnace at 500^0C, cooled in a dessicator and weighed. Oven dried sample from the moisture content determination was used. The crucible containing the dry sample was placed in a muffle furnace and temperature was allowed to rise to 500^0C. After 3hours, it was removed and allowed to cool in a dessicator and weighed (Allen *et al.,* 1974).

Calculation

$$\text{Ash (\%)} = \frac{Ash\ Weghed\ (g)}{Oven\ Dry\ Weight g)} \times 100 \quad \text{.....(3.3)}$$

3.2.4 pH Measurements

1% solution of the sample was made using distilled de – ionized water. pH of the supernatant was obtained after 1hour with an Electric pH Meter.

3.2.5 Bulk Density

This was done by measuring the volume of distilled de – ionized water displaced when a known weight (2grams) of the sample was dropped into a graduated measuring cylinder (Allen *et al.,* 1974; Nwakwere, 2010).

Procedure

A 100cm^3 calibrated measuring cylinder was washed and dried. 50cm^3 of the water was added to the measuring cylinder and the volume was noted. 2grams of the sample was dropped into the measuring cylinder and volume of the increased water level was recorded. Care was taken to ensure there were no air bubbles before taking volume measurements.

Calculation

Volume of water displaced (cm^3) = Final volume – initial volume.

Bulk Density (g/cm^3) = $\frac{M_a}{V_a}$……..(3.4)

Where

M_a = Mass of the biosorbent (g)

V_a = Volume of water displaced (cm^3)

3.2.6 Elemental Analysis

To study the elemental composition of the Native algal biomass, 0.2712g and 0.1734g of the dried biomass were weighed using sterilized tubes, for short-lived and long-lived elemental analysis respectively, at the reactor facility section, Centre for Energy Research and Training (CERT) A. B. U, Zaria by Neutron Activation Analysis.

3.2.7 Scanning Electron Microscope (SEM) and Energy Dispersive X – ray (EDAX) Analysis

The effect of metal biosorption on the surface and elemental properties of the algal biomass *Cosmarium panamense* was analyzed using SEM and EDAX. Surface micrographs of the Native and different Heavy metal treated algal biomass powder were recorded using SEM apparatus of model number: Hitachi s – 540 with Oxford Instruments 7497 EDAX, Using link ISIS Computer Software. U = 10kv for SEM and 14kv for EDAX; at Chemistry Laboratory of Chemistry Department, School of Chemistry, Bangor University North Wales, United Kingdom. 21 Scans were collected . Samples were fixed onto an aluminum holder by

conductive Carbon tape and Sputtered with gold in plasma (Polaron Equipment Ltd, SEM Coating Unit E5000) under nitrogen for 5 minutes at 0.1 Torr. 1kv and 8mA.

3.2.8 Simultaneous Thermal Analysis (STA) (TGA – DSC)

The effect of metal biosorption on the thermophysical properties of the algal biomass *Cosmarium panamense* was analyzed using Simultaneous Thermal Analysis (STA) {generally refered to as Simultaneous Application of Thermo – Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC)} instrument with model number: SDT Q600 V4.1 Build 59 at 50^{o}C/min under nitrogen. Weights of 38.2820mg. 21.6020mg, 40.8060mg, 22.0230mg, 32.1410mg and 27.2750mg were weighed for the Native, Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} treated algal biomass analysis respectively within the temperature range of 50 – 600^{o}C and DSC scanning range of 0 – 120mcal/sec, at Chemistry Laboratory of Chemistry Department, School of Chemistry, Bangor University North Wales, United Kingdom. 6 Scans were collected.

3.2.9 Fourier Transform Infrared Analysis

To study the mechanism of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} removal by algal biomass *Cosmarium panamense*, the active chemical groups on the biomass surface before and after respective metal sorption were evaluated by FTIR Spectroscopy. 30mg of finely grounded algal biomass was encapsulated in 300mg of KBr (sigma) in order to prepare the translucent sample disks used for the FTIR spectra analysis. The FTIR spectra was recorded on a Shimatzu Fourier Transform Infrared Spectrometer (FT – IR) at the National Research Institute for Chemical Technology (NARICT) Zaria. 6 scans were collected, for each measurement the spectra range of 4500 – 400cm-^{1} with resolution of 4cm^{-1}.

3.3 Preparation of Reagents

3.3.1 Preparation of 0.1M HNO_3

4.44cm^3 of Concentrated $HNO_{3(aq)}$ with specific gravity 1.42g/cm^3, was measured into a 1000cm^3 volumetric flask and made up to mark using distilled de – ionized water.

3.3.2 Preparation of 0.1M NaOH

4g of sodium hydroxide pellets was dissolved in distilled de – ionized water in a volumetric flask and made up to 1000cm^3 mark with the distilled de – ionized water.

3.3.3 Preparation of 0.01M $NaNO_3$

0.85g of sodium nitrate was dissolved in distilled de – ionized water in a volumetric flask and made up to 1000cm^3 mark with the distilled de – ionized water.

3.3.4 Preparation of Cadmium Stock Solution (1000mg/l)

2.7436g of cadmium nitrate tetrahydrate was dissolved in 0.01M $NaNO_3$ in a volumetric flask and made up to 1000cm^3 mark with the 0.01M $NaNO_3$. 0.25, 0.50, 0.75, 1.00, and 1.25cm^3 of the stock solution was measured into a 50cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 5, 10, 15 , 20, and 25mg/l working concentration respectively. 0.1, 0.2, 0.3, and 0.4cm^3 of the stock solution was measured into a 100cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 1, 2, 3, and 4mg/l standard solution respectively.

3.3.5 Preparation of Chromium Stock Solution (1000mg/l)

2.829g of potassium dichromate was dissolved in 0.01M $NaNO_3$ in a volumetric flask and made up to 1000cm^3 mark with the 0.01M $NaNO_3$. 0.25, 0.50, 0.75, 1.00, and 1.25cm^3 of the stock solution was measured into a 50cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 5, 10, 15 , 20, and 25mg/l working concentration respectively. 0.1, 0.2, 0.3, and

0.4cm^3 of the stock solution was measured into a 100cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 1, 2, 3, and 4mg/l standard solution respectively.

3.3.6 Preparation of Cobalt Stock Solution (1000mg/l)

4.9383g of cobalt nitrate hexahydrate was dissolved in 0.01M $NaNO_3$ in a volumetric flask and made up to 1000cm^3 mark with the 0.01M $NaNO_3$. 0.25, 0.50, 0.75, 1.00, and 1.25cm^3 of the stock solution was measured into a 50cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 5, 10, 15 , 20, and 25mg/l working concentration respectively. 0.2, 0.4, 0.6, and 0.8cm^3 of the stock solution was measured in a 100cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 2, 4, 6, and 8mg/l standard solution respectively.

3.3.7 Preparation of Lead Stock Solution (1000mg/l)

1.599g of lead nitrate was dissolved in 0.01M $NaNO_3$ in a volumetric flask and made up to 1000cm^3 mark with the 0.01M $NaNO_3$. 0.25, 0.50, 0.75, 1.00, and 1.25cm^3 of the stock solution was measured into a 50cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 5, 10, 15 , 20, and 25mg/l working concentration respectively. 0.2, 0.4, 0.6, and 0.8cm^3 of the stock solution was measured into a 100cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 2, 4, 6, and 8mg/l standard solution respectively.

3.3.8 Preparation of Nickel Stock Solution (1000mg/l)

4.953g of nickel nitrate hexahydrate was dissolved in 0.01M $NaNO_3$ in a volumetric flask and made up to 1000cm^3 mark with the 0.01M $NaNO_3$. 0.25, 0.50, 0.75, 1.00, and 1.25cm^3 of the stock solution was measured into a 50cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 5, 10, 15 , 20, and 25mg/l working concentration respectively. 0.05, 0.1, 0.2, and 0.4cm^3 of the stock solution was measured into a 100cm^3 volumetric flask and made up to mark with 0.01M $NaNO_3$ to give 0.5, 1, 2, and 4mg/l standard solution respectively.

3.4 Adsorption Experiment

3.4.1 Effect Of Initial Metal Ion Concentration

The sorption experiment was by batch method. The experiment was carried out at ambient temperature (25^{o}C) in a shaker water bath using 100cm^3 conical flask as the reactor. 1000mg/l stock solution of each of the metals was prepared, from where the working solutions was prepared by serial dilution method. The metals of interest are: (1) Lead from Lead Nitrate ($Pb(NO_3)_2$) (2) Chromium from Potassium dichromate ($K_2Cr_2O_7$) (3) Nickel from Nickel Nitrate ($Ni(NO_3)_2.6H_2O$) (4) Cobalt from Cobalt Nitrate Hexahydrate ($Co(NO_3)_2 .6H_2O$); the working concentrations in mg/l for all the metals are 5, 10, 15, 20, and 25mg/l.

All the solutions was prepared in 0.01M $NaNO_3$ solution. The $NaNO_3$ serve as background inert electrolyte that provides stability in the ionic strength of the solution throughout the experiment. The pH of the solution was adjusted to the required value throughout the experiment with 0.1M NaOH and 0.1M HNO_3. These gave only nitrate ion and sodium ion which are already in the medium without altering the chemistry of the ion of interest.

The experiment was carried out as follows:

50cm^3 of each of the working solution was measured using standard flask into three 100cm^3 conical flask, which gave a triplicate for a given concentration i.e a set. 0.1g of the adsorbent was transferred into each of these conical flasks; the pH of the mixture was measured and adjusted to the desired pH. Chromium pH – 2.05, Cadmium – 5.05, Nickel – 5.02, Lead pH – 4.02, Cobalt – 4.05. Each set was agitated in the shaker at the same time for 3hr and the set was left undisturbed on the desk for 24hr to allow the system to equilibrate. After 24hr the mixture was filtered through a whatman filter paper into 120cm^3 polyethylene bottle. The first 5cm^3 of the filtrate was discarded. This allows the filter paper to saturate with the solution. The

concentration of the residual metal ion remaining in the solution was determined using AAS. The amount of the metal adsorbed was calculated using the equation:

$$q_e = \frac{V\,(C_o - C_e)}{M}$$

Where q_e is the amount of adsorbate ion adsorbed in milligram per gram of the adsorbent, C_o is the initial concentration of the metal ion before adsorption process, C_e is the equilibrium concentration of the metal ion in the filtrate after adsorption process and M is the mass in gram of the adsorbent, V is the volume of the solution. The percentage metal ion removal (%Rem) was calculated using the equation:

$$\%\text{Rem} = \frac{(c_o - c_e)}{c_o} \times 100$$

A table showing the result obtained was constructed as shown below

Initial conc.	Final conc.(I)	Final conc.(II)	Final conc.(III)	Average conc.	q_e(mg/l)	% of Metal

A graph of the amount of the metal ion adsorbed by the adsorbent was plotted against the initial metal ion concentration. This will depict at a glance initial metal ion that give maximum adsorption (Anurag *et al.,* 2007).

3.4.2 Effect of Biosorbent Dose

The effect of biosorbent dose was studied by varying the biosorbent dose used for the adsorption at a specific pH, concentration, time and temperature. The dosages used in grams are 0.05, 0.1, 0.15, 0.2, and 0.25.

Procedure

50cm^3 of each of the working solution (20mg/l) was measured using standard flask into three 100cm^3 conical flask. This gave a triplicate for a given biosorbent dose i.e a set. Required biosorbent dose was transferred into each of these conical flasks; the pH of the mixture was measured and adjusted to the desired pH. Chromium pH – 2.02, Cadmium – 5.05, Nickel – 5.02, Lead pH – 4.02, Cobalt – 4.05. Each set was agitated in the shaker at the same time for 3hr and the set was left undisturbed on the desk for 24hr to allow the system to equilibrate. After 24hr the mixture was filtered through a whatman filter paper into 120cm^3 polyethylene bottle. The first 5cm^3 of the filtrate was discarded. This allows the filter paper to saturate with the solution. The concentration of the residual metal ion in the solution was determined using AAS. The amount of the metal adsorbed was calculated using the equation:

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Where q_e is the amount of adsorbate ion adsorbed in milligram per gram of the adsorbent, C_o is the initial concentration of the metal ion before adsorption process, C_e is the equilibrium concentration of the metal ion in the filtrate after adsorption process and M is the mass in gram of the adsorbent, V is the volume of the solution. The percentage metal ion removal was calculated using the equation:

$$\%\text{Rem} = \frac{(c_o - c_e)}{c_o} \times 100$$

A table showing the result obtained was constructed as shown below

Initial conc.	Final conc.(I)	Final conc.(II)	Final conc.(III)	Average conc.	q_e(mg/l)	% of Metal

A graph of the amount of the metal ion adsorbed by the adsorbent was plotted against the biosorbent dose. This will depict at a glance the biosorbent dose for optimum adsorption(Anurag *et al.,* 2007).

3.4.3 The Effect of Contact Time

The effect of contact time is necessary in order to determine the time required for maximum adsorption to take place. Data generated from this study would be used to describe the kinetics of the adsorption study. Using the constant concentration, biosorbent dose, the desired pH and at room temperature, the effect of contact time would be studied by varying the agitation time. The following agitation time in minutes that was tested is: 30, 60, 90, 120, and 150.

Procedure

$50cm^3$ of each of the working solution (20mg/l) was measured using standard flask into three $100cm^3$ conical flask. This gave a triplicate for a given time i.e a set. 0.1g of the adsorbent was transferred into each of these conical flasks; the pH of the mixture was measured and adjusted to the specified pH and this was agitated for the desired time. After the specified time, the mixture was filtered immediately using suction pump and buckner funnel into which a filter paper is fitted. The concentration of the residual metal ion remaining in the solution was determined using AAS.

The amount of the metal adsorbed was calculated using the equation:

$$q_e = \frac{V\,(C_o - C_t)}{M}$$

Where q_e is the amount of adsorbate ion adsorbed in milligram per gram of the adsorbent, C_o is the initial concentration of the metal ion before adsorption process, C_t is the equilibrium concentration of the metal ion in the filtrate after adsorption process at a given time t and M is the mass in gram of the adsorbent, V is t

he volume of the solution. The percentage metal ion removal was calculated using the equation:

$$\%\text{Rem} = \frac{(c_o - c_t)}{c_o} \times 100$$

A table showing the result obtained was constructed as shown below

Initial conc.	Final conc.(I)	Final conc.(II)	Final conc.(III)	Average conc.	q_e(mg/l)	% of Metal

A graph of the amount of the metal ion adsorbed by the adsorbent was plotted against the contact time. This will depict at a glance contact time required for optimum adsorption(Anurag *et al.,* 2007). This equilibrium data obtained will be used for kinetics and biosorption modeling

3.4.4 The Effect of Initial Solution pH

The pH of solution is important in adsorption study, in that pH of a solution determines the speciation of the metal ion in solution and also dictates the surface charge of adsorbents with variable charge characteristics. Using constant concentration of 20mg/l of each solution, at

ambient temperature the pH of the solution would be varied. The pH that was tested are: 2, 4, 6, and 8.

Procedure

50cm^3 of each of the working solution was measured using standard flask into three 100cm^3 conical flask. This gave a triplicate for a given pH i.e. a set. 0.1g of the adsorbent was transferred into each of these conical flasks; the pH of the mixture was measured and adjusted to the desired pH. Each set would be agitated in the shaker at the same time for 3hr and the set was left undisturbed on the desk for 24hr to allow the system to equilibrate. After 24hr the mixture was filtered through a whatman filter paper into 120cm^3 polyethylene bottle. The first 5cm^3 of the filtrate was discarded. This allows the filter paper to saturate with the solution. The concentration of the residual metal ion remaining in the solution was determined using AAS. The amount of the metal adsorbed was calculated using the equation:

$$q_e = \frac{\mathrm{V}\,(\mathrm{C_o} - \mathrm{C_e})}{\mathrm{M}}$$

Where q_e is the amount of adsorbate ion adsorbed in milligram per gram of the adsorbent, C_o is the initial concentration of the metal ion before adsorption process, C_e is the equilibrium concentration of the metal ion in the filtrate after adsorption process and M is the mass in gram of the adsorbent, V is the volume of the solution. The percentage metal ion removal was calculated using the equation:

$$\%\mathrm{Rem} = \frac{(\mathrm{c_o} - \mathrm{c_e})}{\mathrm{c_o}} \times 100$$

A table showing the result obtained was constructed as shown below

Initial conc.	Final conc.(I)	Final conc.(II)	Final conc.(III)	Average conc.	q_e(mg/l)	% of Metal

A graph of the amount of the metal ion adsorbed by the adsorbent was plotted against pH. This will depict at a glance the pH that corresponds to the pH at which the adsorption is best carried out for each ion using the adsorbent(Anurag *et al.,* 2007).

3.4.5 Effect of Temperature

The effect of temperature was studied by varying the temperature at which the reaction takes place at specific concentration, pH, biosorbent dose and time. The temperature range used are: 300, 313, 323, and 333K.

Procedure

50cm^3 of each of the working solution (20mg/l) was measured using standard flask into three (3) 100cm^3 conical flask. This gave a triplicate for a given temperature i.e a set. 0.1g of the adsorbent was transferred into each of these conical flasks; the pH of the mixture measured and was adjusted to the desired pH. Chromium pH – 2.02, Cadmium – 5.05, Nickel – 5.02 , Lead pH – 4.02, Cobalt – 4.05. Each set was agitated in the shaker at the same time for 3hr and the set was left undisturbed on the desk for 24hr to allow the system to equilibrate. After 24hr the mixture was filtered through a whatman filter paper into 120cm^3 polyethylene bottle. The first 5cm^3 of the filtrate was discarded. This allows the filter paper to saturate with the solution. The concentration of the residual metal ion in the solution was determined using AAS. The amount of the metal adsorbed was calculated using the equation:

$$q_e = \frac{V\,(C_o - C_e)}{M}$$

Where q_e is the amount of adsorbate ion adsorbed in milligram per gram of the adsorbent, C_o is the initial concentration of the metal ion before adsorption process, C_e is the equilibrium concentration of the metal ion in the filtrate after adsorption process and M is the mass in gram of the adsorbent, V is the volume of the solution. The percentage metal ion removal was calculated using the equation:

$$\%\text{Rem} = \frac{(c_o - c_e)}{c_o} \times 100$$

A table showing the result obtained was constructed as shown below

Initial conc.	Final conc.(I)	Final conc.(II)	Final conc.(III)	Average conc.	q_e(mg/l)	% of Metal

A graph of the amount of the metal ion adsorbed by the adsorbent was plotted against the temperature. This will depict at a glance the temperature t required for optimum adsorption (Anurag *et al.,* 2007). The equilibrium data obtained will be used for thermodynamic parameters calculation.

3.5 Atomic Absorption Spectrometry (AAS)

The Bulk Scientific Atomic Absorption Spectroscopy machine with model number: VGP 210 was used in the determination of the equilibrium or residual concentrations of the metal ion in the filtrates after adsorption process. The operating conditions are as provided in the operational manual (Table 3.1). The Air – acetylene flame was used in the determination of the equilibrium residual metal ion concentration.

Table 3.1: Operating Conditions of atomic absorption spectrometer

Metal	Wave – length(nm)	Band – Pass	Flame Type
Pb	283.3	0.2	Air/C_2H_2
Cr	357.9	0.5	Air/C_2H_2
Cd	228.8	0.2	Air/C_2H_2
Co	240.7	0.2	Air/C_2H_2
Ni	232.0	0.2	Air/C_2H_2

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CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Preliminary Studies

Before the adsorption studies were conducted, some preliminary investigations on the properties of the algal biomass *Cosmarium panamense* were examined. These include some physicochemical characteristics of algal biomass as shown in Table 4.1.

The result showed that the percent dry matter and percent moisture of the dry algal biomass *Cosmarium panamense* are 92.5% and 7.53% respectively. The moisture content of a sample refers to the water content of that sample. The determination of the moisture content was carried out during the dry season when the moisture content of the atmosphere was very low because seasons affect the moisture content, for the adsorbent will absorb moisture from the environment.

The percent moisture was higher than 5.75% reported by Shakirullah *et al* (2006) for Sawdust of *Dalbergia sissoo* and 6.48%, 7.18%, 7.21% reported by Erhan *et al* (2004) for Cornelian Cherry(CC), Apricot Stone(AC) and Almond Shells(ACS) respectively. This may be as a result of its hydrophilic nature.

The pH reported in the literature ranges 5.80 – 7.10: (Shakirullah *et al.,* 2006; Erhan *et al.,* 2004). The pH of 1% solution of the algal biomass *Cosmarium panamense* is 6.73 which falls within the acceptable pH conditions Most applications generally accept pH of 6 – 8 (Okieimen *et al.,* 2004).. Adsorption capacity increases under pH conditions that decreases the solubility

of the adsorbate. In highly acidic media, the adsorbent surfaces might be highly protonated and favour the uptake of metal ions in the anionic form (Selvi, *et al.,* 2001).

Bulk density is another important physical parameter especially when an adsorbent is to be investigated for its filterability because it determines the amount of adsorbent that can be contained in a filter of given solids capacity and the amount of treated liquid that can be retained by the filter cake (Okieimen *et al.,* 2004). Higher density provides greater volume activity and normally indicates better quality adsorbent. Bulk density determines to a large extent the length of the filtration cycle of activated carbon (Abram, 1973). Holan and Volesky (1993) reported 0.761g/cm^3 for *Ascophyllum nodosum* and 0.478g/cm^3 for *Fucus vesiculosus*; Shakirullah *et al* (2006) reported 0.26g/cm^3 for Sawdust of *Dalbergia sissoo*; Erhan *et al* (2004) reported 0.46, 0.43 and 0.51g/cm^3 for cornelian cherry(CC), apricot stone(AC) and almond shells(ACS) respectively while bulk density obtained from algal biomass of *Cosmarium panamense* is 0.687g/cm^3. This suggests that the algal biomass of *Cosmarium panamense* is a good adsorbent in terms of volume activity.

The percent ash content, 29.3%, of the sample was found to be very high compared to results by other researchers (Shakirullah *et al* (2006), reported ash content of 0.1% for saw dust of *Dalbergia sissoo*; Erhan *et al.* (2004), reported ash content of 2.08%, 2.21% and 2.14% for cornelian cherry(CC), apricot stone(AC) and almond shells(ACS) respectively). The ash content of a sample is the inorganic residue left after the organic matter has been burnt off. This is the measure of the mineral content of the sample (Allen *et al*., 1974). The amount of ash depends on the carbon sources (OMRI, 2002), most carbons sources have varying quantities of ash components, high ash content suggest good presence of inorganic constituents and hence, reduces the overall activity of activated carbon and also reduces the efficiency of

reactivation. This therefore suggests that there is high presence of inorganic constituents in the algal biomass of *Cosmarium panamense*. Hence, the poor activity of its carbon predicted.

Table 4.1:
Physicochemical parameters of the algal biomass *Cosmarium panamense*

Parameters	Native algal Biomass
pH of 1% Solution	6.73
Bulk Density (g/cm^3)	0.687
Moisture content (%)	7.53
Dry Matter (%)	92.6
Ash Content (%)	29.3

4.2 Characterization of the Biosorbent

4.2.1 Elemental Analysis.

This was done on the native algal biomass alone using Neutron Activation Analysis (NAA) to determine the elements present. Energy Dispersive X-ray was further used to further conduct elemental analysis qualitatively on the native and different heavy metal (Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+}) ion treated algal biomass to determine the effect of biosorption on availability of elements for detection. The EDAX graphical representation of all the elements detected in the native, Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} treated algal biomass are presented in figures 4.1,4.2, 4.3, 4.4, 4.5, and 4.6 respectively. The result of the elemental analysis on the biosorbent using NAA is presented in Table 4.2.

The result shows that the elemental quantities of short-lived elements (Mg, Al, Ca, V, Mn, Sc, Cr, Fe, Co, and Rb) in the sample were comparatively higher than the amounts of long-lived elements. Native sample showed high elemental quantities for Aluminum(Al), Magnesium(Mg), Calcium(Ca), Iron(Fe), and Potassium(K), Manganese(Mn), Vanadium(V), Barium(Ba), Bromine(Br), (La) while Arsenic(As), (Ta), Hafnium (Hf), Zinc(Zn), Europium (Eu), Cesium (Cs), Thorium(Th), Uranium (U), Dysprosium (Dy) and Thallium (Ti) are Below Detection Limit(BDL). Hence, the sample showed high metal content. The metal content is usually the non-volatile inorganic constituents present in the carbons. Al, K, Na, Mg, Ca, and Fe are the metals that were found to be high in the sample. This suggests the reason why the ash content was extremely high. It is important to state here that the chemical nature and

concentration of these metal constituents may sometimes interfere with the carbonization and/or activation process either positively or negatively.

From the EDAX graphical representations it was observed that most of the elements detected in the native biomass using NAA were not detected by Energy Dispersive X – ray. This is due to the fact that Energy Dispersive X – ray detect only quantities up to 0.1% and most of the elements detected by NAA when converted to percentage are not up to this amount that can be detected by EDAX. Table 4.3 Shows elements detected by Energy Dispersive X – ray on the native and heavy metal(Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+}) ions treated algal biomass and it was observed that the number of elements detected by the Energy Dispersive X – ray on the biomass before and after biosorption varies. This is due to the surface interactions of each of these heavy metals (Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+}) ions on the surface binding sites of the algal biomass during biosorption. This surface interaction which may lead to either sequestering or masking some of these elements, making them unavailable for detection and releasing some of these elements, making them available for detection. In other words, complexation and ion – exchange plays major role in the variation of elements on the biomass before and after biosorption. This agrees with reports by Sannasi *et al* (2006), that Non – metabolism dependent biosorption mechanism is a rapid process that include precipitation, physical adsorption, ion – exchange, and complexation.

Table 4.3 shows the presence of C, O, Al, Cl, Cu and Nb in the native algal biomass. After biosorption with Pb^{2+}; C, O, Al, Cu and Pb was detected in the biomass. Cd^{2+}treated algal biomass showed the presence of C, Al, Cu, Nb and Cd. Ni^{2+} and Cr(VI) ion treated biomass showed their presence in addition to C, O, Al, Cl, Cu, Si, and Nb in their respective treated algal biomass while Co^{2+} biomass displayed all the above mentioned metals in addition to Fe

and itself(Cobalt). Pb^{2+} treated algal biomass showed the least number of elements detected after biosorption while Co^{2+} biomass showed highest number of elements. This may be attributed to high complexation reaction on the native algal biomass surface during sorption of Pb^{2+} and $Cd^{2+}$and high ion – exchange reaction during sorption of Co^{2+}, Ni^{2+} and Cr(VI). This observation suggests that the sorption of these metals could be chemisorption. From visual analysis of the EDX profile of native, Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} treated algal biomass, it was that observed carbon had the highest concentration of all the elements detected followed by oxygen (except for Cd^{2+}).

Table 4.2: Elemental analysis of the algal biomass *Cosmarium panamense* using NAA

Elements	Native Algal Biomass	Elements	Native Algal Biomass
Mg (%)	0.70 ± 0.07	Sc (ppm)	3.34 ± 0.10
Al (%)	2.44 ± 0.07	Cr (ppm)	4.50 ± 0.70
Ca (%)	0.40 ± 0.04	Fe (%)	1.00 ± 0.03
Ti (ppm)	BDL	Co (ppm)	6.60 ± 0.4
V (ppm)	16.00 ± 2.00	Zn (ppm)	BDL
Mn (ppm)	70.00 ± 7.00	Rb (ppm)	8.50 ± 2.20
Dy (ppm)	BDL	Cs (ppm)	BDL
Na (%)	0.06 ± 0.001	Ba (ppm)	27.00 ± 4.00
K (%)	0.32 ± 0.03	Eu (ppm)	BDL
As (ppm)	BDL	Tb (ppm)	NA
Br (ppm)	20.00 ± 0.40	Lu (ppm)	0.03 ± 0.004
La (ppm)	13.4 ± 0.40	Hf (ppm)	BDL
Sm (ppm)	3.10 ± 0.20	Ta (ppm)	BDL
Yb (ppm)	0.26 ± 0.03	Sb (ppm)	0.15 ± 0.03
U (ppm)	BDL	Th (ppm)	BDL

Table 4.3: Summary of Elemental Analysis of Native and Heavy Metal Treated Algal Biomass by EDAX.

Native Biomass	Cadmium Treated Biomass	Chromium Treated Biomass	Cobalt Treated Biomass	Lead Treated Biomass	Nickel Treated Biomass
Carbon	Carbon	Carbon	Carbon	Carbon	Carbon
Oxygen	----------	Oxygen	Oxygen	Oxygen	Oxygen
Aluminium	Aluminium	Aluminium	Aluminium	Aluminium	Aluminium
Niobium	Niobium	Niobium	Niobium	---------	Niobium
Chlorine	----------	Chlorine	Chlorine	---------	Chlorine
Copper	Copper	Copper	Copper	Copper	Copper
----------	----------	Silicon	Silicon	----------	Silicon
----------	----------	----------	Iron	----------	--------
	Cadmium	Chromium	Cobalt	Lead	Nickel

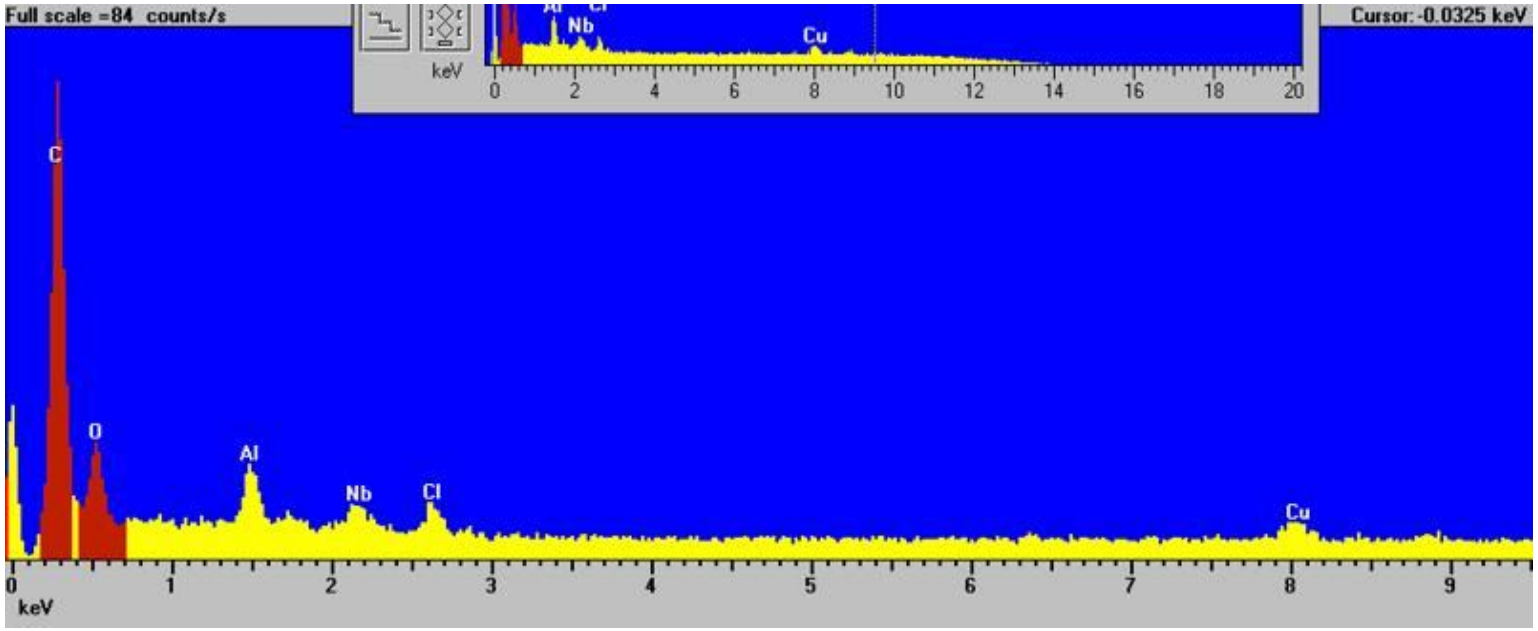


Figure 4.1: EDAX Profile for Native Algal Biomass.

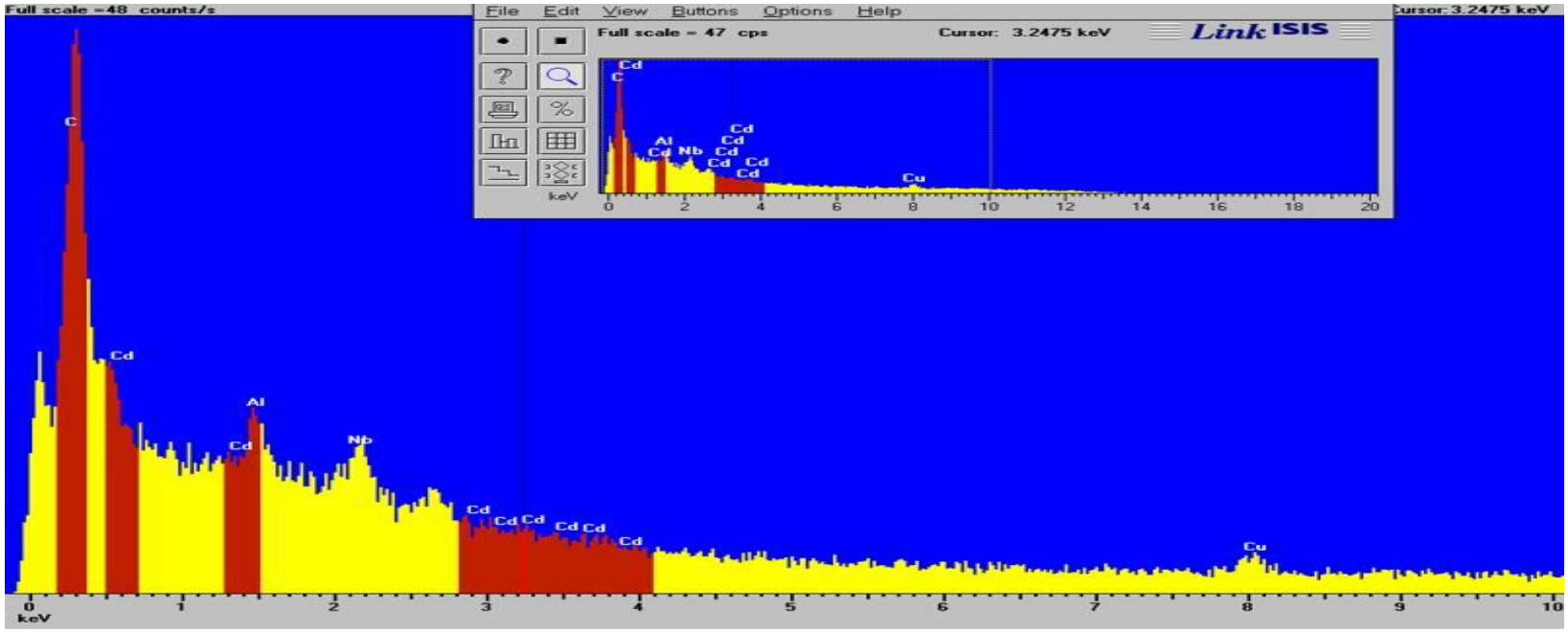


Figure 4.2: EDAX Profile for Cd^{2+} Treated Algal Biomass.

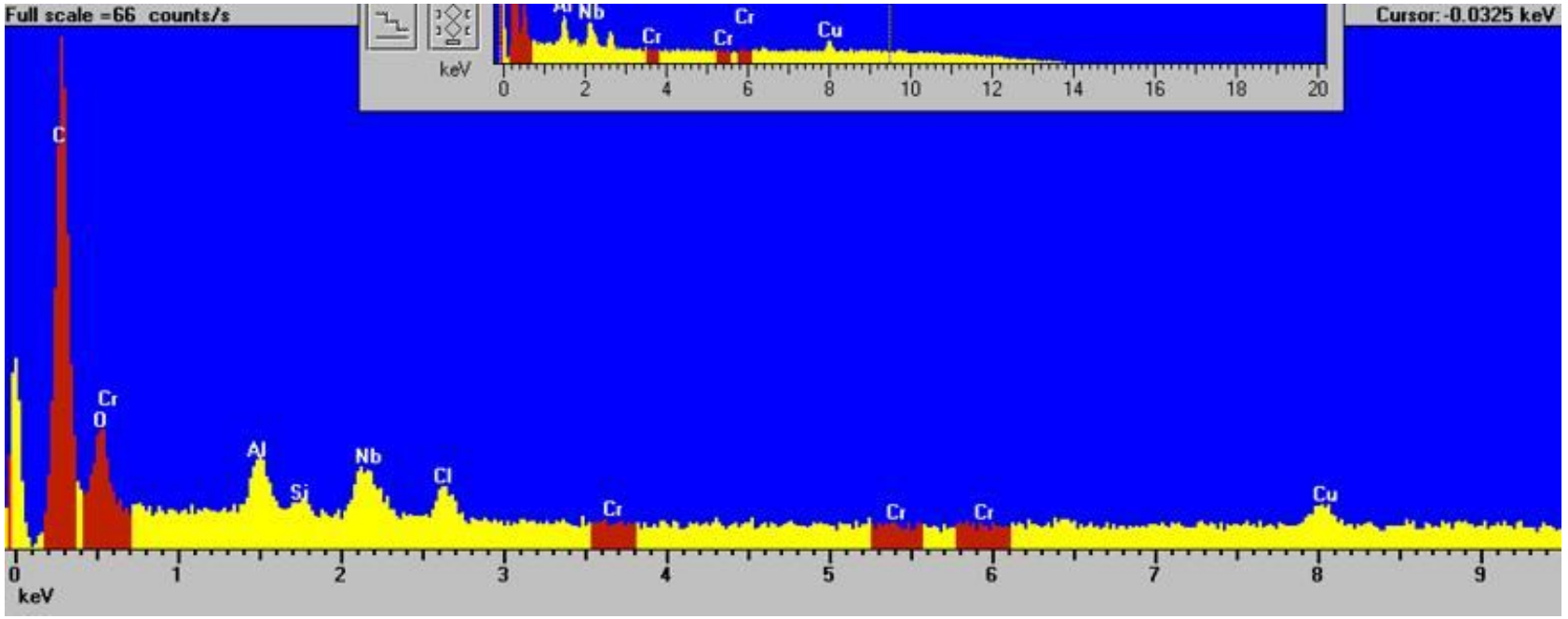


Figure 4.3: EDAX Profile for Cr(VI) Treated Algal Biomass.

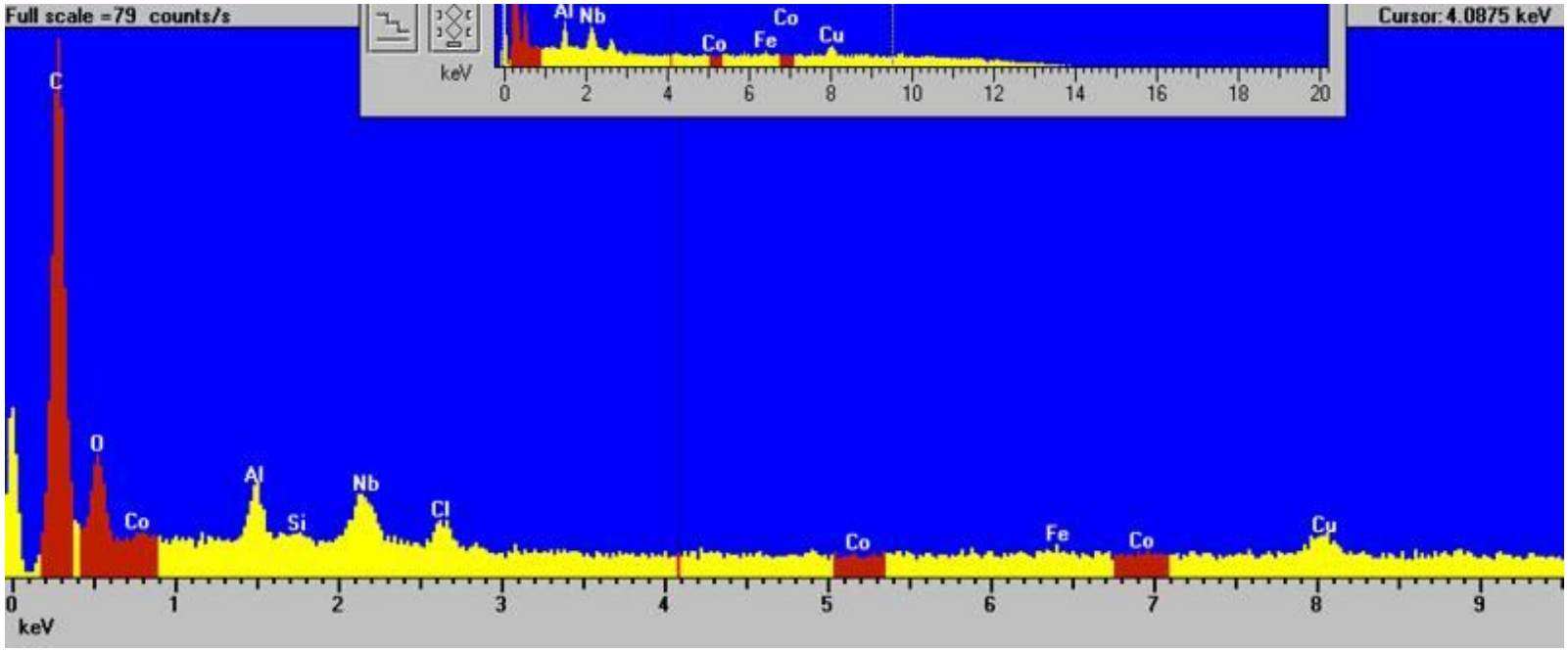


Figure 4.4: EDAX Profile for Co^{2+} Treated Algal Biomass.

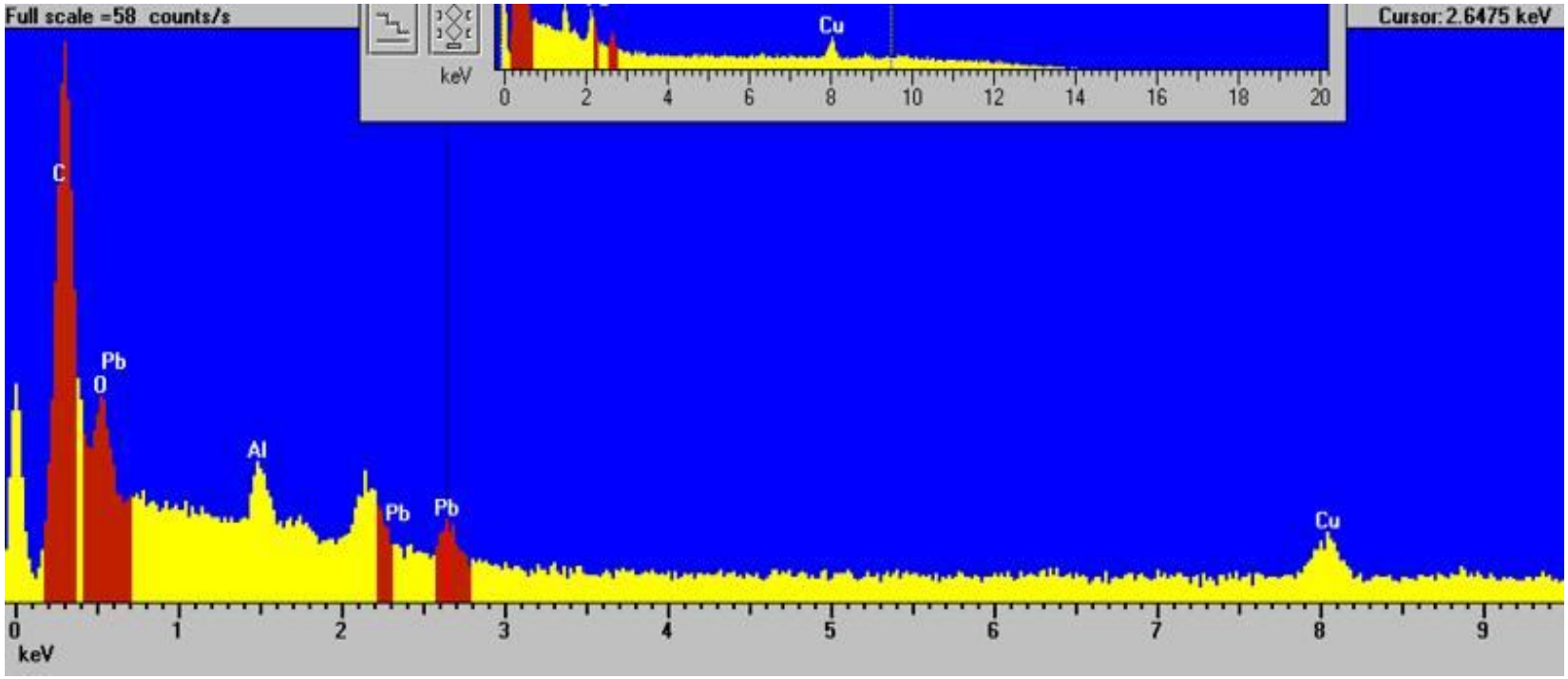


Figure 4.5: EDAX Profile for Pb^{2+} Treated Algal Biomass.

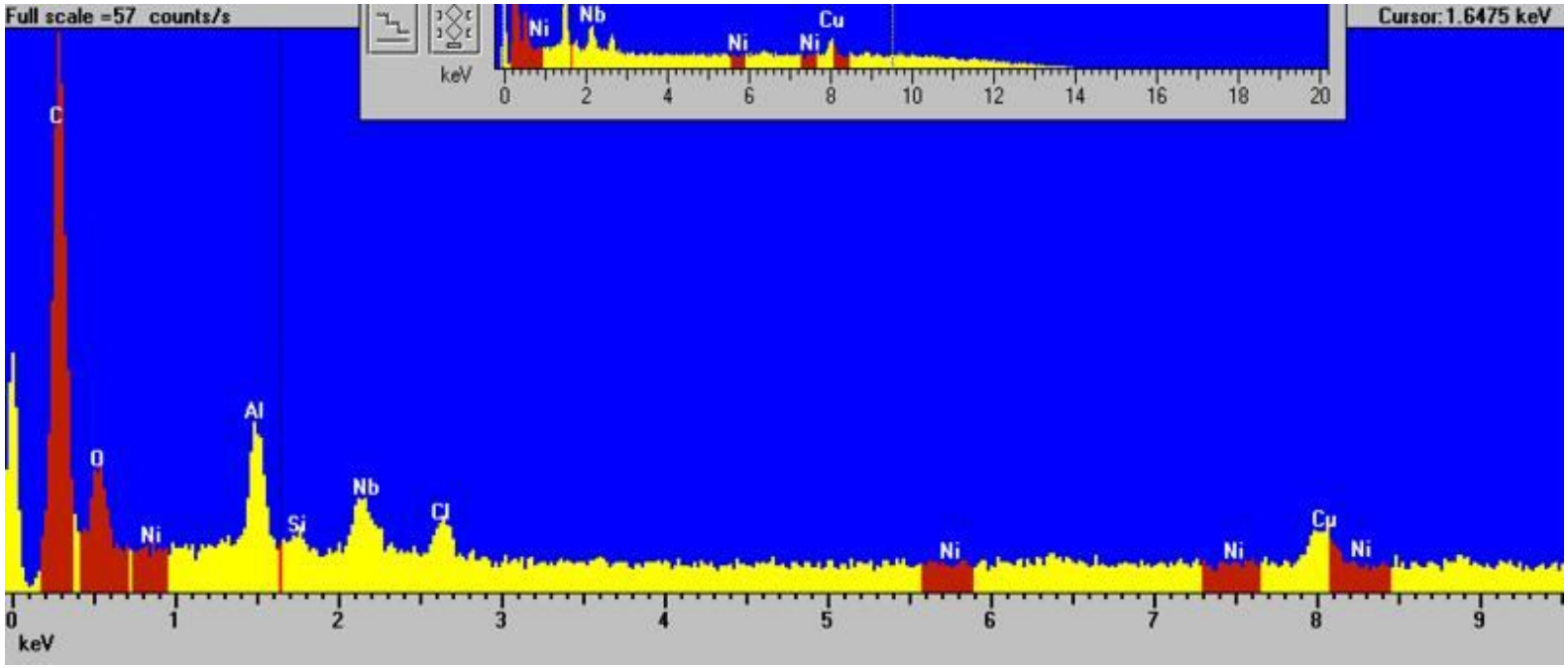
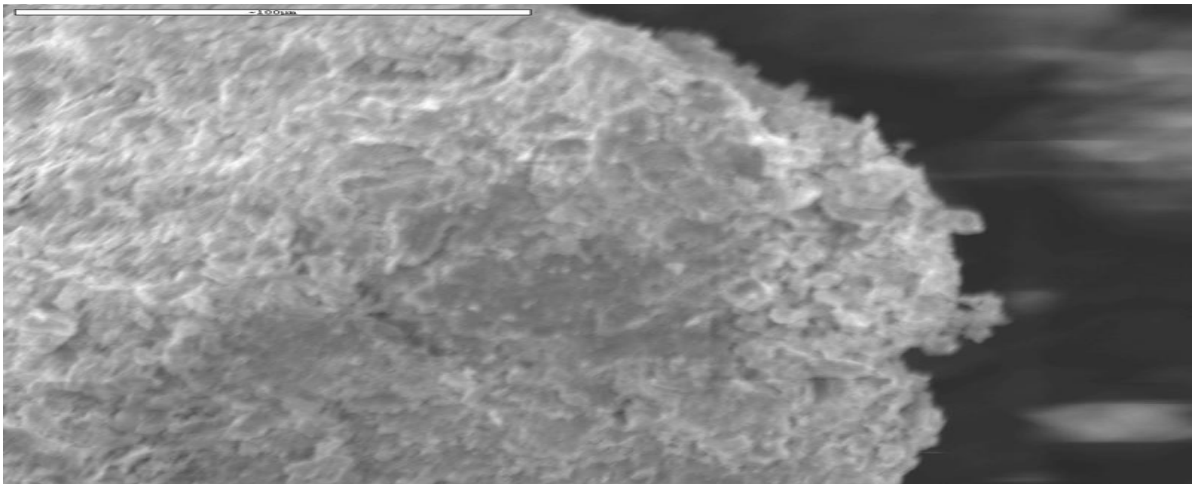


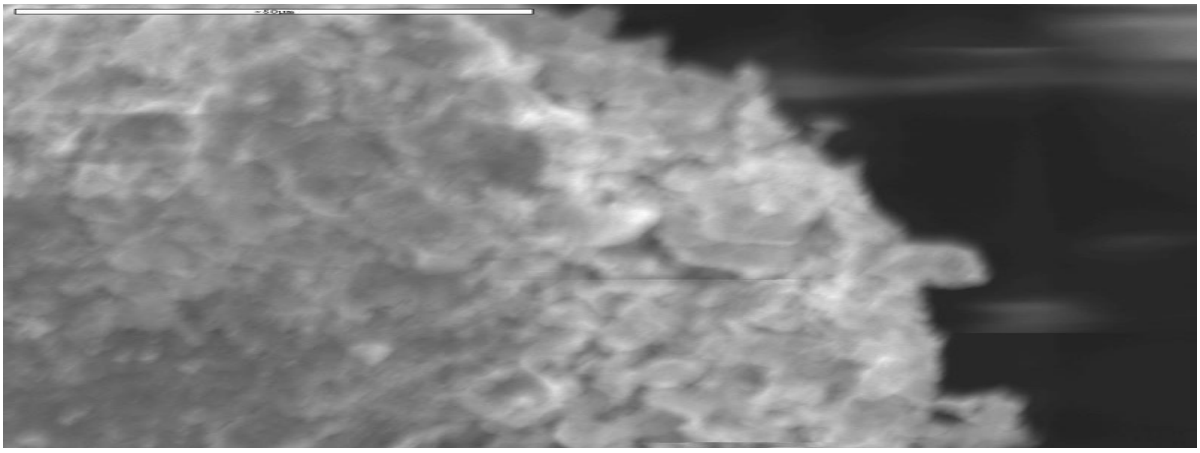
Figure 4.6: EDAX Profile for Ni^{2+} Treated Algal Biomass.

4.2.2 Scanning Electron Microscope (SEM)

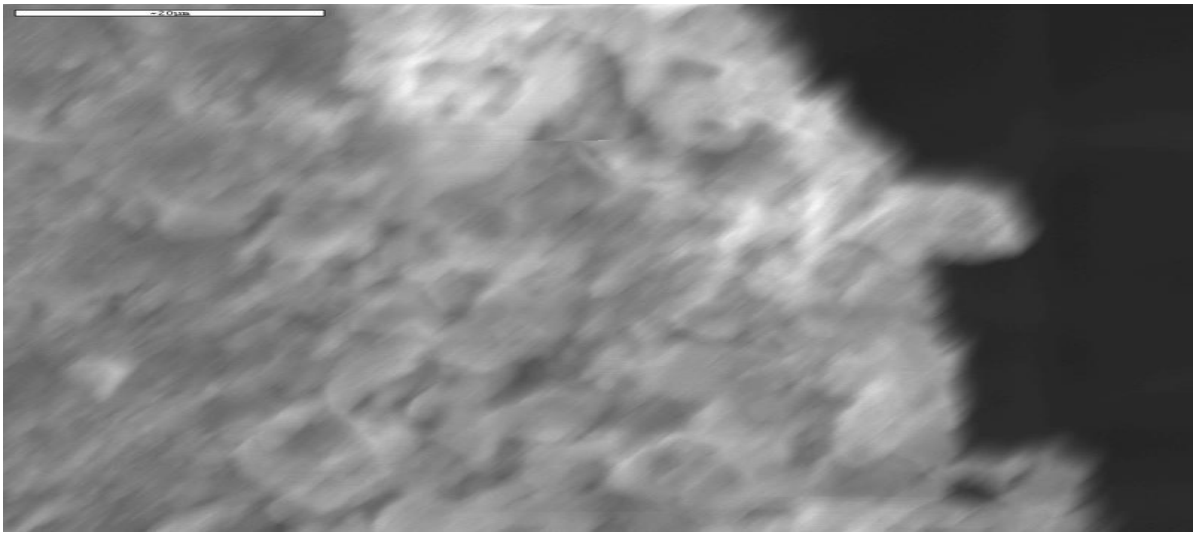
Scanning Electron Microscope clearly revealed the surface texture and morphology of the algal biomass before and after biosorption at different magnifications(500x, 1000x, and 1500x). The SEM micrograph of native and Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} treated algal biomass are shown in Figures 4.7, 4.8, 4.9, 4.10, 4.11, and 4.12 respectively. At the different magnifications of the native algal biomass, uneven surface texture along with a lot irregular surface format was observed. These figures displayed evidently that over the biosorption period, the surface morphology of the native algal biomass had undergone remarkable physical disintegration resulting to emergence of protrusions and rough surface area. SEM micrograph for Ni^{2+} treated algal biomass in Fig.4.12 showed more disintegrations and rough surface than the other heavy metal ion treated biomass SEM micrographs. This is due to the high surface interactions between the binding sites on the surface of the algal biomass and Ni^{2+} thereby supporting the finding that the FTIR spectra of Ni^{2+} treated algal biomass had the highest number of absorption peaks. Similar SEM observations have been reported (Wilke *et al.,* 2006; Shakirullah *et al.,* 2006; Gupta and Rastogi, 2007; Sethuraman and Balasubramanian, 2010).



500x

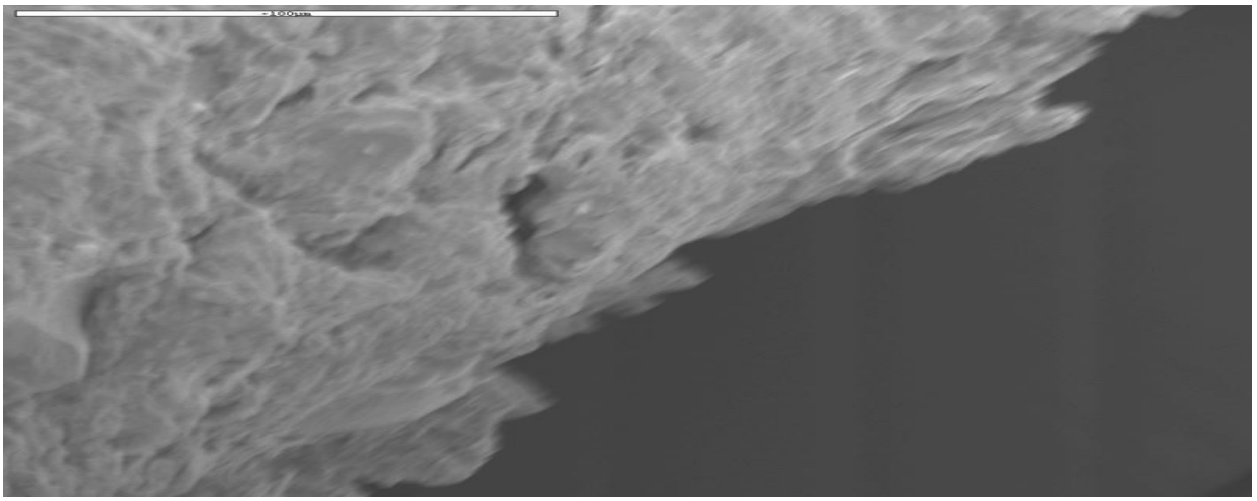


1000x

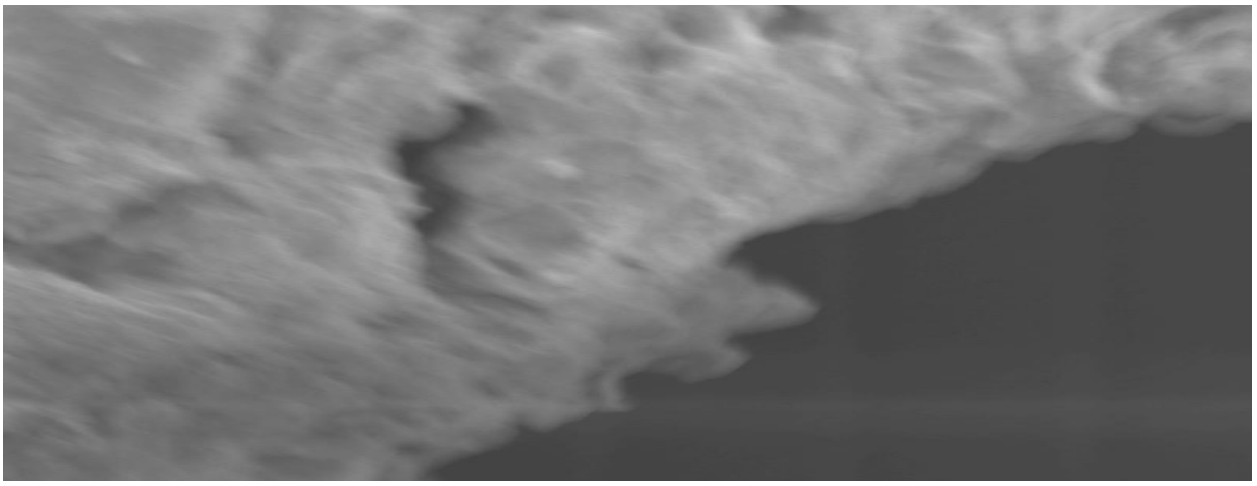


1500x

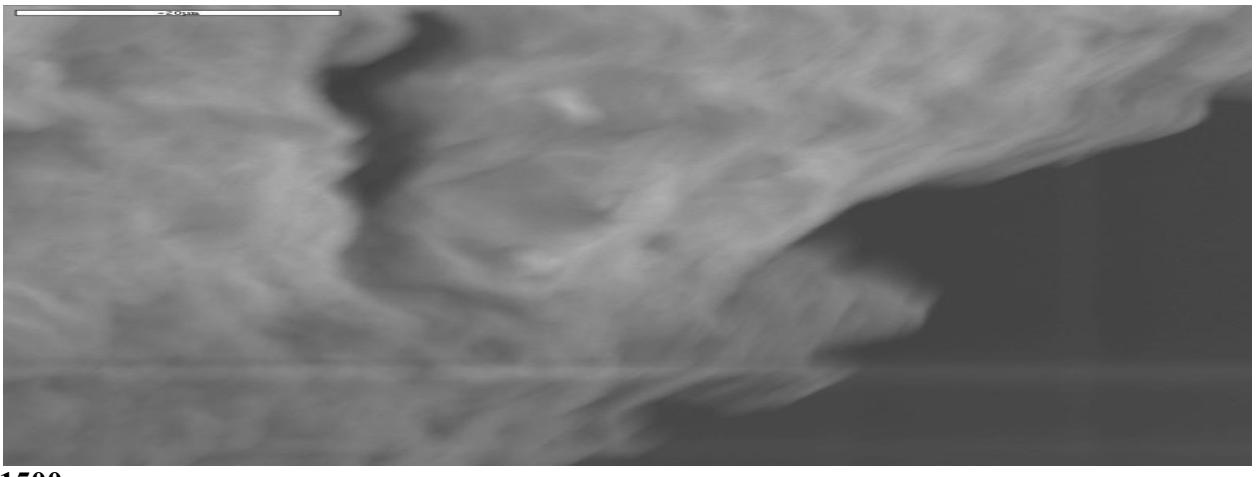
Figure 4.7: SEM Micrograph for Native Algal Biomass.



500x

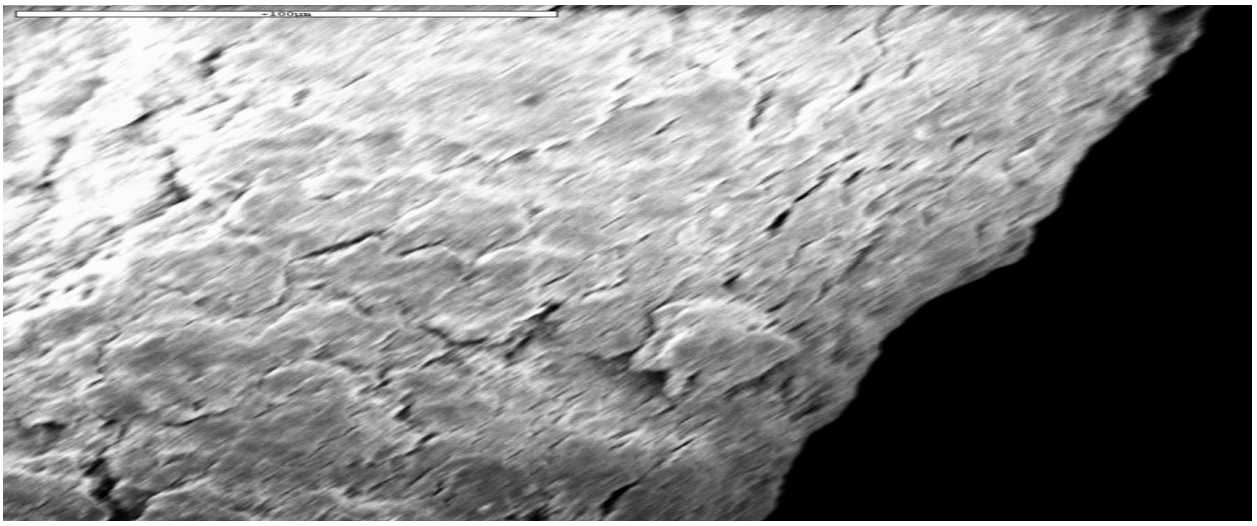


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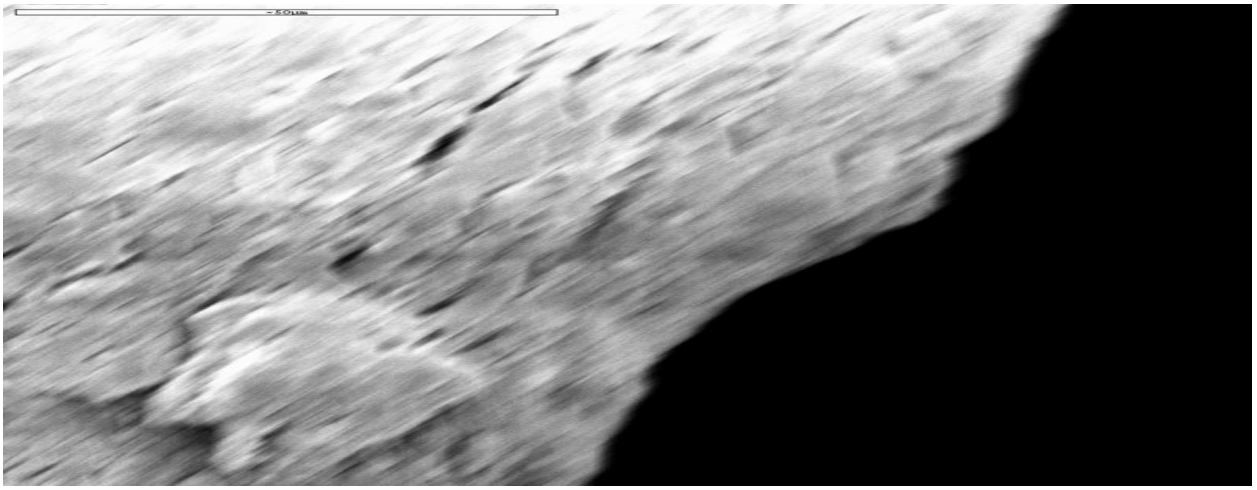


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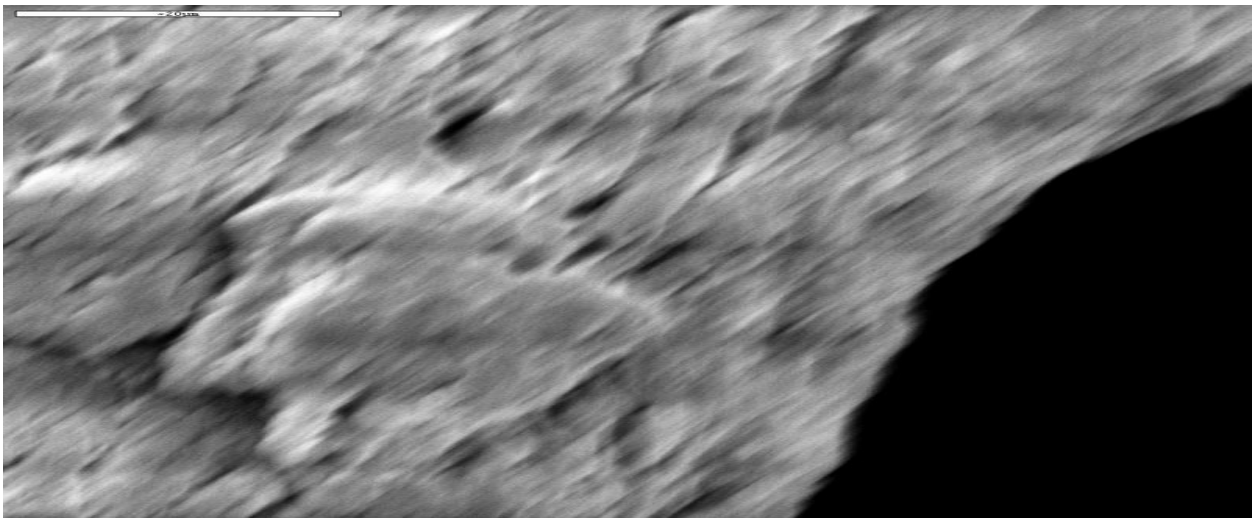
Figure 4.8: SEM Micrograph for Cd^{2+} Treated Algal Biomass.



500x

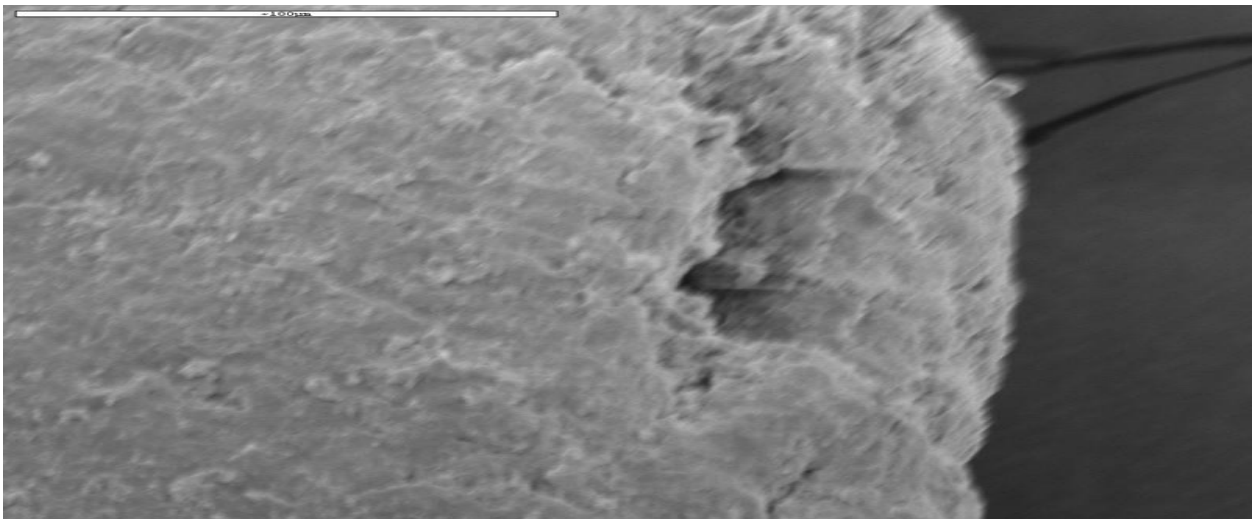


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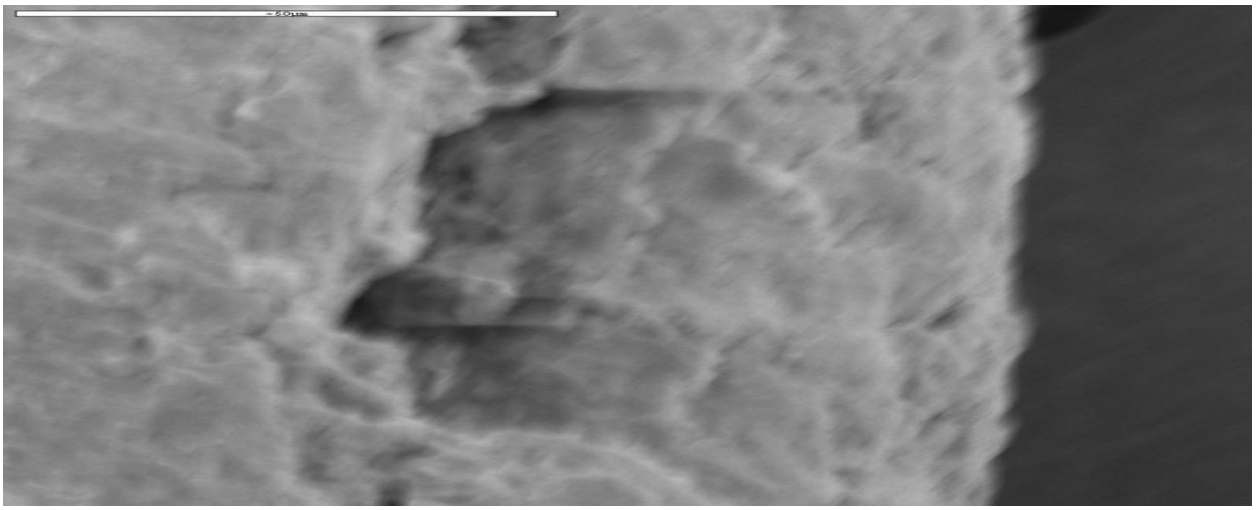


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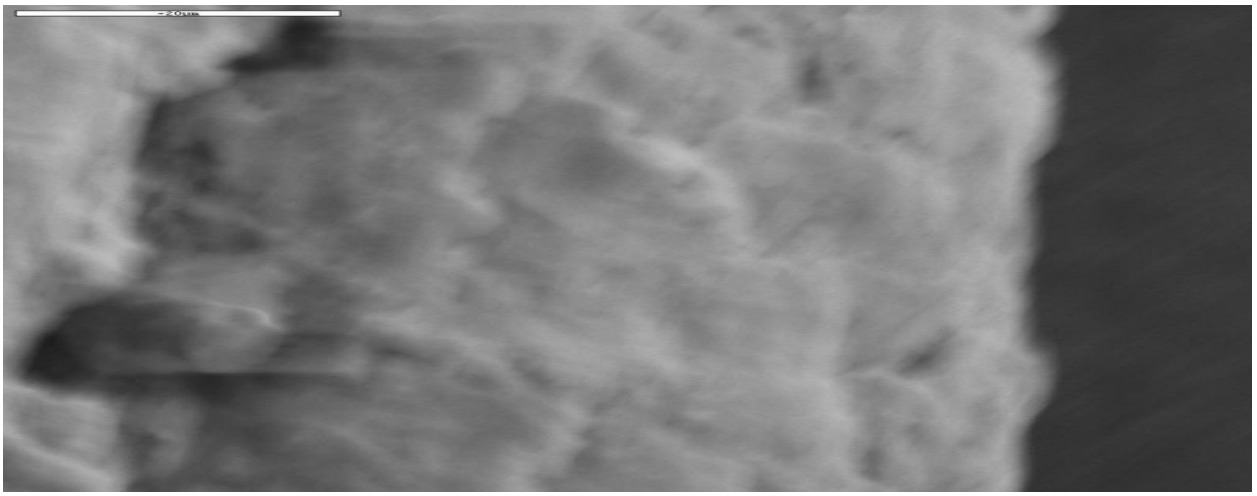
Figure 4.9: SEM Micrograph for Cr(VI) Treated Algal Biomass.



500x

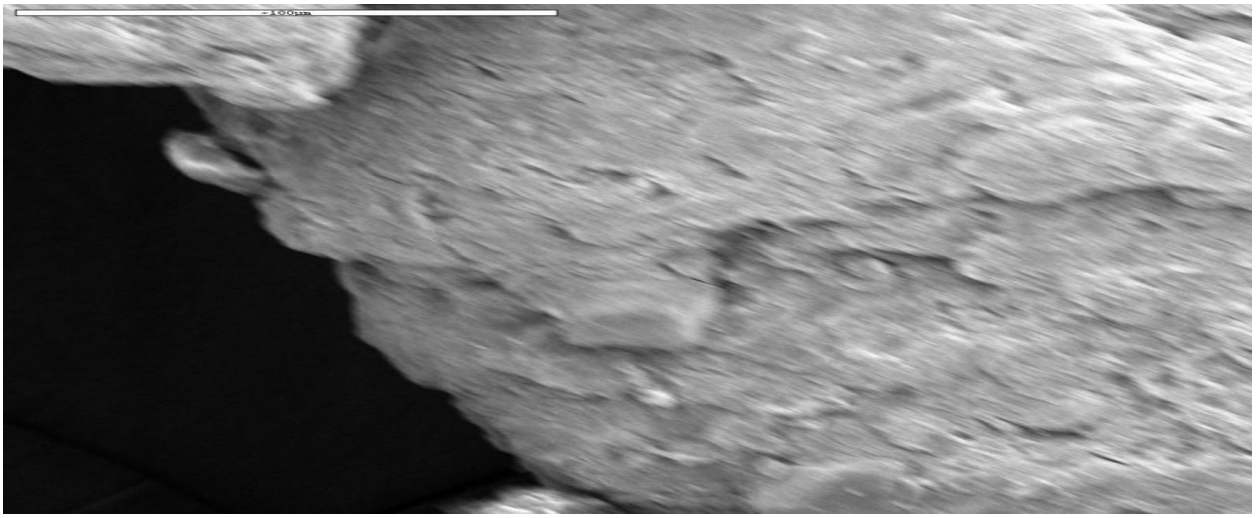


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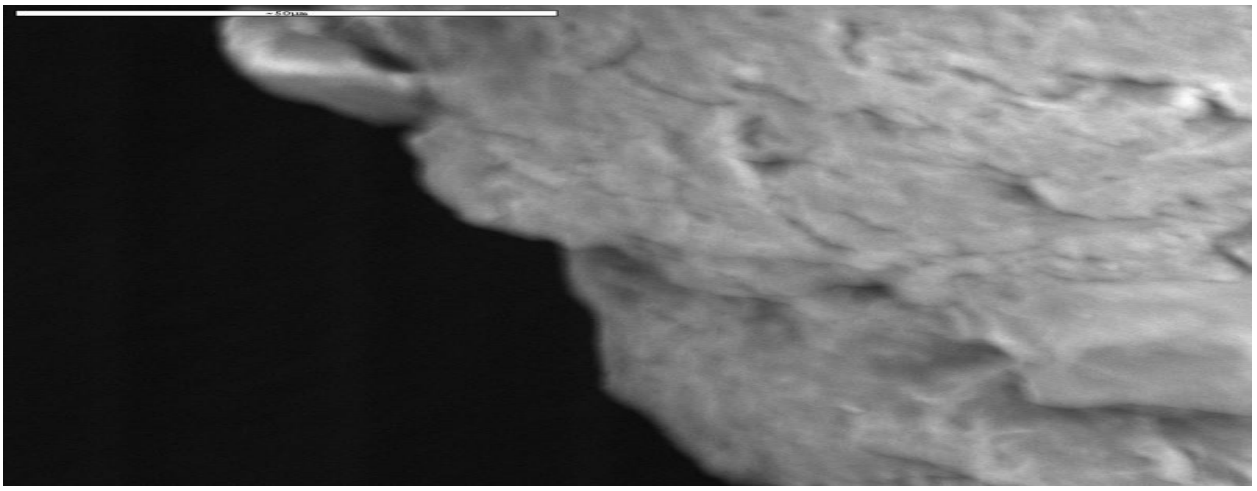


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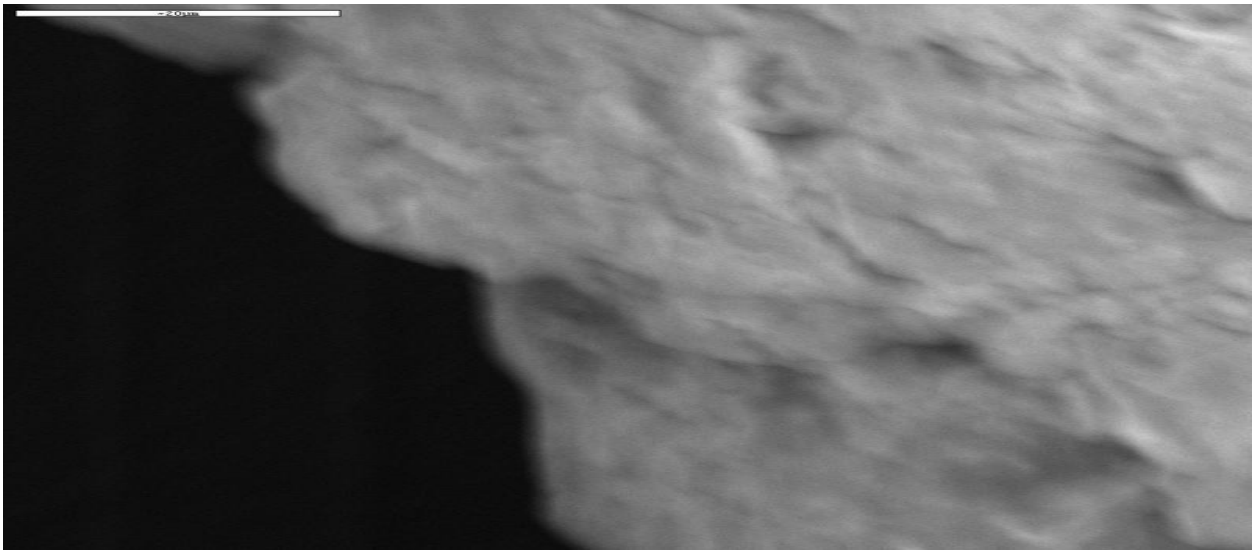
Figure 4.10: SEM Micrograph for Co^{2+} Treated Algal Biomass.



500x

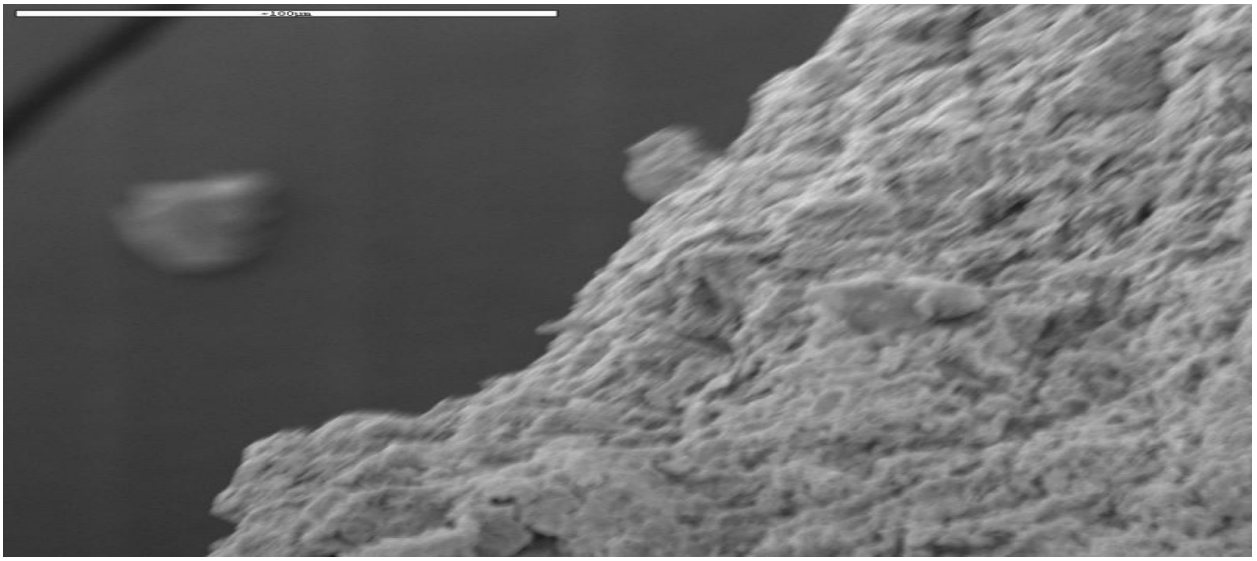


1000x

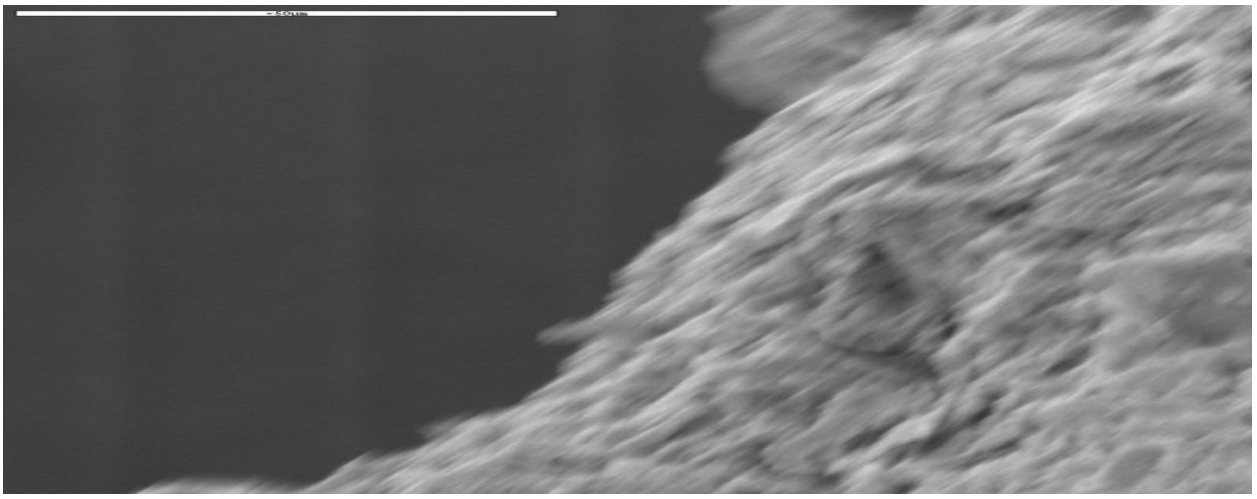


1500x

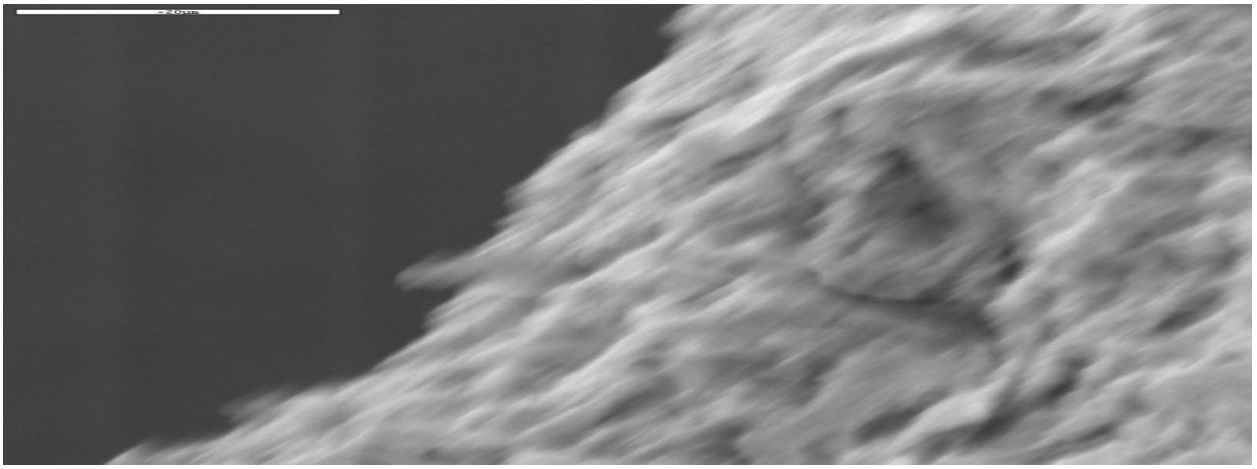
Figure 4.11: SEM Micrograph for Pb^{2+} Treated Algal Biomass.



500x



1000x



1500x

Figure 4.12: SEM Micrograph for Ni^{2+} Treated Algal Biomass.

4.2.3 Simultaneous Thermal Analysis (STA)

The thermal degradation analysis was carried out to provide information on the bulk composition of the compound (Hussein *et al.*, 2008). Simultaneous Thermo – Gravimetric analysis/Differential Scanning Calorimetry provide information on both the heat flow and weight changes in a material as a function of temperature or time in a controlled atmosphere (Mansfield, 2010). The results obtained for thermal properties of native, Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+}treated algal biomass are shown in figures 4.13, 4.14, 4.15, 4.16, 4.17 and 4.18 respectively.

All the samples showed a two step decomposition process. This similar observation was reported by Gupta and Rastogi (2007). The first decomposition step was the same in all the samples as small heat flow of 21.8mcal/sec absorbed by the samples increased their temperature to 110^{o}C resulting to a minor weight loss of 11.2% in all the samples at 110^{o}C. This observation is attributed to water desorption or dehydration (Li *et al*., 2009).

Hussein *et al* (2008) reported that during thermal analysis, different regions can be associated with weight loss, retained water at 100^{o}C, hemicelluloses degradation at 200^{o}C – 260^{o}C, cellulose degradation at 280 – 500^{o}C. The thermograms clearly showed that the second decomposition was a thermal degradation because large amount of heat was absorbed. This effect is obviously due to the disintegration of intra – molecular interactions such as hydrogen bonds between molecules (Sun *et al.*, 2004). All the samples started decomposing at 310^{o}C. The increasing decomposition temperature for all the biomass proved that they are all thermally stable. This suggests that the main structure of native algal biomass was not altered during biosorption of these heavy metal ions. However, the thermograms showed that the heat flow absorbed by the various samples to decompose as

well as weight loss after decomposition varied for the different samples. The thermogram (Figure 4.13) revealed that the native algal biomass absorbed heat at flow rate of 99.4mcal/sec to begin decomposition and percent residue weight of 27.6 remained after the decomposition process. This supports earlier finding of 29.3% ash content from the Ash Content of the native algal biomass. Thermograms of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} treated algal biomass showed that they received heat flow of 89, 112, 105, 107, 108mcal/sec respectively to begin decomposition or thermal degradation and percent weight of 22.1 for cadmium; 23.9 for chromium, 22.8 for cobalt, 23.4 for lead and 21.2 for nickel; remained constant after the decomposition process despite increase in temperature. Hence it was observed that heat absorbed by various samples to decompose at 310^{o}C was higher in the heavy metal ions treated algal biomass than the native algal biomass. This could be attributed to ionic bonds formed on the surface of the algal biomass after adsorption of the metal ions by the native biomass. These ionic bonds possess high energy and therefore result to the high amount of heat required for the thermal degradation of heavy metal ion treated algal biomass. Also, percent residue weight of the native biomass remaining after decomposition process was found to be higher than that remaining for the different heavy metal ion treated algal biomass and this may be due to the interaction of these heavy metal ions with the surface binding sites of the cell wall structure of the native biomass, altering quantitatively the inorganic constituents of the biomass through complexation and ion exchange process, this then results in reduction in the percent residue weight when subjected to heat after adsorption. These observations also support the findings made in this study using adsorption models, that the sorption of these heavy metals ions are chemisorption process.

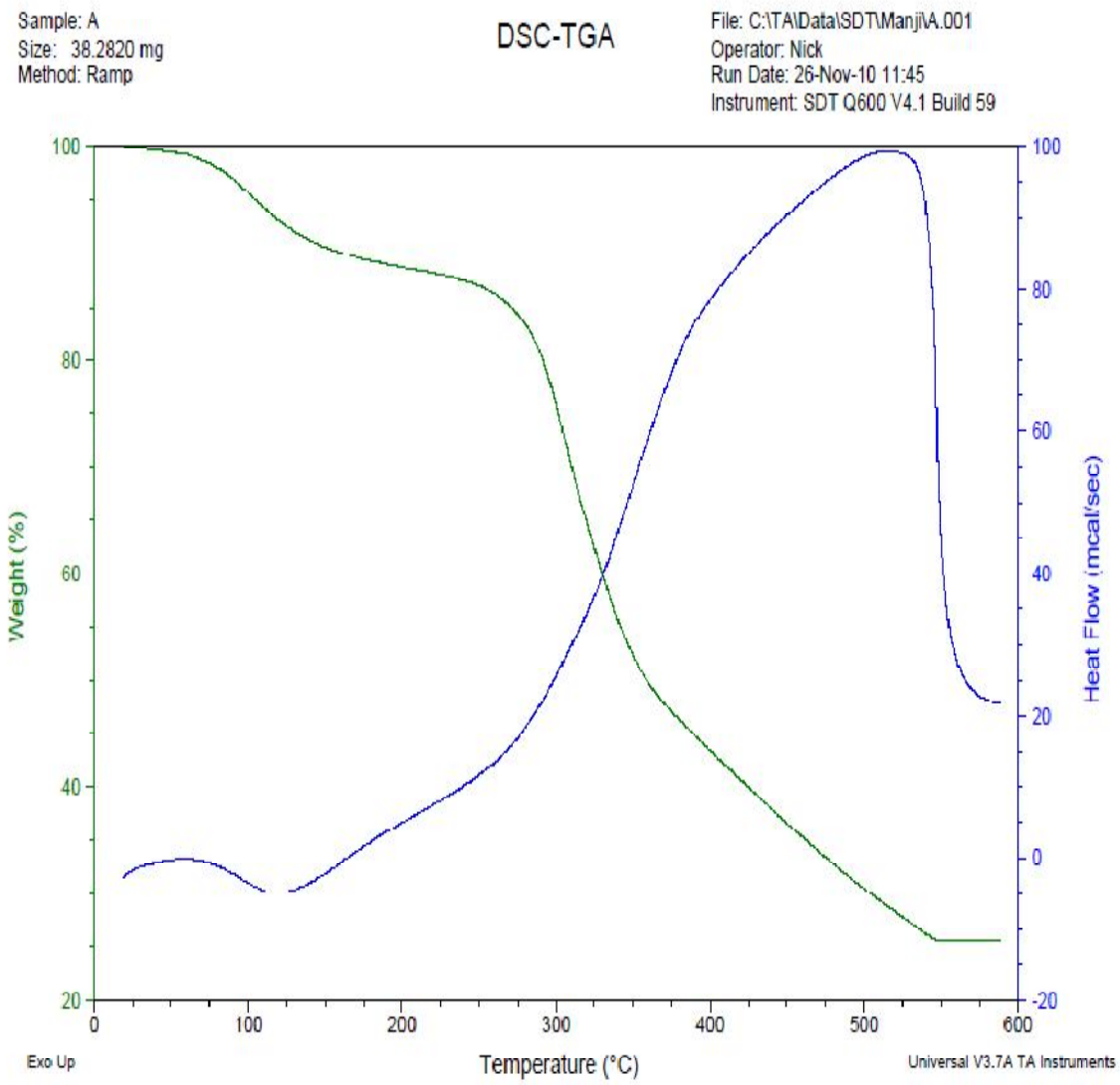


Figure 4.13: Thermogram of Native Algal Biomass Degradation.

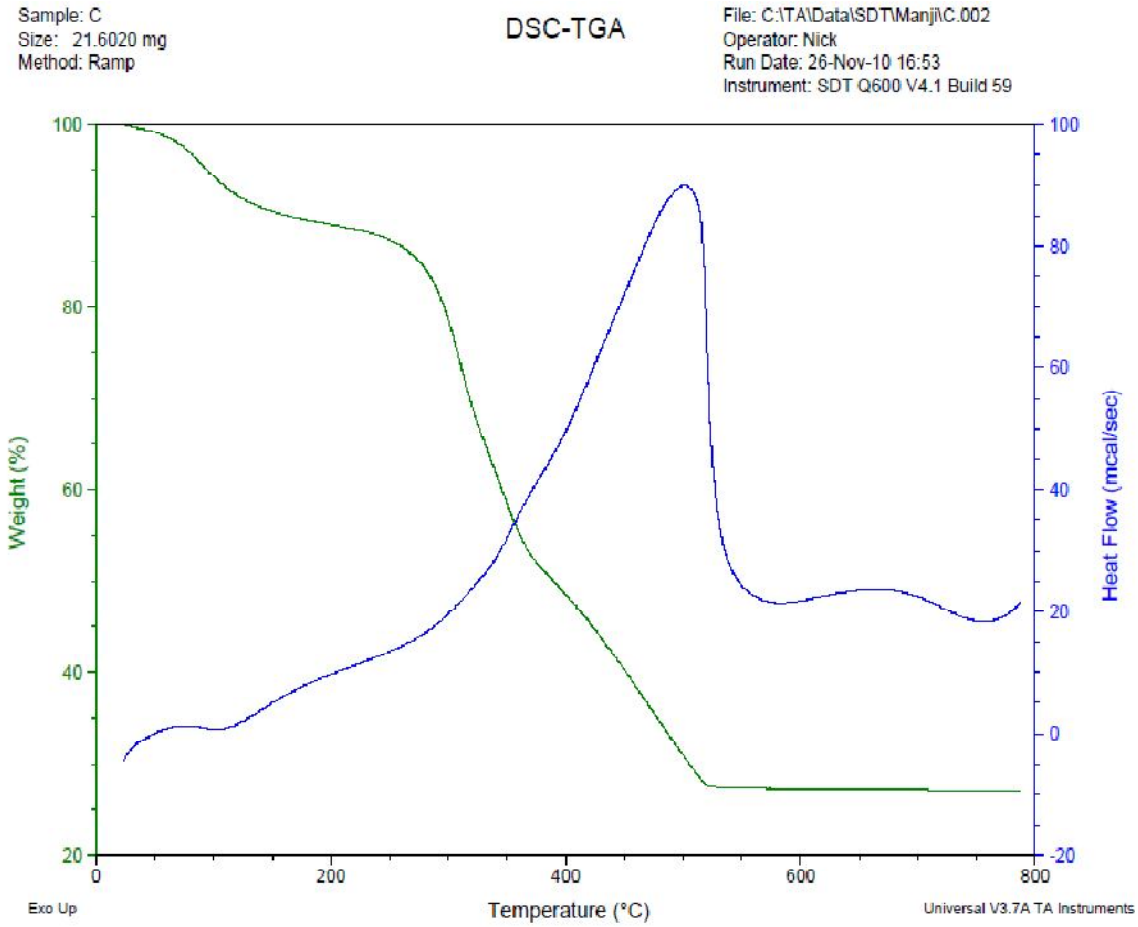


Figure 4.14: Thermogram of Cd^{2+} Treated Algal Biomass Degradation.

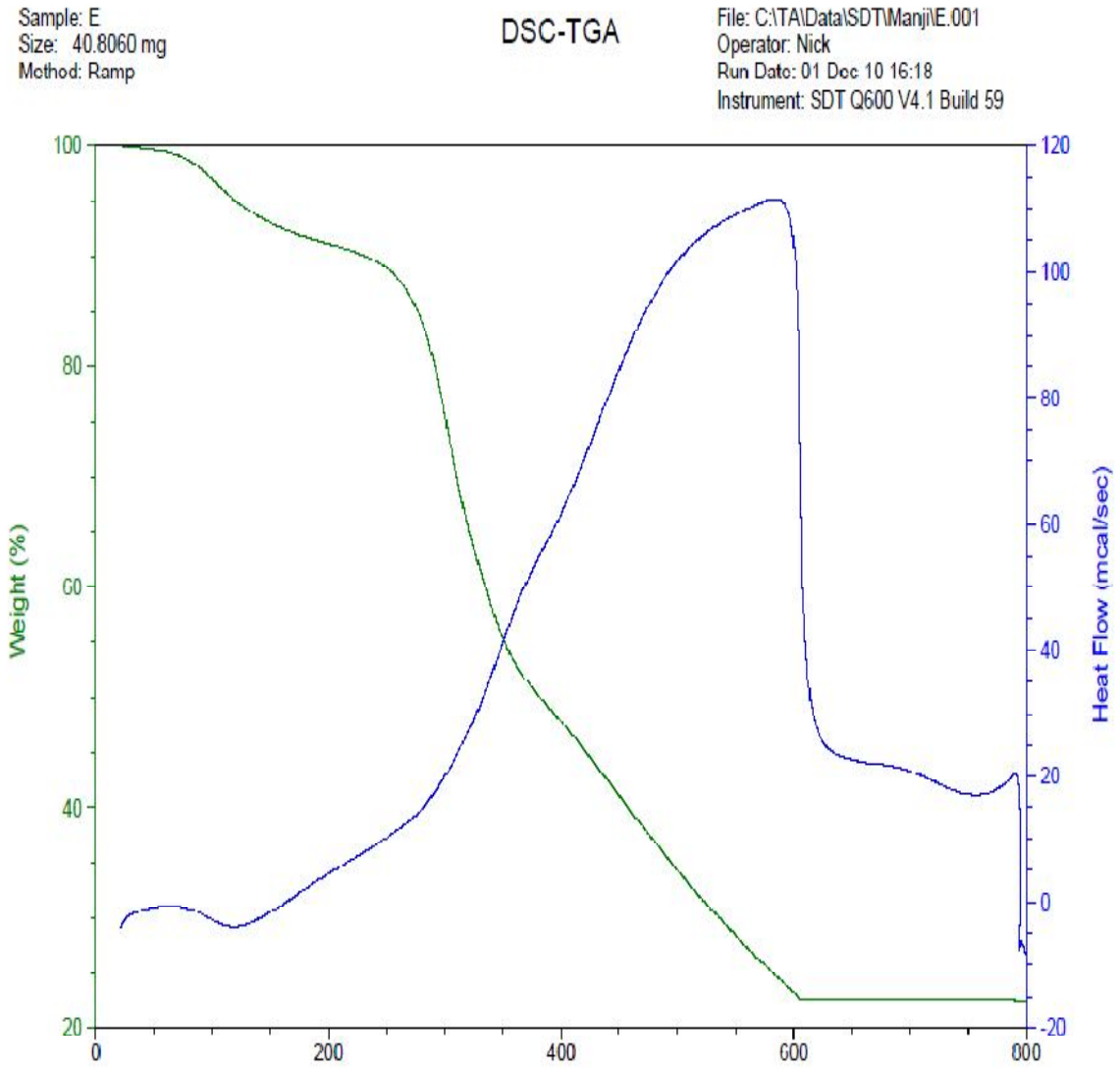


Figure 4.15: Thermogram of Cr(VI) Treated Algal Biomass Degradation.

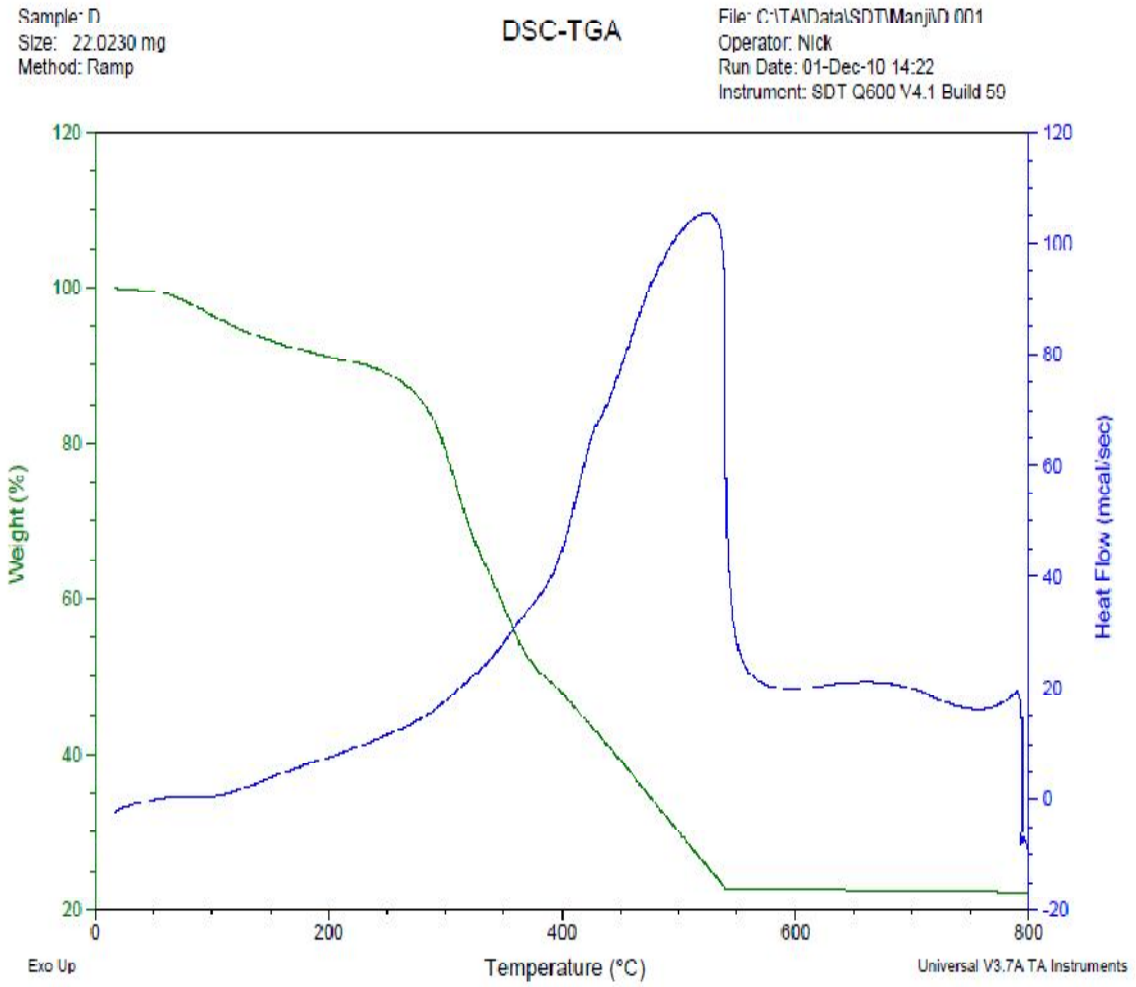


Figure 4.16: Thermogram of Co^{2+} Treated Algal Biomass Degradation.

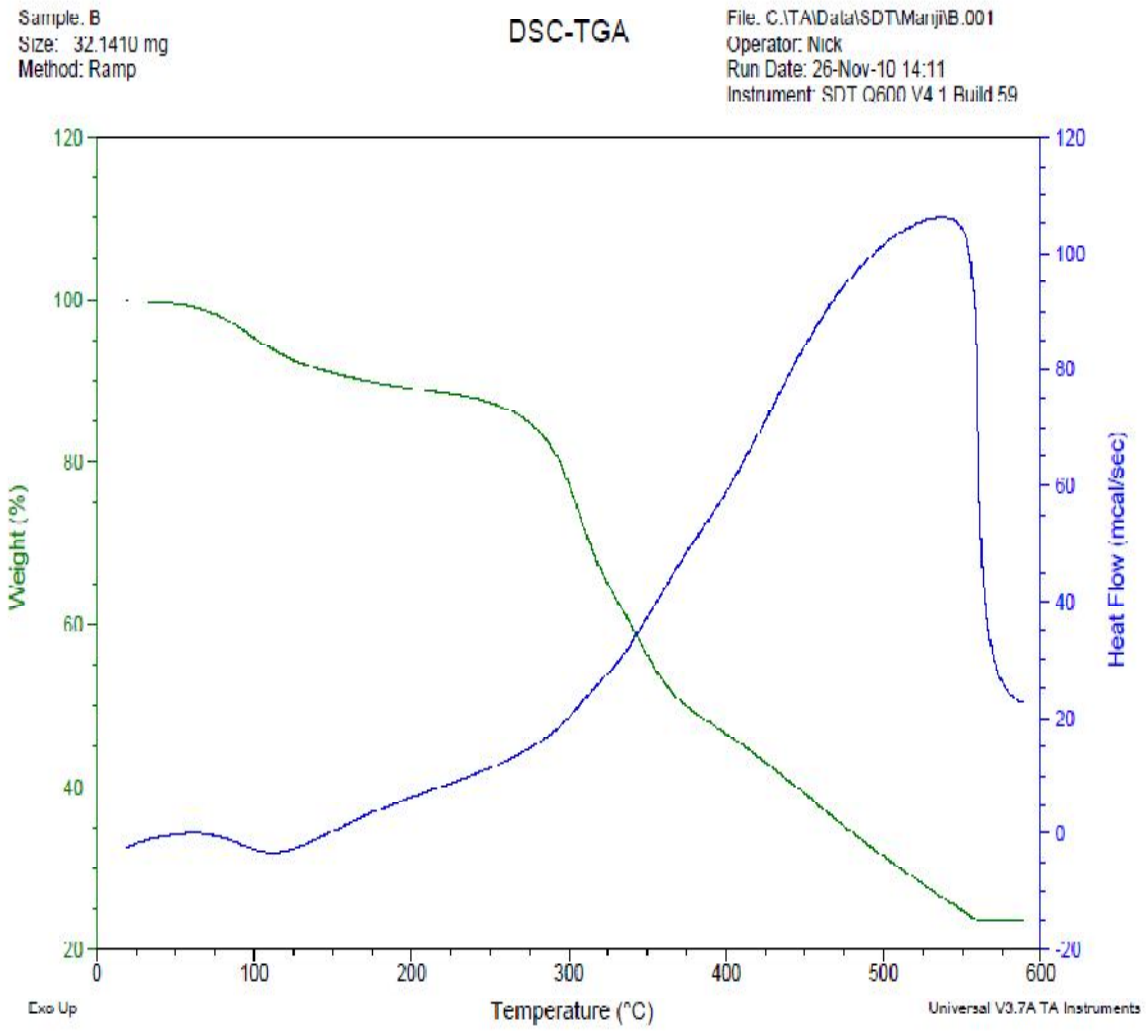


Figure 4.17: Thermogram of Pb^{2+} Treated Algal Biomass Degradation.

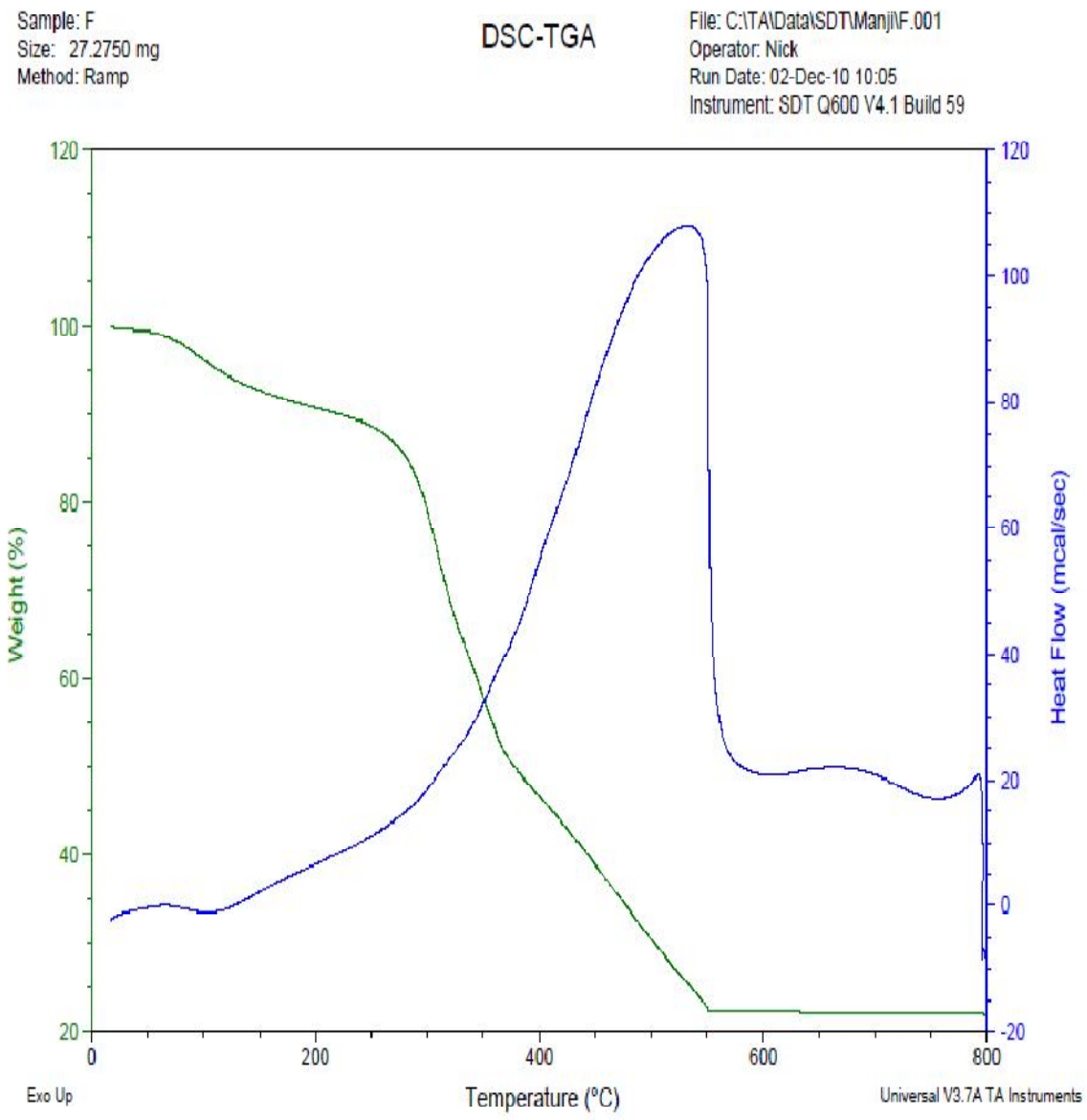


Figure 4.18: Thermogram of Ni^{2+} Treated Algal Biomass Degradation.

4.2.4 Fourier Transform Infra – red (FTIR) Spectra Analysis

The FTIR spectra of native and different heavy metal ions (Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+}) treated algal biomass in the range of 500 – 5000cm^{-1} were performed to have an idea of which functional groups were responsible for the biosorption process. The FTIR spectra of native, Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} treated algal biomass are presented in Figures 4.19, 4.20, 4.21, 4.22, 4.23 and 4.24 respectively. Absorbance peaks that appeared in all the FTIR spectra are summarized in Table 4.5 while assignment of IR bands of functional groups in the algal biomass is presented in Table 4.4.

The spectra analysis indicate the presence of ionizable functional groups namely amino, amide, carboxyl, phosphonate, hydroxyl, silica, methyl and methylene group which are able to interact with proton or metal ions.

It was observed from Table 4.5 that the most absorbance peaks of the different heavy metal ions treated algal biomass were slightly lower than the absorbance peaks of native algal biomass.

The native biomass displays a number of absorption peaks reflecting the complex nature of the biomass as shown in Fig.4.19. From Fig.4.19, band shift that appeared at 534.3cm^{-1} represents the C – N – S scissoring which are found in polypeptide structure (Gupta and Rastogi, 2007) and Si – O – Si bend of the silica group (Nwankwere, 2010). This supports the detection of silicon in the algal biomass using EDAX. After Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+} biosorption, this band decreased to 470.65, 484.15, 461, and 527.55cm^{-1} respectively while Cd^{2+} biosorption showed an increase to 538.16cm^{-1}. This suggests interaction between these functional groups and the heavy metal ions.

The absorption band observed at 1030.99cm^{-1} could be assigned to C – N, C – OH and P – O – C stretching vibrations of amines and proteins fractions (Park *et al.*, 2005; Ansari *et al*; 2009; Gochev *et al.*, 2010); polysaccharides (Krishnaiah *et al*., 2008) and phosphonate groups respectively(Choi and Yun, 2006; Pagnanelli *et al.,* 2000). This increased to 1033.88, 1033.88, 1032.92 and 1049.31cm^{-1} after biosorption of Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+} by the biomass respectively but decreased to 1023.27cm^{-1} over Cd^{2+} adsorption, suggesting interaction between these functional groups and the heavy metal ions

The peak appearing at 1435.09cm^{-1} could be attributed to C – H bending of methyl and methylene functional group (Morrison and Boyd, 2002; Yee *et al.*, 2004; Krishnaiah *et al.,* 2008). This peak decreased to 1412.9, 1387.83, 1387.83, 1378.18 and 1381.08cm^{-1} after Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+} respectively, which suggest interaction between this functional group and the heavy metal ions.

The absorption band at 1654.01cm^{-1} may be as a result of –C = C (characteristics of alkene), - C = N, - C = O stretching and N – H bending of amines or amide I and amide II band of amide bond of protein peptide bonds (Gupta and Rastogi, 2007; Ansari *et al*, 2009; Yee *et al.*, 2004; Gochev *et al.*, 2010; Morrison and Boyd, 2002). This band remained unchanged on adsorption of Pb^{2+}, but decreased to 1649.19, 1646.3, 1645.33 and 1636.65cm^{-1} on biosorption of Cd^{2+}, Cr(VI), Co^{2+}, and Ni^{2+} respectively. This suggests no interaction between this functional group, at this characteristic peak, and Pb^{2+} ions, hence, not responsible for its adsorption.

The peak observed at 2927.08cm^{-1} could be assigned C – H stretching of methyl and methylene group in cell wall structure (Park *et al*, 2005; Ansari *et al*, 2009; Sethuraman and

Balasubramanian, 2010) and asymmetric stretching of carboxylate anion (- COO^-) (Choi and Yun, 2006; Gupta and Rastogi, 2007). This increased to 2929.18cm^{-1} after Cd^{2+} biosorption but over Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+} adsorption, it decreased to 2925.15, 2926.11, 2926.11 and 2924.18cm^{-1} respectively.

The display of strong broad – OH (H bonded) and – NH stretch of carboxylic and amine band respectively were also observed in the region of 3453.66cm^{-1} (Ansari *et al*, 2009). This increased on nickel adsorption to 3461.38cm^{-1} but decreased on adsorption of Cd^{2+}, Cr(VI), Co^{2+}, and Pb^{2+} to 3426.66, 3429.55, 3424.73, and 3412.19cm^{-1} respectively

The two peaks that emerged at 621.1 and 3335.03cm^{-1} on adsorption of nickel could be attributed to – NH bending of amines and – NH and – C – H stretching of proteins and alkynes respectively (Gochev *et al.*, 2010; Morrison and Boyd, 2002).

The FTIR spectra result obtained is consistent with published FTIR spectra reports by other researchers (Yee *et al.*, 2004 ; Choi and Yun, 2006 ; Pagnanelli *et al.,* 2000 ; Krishnaiah *et al*., 2008; Morrison and Boyd, 2002 ; Park *et al*., 2005; Gochev *et al*., 2010; Ansari *et al*., 2009, Gupta and Rastogi, 2007).

.

Table 4.4: Assignment of IR bands of Functional Groups in Native and Heavy Metals treated algal Biomass

Band Position (cm^{-1})	
534.3 – 461.1	Si – O – Si bend (Silica), C – N – S scissoring (polypeptide)
621	-NH bend (amines)
1049.31 – 1023.27	C – N(amine), C – OH(polysaccharides) and P – O – C (phosphonate - groups) stretch
1435.09 – 1378.18	C – H bend (methyl and methylene groups)
1654.01 – 1636.65	- C = C (alkene), C = N, - C = O stretch and N – H bend (amides)
2929.18 – 2924.18	C – H stretch (methyl and methylene groups)
3335.03	- NH (proteins) and C – H (alkynes) stretch
3461.38 – 3412.19	- OH (carboxylic) and – NH (amine) stretch

(Ref :Yee *et al.*, 2004 ; Choi and Yun, 2006 ; Pagnanelli *et al.,* 2000 ; Krishnaiah *et al*., 2008; Morrison and Boyd, 2002 ; Park *et al*., 2005; Gochev *et al*., 2010; Ansari *et al*., 2009, Gupta and Rastogi, 2007)

Table 4.5: IR bands in cm^{-1} of Functional groups in Algal biomass and the various Heavy treated alga biomass.

Native Biomass	Cadmium Treated Biomass	Chromium Treated Biomass	Cobalt Treated Biomass	Lead Treated Biomass	Nickel Treated Biomass
534.3	538.16	470.65	484.15	461.00	527.55
-----	-----	-----	-----	-----	621.10
1030.99	1023.27	1033.88	1033.88	1032.92	1049.31
1435.09	1412.9	1387.83	1387.83	1378.18	1381.08
1654.01	1649.19	1646.30	1645.33	1654.01	1636.65
2927.08	2929.18	2925.15	2926.11	2926.11	2924.18
-----	-----	-----	-----	-----	3335.03
3453.66	3426.66	3429.55	3424.73	3412.19	3461.38

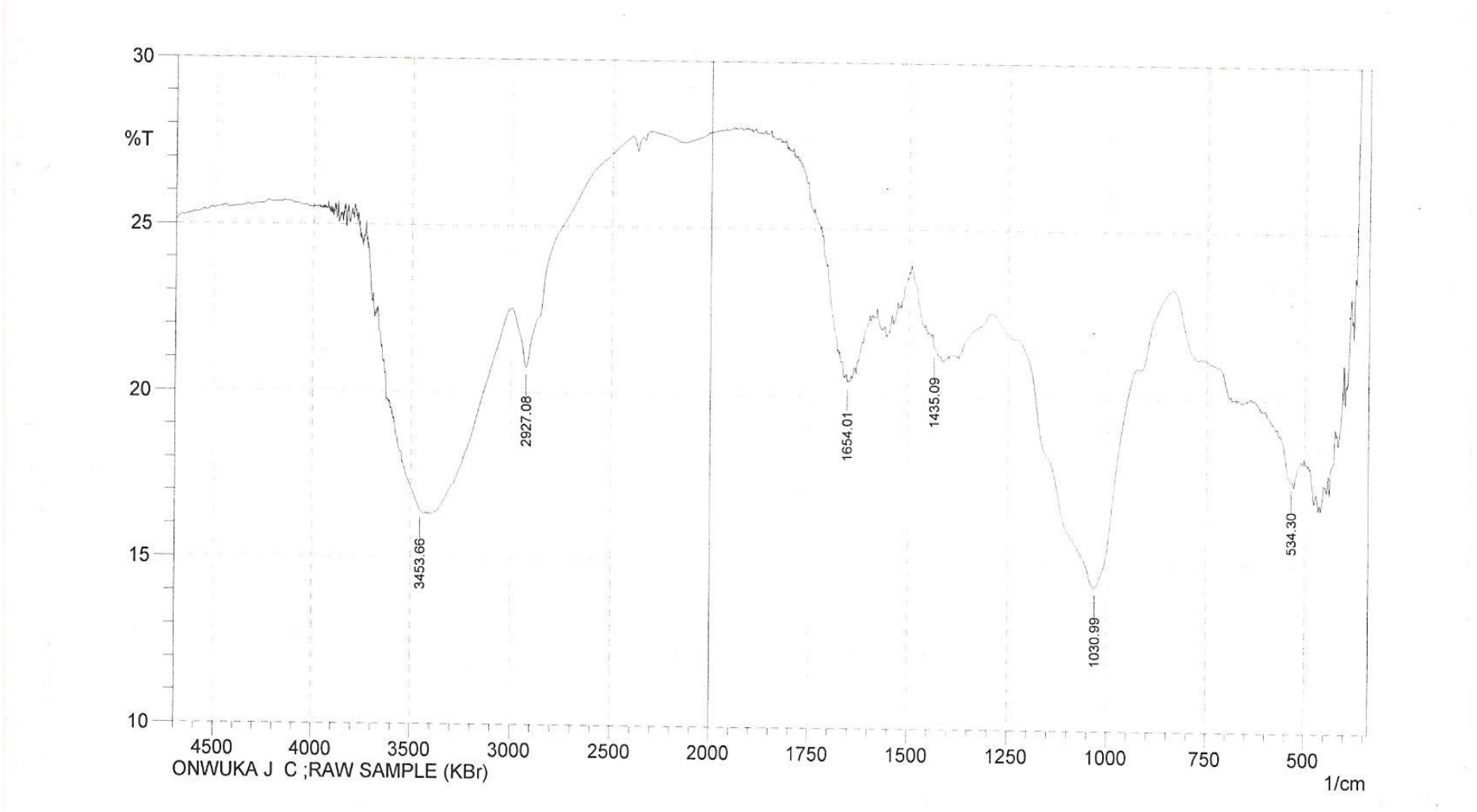


Figure 4.19: FTIR Spectra of Native Algal Biomass.

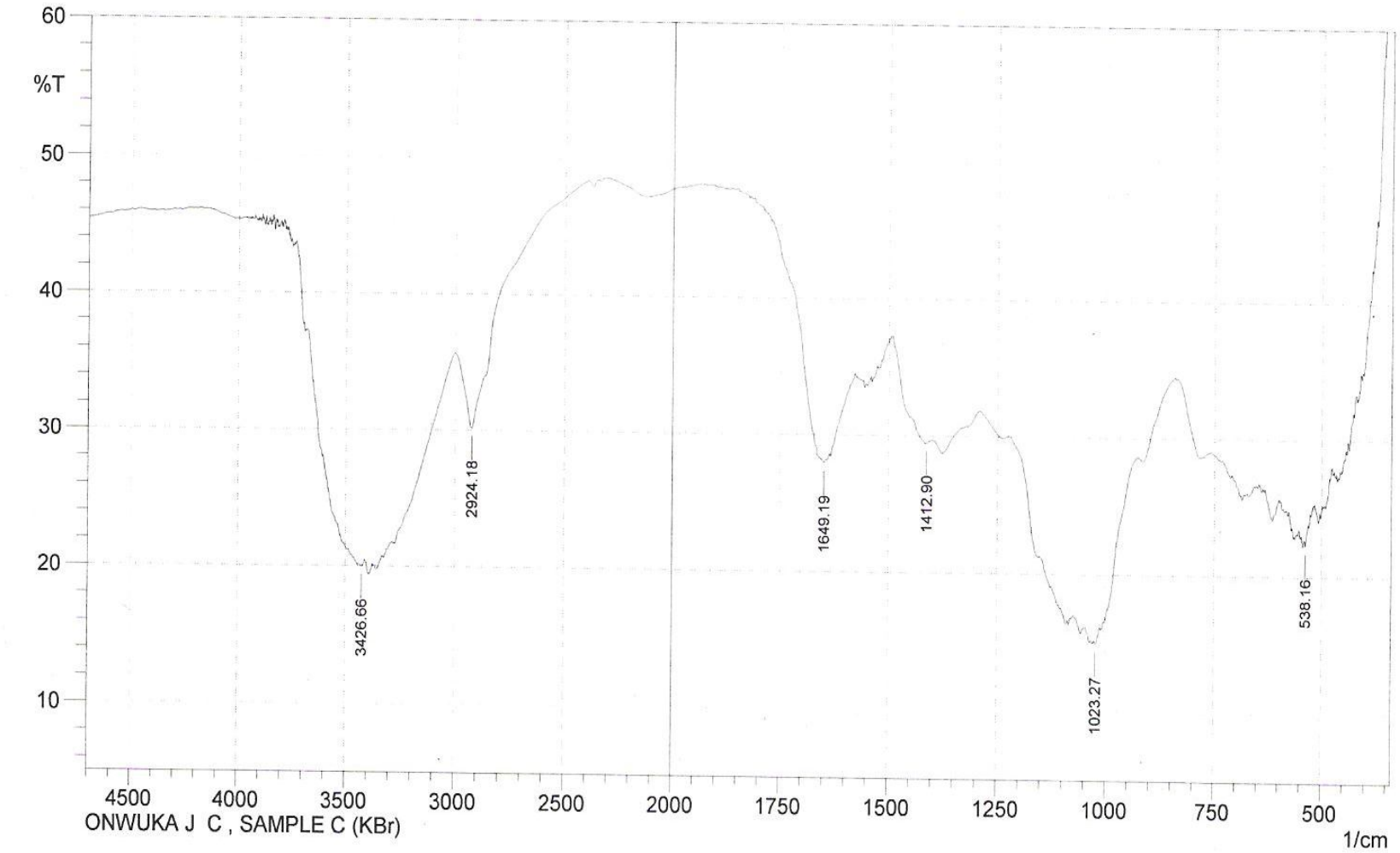


Figure 4.20: FTIR Spectra of Cd^{2+} Treated Algal Biomass.

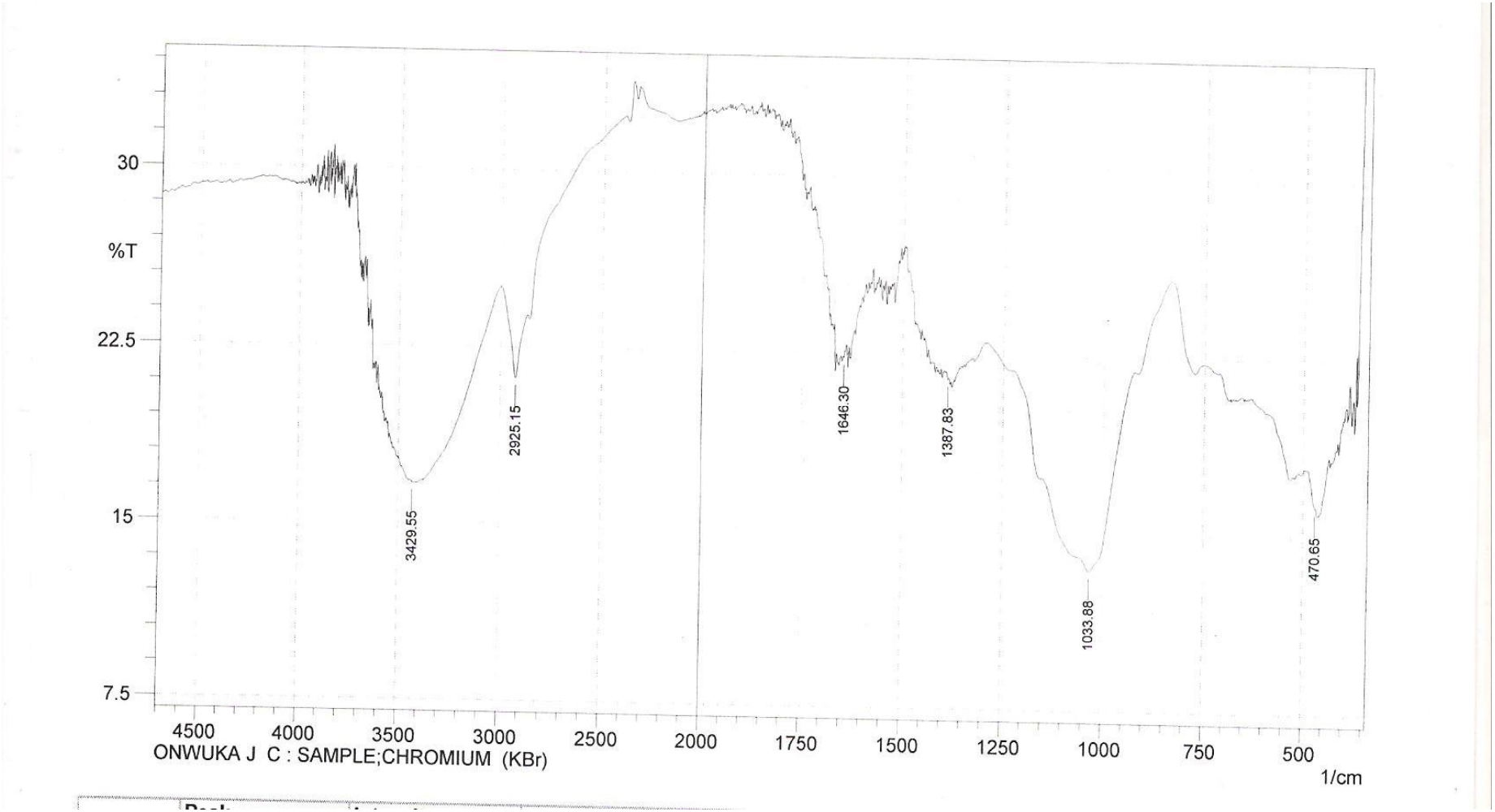


Figure 4.21: FTIR Spectra of Cr(VI) Treated Algal Biomass.

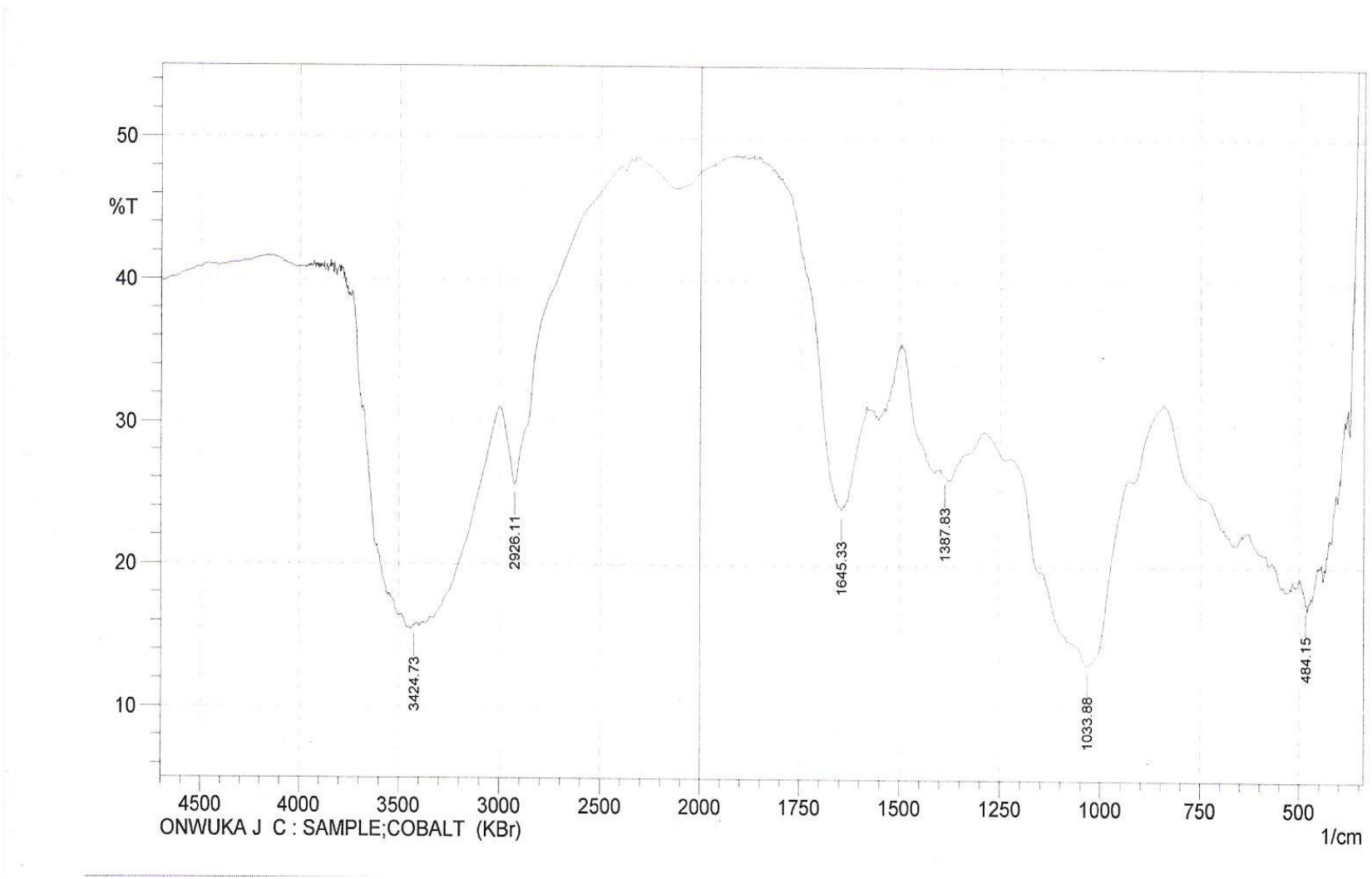


Figure 4.22: FTIR Spectra of Co^{2+} Treated Algal Biomass.

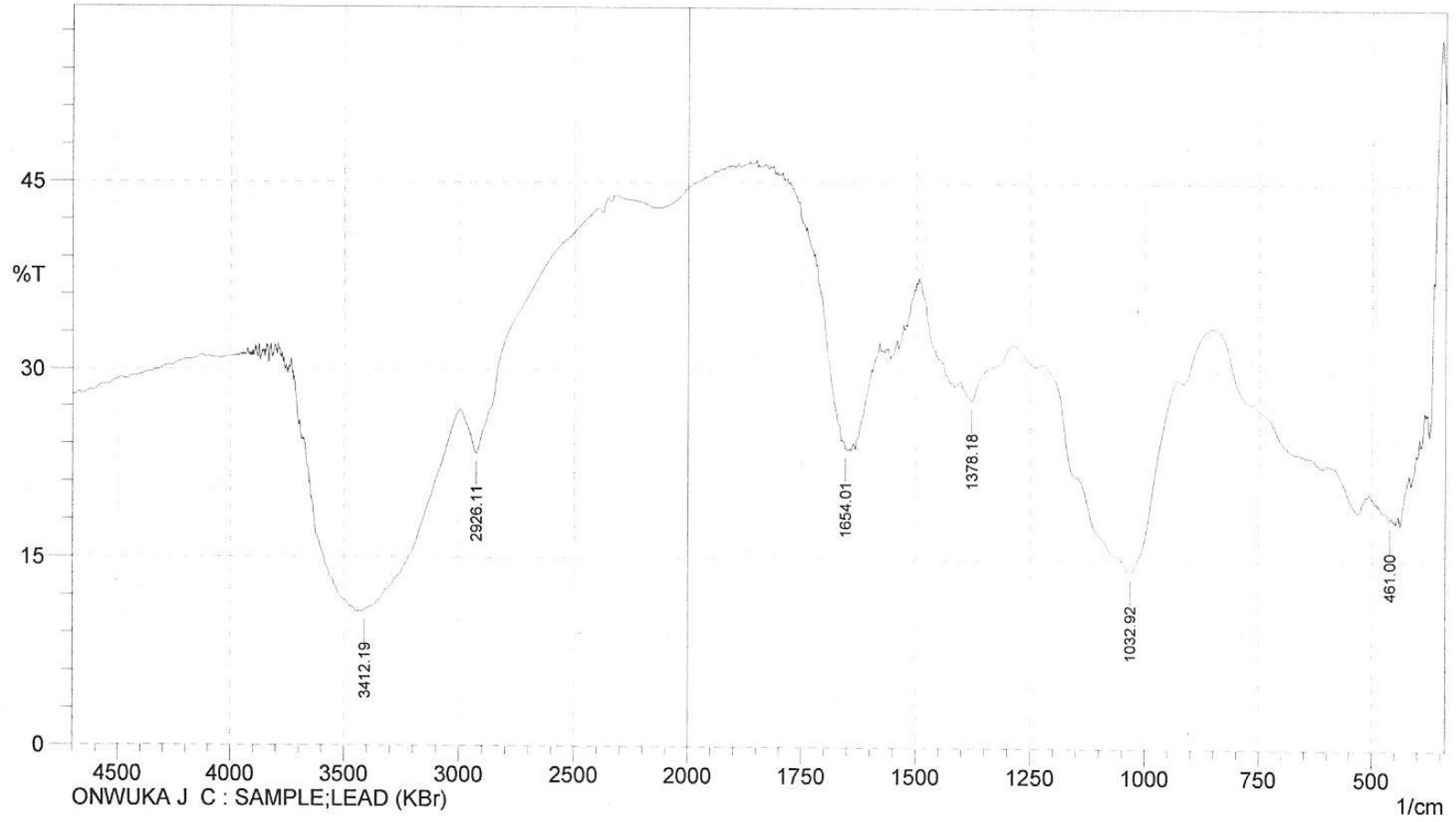


Figure 4.23: FTIR Spectra of Pb^{2+} Treated Algal Biomass.

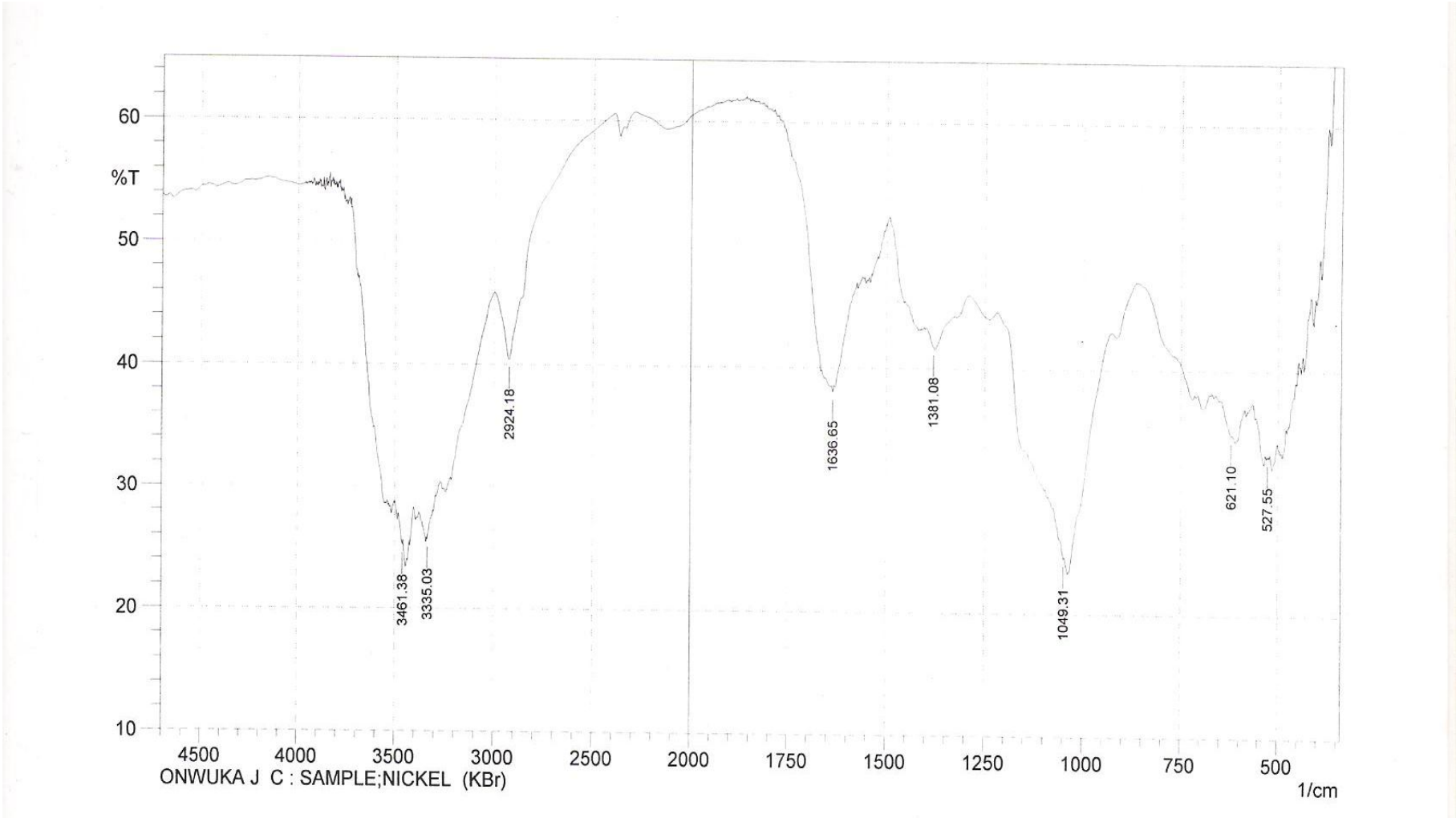


Figure 4.24: FTIR Spectra of Ni^{2+} Treated Algal Biomass.

4.3 Biosorption of Heavy Metal ions

4.3.1 Effect of Initial Metal ion Concentration

The percentage removal of metal and metal uptake is dependent on the initial metal ion concentration of the solution (Saradhi *et al*., 2010). Biosorption of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} at constant biomass concentration, pH, and temperature were studied by varying the initial metal ion concentration (5, 10, 15, 20, and 25mg/l) of each of the metal. Figure 4.25 represents effect of initial metal ion concentration on Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} biosorption respectively.

All the metals showed highest metal uptake at initial metal concentration of 25mg/l. Of all the metals, Cr(VI) uptake was the highest (12.11mg/g), followed by Pb^{2+}, Cd^{2+}, Ni^{2+} and Co^{2+} with metal uptakes of 8.93mg/g, 7.02mg/g, 6.47mg/g and 5.97mg/g respectively. Fig.4.25 also revealed that all the metals exhibit unique sorption capacity characteristics. It was observed that as the initial metal ion concentration of the various metals was increased, the respective metal uptake also increased. This sorption characteristics represent that surface saturation was dependent on the initial metal ion concentration (Bhatti *et al.,* 2006). It has been reported that with increasing metal ion concentration, the specific sites are saturated and vacant sites are filled and at low concentrations adsorption sites took up the available metal more rapidly or quickly while at higher concentrations metal ions need to diffuse to the biomass surface by intra particle diffusion and greatly hydrolyzed ions will diffuse at a slower rate (Jnr and Spiff, 2004; Bhatti *et al.*, 2006; Saradhi *et al.*,2010). To facilitate the estimation of the sorption capacity of the algal biomass, the isotherm data were analyzed using adsorption type isotherm models.

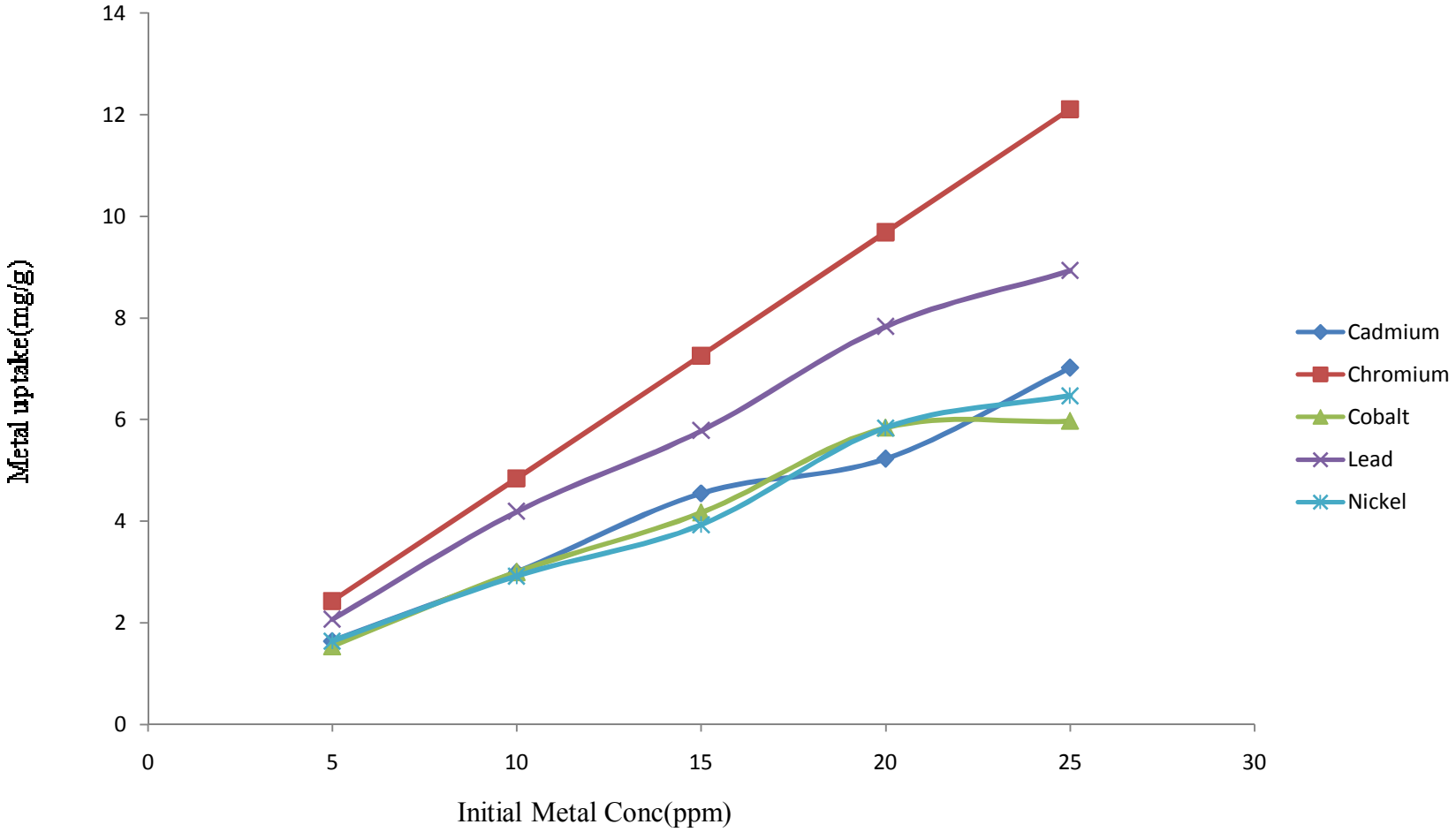


Figure 4.25: Effect of Initial Metal Ion Concentration on Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+} Biosorption.

4.3.2 Biosorption Modeling

Adsorption isotherms are important to describe how adsorbates will interact with adsorbents and are critical for design purposes, the correlation of the equilibrium data using an equation is essential for practical sorption operations (Nwankwere, 2010). The equilibrium data obtained on effect of initial metal ion concentration were applied on linearized Langmuir and Freundlich isotherm equation. The Langmuir model was chosen for the estimation of maximum sorption capacity to biomass surface saturation while Freundlich model was chosen to estimate the adsorption intensity of heavy metal ions towards the algal biomass.

The Langmuir and Freundlich isotherms are the most commonly used for solid – liquid phase isotherms. These isotherms relate the amount of metal ion sorbed at equilibrium per unit weight of the sorbent, q_e(mg/g) to the sorbate concentration at equilibrium, C_e(mg/l). According to the Langmuir model, adsorption occurs uniformly on the active sites of the biosorbent, and once an adsorbate occupies a site, no further sorption can take place at that site. The Langmuir isotherm is defined as:

$$q_e = \frac{q_{max} b C_e}{1 + b C_e} \quad \text{.....(4.1)}$$

The linearized form is expressed as

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \frac{1}{b q_{max} C_e} \quad \text{.....(4.2)}$$

Where; q_e is the amount of adsorbate adsorbed per gram of dried adsorbent at equilibrium (mg adsorbate/g of dried adsorbent), q_{max} is the constant relating to the maximum amount of adsorbate ion bound per g of adsorbent for a monolayer (mg/g), b is Langmuir constant or adsorption coefficient or the adsorption affinity (l/mg) for binding of adsorbate on the adsorbent sites and C_e is equilibrium (residual) adsorbate concentration in solution after

sorption (mg/l). The values of q_{max} and b can be calculated from the intercept $\left(\frac{1}{q_{max}}\right)$ and slope $\left(\frac{1}{q_{max}b}\right)$ of the plot $\frac{1}{q_e}$ against $\frac{1}{C_e}$ as illustrated in Figures 4.26, 4.28, 4.30, 4.32, and 4.34 (Gupta and Rastogi, 2007). The isotherm constant and their coefficients of determination, R^2 are listed in Table 4.6.

The Freundlich isotherm model describes non – ideal sorption onto heterogeneous surfaces involving multilayer sorption. The isotherm model is defined as

$$q_e = K_F\, C_e^{1/n} \quad \text{.....(4.3)}$$

Its linearized form is given as

$$lnq_e = lnK_F + \frac{1}{n}\, ln\, C_e \quad \text{..... (4.4)}$$

Where, q_e is the amount of adsorbate adsorbed per unit weight of biosorbent, K_F is Freundlich Constant measuring adsorption capacity(L/mg), C_e is equilibrium concentration of the adsorbent in solution(mg/l), n is constant related to adsorption efficiency and energy of adsorption or adsorption intensity of the adsorbent.

Figures 4.27, 4.29, 4.31, 4.33 and 4.35 shows Freundlich isotherm model the different heavy metal ions biosorption and the isotherm constants and correlation coefficients, R^2, are also listed in Table 4.6. A plot of lnq_e against lnC_e gives a straight line with a slope, 1/n and an intercept of lnK_F. The K_F value increases with the total adsorption capacity of the adsorbent to bind the adsorbate. The numerical value of n is a useful index to determine favorability of the adsorption.

Figures 4.25 – Fig.4.35 represents Langmuir and Freundlich fitting of adsorption isotherms of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+}respectively on algal biomass. Table 4.6 presents the

Langmuir and Freundlich isotherm parameters for the biosorption of these metal ions on algal biomass *Cosmarium panamense* at 27^{o}C.

The values of the maximum metal uptake (q_{max}) for Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} are 12.89mg/g, 109.9mg/g, 16.69mg/g, 19.72mg/g and 10.73mg/g respectively. Therefore, the order of sorption can be deduced from Table 4.6 as follows: Cr> Pb> Co> Cd> Ni. This can be attributed to many factors ranging from types of biosorbent, available binding sites, binding sites affinity to the metal to be sorbed etc. Cr(VI) obtained was higher than 60mg/g reported by Barkhoudar and Ghaiseddin (2004) for *Sargassum seaweed*. Cd^{2+} and Ni^{2+} falls within the range (8.26 – 115.36mg/g) for Cd^{2+} (Yu and Matheickal, 1999; Davis *et al.*, 2000; Gonzalez *et al.*, 2006; Hajar, 2009; Kumar *et al.,* 2009) and (1.43 – 70.2mg/g) for Ni^{2+} (Davis *et al.*, 2000; Gonzalez *et al.*, 2006). Pb^{2+} and Co^{2+} were found to be below 51.5 – 204.1mg/g (Gupta and Rastogi, 2007; Gonzalez *et al.*, 2006; Jalali *et al.*, 2002; Ramin *et al.*, 2006) and 100.3mg/g (Davis *et al.*, 2000) respectively.

It was observed that the linearized forms of the isotherms (Langmuir and Freundlich isotherm) were linear over the whole concentration range studied and the metals sorption closely followed Langmuir model of sorption. In other words, the equilibrium data were well represented by Langmuir isotherm equation when compared to Freundlich isotherm because Langmuir isotherm averagely possess higher correlation coefficient than Freundlich isotherm. The high degree of correlation for the linearized Langmuir relationship suggest monolayer sorption on specific sites or single surface reaction (Gupta and Rastogi, 2007).

All the values of $1/n$ in Table 4.6 falls within 0 – 1 range which strongly suggest favorable adsorption. The essential features of the Langmuir isotherm was expressed in terms of a dimensionless constant called the equilibrium parameter, R_L.

Table 4.6: Langmuir and Freundlich Isotherm Constants for the Biosorption of all The Selected Metal ions on Algal Biomass (*Cosmarium panamense*).

Metals	Langmuir Constant				Freundlich Constant		
	q_{max}(mg/g)	b(Lmg^{-1})	b(Lmol^{-1})	R^2	$1/n$	K_f(mg/g)	R^2
Cadmium	12.89	0.08	8960	0.9893	0.75	1.10	0.9743
Chromium	109.9	0.15	7800	0.9969	0.97	14.50	0.9991
Cobalt	16.69	0.05	2950	0.9919	0.75	1.01	0.9572
Lead	19.72	0.14	28980	0.9813	0.69	2.56	0.9525
Nickel	10.73	0.10	5900	0.9778	0.73	1.06	0.9667

(q_{max}, is the maximum metal uptake (mg/g), *b,* is Langmuir constant (l/mg) K_F, is Freundlich Constant measuring adsorption capacity(L/mg), and n, is constant related to adsorption efficiency and energy of adsorption or adsorption intensity of the adsorbent.)

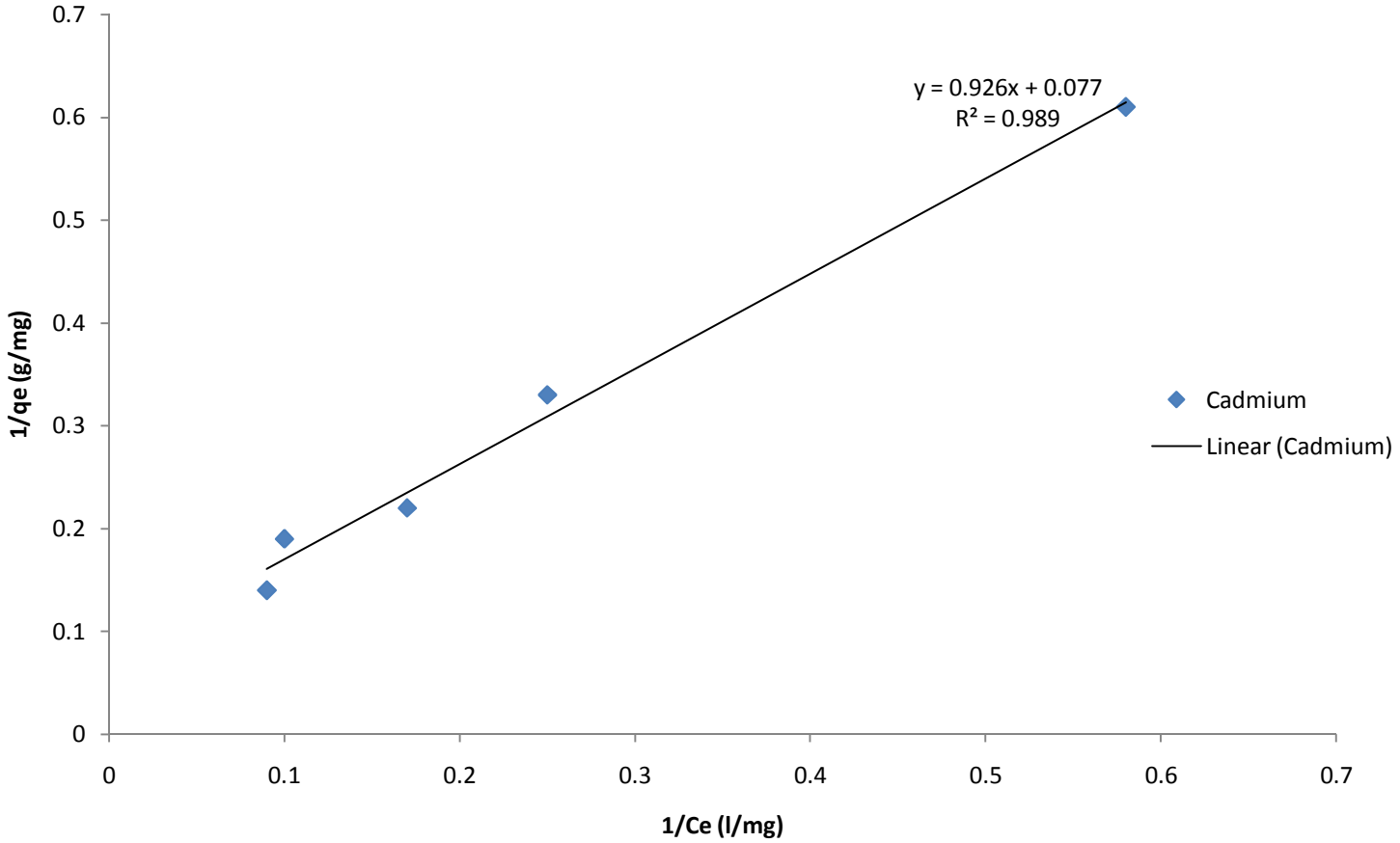


Figure 4.26: Langmuir Adsorption Isotherm for Cd^{2+} Biosorption.

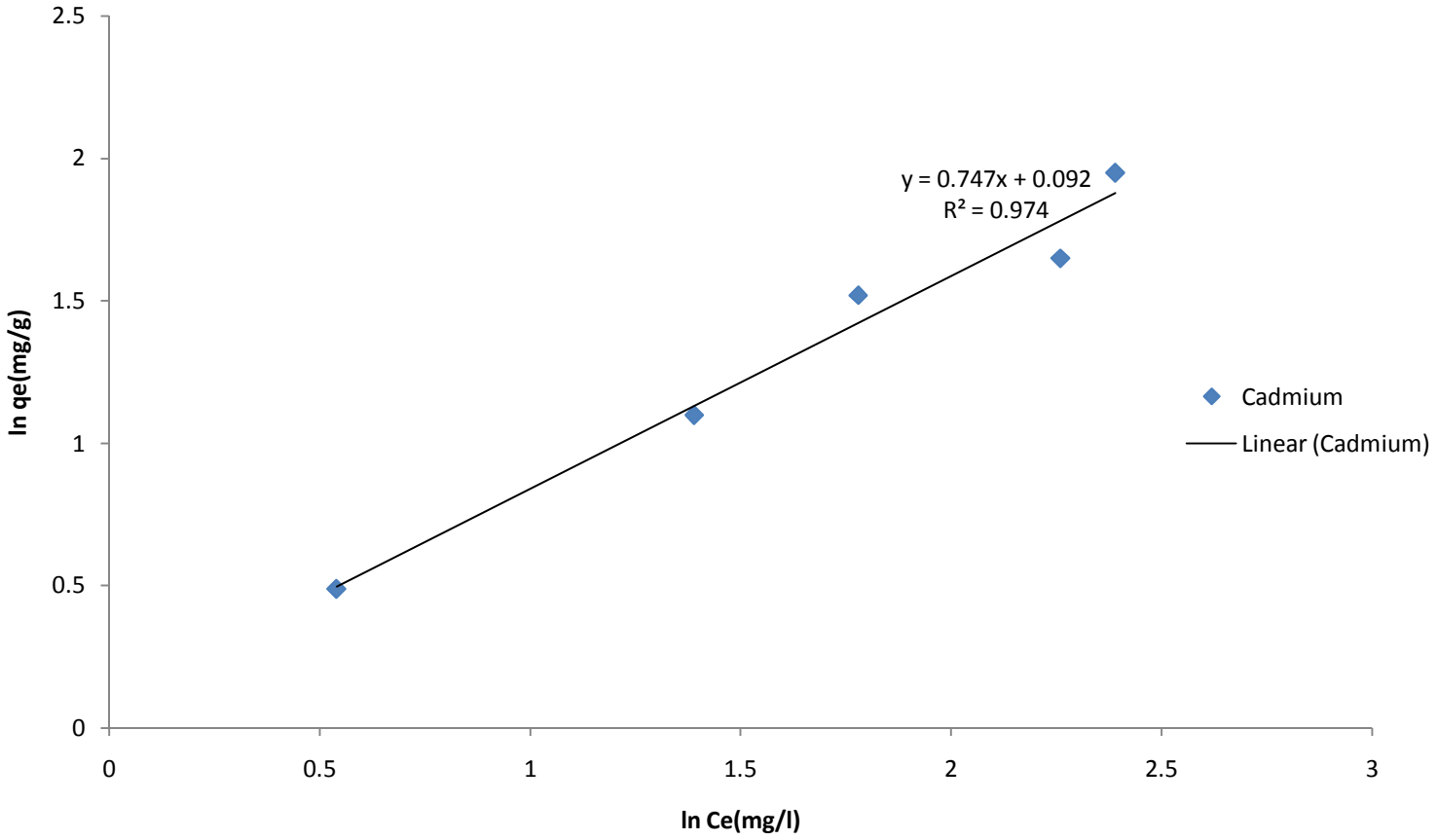


Figure 4.27: Freundlich Adsorption Isotherm for Cd^{2+} Biosorption.

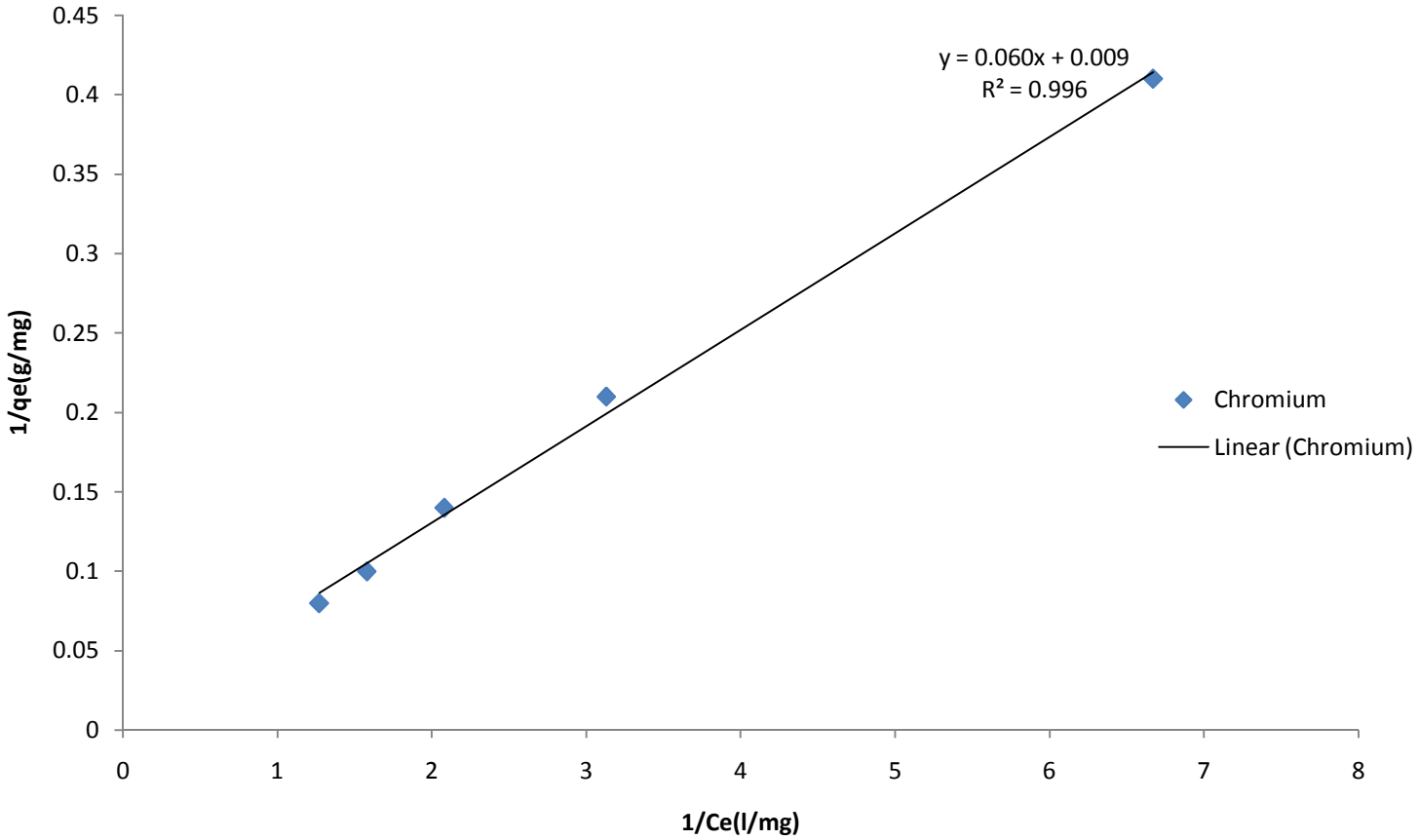


Figure 4.28: Langmuir Adsorption Isotherm for Cr(VI) Biosorption.

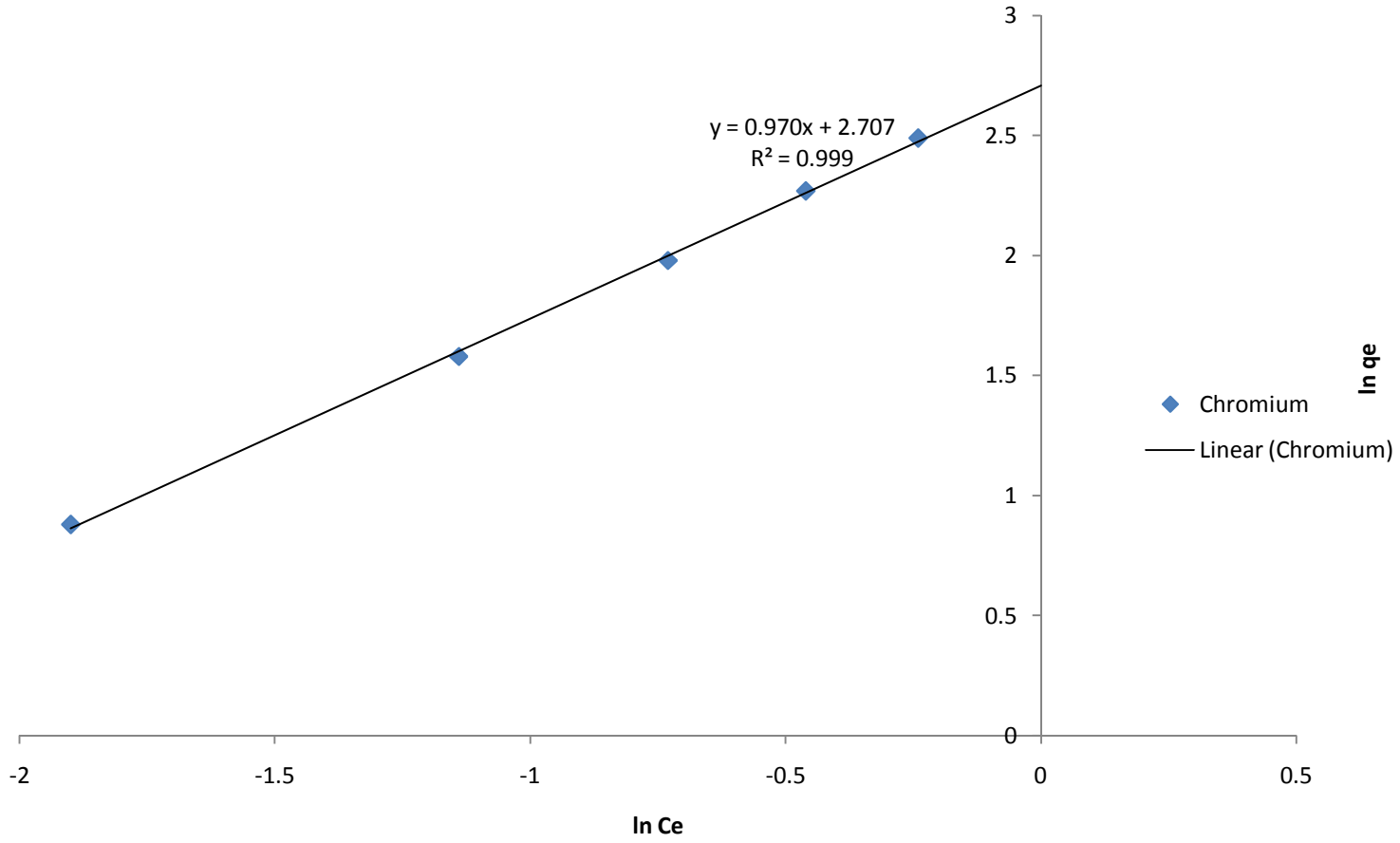


Figure 4.29: Freundlich Adsorption Isotherm for Cr(VI) Biosorption.

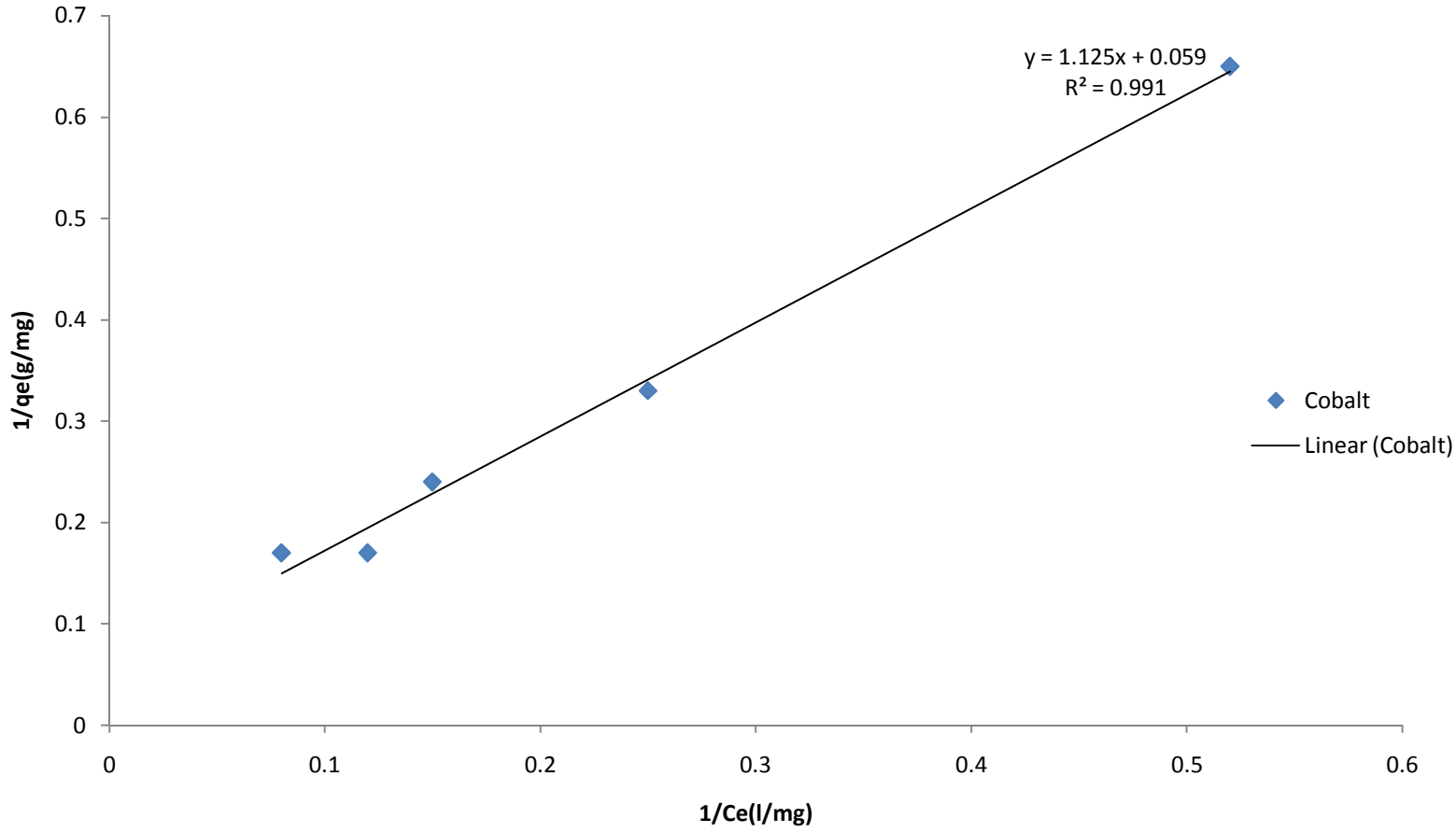


Figure 4.30: Langmuir Adsorption Isotherm for Co^{2+} Biosorption.

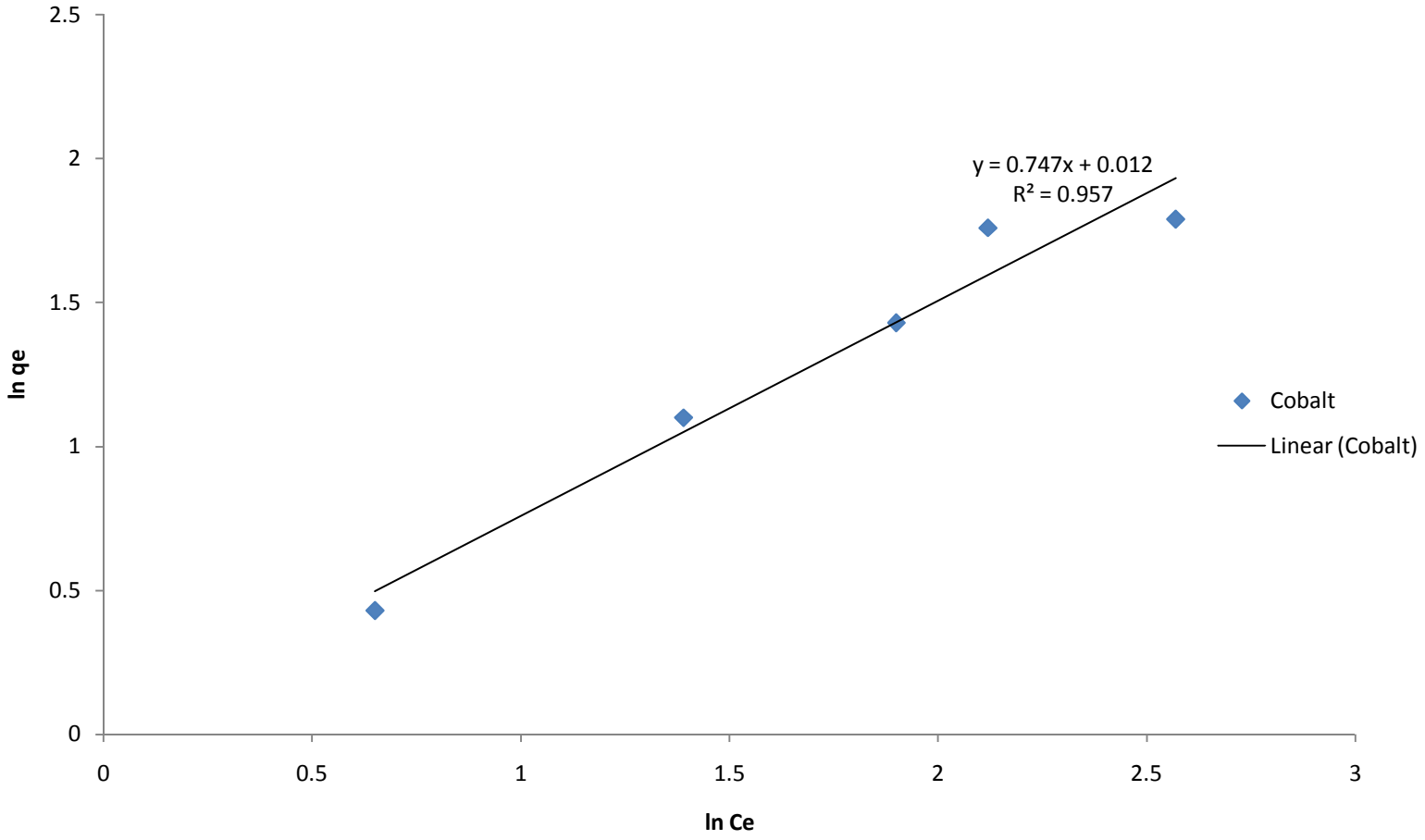


Figure 4.31: Freundlich Adsorption Isotherm for Co^{2+}Biosorption.

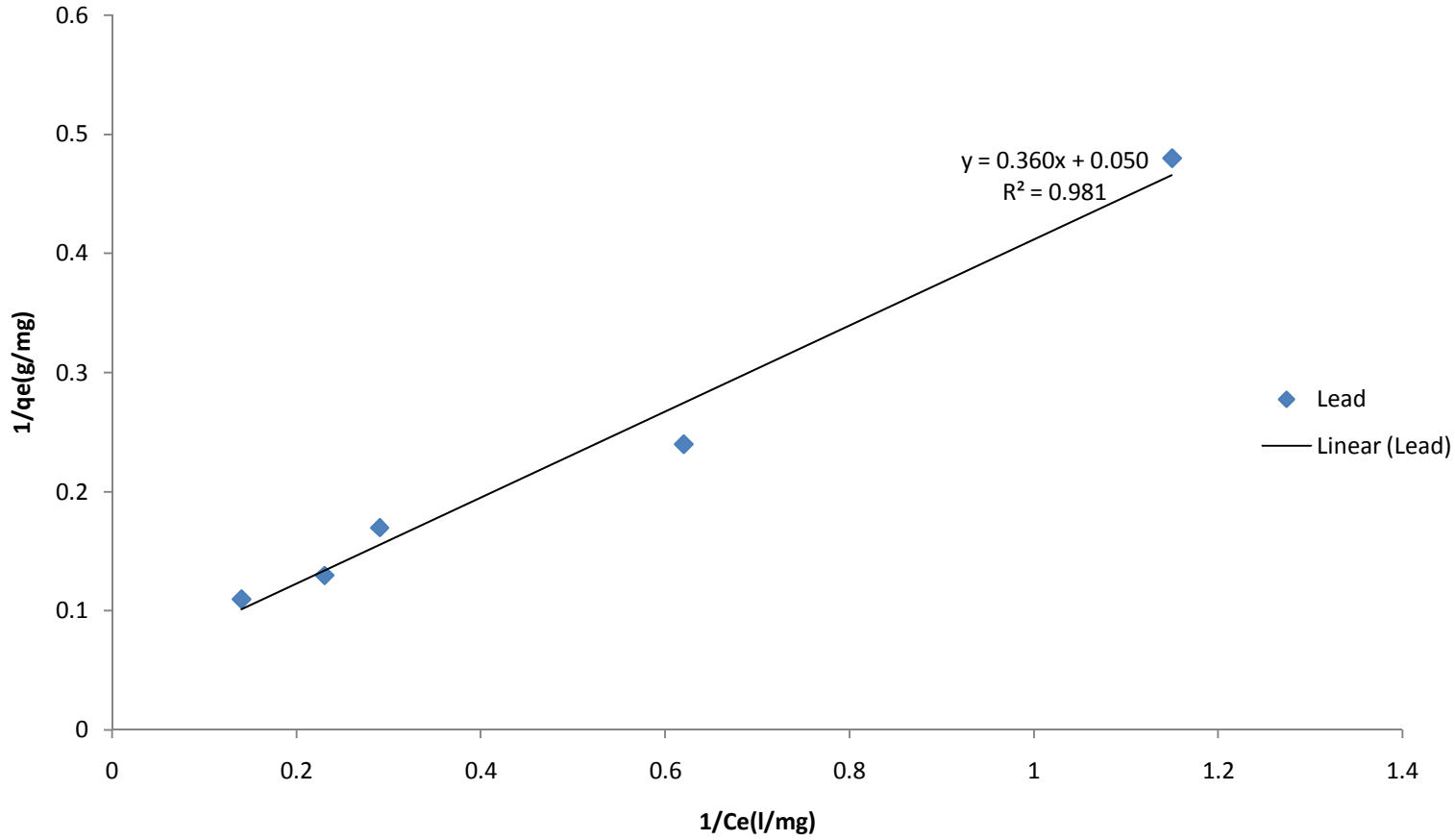


Figure 4.32: Langmuir Adsorption Isotherm for Pb^{2+} Biosorption.

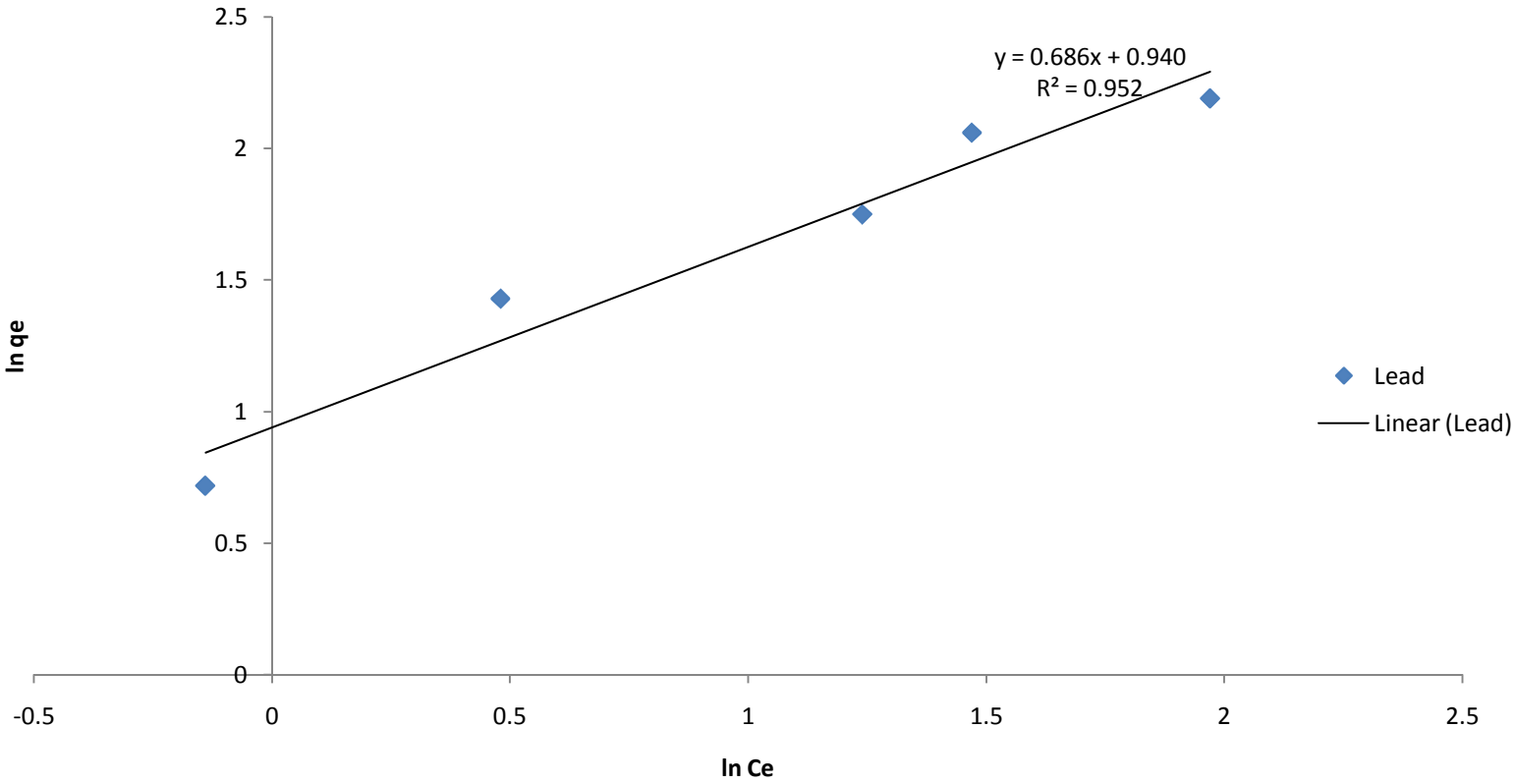


Figure 4.33: Freundlich Adsorption Isotherm for Pb^{2+}Biosorption.

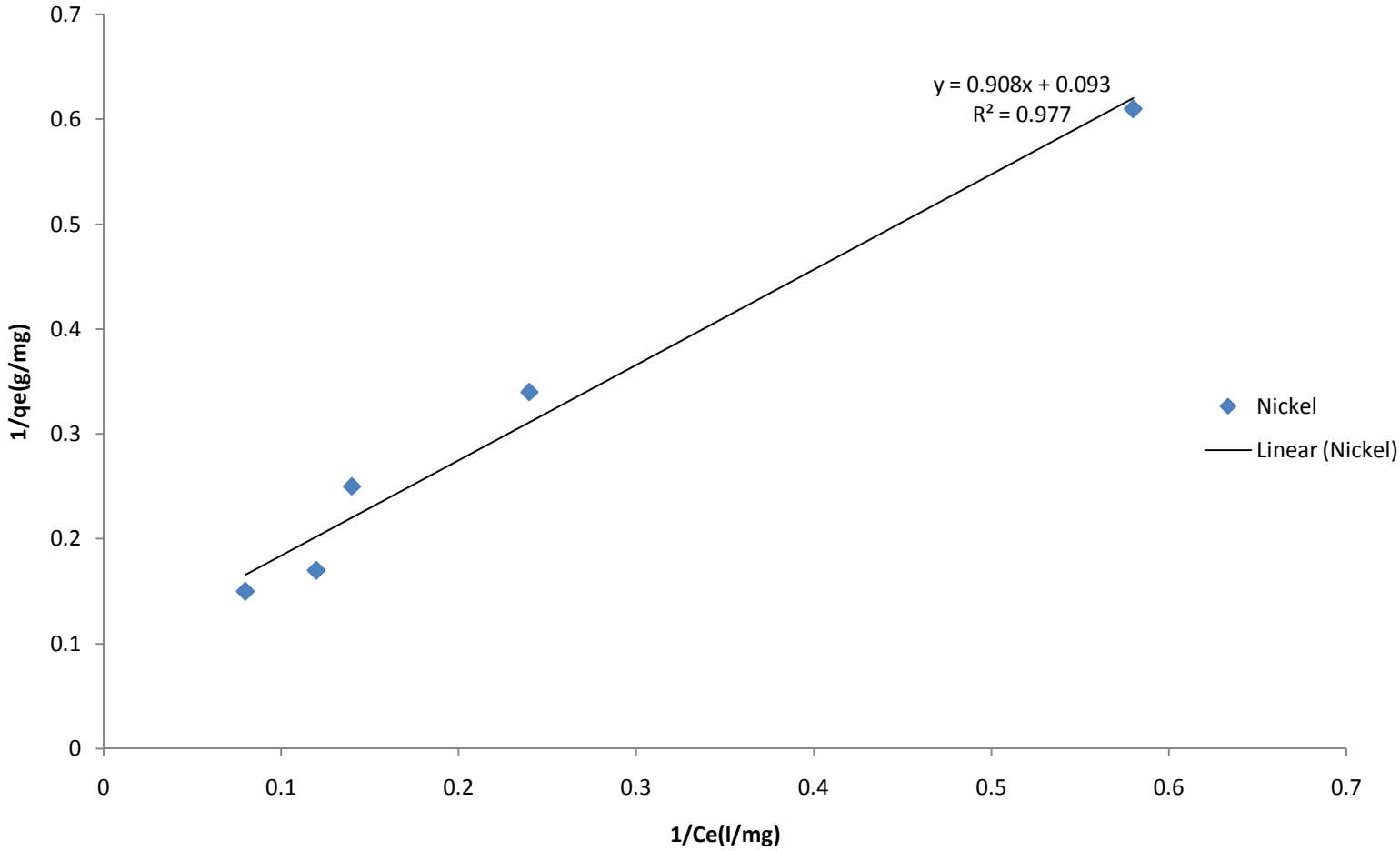


Figure 4.34: Langmuir Adsorption Isotherm for Ni^{2+} Biosorption.

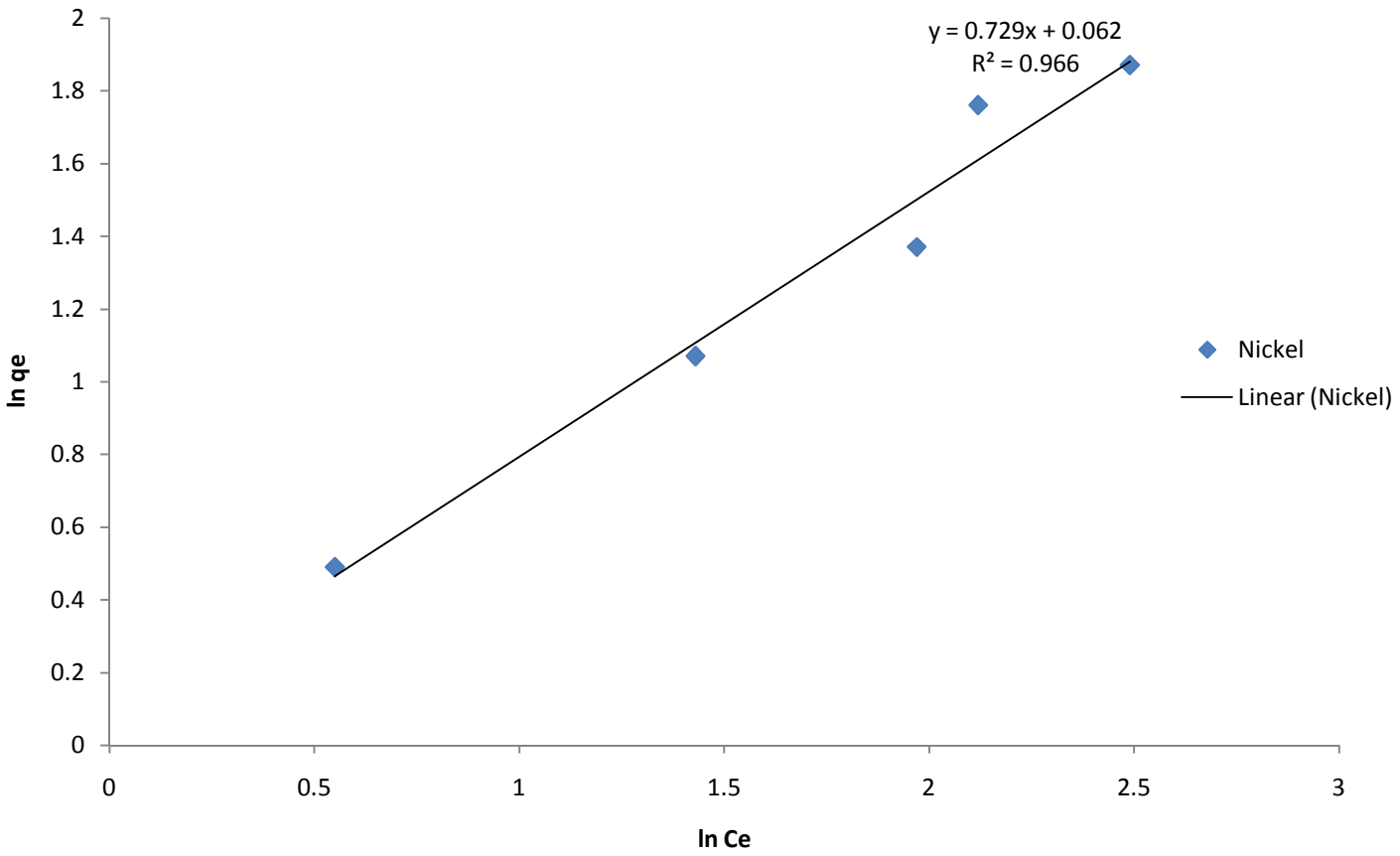


Figure 4.35: Freundlich Adsorption Isotherm for Ni^{2+} Biosorption.

4.3.3 Separation Factor, R_L.

The shape of Langmuir isotherm can be used to predict whether the sorption system is favourable or unfavourable in batch process. Separation factor is a dimensionless constant used in expressing the essential characteristics of Langmuir isotherm. Separation constant, R_L, which is defined as

$$R_L = \frac{1}{1+bC_o} \quad \text{.....(4.5)}$$

Where: b is the Langmuir constant (l/mg), C_o is the initial metal ion concentration of the selected metal (mg/l), It is known that R_L values between 0 and 1 indicate favourable adsorption (Mckay and Roberts, 1982; Raghavarao *et al.,* 2009).

Figure 4.36 shows the separation factor for biosorption of these metal ions on to algal biomass over the whole concentration range studied. It was observed that as the concentration of the metals are increased, the separation factor decreases for each metal. The lower the separation factor the more effective the adsorption (Shakirullah *et al.*, 2006). The separation factor values were all found to fall between 0 – 1 which indicate favorable adsorption. Hence, the most effective adsorption was achieved at 25ppm for all the metals and the sorption of Cr(VI) was the most effective since it has the least separation factor (0.21). From Figure 4.36, the order of effective separation can be deduced as Cr> Pb> Ni> Cd> Co.

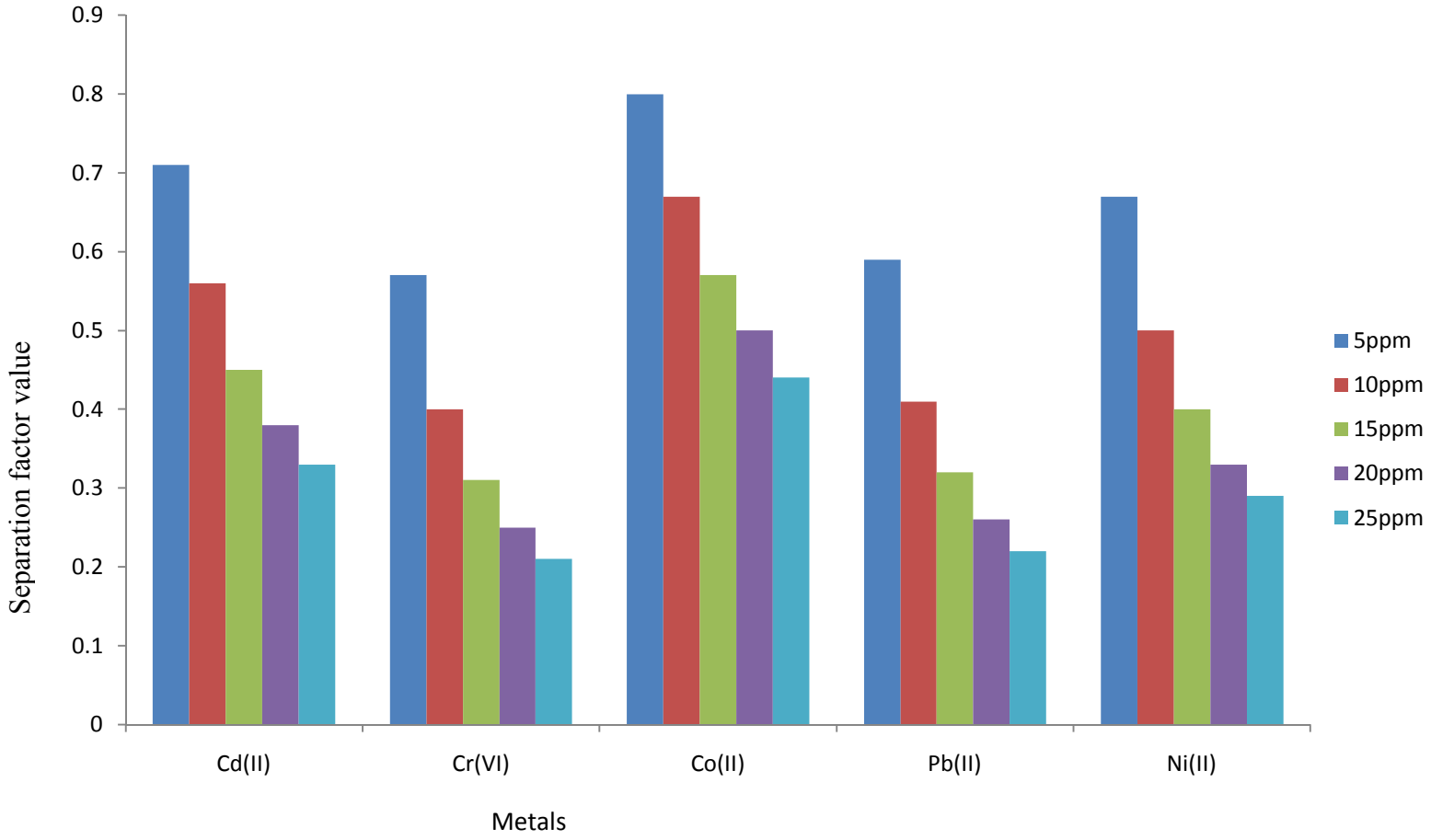


Figure 4.36: Separation Factor, R_L, Variation with Concentration(mg/l) on Biosorption of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+}

4.3.4 Effect of Biosorbent Dose

The amount of adsorbent significantly influenced the extent of heavy metal adsorption (Gupta and Rastogi, 2007).

Figure 4.37 shows a graphical representation of effect of biosorbent dose on Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} uptake respectively. From Fig. 4.37, it was observed in this study that all the heavy metals showed a unique biosorption behaviour at the various biosorbent dosage. As the biosorbent dose increases, the metal uptake decreases. Therefore, 0.05g of the adsorbent comparatively showed the highest adsorption capacity while 0.25g of the adsorbent gave the lowest adsorption capacity for all the metals. It has been reported by Pattabhi *et al* (2008) that increase in biomass concentration increases the level of biosorption due to the overall increase in surface area of the biomass which in turn increases the number of binding sites. Saradhi *et al* (2010) explained that the metal uptake decreases with increase in biosorbent dose due to the interference of inter – particle cohesive forces, interference of binding sites, desorption due to abrasion, turbulence and reduce mixing due to large mass of the biosorbent while Rastogi and Gupta (2007) attributed this to the fact that at higher adsorbent dose, the availability of higher energy sites decreases with a larger fraction of lower energy sites occupied. It has been suggested that electrostatic interaction between cells can be a significant factor in the relationship between biomass concentration and metal sorption, in this connection, at a given metal ion concentration, the smaller the amount of the biomass in suspension, the higher will be metal/biomass ratio and the metal retained by sorbent unit unless the biomass reaches saturation. High biomass concentration can exert a shell effect protecting the active sites from being occupied by metal and this results to lower specific metal uptake (Gonzalez *et al.*, 2006).

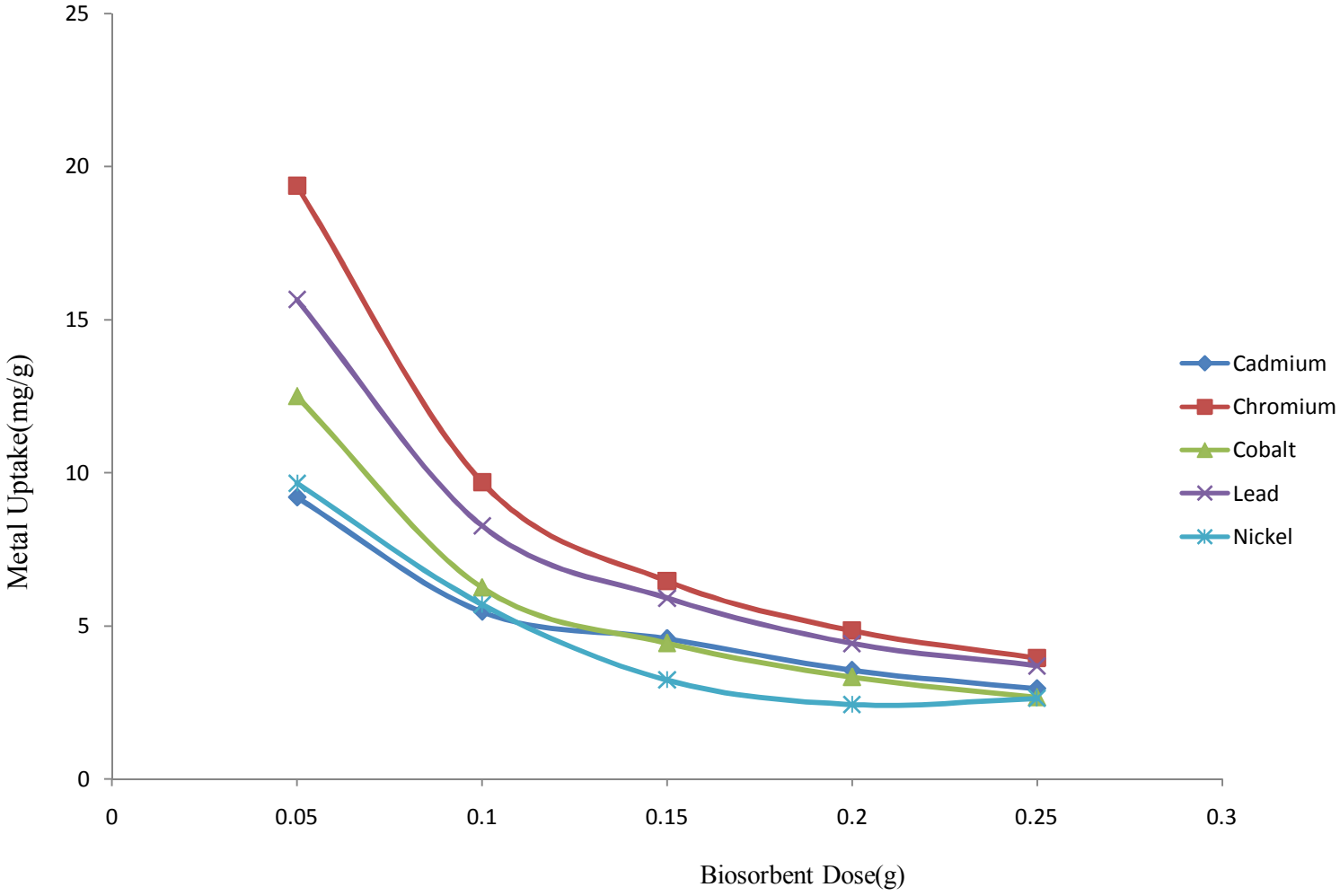


Figure 4.37: Effect of Biosorbent Dose

4.3.5 Effect of Contact Time

The contact time was evaluated as one of the important parameters affecting the biosorption efficiency (Hajar, 2009). The percentage removal of metal ion increases with the time of shaking (Lokeshwari and Keshava, 2009). Figure 4.38 is a graphical representation of effect of contact time on the uptake or sorption of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} respectively.

It was observed that maximum adsorption of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} took place within the first 120, 90, 120, 120, and 90min respectively. From Figure 4.38, for contact time of 90min, uptake of 9.84mg/g and 5.69mg/g for Cr(VI) and Ni^{2+} respectively, was achieved at their initial concentration of 20mg/l while for 120min, uptake of 5.87mg/g, 6.67mg/g and 9.43mg/g for Cd^{2+}, Co^{2+} and Pb^{2+} respectively was achieved at their initial concentration of 20mg/l.

It was also observed from Fig.4.38 that the process of adsorption was high at the initial stage and became slower while approaching the equilibrium stage. This is obvious due to the fact that more number of vacant negatively charged sites are available initially on the surface of the adsorbent and the sites are gradually filled up while approaching equilibrium and completely filled at equilibrium (Saradhi *et al*., 2010). Contact time were used to generate the rate of the reaction (Gupta and Rastogi, 2007). To generate the rate of adsorption, pseudo first order and pseudo second order were used on the experimental data obtained from the effect of contact time.

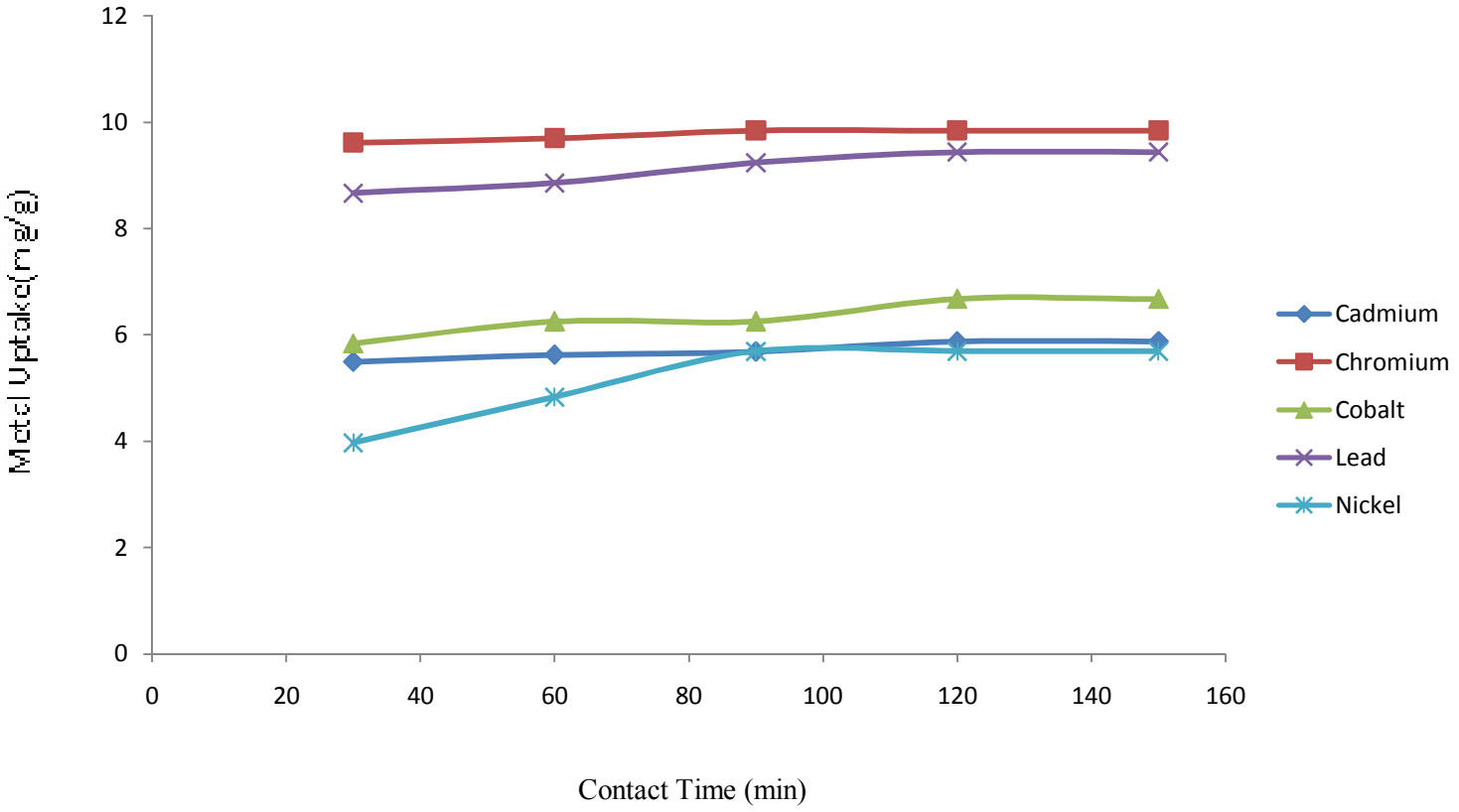


Figure 4.38: Effect of Contact Time

4.3.6 Biosorption Kinetics

In order to investigate the mechanism of biosorption and potential rate controlling step, such as mass transport and chemical reaction processes, pseudo first order and pseudo second order kinetic models were used to test the equilibrium data obtained from effect of contact time. Moreover, information on the kinetics of metal uptake is required for selecting optimum conditions for full scale batch metal removal processes. A kinetic study with different time intervals, fixed metal and biosorbent concentration was performed and the obtained results are presented in Table 4.7.

Pseudo first order and pseudo second order model proposed by Rastogi and Gupta (2007); were used to test adsorption kinetics data to investigate the mechanism of the heavy metals biosorption. The pseudo first-order equation (Lagergren, 1898) is generally expressed as follows:

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \quad \text{.....(4.6)}$$

Where q_e and q_t are the amount of adsorbate adsorbed on adsorbent at equilibrium and time *t*, respectively (mg/g), and k_1 is the rate constant of first order adsorption (min^{-1}).

The plot of $\log(q_e - q_t)$ versus t will give a straight line and the value of k_1 can be evaluated from the slope of the graph, - $\frac{k_1}{2.303}$, while $q_{e(\text{calculated})}$ is obtained from the intercept, log q_e, of the graph as illustrated in Figures 4.39, 4.41, 4.43, 4.45, and 4.46 (Gupta and Rastogi, 2007).

The linearized second-order kinetic model is expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad \text{.....(4.7)}$$

Where, k_2 is the pseudo-second-order rate constant of adsorption (g $mg^{-1}min^{-1}$). The plot of $\frac{t}{q_t}$ versus t will give a linear relationship with $\frac{1}{q_e}$ and $\frac{1}{k_2 q_e^2}$ as a slope and intercept, respectively. The values of q_e and k_2 can be determined from the slope and intercept as illustrated in Figures 4.40, 4.42, 4.44, 4.46 and 4.48. The pseudo second-order kinetics model has been successfully applied to several biosorption systems as reported by McKay and Ho (1999).

Table 4.7 shows comparison between adsorption rate constants, q_e estimated and correlation coefficient associated to the lagergren pseudo – first and – second order adsorption all the metals. Figures 4.39 – 4.49 represents pseudo – first and – second order kinetics modeling of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+}, and Ni^{2+} adsorption on *Cosmarium panamense* algal biomass respectively.

The experimental adsorption capacities of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} are 5.87mg/g, 9.84mg/g, 6.67mg/g, 9.23mg/g and 5.69mg/g respectively. Their calculated adsorption capacities by using pseudo first – order model are 0.16mg/g, 0.12mg/g, 0.48mg/g, 0.40mg/g and 1.44mg/g respectively while that of pseudo second - order model are 6mg/g, 9.95mg/g, 6.96mg/g, 9.43mg/g, and 6.49mg/g. The calculated adsorption capacities of all the metals by pseudo second order model are very close to their respective experimentally determined adsorption capacities unlike pseudo first order, where there is large difference between the experimental and calculated adsorption capacities of all the metals. The correlation coefficients, R^2 values for the pseudo second order adsorption model of all the metals are higher (>0.9948) than that of pseudo first order adsorption model (<0.7). However, the correlation coefficient values and calculated adsorption capacities of all the metals by pseudo first order model are not satisfactory, which suggest that they are dependent on initial

concentration. Hence, Pseudo second order adsorption model is more suitable to describe the adsorption kinetics of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} on the algal biomass and this relies on the assumption that biosorption may be the rate-limiting step (Gupta and Rastogi, 2007). The obtained kinetic information has a significant practical value for technological applications, since kinetic modeling successfully replaces time and material consuming experiments, necessary for process equipment design (Bhatti *et al.*, 2006; Gupta and Rastogi, 2007; Mahamadi and Torto, 2007; Krishnaiah *et al.*, 2008; Anasri *et al.*, 2009).

Table 4.7: Comparison between adsorption rate constants, q_e estimated and correlation coefficient associated to the Lagergren pseudo first – and second – order adsorption.

	Pseudo First – Order Model			$q_{e\,\text{exp.}}$	Pseudo Second – Order Model		
	$q_{e(\text{cal})}$	$K_1(\text{min}^{-1})$	R^2	(mg/g)	$q_{e(\text{cal})}$	$K_2(\text{gmg}^{-1}\text{min}^{-1})$	R^2
Cadmium	0.160	- 0.011	0.4615	5.87	6.00	0.045	0.9995
Chromium	0.120	- 0.016	0.6726	9.84	9.95	0.088	0.9999
Cobalt	0.480	-0.002	0.1893	6.67	6.96	0.021	0.9983
Lead	0.400	-0.007	0.5515	9.23	9.43	0.037	0.9997
Nickel	1.440	0.003	0.3066	5.69	6.49	0.009	0.9948

(key: k_1 rate constant for pseudo first - order adsorption ,k_2 is the pseudo-second-order rate constant of adsorption, $q_{e(\text{cal})}$ is Metal Uptake (mg/g) calculated using pseudo first and second – order equation, $q_{e\,\text{exp.}}$ is Metal Uptake obtained experimental during biosorption of the metal ions)

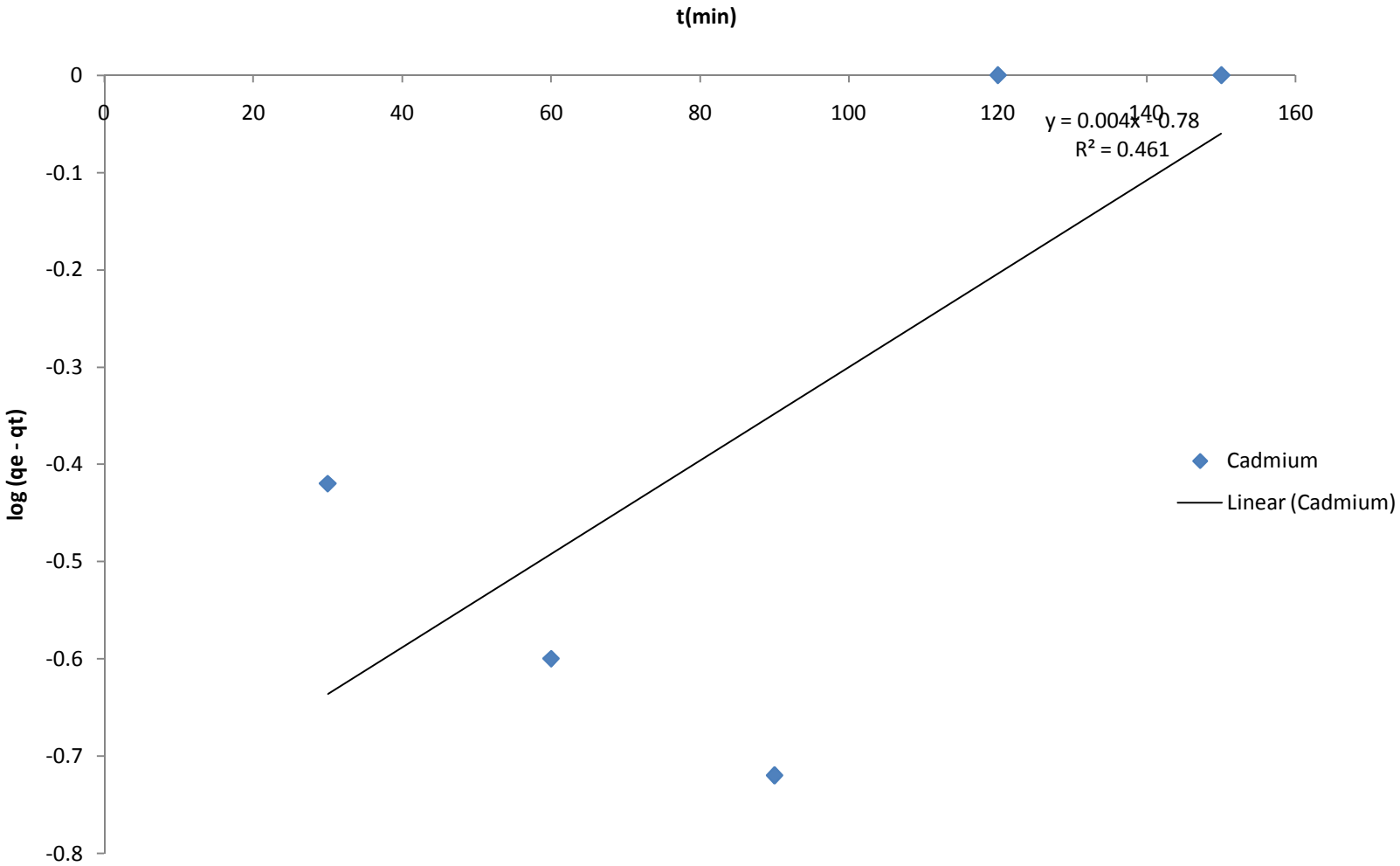


Figure 4.39: Pseudo First – Order Model for Cd^{2+} Biosorption.

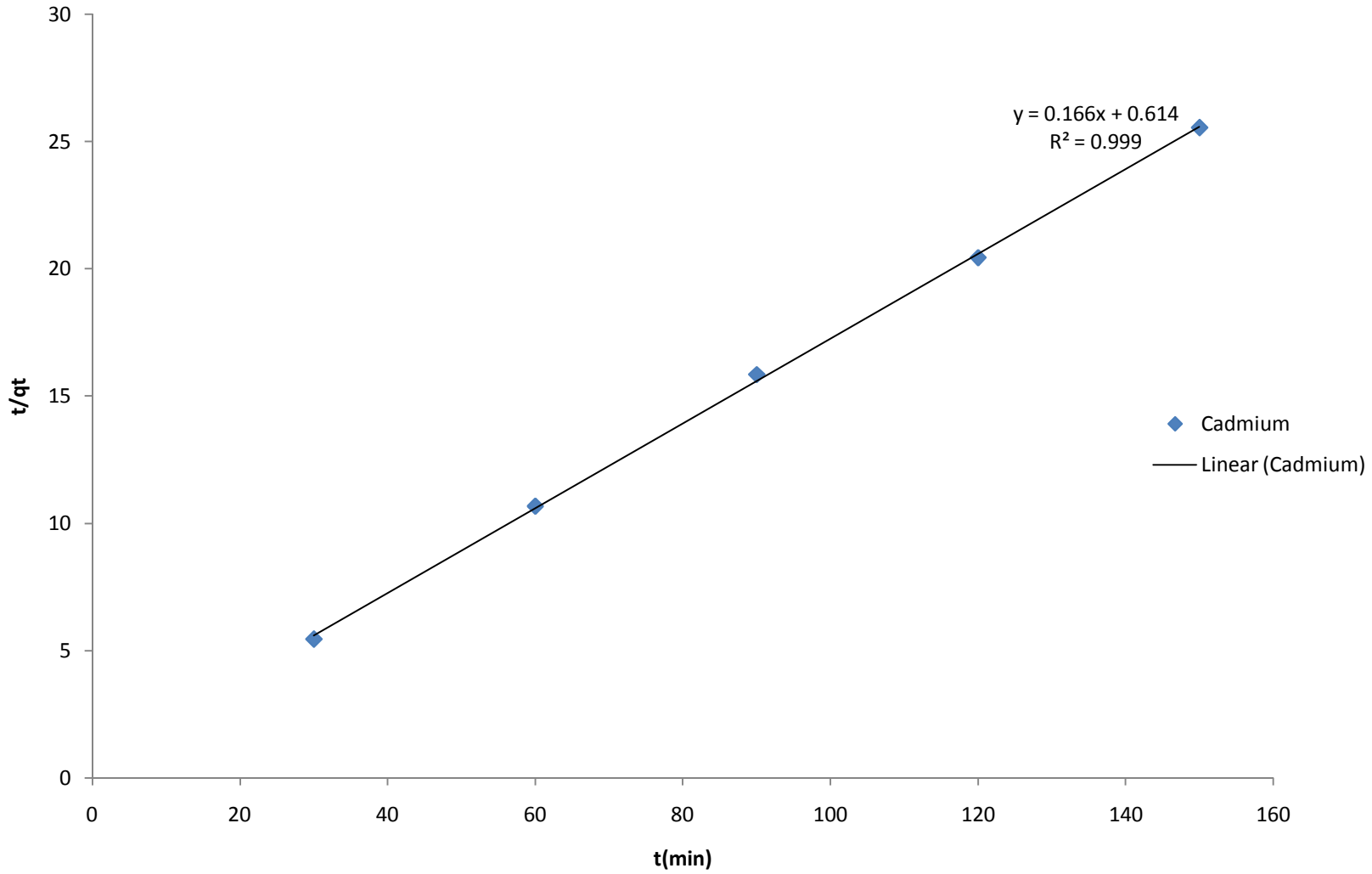


Figure 4.40: Pseudo Second – Order Model for Cd^{2+} Biosorption.

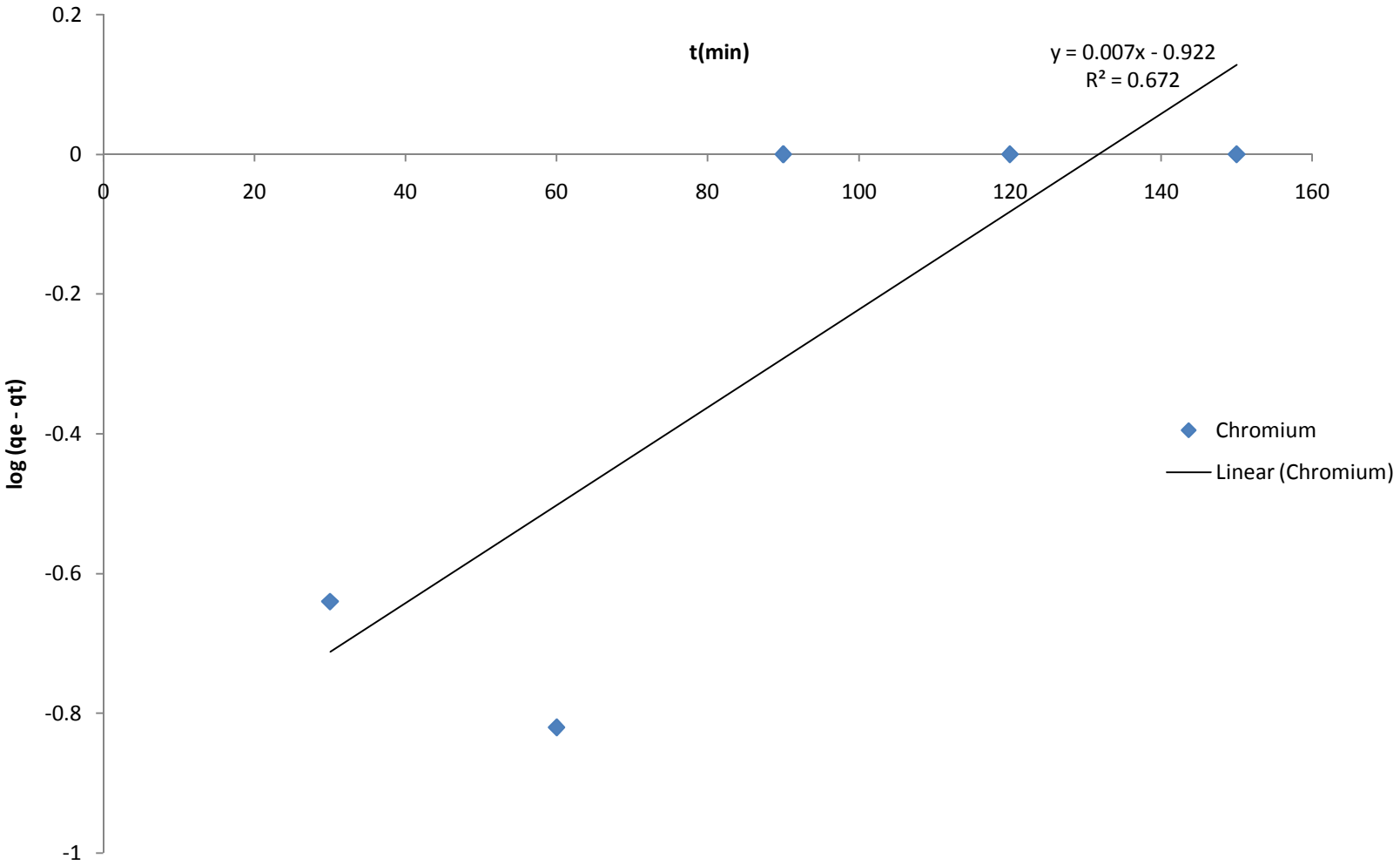


Figure 4.41: Pseudo First – Order Model for Cr(VI) Biosorption.

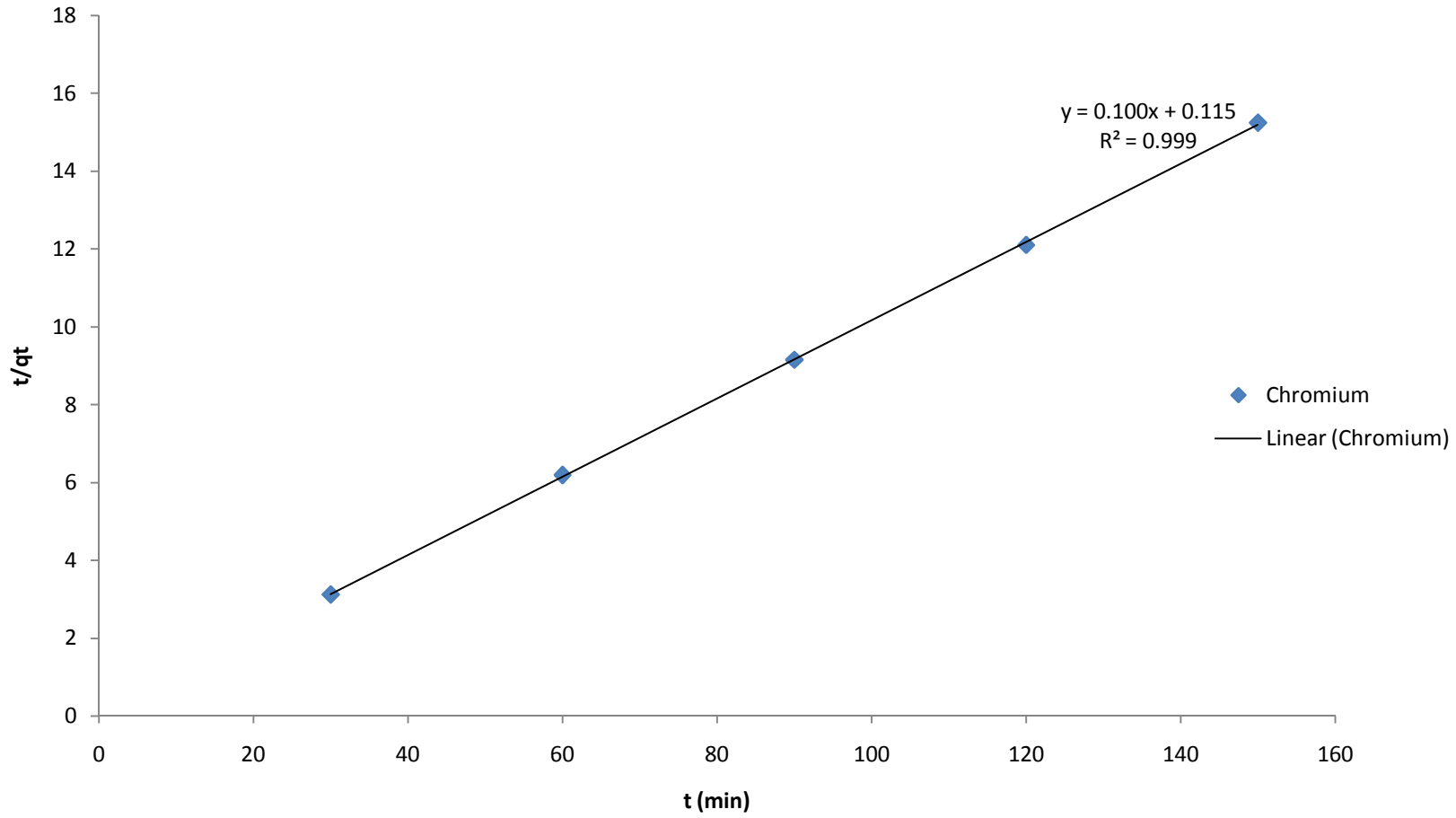


Figure 4.42: Pseudo Second – Order Model for Cr(VI) Biosorption.

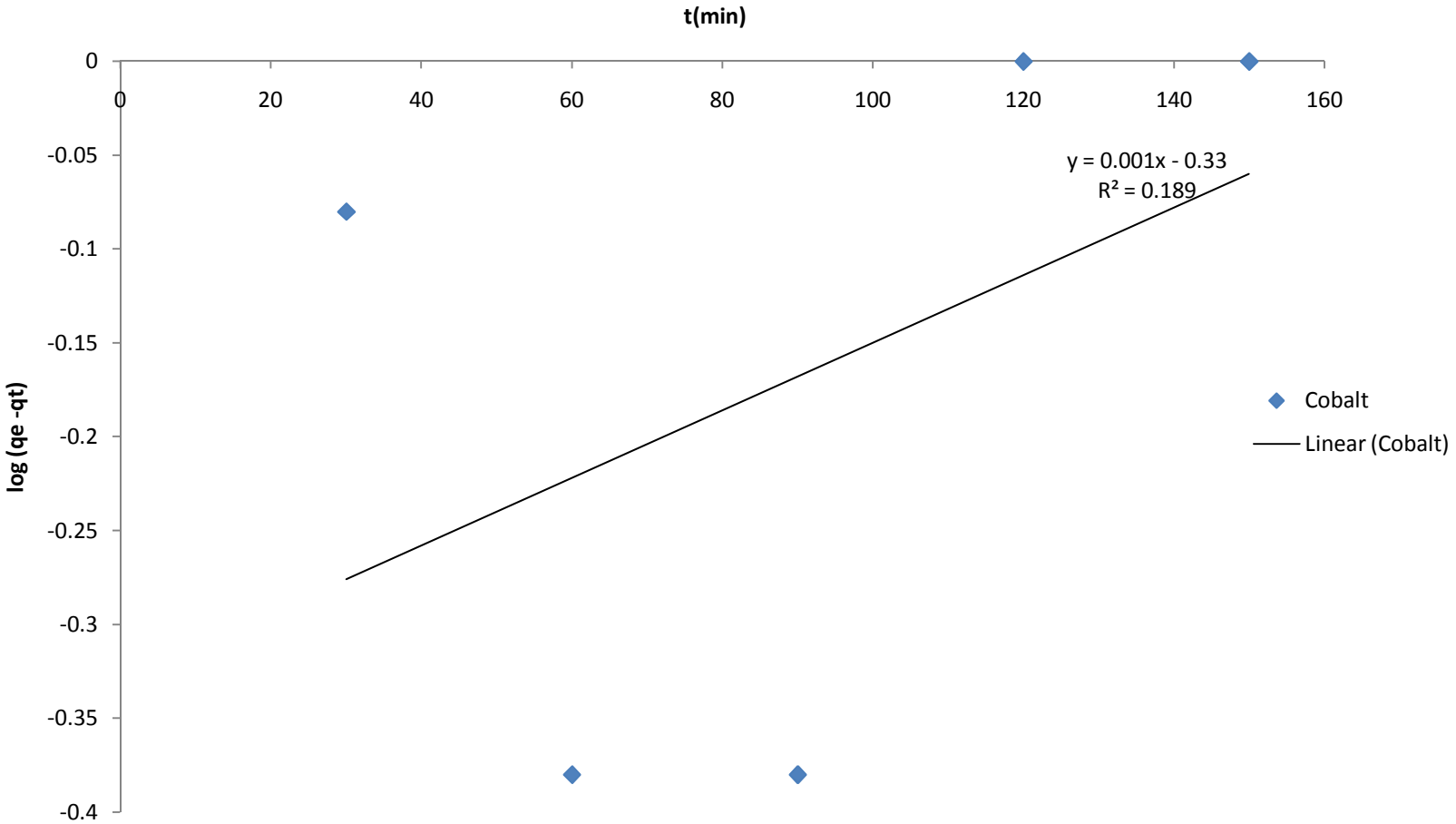


Figure 4.43: Pseudo First – Order Model for Co^{2+} Biosorption.

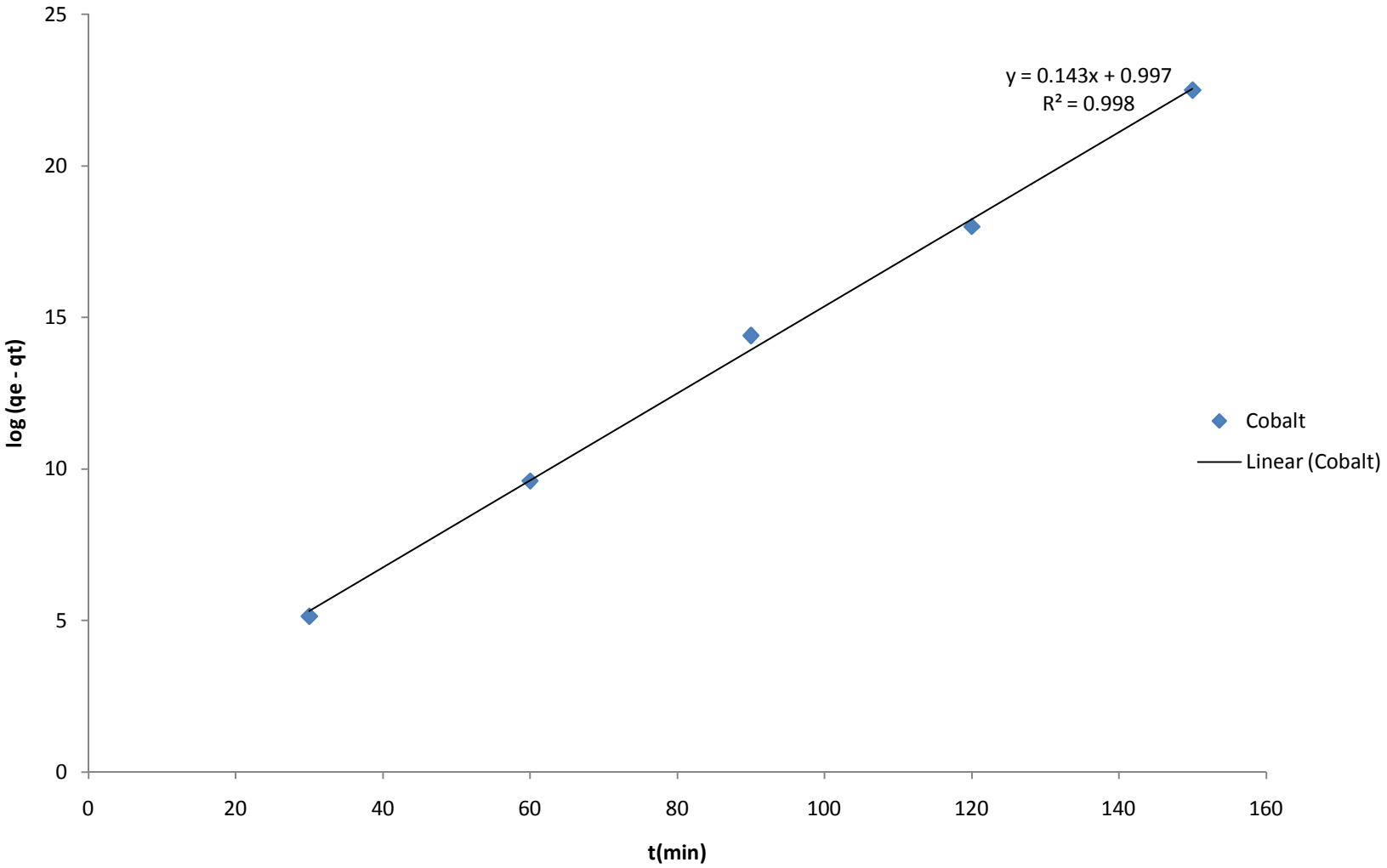


Figure 4.44: Pseudo Second – Order Model for Co^{2+} Biosorption.

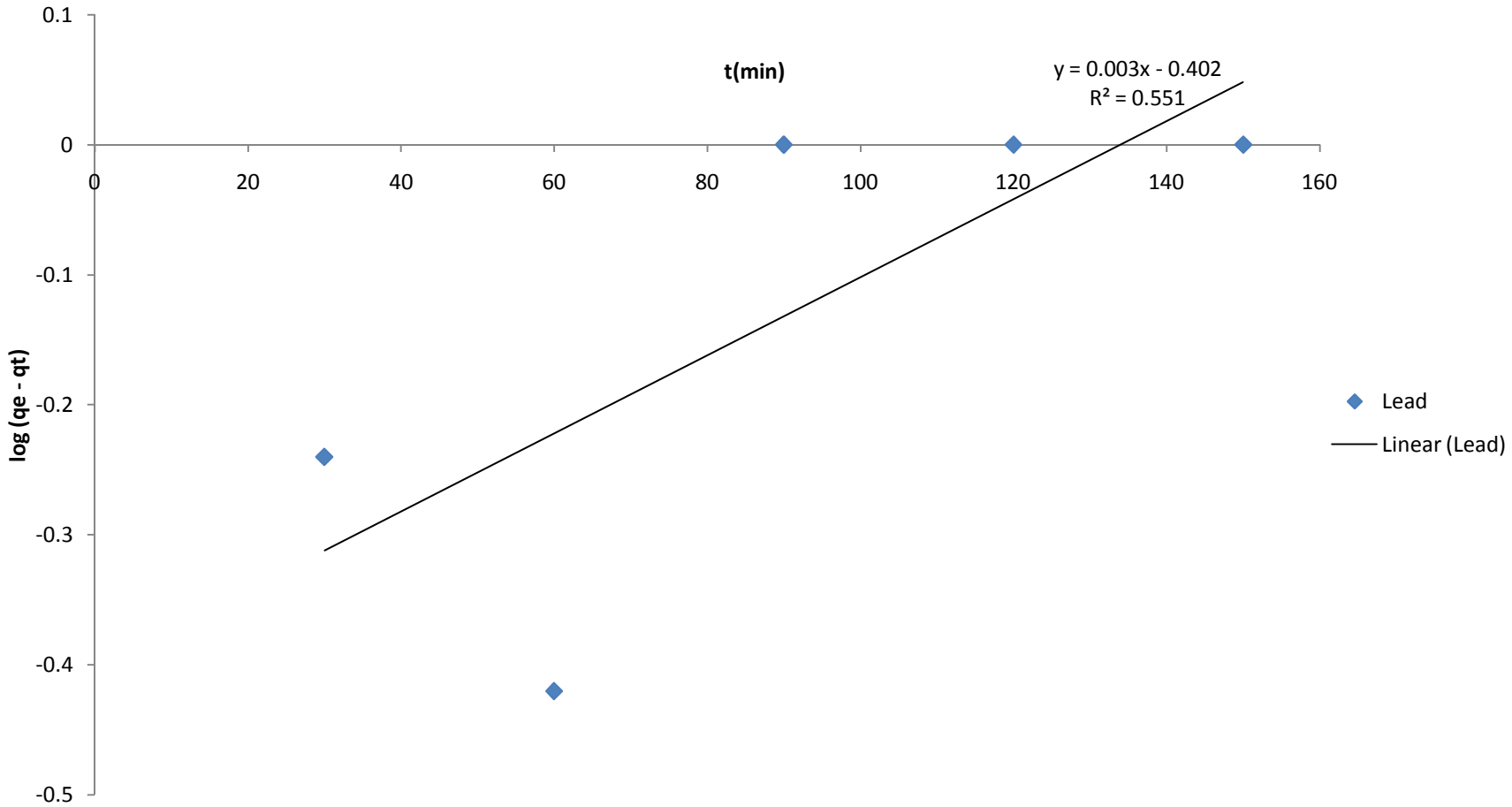


Figure 4.45: Pseudo First – Order Model for Pb^{2+} Biosorption.

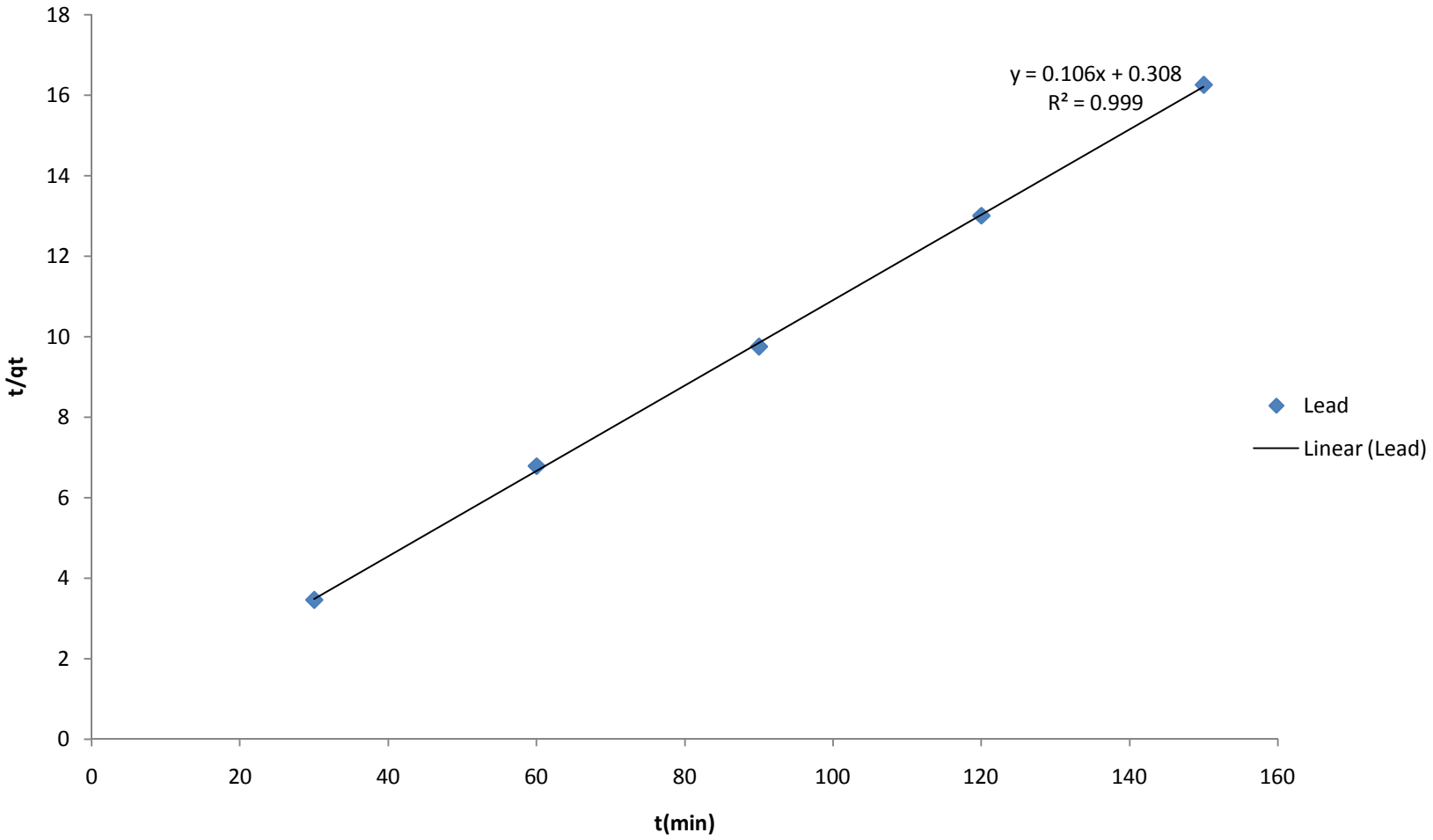


Figure 4.46: Pseudo Second – Order Model for Pb^{2+} Biosorption.

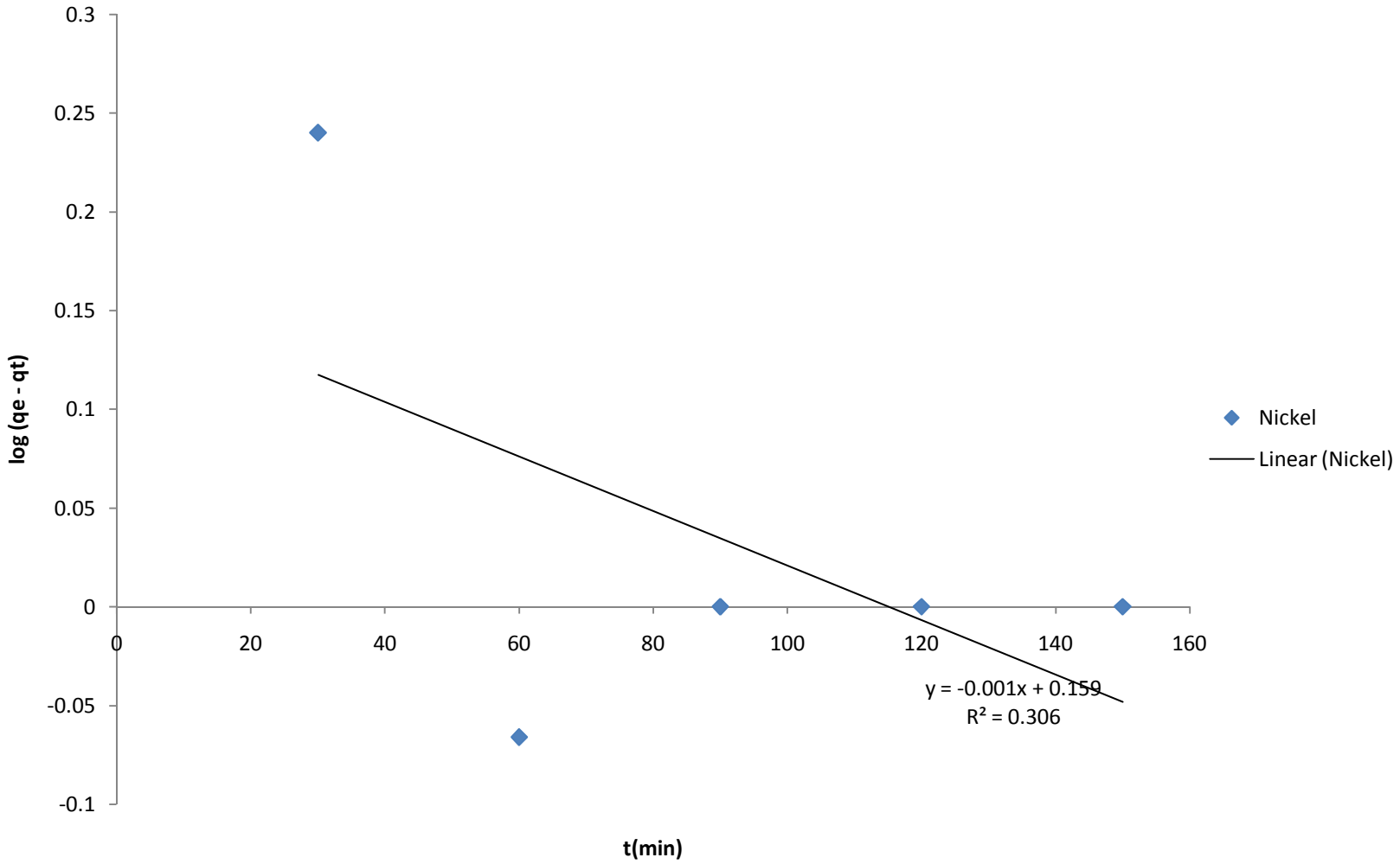


Figure 4.47: Pseudo First – Order Model for Ni^{2+} Biosorption.

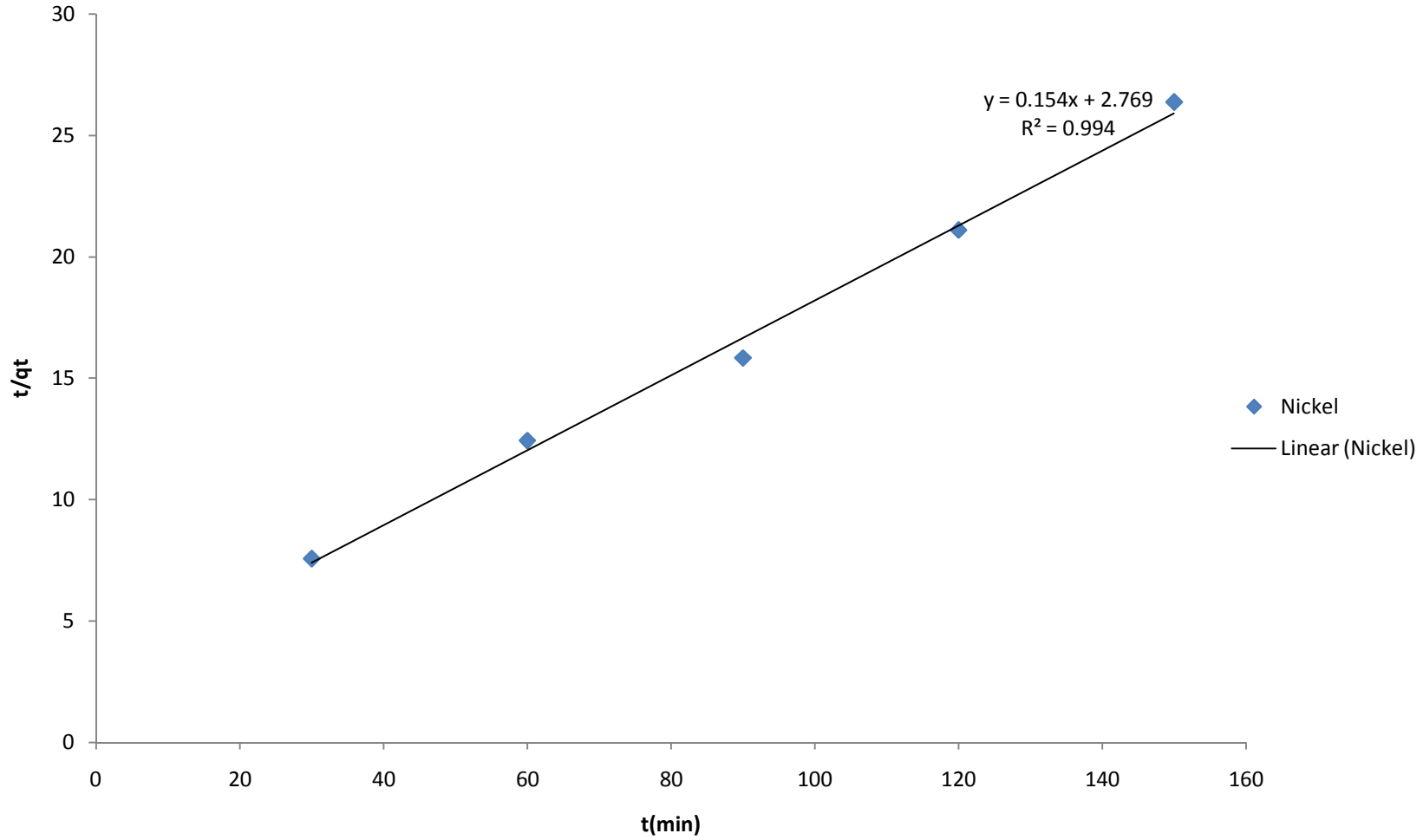


Figure 4.48: Pseudo Second – Order Model for Ni^{2+} Biosorption.

4.3.7 Effect of Initial Solution pH

Biosorption of heavy metal ions depends on the pH of the solution as it affects biosorbent surface charge, degree of ionization, and the species of biosorbent (Ahmady – Asbchin *et al.,* 2008). The pH of the solution influence both metal binding sites on the cell surface and the chemistry of metal in solution (Dursun, 2006). In order to demonstrate the effect of initial solution pH on biosorption capacity, uptake of each of Cd(II), Cr(VI), Co(II), Pb(II), and Ni(II) on to the algal biomass as a function of pH was studied in the pH range of 2 to 8.

Figure 4.49 is graphical representation on effects of initial solution pH on uptake of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} respectively. Cr(VI) showed highest adsorption capacity of 9.84mg/g at pH 2. From Figure 4.49, it was observed that the adsorption capacity of Cr(VI) decreases as the pH of the solution increases. The increased binding of hexavalent chromium at low pH can be explained by two factors. First, adsorption of Cr(VI) at pH 2.0 suggests that the negatively charged chromium species (chromate/dichromate in the medium) bind through electrostatic attraction to positively charged functional groups on the surface of biosorbents. As the pH increased, the overall surface charge on the cells became negative and biosorption decreased. In alkali conditions, carboxylate group exists in deprotonated form and has net negative charge. As a result, the surface charge of the biosorbents become negative and biosorption of Cr(VI) decreases. Secondly, the solution chemistry of Cr(VI) ions can affect the biosorption process. Previous studies showed that chromium exhibits different types of pH dependent equilibria in solutions. Sorbate and chromium form stable complexes such as $Cr_2O_7^{2-}$, $HCrO_4^-$, CrO_4^{2-}, and $HCr_2O_7^-$, the fraction of any particular species is dependent on chromium concentration and pH. In low chromium concentration, the main fraction is $HCrO_4^-$ with pH below 5.0, whereas the CrO_4^{2-} increases with increase of pH value and becomes the

main form with pH above 7.0 (Sethuraman and Balasubramanian, 2010; Park *et al.*, 2005; Raghavarao *et al*., 2009).

At pH 6, the highest adsorption capacity of Cd^{2+}, Co^{2+}, Pb^{2+} and Ni^{2+} were found to be 5.97mg/g, 6.67mg/g, 9.23mg/g, and 7.42mg/g respectively. Figure 4.49 showed that Cd^{2+}, Co^{2+}, Pb^{2+}, and Ni^{2+} showed a unique biosorption behavior at their different solution pH, that is, as their metal solution pH was increased, their adsorption capacity first increased from 2 pH to pH 6 and then decreased at pH 8. At pH higher than 6, formation of insoluble metal hydroxides or anionic hydroxide complexes of metal ions takes place restricting the true biosorption studies. This is consistent with the results obtained for the other adsorbent systems (Holan and Volesky, 1993; Mahamadi and Torto, 2007; Gonzalez *et al*., 2006; Ansari *et al*., 2009; Pattabhi *et al*., 2008).

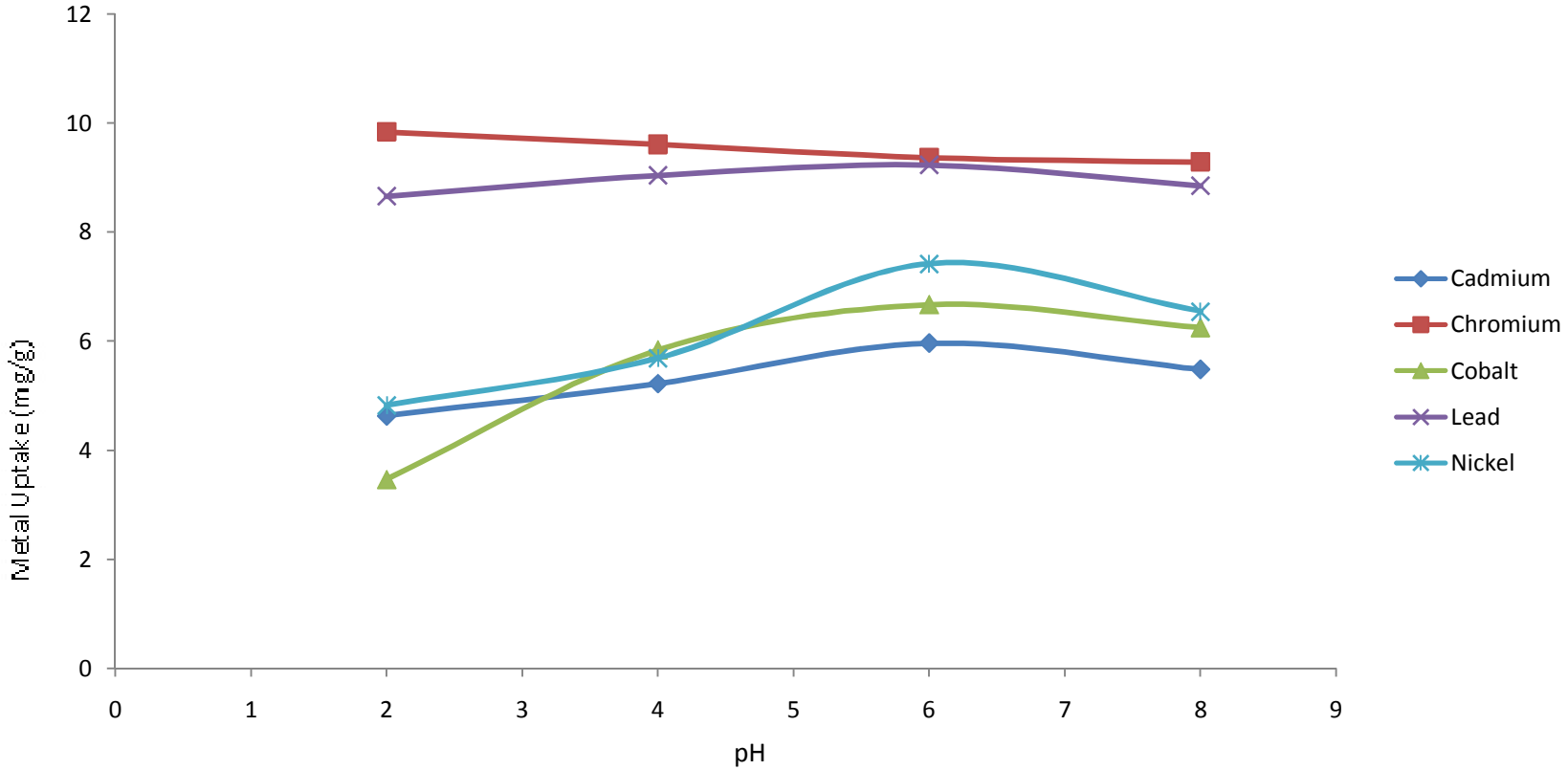


Figure 4.49: Effect of Initial Solution pH

4.3.8 Effect of Temperature

Metal uptake and removal efficiency are sometimes dependent on temperature owing to the fact that biosorption is reported to be metabolism dependent (Ahalya *et al.,* 2003). Figure 4.50 is a graphical representation of effect of temperature Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} biosorption respectively.

It was observed that all the metals showed increase in adsorption capacity with increase in temperature. This could be due to increase in average kinetic energy of the metal ions in solutions containing the adsorbent which increases the number of metal ions interacting with the adsorbent surface by increasing the rate at which the metal ions hit the binding sites at the surface of the adsorbent thus increasing the adsorption capacities. Cadmium showed an increase from 5.62mg/g at 27^{o}C to 7.37mg/g at 60^{o}C. Chromium showed slight increase from 9.84mg/g at 27^{o}C to 9.92mg/g at 40 – 60^{o}C, i.e at 40^{o}C maximum adsorption chromium was achieved. Cobalt and lead showed maximum adsorption capacity of 8.85mg/g and 6.67mg/g respectively at 27^{o}C, then decreased at 40^{o}C after which they increased again as the temperature was increased to 60^{o}C. This suggests that adsorption of cobalt and lead did not show temperature dependence. Nickel showed a constant metal uptake or removal efficiency of 5.69mg/g until at 60^{o}C where there is an increase to 6.55mg/g.

The equilibrium data presented obtained from the effect of temperature, was used for thermodynamic modeling to determine the enthalpy, entropy and free energy of the adsorption reaction at different temperatures.

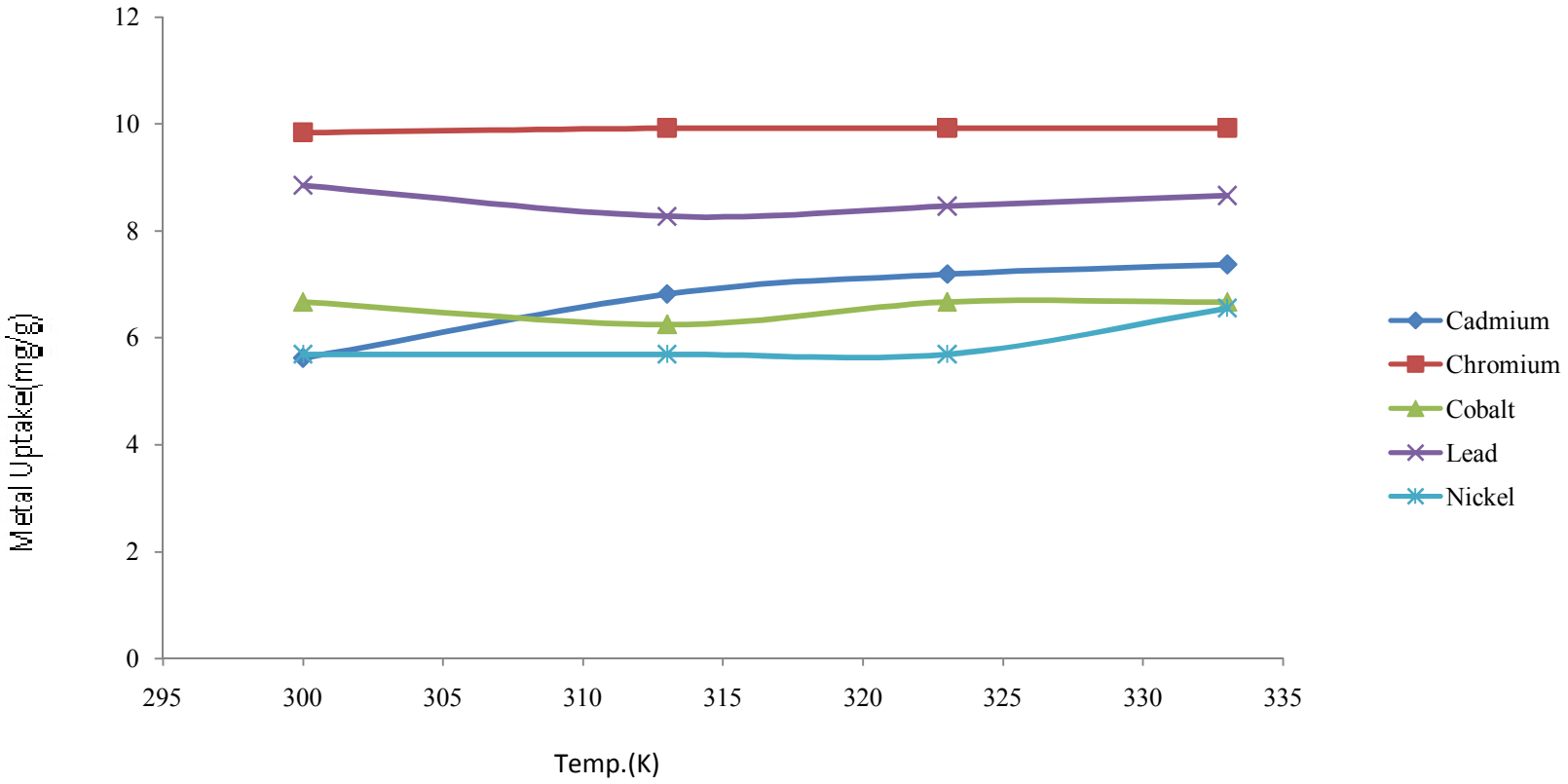


Figure 4.50: Effect of Temperature.

4.3.9 Thermodynamic Modeling

Thermodynamic model proposed by Rastogi and Gupta (2007) was applied in this study. The thermodynamic parameters such as change in standard free change energy (ΔG^o), enthalpy change (ΔH^o) and entropy change (ΔS^o) for adsorption process are calculated from binding energy constant, *b*, obtained from Langmuir equation using the following equation:

$$\Delta G^o = -RT\, ln(b) \quad \text{.....(4.8)}$$

$$\Delta G^\circ_{ads} = \Delta H^\circ_{ads} - T\Delta S^\circ_{ads} \quad \text{.....(4.9)}$$

Where: R (8.314 J/mol K) is the gas constant, T (K) the absolute temperature and *b* (L/mol) is the Langmuir constant related to free energy or net enthalpy of adsorption. By plotting a graph of ΔG°_{ads} versus *T,* the values ΔH^o and ΔS^o can be estimated from the intercept and slope respectively as illustrated in Figures 4.51 – 4.55. Table 4.8 presents calculated values of Gibbs free energy change of all the metal ion sorption process over the temperature range studied. Figures 4.51, 4.52, 4.53, 4.54 and 4.55 represents the free energy change in the biosorption of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} respectively.

Free energy change of all the metal ions adsorption process was found to be negative, suggesting spontaneous process. In general, it is of note that free energy up to −20 kJ/mol are consistent with electrostatic interaction between charged molecules and surface indicative of physiosorption while more negative values −40 kJ/mol involve chemisorption; free energy change values between −20 to −40 kJ/mol indicate that both physisorption and chemisorption are responsible for adsorption (Bhatti *et al.,* 2006). Thus, the magnitude of the free energy change obtained in this study indicates both physical and chemical mechanism for the

adsorption of Cd(II), Cr(VI), Co(II), Pb(II) and Ni(II) ions on to the *Cosmarium panamense* algal biomass, hence physiosorption and chemisorption were responsible for their adsorption.

Table 4.9 presents thermodynamic parameters for the biosorption of these metals at different temperatures. Lead had the highest entropy value of 85.4 $Jmol^{-1}$ K^{-1}, followed by cadmium, 75.7 $Jmol^{-1}$ K^{-1}; chromium, 74.5 $Jmol^{-1}$ K^{-1}; nickel, 72.2 $Jmol^{-1}$ K^{-1}; and cobalt, 66.4 $Jmol^{-1}$ K^{-1}. The maximum value of enthalpy change occurred at cadmium biosorption (0.7614 $Jmol^{-1}$), followed by cobalt (0.4912 $Jmol^{-1}$), chromium (0.0474 $Jmol^{-1}$), nickel (0.0106 $Jmol^{-1}$) and lead (0.0071 $Jmol^{-1}$). The values of enthalpy change for the sorption of all the metals were positive, which suggest endothermic reaction and could be attributed to increase in adsorption on successive increase in temperature while the positive values of entropy reveals increase in randomness at the solid – solution interface during the fixation of the different metal ions on the active sites of the biosorbent (Gupta and Rastogi, 2007). This could be as a result of high affinity for these metal ions by the binding sites on the surface of the algal biomass. Further, Gupta and Rastogi (2007) reported that if adsorption process is endothermic, it means under the conditions the process becomes spontaneous because of the large positive entropy change.

Table 4.8: Calculated Values of Gibbs Free Energy in $kJmol^{-1}$ of all the Selected Metals Sorption Process over the Temperature Range Studied.

Temp.(K)	Cadmium	Chromium	Cobalt	Lead	Nickel
300	-22.698	-22.353	-19.928	-25.626	-21.656
313	-23.682	-23.321	-20.791	-26.737	-22.595
323	-24.439	-24.066	-21.455	-27.591	-23.317
333	-25.195	-24.811	-22.119	-28.445	-24.039

Table 4.9: Thermodynamic Parameters for the Biosorption of all the Selected Metals on The Algal Biomass

	Cadmium	Chromium	Cobalt	Lead	Nickel
ΔH_{ads} (Jmol^{-1})	0.7614	0.0474	0.4912	0.0071	0.0106
ΔS_{ads} (Jmol^{-1} K^{-1})	75.664	74.509	66.427	85.421	72.188

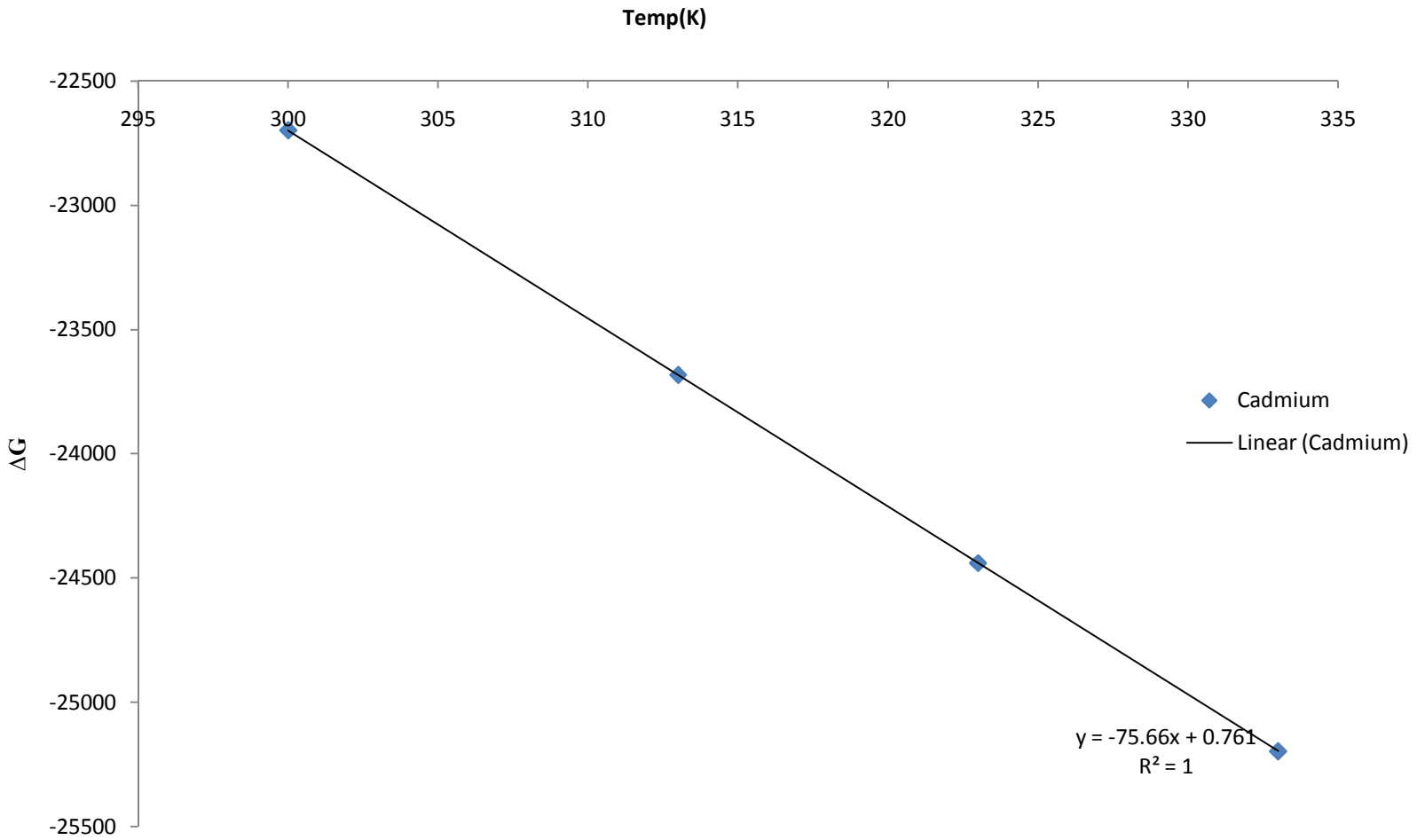


Figure 4.51: Variation of Free Energy Change with Temperature on Cd^{2+} Adsorption.

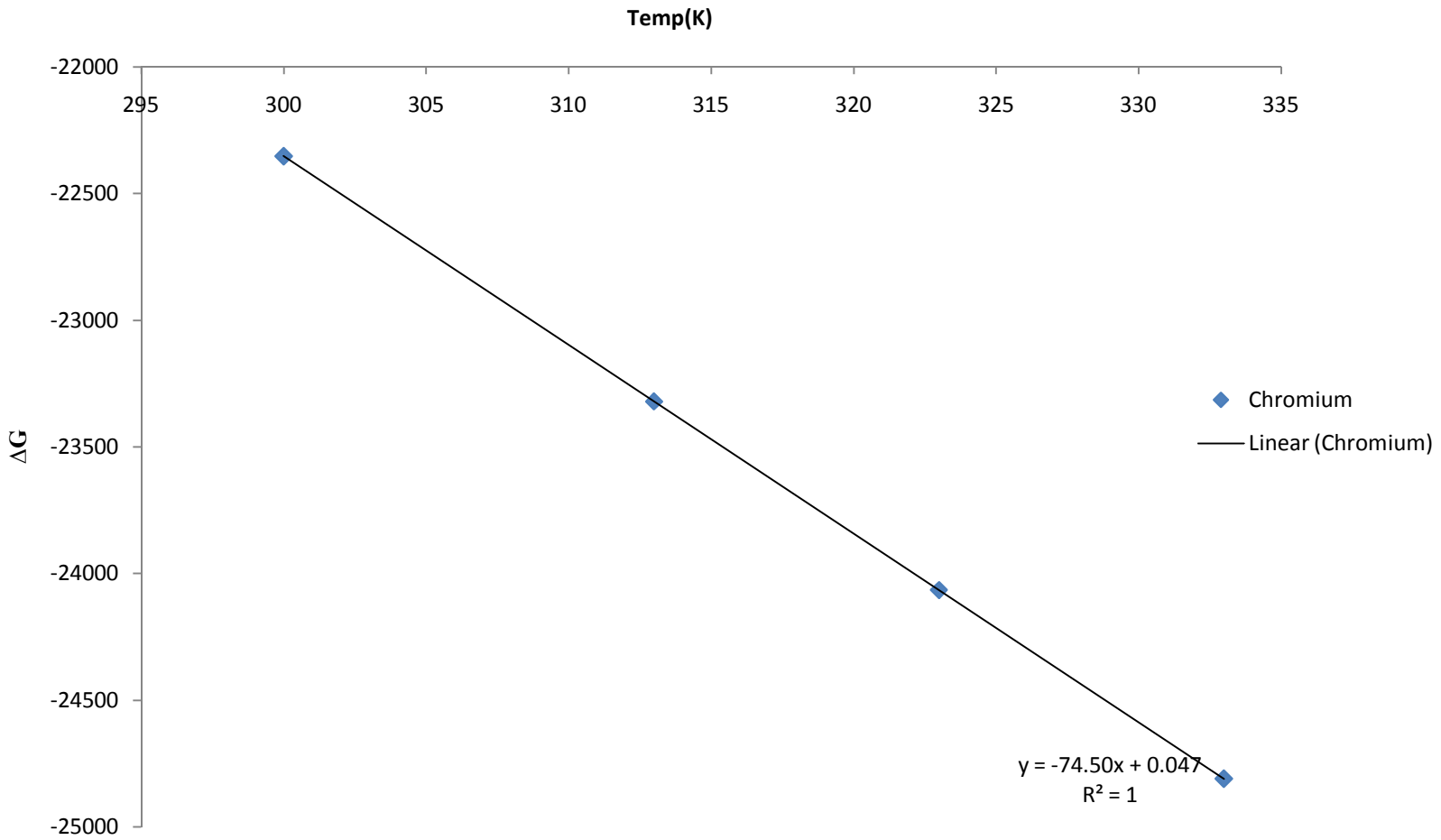


Figure 4.52: Variation of Free Energy Change with Temperature on Cr(VI) Adsorption.

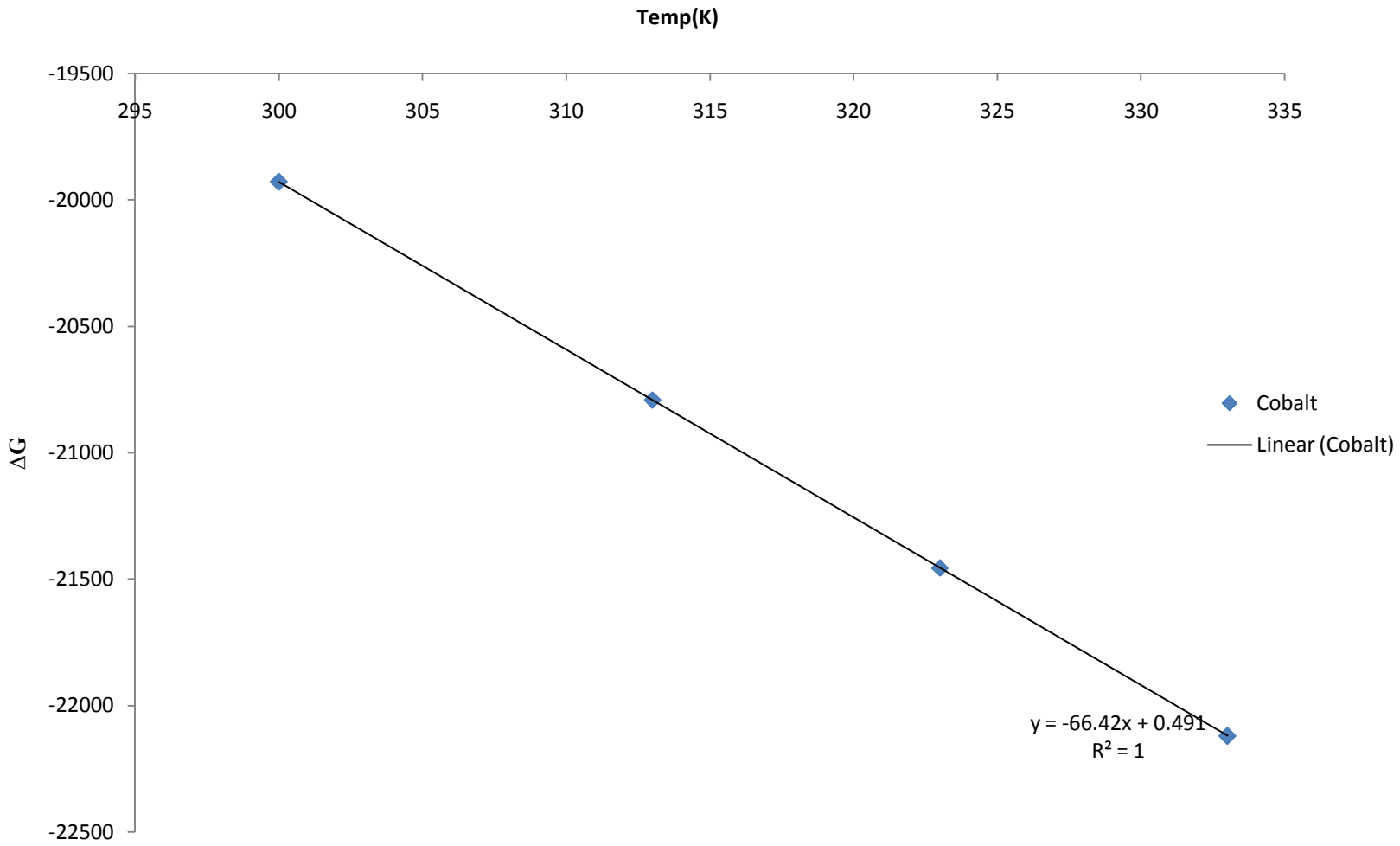


Figure 4.53: Variation of Free Energy Change with Temperature on Co^{2+} Adsorption.

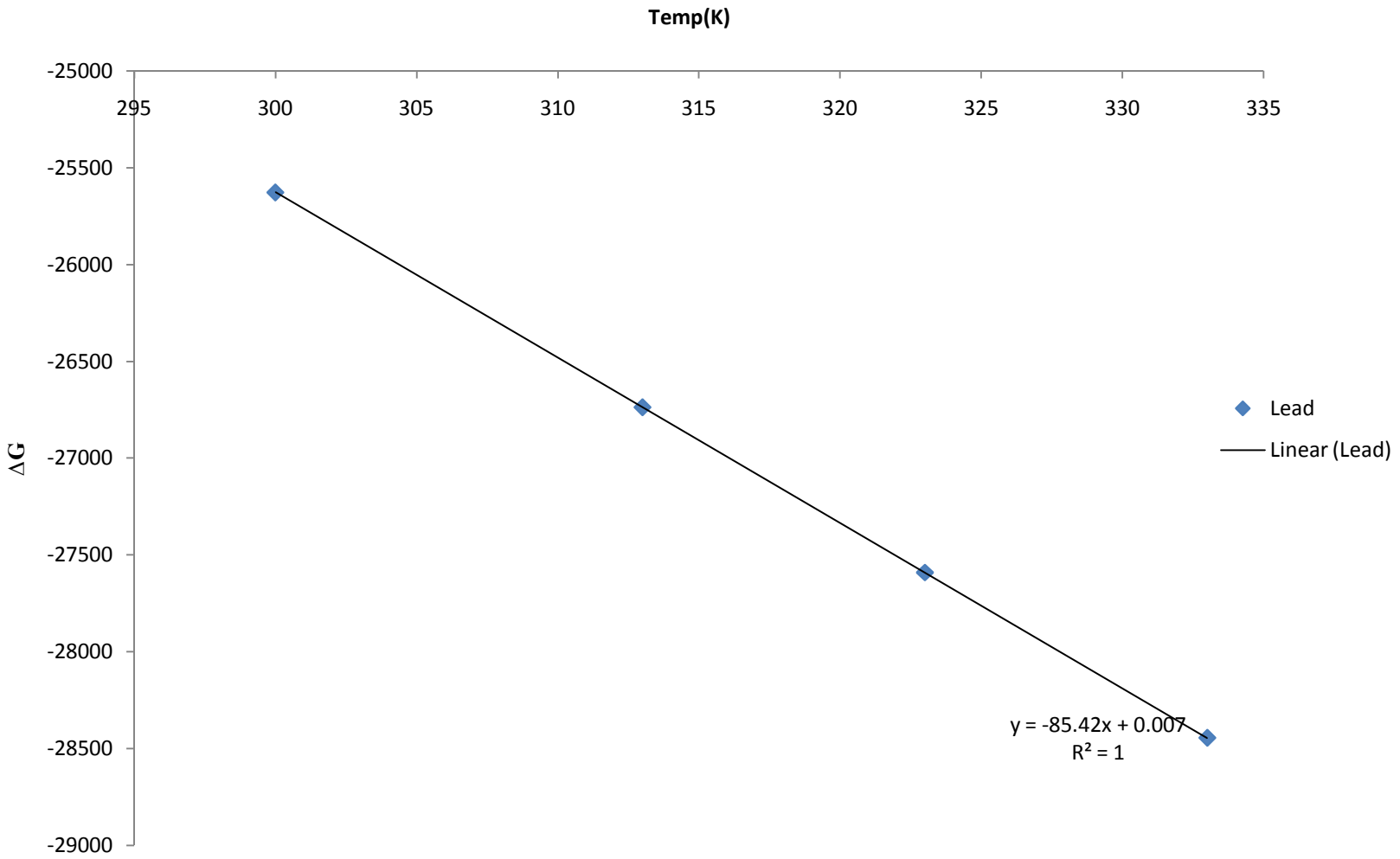


Figure 4.54: Variation of Free Energy Change with Temperature on Pb^{2+} Adsorption.

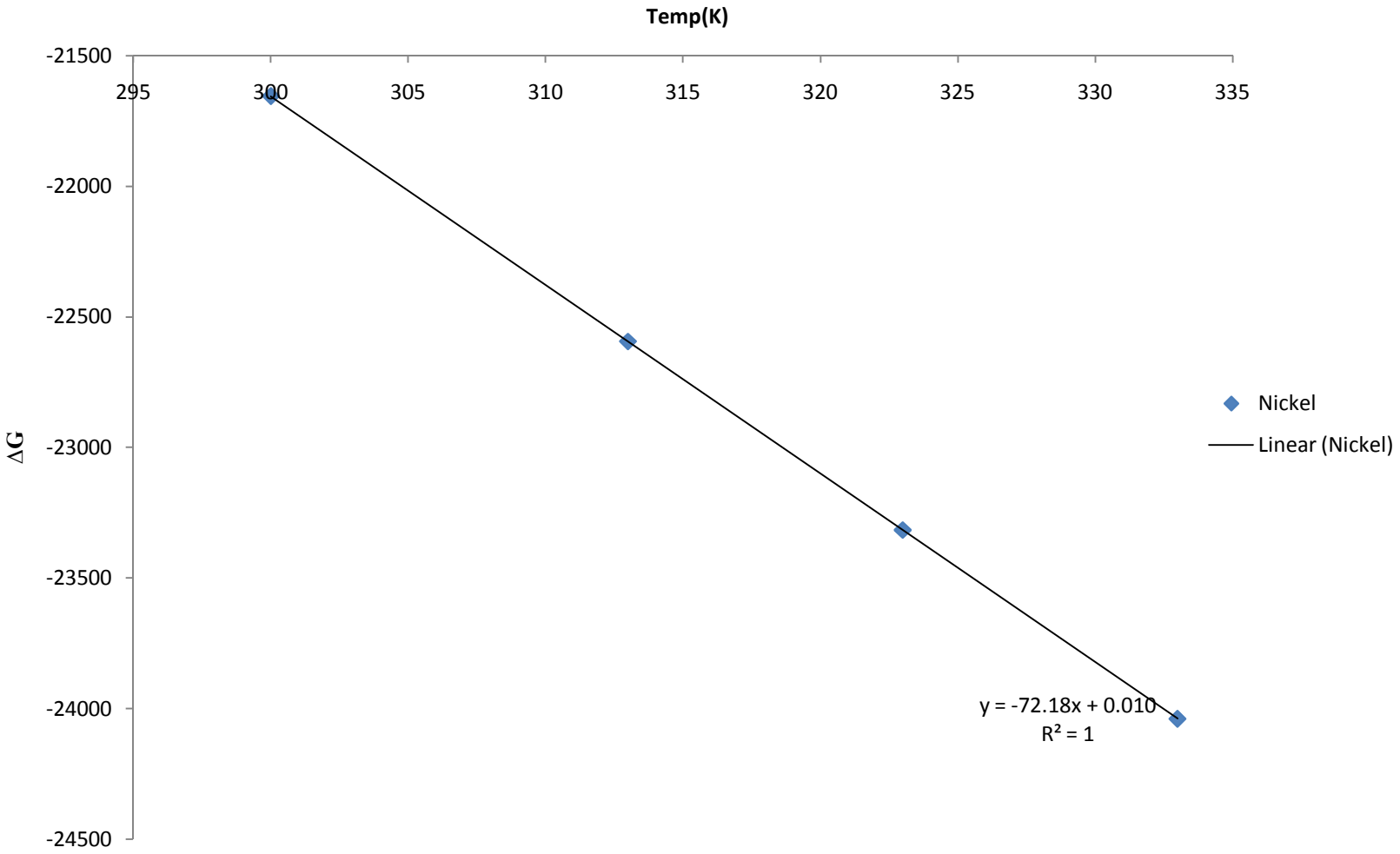


Figure 4.55: Variation of Free Energy Change with Temperature on Ni^{2+}Adsorption.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

This study indicated that dead biomass of green algae *Cosmarium panamense* is an effective biosorbent for Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} removal from aqueous solution. The uptake of all metal ions by algal biomass increases with increasing initial metal ion concentration and temperature but decreases with increasing biosorbent dosage. The uptake of the selected heavy metal ions of interest increases with increase in contact time till equilibrium is achieved. The uptake of Cr(VI) decreases as pH is increased from 2 – 8 while that of Cd(II), Co(II), Pb(II), and Ni(II) increased with increase in pH from 2 – 6.

Langmuir and Freundlich isotherm models were used for the mathematical description of biosorption of the selected heavy metal ions on to algal biomass and it was found that the adsorption equilibrium data of all the metals fitted well to the Langmuir model. The maximum adsorption capacities of Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} were determined to be 12.89mgCd/g, 109.9mgCr/g, 16.69mgCo/g, 19.72mgPb/g and 10.73mgNi/g of the dry weight of the algal biomass respectively at algal dose of 2g/l, initial metal ion concentration of 20ppm and pH 5.05, 2.05, 4.05, 4.02, and 5.02 respectively. The order of biosorption of these heavy metal ions are Cr>, Pb>, Co> Cd> Ni. Also, the biosorption of these heavy metal ions on the algal biomass follows pseudo - second order biosorption kinetics.

The values of the calculated Gibbs Free Energy are negative and falls between -20kJmol^{-1} and -40kJmol^{-1}, the enthalpy and entropy values are positive. Analysis with NAA and EDAX showed that not all the elements in the algal biomass detected by NAA was detected

by EDAX because of their quantity variation. The number of elements detected by EDAX in the native, Cd(II), Cr(VI), Co(II), Pb(II), and Ni(II) ion treated algal biomass varied. Simultaneous thermal analysis reveals that both the native and the different heavy metal treated algal biomass starts decomposition(phase transition) at the same temperature but with different amount of heat absorbed and different percent residue weight of the various biomass remaining after decomposition. Biosorption did not destroy the main structure of the biomass but alters the peripheral surface structure through surface interaction, after which a new biomass of different energy status with respect to the metal adsorbed is formed. Also the inorganic constituent of the algal biomass are being altered during biosorption which result to decrease in the percent residue weight after biosorption

FTIR spectra analysis confirms the occurrence of a successful biosorption of these metals by algal biomass. FTIR spectra display characteristics absorbance peaks of carboxyl, hydroxyl, amines, amides, alkenes, alkynes, phosphonate, silica, methyl and methylene functional groups. Hence, these functional groups are responsible for the adsorption of the selected heavy metals.

With advantage of high metal biosorption capacity, the biomass of *Cosmarium panamense* has the potential to be used as an efficient and economic biosorbent material for the removal of Cd(II), Cr(VI), Co(II), Pb(II), and Ni(II) ions from aqueous solutions.

5.2 Conclusion

The batch studies conducted in the present study provides significant information, that biosorption of Cd(II), Cr(VI), Co(II), Pb(II), and Ni(II) ions on dead biomass of green algae

Cosmarium panamense species is dependent of initial solution pH, Initial metal ion Concentration, biosorbent dosage, contact time, and temperature.

Langmuir and Freundlich isotherm models were used for the mathematical description of biosorption equilibrium of the selected heavy metal ions on to algal biomass and result obtained showed that the adsorption equilibrium data of all the metals fitted well to the Langmuir model. Hence, biosorption of these heavy metals on the algae biomass is a monolayer sorption process and is also a rate limiting step as well as initial concentration dependent since it follows pseudo - second order biosorption kinetics.

Furthermore, from thermodynamic modeling, it can be deduce that the biosorption of these metals are spontaneous and endothermic while physical and chemical mechanism are responsible for the process.

Also availability of elements in the biomass for detection and the inorganic constituents of the biomass are affected by the biosorption of the different metals on algal biomass, as revealed by NAA, EDAX and STA respectively .

Carboxyl, hydroxyl, amines, amides, alkenes, alkynes, phosphonate, silica, methyl and methylene functional groups are responsible for the adsorption of the selected heavy metals. Thus, *Cosmarium panamense* is an effective biosorbent for Cd^{2+}, Cr(VI), Co^{2+}, Pb^{2+} and Ni^{2+} removal from aqueous solution.

5.3 Recommendations on Further Studies

It is now proven without doubt that dead algal biomass *Cosmarium panamense* has the potential to remove heavy metal ions from aqueous solution. Bearing this in mind, the following recommendations are necessary:

- Further research should be done on living specie of this biomass to determine its potential to remove these heavy metal ions from aqueous solution since it has been reported that biosorption is metabolism dependent.
- Research should be carried out on the potential of this algal biomass *Cosmarium panamense* to absorb other metals not studied in this research and the ability or potential of the chemically modified form of the algal biomass *Cosmarium panamense* to remove the heavy metals from waste water
- Nigeria is an oil producing country, pollution caused by oil spillage is a major problem globally. In view of this, research into the capability of this biomass or any of its modified form to absorb oil is recommended.
- Northern part of Nigeria is famously known for its textile industries with Zaria and Kano being dominated with these industries. Pollution caused to the receiving environment by the wastewater discharge from these industries makes it paramount to recommend further research in to the ability of this biomass to absorb synthetic dyes.

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APPENDIX

Initial Conc. (ppm)	Average Conc.(I) (ppm)	q_e (mg/g)	%Removal	
5	1.72	1.64	65.6	
10	4.01	3.00	59.9	
15	5.91	4.55	60.6	
20	9.54	5.23	52.3	
25	10.96	7.02	56.2	
Dosage				
20	10.80	9.2	46.0	0.05
20	9.08	5.46	54.6	0.10
20	6.27	4.58	68.7	0.15
20	5.82	3.55	70.9	0.20
20	5.27	2.95	73.7	0.25
Time				
20	9.02	5.49	54.9	30
20	8.76	5.62	56.2	60
20	8.64	5.68	56.8	90
20	8.26	5.87	58.7	120
20	8.26	5.87	58.7	150
pH				
20	10.72	4.64	46.4	2
20	9.54	5.23	52.3	4
20	8.06	5.97	59.7	6
20		5.49	54.9	8
Temp.				
20	8.76	5.62	56.2	300
20	6.37	6.82	68.2	313
20	5.63	7.19	71.9	323
20	5.27	7.37	73.7	333

(pH – 5.05, Temp. – 27^{o}C, Equilibrium Time – 24hr)

Appendix 1: Experimental Data Obtained on Biosorption of Cadmium

Initial Conc. (ppm)	Average Conc.(I) (ppm)	q_e (mg/g)	%Removal	
5	0.15	2.43	97.0	
10	0.32	4.84	96.8	
15	0.48	7.26	96.8	
20	0.63	9.69	96.9	
25	0.79	12.11	96.8	
Dosage				
20	0.63	19.37	96.9	0.05
20	0.63	9.69	96.9	0.10
20	0.63	6.46	96.9	0.15
20	0.63	4.84	96.9	0.20
20	0.32	3.94	98.4	0.25
Time				
20	0.79	9.61	96.1	30
20	0.63	9.69	96.9	60
20	0.32	9.84	98.4	90
20	0.32	9.84	98.4	120
20	0.32	9.84	98.4	150
pH				
20	0.32	9.84	98.4	2
20	0.79	9.61	96.1	4
20	1.27	9.37	93.7	6
20	1.43	9.29	92.9	8
Temp.				
20	0.32	9.84	98.4	300
20	0.16	9.92	99.2	313
20	0.16	9.92	99.2	323
20	0.16	9.92	99.2	333

(pH – 2.05, Temp. – 27^{o}C, Equilibrium Time – 24hr)

Appendix 2: Experimental Data Obtained on Biosorption of Chromium

Initial Conc. (ppm)	Average Conc.(I) (ppm)	q_e (mg/g)	%Removal	
5	1.92	1.54	61.6	
10	4.00	3.00	60.0	
15	6.67	4.17	55.5	
20	8.33	5.84	58.4	
25	13.06	5.97	47.8	
Dosage				
20	7.50	12.5	62.5	0.05
20	7.50	6.25	62.5	0.10
20	6.67	4.44	66.7	0.15
20	6.67	3.33	66.7	0.20
20	6.67	2.67	66.7	0.25
Time				
20	8.33	5.84	58.4	30
20	7.50	6.25	62.5	60
20	7.50	6.25	62.5	90
20	6.67	6.67	66.7	120
20	6.67	6.67	66.7	150
pH				
20	13.06	3.47	34.7	2
20	8.33	5.84	58.4	4
20	6.67	6.67	66.7	6
20	7.50	6.25	62.5	8
Temp.				
20	6.67	6.67	66.7	300
20	7.50	6.25	62.5	313
20	6.67	6.67	66.7	323
20	6.67	6.67	66.7	333

(pH – 4.05, Temp. – 27^{o}C, Equilibrium Time – 24hr)

Appendix 3: Experimental Data Obtained on Biosorption of Cobalt

Initial Conc. (ppm)	Average Conc.(I) (ppm)	q_e (mg/g)	%Removal	
5	0.87	2.07	82.6	
10	1.62	4.19	83.8	
15	3.45	5.78	77.0	
20	4.35	7.83	78.3	
25	7.14	8.93	71.4	
Dosage				
20	4.35	15.65	78.3	0.05
20	3.46	8.27	82.7	0.10
20	2.31	5.90	88.5	0.15
20	2.31	4.42	88.5	0.20
20	1.54	3.69	92.3	0.25
Time				
20	2.69	8.66	86.6	30
20	2.31	8.85	88.5	60
20	1.54	9.23	92.3	90
20	1.15	9.43	94.3	120
20	1.15	9.43	94.3	150
pH				
20	2.69	8.66	86.6	2
20	1.92	9.04	90.4	4
20	1.54	9.23	92.3	6
20	2.31	8.85	88.5	8
Temp.				
20	2.31	8.85	88.5	300
20	3.46	82.7	82.7	313
20	3.08	8.46	84.6	323
20	2.69	8.66	86.6	333

(pH – 4.02, Temp. – 27^{o}C, Equilibrium Time – 24hr)

Appendix 4: Experimental Data Obtained on Biosorption of Lead

Initial Conc. (ppm)	Average Conc.(I) (ppm)	q_e (mg/g)	%Removal	
5	1.73	1.64	65.4	
10	4.17	2.92	58.3	
15	7.14	3.93	52.4	
20	8.33	5.84	58.4	
25	12.07	6.47	51.7	
Dosage				
20	10.34	9.66	48.3	0.05
20	8.62	5.69	56.9	0.10
20	10.34	3.22	48.3	0.15
20	10.34	2.42	48.3	0.20
20	6.90	2.62	65.5	0.25
Time				
20	12.07	3.97	39.7	30
20	10.34	4.83	48.3	60
20	8.62	5.69	56.9	90
20	8.62	5.69	56.9	120
20	8.62	5.69	56.9	150
pH				
20	10.34	4.83	48.3	2
20	8.62	5.69	56.9	4
20	5.17	7.42	74.2	6
20	6.90	6.55	65.5	8
Temp.				
20	8.62	5.69	56.9	300
20	8.62	5.69	56.9	313
20	8.62	5.69	56.9	323
20	6.90	6.55	65.5	333

(pH – 5.02, Temp. – 27^{o}C, Equilibrium Time – 24hr)

Appendix 5: Experimental Data Obtained on Biosorption of Nickel

Initial Conc. (ppm)	Cadmium		Chromium		Cobalt		Lead		Nickel	
	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem
5	1.64	65.6	2.43	97.0	1.54	61.6	2.07	82.6	1.64	65.4
10	3.00	59.9	4.84	96.8	3.00	60.0	4.19	83.8	2.92	58.3
15	4.55	60.6	7.26	96.8	4.17	55.5	5.78	77.0	3.93	52.4
20	5.23	52.3	9.69	96.9	5.84	58.4	7.83	78.3	5.84	58.4
25	7.02	56.2	12.11	96.8	5.97	47.8	8.93	71.4	6.47	51.7

(Biomass Conc. – 0.1g, pH = Cd(II) – 5.05, Cr(VI) – 2.05, Co(II) – 4.05, Pb(II) –4.02, Ni(II) –5.02, Temp. – 27^{o}C)
(Key: q_e is the metal uptake in mg/g, i. e. the amount of metal ion adsorbed in milligram per gram of the algal biomass while %Rem is the percentage metal ion removed)

Appendix 6: Experimental Data Showing Effect of Initial Metal ion Concentration

C_e(mg/l)	q_e(mg/g)	$1/C_e$(mg^{-1}l)	$1/q_e$(mg^{-1}l)	lnC_e(mg/l)	lnq_e(mg/g)
Cadmium					
1.72	1.64	0.58	0.61	0.54	0.49
4.01	3.00	0.25	0.33	1.39	1.10
5.91	4.55	0.17	0.22	1.78	1.52
9.54	5.23	0.10	0.19	2.26	1.65
10.96	7.02	0.09	0.14	2.39	1.95
Chromium					
0.15	2.43	6.67	0.41	- 1.90	0.89
0.32	4.84	3.13	0.21	-1.14	1.58
0.48	7.26	2.08	0.14	-0.73	1.98
0.63	9.69	1.58	0.1	-0.46	2.27
0.79	12.11	1.27	0.08	-0.24	2.49
Cobalt					
1.92	1.54	0.52	0.65	0.65	0.43
4.00	3.00	0.25	0.33	1.39	1.10
6.67	4.17	0.15	0.24	1.90	1.43
8.33	5.84	0.12	0.17	2.12	1.76
13.06	5.97	0.08	0.17	2.57	1.79
Lead					
0.87	0.48	1.15	0.48	0.24	0.63
1.62	4.19	0.62	0.24	0.48	1.43
3.45	5.78	0.29	0.17	1.24	1.75
4.35	7.83	0.23	0.13	1.47	2.06
7.14	8.93	0.14	0.11	1.97	2.19
Nickel					
1.73	1.64	0.58	0.61	0.55	0.49
4.17	2.92	0.24	0.34	1.43	1.07
7.14	3.93	0.14	0.25	1.97	1.37
8.33	5.84	0.12	0.17	2.12	1.76
12.07	6.47	0.08	0.15	2.49	1.87

Appendix 7: Equilibrium Data for Plotting Langmuir and Freundlich Isotherms for All The Selected Heavy Metals.

Conc.(ppm)	Cadmium	Chromium	Cobalt	Lead	Nickel
5.00	0.71	0.57	0.80	0.59	0.67
10.00	0.56	0.40	0.67	0.41	0.50
15.00	0.45	0.31	0.57	0.32	0.40
20.00	0.38	0.25	0.50	0.26	0.33
25.00	0.33	0.21	0.44	0.22	0.29

Appendix 8: Separation Factor for Biosorption of the Selected Metals on to Algal Biomass over the Whole Concentration Range Studied.

Biomass Dose (g)	Cadmium		Chromium		Cobalt		Lead		Nickel	
	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem
0.05	9.2	46.0	19.37	96.9	12.5	62.5	15.65	78.3	9.66	48.3
0.10	5.46	54.6	9.69	96.9	6.25	62.5	8.27	82.7	5.69	56.9
0.15	4.58	68.7	6.46	96.9	4.44	66.7	5.90	88.5	3.22	48.3
0.20	3.55	70.9	4.84	96.9	3.33	66.7	4.42	88.5	2.42	48.3
0.25	2.95	73.7	3.94	98.4	2.67	66.7	3.69	92.3	2.62	65.5

(Initial Conc. – 20ppm, pH = Cd(II)– 5.05, Cr(VI) – 2.05, Co(II) – 4.05, Pb(II) – 4.02, Ni(II) -5.02, Temp. – 27^{o}C)

(Key: q_e is the Metal Uptake in mg/g, i. e. the amount of metal ion adsorbed in milligram per gram of the algal biomass while %Rem is the Removal Efficiency. i. e percentage metal ion removed)

Appendix 9: Experimental Data Showing Effect of Biosorbent Dose

Time (min)	Cadmium		Chromium		Cobalt		Lead		Nickel	
	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem
30	5.49	54.9	9.61	96.1	5.84	58.4	8.66	86.6	3.97	39.7
60	5.62	56.2	9.69	96.9	6.25	62.5	8.85	88.5	4.83	48.3
90	5.68	56.8	9.84	98.4	6.25	62.5	9.23	92.3	5.69	56.9
120	5.87	58.7	9.84	98.4	6.67	66.7	9.43	94.3	5.69	56.9
150	5.87	58.7	9.84	98.4	6.67	66.7	9.43	94.3	5.69	56.9

(Biomass.– 0.1g,Initial Conc. – 20ppm, pH = Cd(II)– 5.05, Cr(VI) – 2.05, Co(II) – 4.05, Pb(II) – 4.02, Ni(II) - 5.02, Temp. – 27^{o}C)
(Key: q_e is the Metal Uptake in mg/g, i. e. the amount of metal ion adsorbed in milligram per gram of the algal biomass while %Rem is the Removal Efficiency. i. e percentage metal ion removed)

Appendix 10: Experimental Data Showing Effect of Contact Time

t(mins)	q_t(mg/g)	$q_e - q_t$(mg/g)	log $(q_e - q_t)$ (mg/g)
Cadmium			
30.00	5.49	0.38	-0.42
60.00	5.62	0.25	-0.60
90.00	5.68	0.19	-0.72
120.00	5.87	0.00	0.00
150.00	5.87	0.00	0.00
Chromium			
30.00	9.61	0.23	-0.64
60.00	9.69	0.15	-0.82
90.00	9.84	0.00	0.00
120.00	9.84	0.00	0.00
150.00	9.84	0.00	0.00
Cobalt			
30.00	5.84	0.83	-0.08
60.00	6.25	0.42	-0.38
90.00	6.25	0.42	-0.38
120.00	6.67	0.00	0.00
150.00	6.67	0.00	0.00
Lead			
30.00	8.66	0.57	-0.24
60.00	8.85	0.38	-0.42
90.00	9.23	0.00	0.00
120.00	9.23	0.00	0.00
150.00	9.23	0.00	0.00
Nickel			
30.00	3.97	1.72	-0.42
60.00	4.83	0.86	-0.066
90.00	5.69	0.00	0.00
120.00	5.69	0.00	0.00
150.00	5.69	0.00	0.00

Appendix 11: Equilibrium Data for Plotting Pseudo First – Order Model of all the Selected Heavy Metals.

	Cadmium		Chromium		Cobalt		Lead		Nickel	
T (mins)	q_t (mg/g)	$\frac{t}{q_t}$ (mins. $mg^{-1}g$)	q_t (mg/g)	$\frac{t}{q_t}$ (mins. $mg^{-1}g$)	q_t (mg/g)	$\frac{t}{q_t}$ (mins. $mg^{-1}g$)	q_t (mg/g)	$\frac{t}{q_t}$ (mins. $mg^{-1}g$)	q_t (mg/g)	$\frac{t}{q_t}$ (mins. $mg^{-1}g$)
30	5.49	5.46	9.61	3.12	5.84	5.14	8.66	3.46	3.97	7.56
60	5.62	10.68	9.69	6.19	6.25	9.60	8.85	6.78	4.83	12.42
90	5.68	15.85	9.84	9.15	6.25	14.40	9.23	9.75	5.69	15.82
120	5.87	20.44	9.84	12.20	6.67	17.99	9.23	13.00	5.69	21.09
150	5.87	25.55	9.84	15.24	6.67	22.49	9.23	16.25	5.69	26.36

Appendix 12: Equilibrium Data for Plotting Pseudo Second – Order Model for all the Metals Studied

pH	Cadmium		Chromium		Cobalt		Lead		Nickel	
	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem
2	4.64	46.4	9.84	98.4	3.47	34.7	8.66	86.6	4.83	48.3
4	5.23	52.3	9.61	96.1	5.84	58.4	9.04	90.4	5.69	56.9
6	5.97	59.7	9.37	93.7	6.67	66.7	9.23	92.3	7.42	74.2
8	5.49	54.9	9.29	92.9	6.25	62.5	8.85	88.5	6.55	65.5

(Biomass Conc.– 0.1g,Initial Conc. – 20ppm, Temp. – 27^{o}C)
(Key: q_e is the Metal Uptake in mg/g, i. e. the amount of metal ion adsorbed in milligram per gram of the algal biomass while %Rem is the Removal Efficiency. i. e percentage metal ion removed)

Appendix 13: Experimental Data Showing Effect of Initial Solution pH

Temp. (K)	Cadmium		Chromium		Cobalt		Lead		Nickel	
	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem	q_e (mg/g)	%Rem
300	5.62	56.2	9.84	98.4	6.67	66.7	8.85	88.5	5.69	56.9
313	6.82	68.2	9.92	99.2	6.25	62.5	82.7	82.7	5.69	56.9
323	7.19	71.9	9.92	99.2	6.67	66.7	8.46	84.6	5.69	56.9
333	7.37	73.7	9.92	99.2	6.67	66.7	8.66	86.6	6.55	65.5

(Biomass Conc.– 0.1g,Initial Conc –20ppm, pH=Cd(II) -5.05,Cr(VI) –2.05,Co(II) – 4.05, Pb(II) – 4.02,Ni(II)– 5.05)

(Key: q_e is the Metal Uptake in mg/g, i. e. the amount of metal ion adsorbed in milligram per gram of the algal biomass while %Rem is the Removal Efficiency. i. e percentage metal ion removed)

Appendix 14 : Experimental Data Showing Effect of Temperature.