Biosorption of selected heavy metals by green algae, *spirogyra species* and its potential as a pollution biomonitor

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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Thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

This work is dedicated to my family. To my excellent wife Christine, my son Jim and my small daughters Abi and Vicky. Special dedications also go to my dad and role model, Matei Snr and my mum, Naomi for their moral support. These people made this work worth doing. God be with them always.

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FAAS	Flame Atomic Absorption Spectrometry
FTIR	Fourier Transform Infrared Spectroscopy
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectrometry
ppm	parts per million
ppb	parts per billion
USEPA	United States Environmental Protection Agency
USPHS	United States Public Health Service
rpm	revolutions per minute

ABSTRACT

Control of heavy metal pollution is becoming increasingly important as industrialization becomes the main economic activity of many nations. A number of strategies have been employed to control environmental pollution. The aim of this work was to study the biosorption parameters of green algae for cadmium, chromium, copper and lead and the possibility of using the algae as a biomonitor of environmental pollution by the selected metals. Biosorption studies were conducted to determine the adsorption parameters (pH, contact time and adsorption capacity) of the selected metals in model aqueous solutions using green algae, *spirogyra* species. The optimum pH values were found to be 5.0, 5.5, 5.8, and 5.9 for lead, cadmium, chromium and copper, respectively. The adsorption process was second order and fitted the Langmuir isotherm better than the Freundlich isotherm. Adsorption capacities on green algae were found to be 22.52, 35.59, 38.19and 94.34 mg/g for cadmium, copper, chromium and lead, respectively. The time required for quantitative uptake of each metal from model solution was investigated over a period of 140 minutes for all metals and found to be 15 minutes for cadmium, 40 minutes for chromium and copper and 50 minutes for lead. The optimum initial concentrations for metal adsorption ranged from 500 – 700 mg/L. Adsorption kinetics of the metals on green algae were also investigated. The experimental data was tested using first and second order kinetic models and was found to follow second order kinetics. The algae was also used as biomonitor of water pollution by the selected heavy metals. Concentration of the selected metals was determined in algae and in the parent water. The results in both samples by ICP – OES were 1.81 ± 0.11, 12.08 ± 1.80, 17.14 ± 0.155 and 64.33 ± 0.35 µg/L in water and 2.30 ± 0.09,

12.17 ± 0.20, 25.61 ± 0.74 and 60.50 ± 1.57 µg/g for cadmium, lead, copper and chromium in algae, respectively. The average concentration factors were Cd (2547.01), Cr (367.02), Cu (1843.59) and Pb (7154.95). These results point to the dominance of sorption process vis-à-vis diffusion in metal uptake. This is seen in the fact the algae can maintain a high internal concentration of metal against the large concentration gradient between it and the water. This demonstrates the potential of green algae as both a biosorbent and biomonitor of water pollution by the selected heavy metals.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

A heavy metal is a collective term for metals of high atomic mass, particularly those that are toxic and cannot be processed by living organisms. These include lead, mercury and cadmium among others. Many other definitions of heavy metals have been proposed based on density, atomic number and atomic weight. Depending on the context, the term can include elements lighter than carbon and can exclude some of the heaviest metals (Duffus, 2002). Any element that exhibits metallic properties, and belongs to the transition metals, metalloids or lanthanides and actinides can pass as a heavy metal. At one time, an IUPAC technical report described the term *heavy metal* as a "meaningless and misleading term" due to its contradictory definitions, lack of a "coherent scientific basis" and unclear boundaries (Duffus, 2002). Recently, the definition has been based on chemical properties particularly toxicity. Heavy metals have thus been defined collectively as metals of high atomic mass, particularly those transition metals that are toxic and cannot be processed by living organisms (Harrison and Waites, 1998).

Heavy metals can be broadly classified into three groups; those that are essential for certain biochemical processes, but are toxic when their concentration exceeds certain thresholds. These include copper, zinc, cobalt, selenium and iron. The second group consists of metals with no known biological function and toxic if present in concentrations above trace amounts. These include arsenic, bismuth, indium, antimony and thallium. The last and evidently the most dangerous group includes lead, cadmium and mercury which serve no known biological function and

are toxic at all concentrations (Fernandez *et al.,* 1992). Within the European community the 11 elements of highest concern are As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Tl. Some of these elements are actually necessary for human health in trace amounts (Co, Cu, Cr, Ni) while others are carcinogenic or toxic, mainly affecting the central nervous system (Hg, Pb, As), the kidneys or liver (Hg, Pb, Cd, Cu), skin, bones or teeth (Ni, Cd, Cu, Cr), (Zevenhoven and Kilpinen, 2001).

The toxicity of trace metals arises from their interference with an organisms' uptake of essential metal ions such as sodium and calcium. For instance, cadmium and zinc block the uptake of calcium ions which is essential for bone and teeth development. The interaction of some heavy metals with enzymes and their tendency to bind to protein and other biological tissues also cause trace metal poisoning in organisms (Campbell, 1995). The common results of trace metal toxicity to living organisms include brain disorder, gross deformities in development, carcinogenic effects and generally, disruption of biological processes.

In most cases these elements find their way into the environment through human industrial processes such as mining, electroplating, battery manufacture, leather tanning, and manufacture of printing pigments and paints, among others. A high concentration of heavy metals in the environment is of great health concern because they are non-biodegradable and end up accumulating in food chains in various forms such as organic, inorganic or organometallic species (Cordero *et al.*, 2004) with disastrous consequences. Due to the toxicity of trace metals, it is important to remove them from water in particular and the environment in general.

1.1 Removal of Heavy Metals from Wastewater

To contain the proliferation of heavy metals into the environment, governments and local authorities have passed legislations to control the levels of trace elements in industrial, domestic and agricultural effluent. This has led to the development of various technologies for removing heavy metals from industrial and agricultural wastewater before it is discharged to the environment. The common technologies in use include precipitation, ion exchange and reverse osmosis (Deng *et al.*, 2006).

1.1.1 Precipitation

Metal precipitation from contaminated water involves the conversion of soluble heavy metal salts to insoluble salts that will precipitate. The precipitate can then be removed from the treated water by physical methods such as filtration or sedimentation. The process usually involves pH adjustment, addition of a chemical precipitant, and flocculation. Typically, metals precipitate from the solution as hydroxides, sulfides, or carbonates (Kuyucak *et al.*, 1998). However, the precipitation processes is non-specific to metal ions, inefficient at low ion concentrations and presents the problem of disposal of the toxic metal sludge formed in the process. Recovery of metals from the sludge is also not easy (Kuyucak *et al.*, 1998).

1.1.2 Ion Exchange

This method involves passing the wastewater through an ion-exchange column in the presence of an oxidizing agent. On contact, the resin exchanges selected metal ions in the solution for preferred ions in the resin thus reducing the concentration in wastewater of the target metal ions. Ion exchange uses mainly

hydrocarbon-derived polymeric resins (Khopkar, 1988). The hydrocarbon basis of ion exchangers makes them dependent on the price of crude oil, hence generally expensive. Ion exchangers are not very specific and are generally ineffective at low metal ion concentration.

1.1.3 Reverse Osmosis

Osmosis is the movement of solvent molecules from the pure solvent to a solution of the same solvent through a semi-permeable membrane. In reverse osmosis, the wastewater is put on one side of the membrane and pure water on the other side and pressure is applied on the solution to stop, and then reverse the osmotic process so that the solvent passes from the solution through the membrane into the pure solvent leaving behind the polluting solutes, hence the term reverse osmosis. The contaminant becomes more concentrated in the wastewater. It generally takes a lot of pressure and is fairly slow. Though this method is effective in removing heavy metal contaminants even at very low concentrations it is quite expensive because the semi – permeable membranes are costly to procure and the high pressure process is expensive to maintain. The resulting concentrated by-product solutions make eventual recovery of metals feasible (http://www.ag.ndsu.edu, 07/08/2007).

1.1.4 Biosorption

Biosorption is a term that describes the removal of heavy metals from an aqueous solution by passive binding to non-living biomass. This implies that the removal mechanism is not metabolically mediated. Bioaccumulation on the other hand, describes an active process where removal of metals requires the

metabolic activity of a living organism that is active transport (Davies *et al.*, 2003).

In recent years research on biosorption has intensified with a view to using biomass to remove heavy metals from industrial effluents or to recover precious metals from processing wastewater. Of the many types of biosorbents recently investigated, algal biomass has proven to be highly effective, reliable and predictable in the removal of heavy metal ions from aqueous solutions (Davies *et al.*, 2003). It is particularly the cell wall structure of certain algae which is responsible for this phenomenon (http://www.biosorption.mcgill.ca, 15/08/1998). While having a performance similar to ion exchange, biosorption is more advantageous because biosorbents are quite abundant, less costly and easily biodegradable. Additional cost reduction results from the possible recovery of heavy metals which have a high market value. The metal recovery process by algae is even economically viable as a metal recovery industry by itself for the more precious metals. Besides the low cost and abundance, biosorbents can be recycled. This makes them even more attractive (Volesky, 2003). Besides algae, other biosorbents have been successfully used. Table 1 gives some examples.

Table 1. Some biosorbents in literature

Biosorbent	Pollutant	Uptake capacity, mg/g	pH	Reference
Pinus sylvesteris (red pine)	Cd(II)	19.10	6.0	Rakhshaee *et al.*, 2006
Mucor rouxii (fungi)	Cd(II)	8.50	5.0	Yan *et al.*, 2003
Cystine-modified biomass	Cd(II)	11.60	5.2	Yu *et al.*, 2007
Chlorella minutissima (green algae)	Cd(II)	11.10	5.5	Roy *et al.*, 1993
Sargassum glaucescens (brown algae)	Cr(III)	6.08	6.0	Yang and Chen, 2008
P. palmate (red algae)	Cu(II)	6.65	5.0	Holan *et a1.*, 1993
O. angustissima (green algae)	Cu(II)	7.62	5.4	Fraile *et al.*, 2005
P. aerogenosa (bacteria)	Cu(II)	23.00	5.0	Chang *et al.*, 1997
Crab shell	Pb(II)	19.80	5.5	Dahiya *et al.*, 2006
Chaff	Pb(II)	12.40	5.5	Han *et al.*, 2005
Gelidium algae (red algae)	Pb(II)	64.0	5.0	Vilar *et al.*, 2005
Rhizopus Arrhizus (fungi)	Pb(II)	26.43	4.5	Bahadir *et al.*, 2007
Spirogyra sp. (green algae)	Cd(II)	22.52	5.5	This work
Spirogyra sp. (green algae)	Cr(III)	38.19	5.8	This work
Spirogyra sp. (green algae)	Cu(II)	35.59	5.9	This work
Spirogyra sp. (green algae)	Pb(II)	94.34	5.0	This work

1.2 Biosorption by green Algae

Green algae are the most diverse group of algae, with more than 7000 species growing in a variety of habitats. *Spirogyra* species are unbranched filamentous freshwater green algae. Their cell wall is characteristically straight and parallel-sided. Green algae are commonly found in clean water and produce food through the process of photosynthesis. They can be easily identified from their green filamentous structure and fresh water habitat. The most striking characteristic of this genus is a single chloroplast in the form of a spiral ribbon which usually fills almost the entire length of the cell, (Figure 1).

Figure 1: Green algae, *spirogyra* species

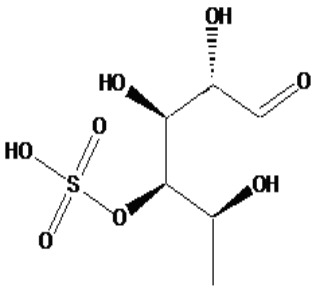
The uptake of trace metals by green algae occurs through biosorption and bioaccumulation processes (Davis *et al.,* 2003). Bioaccumulation is the most important process, *in vivo*. In streams and ponds metal uptake is mainly by active transport by the viable weeds. Carboxylate groups are generally the most abundant acidic functional groups in the algae. They constitute the highest

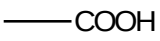
percentage of titrable sites (typically greater than 70%) in dried algal biomass. The adsorption capacity of dried (non-viable) algae is directly related to the presence of these sites on the algal cells (algin molecule), which itself comprises up to 40% of the dry weight of seaweed biomass (Percival and McDowell, 1967). The second most abundant acidic functional group in algae is the sulfonic acid of fucoidan. Fucoidan is a branched polysaccharide sulfate ester with L-fucose 4-sulfate building blocks (Figure 2) as the major component. Sulfonic acid groups typically play a secondary role, except when metal binding takes place at low pH (Davis *et al.,* 2003). Hydroxyl groups are also present in all polysaccharides but they are less abundant and only become negatively charged at pH >10, thereby also playing a secondary role in metal binding at high pH (Davis *et al.*, 2003). Green algae has been reported to have high metal binding capacity due to the presence of polysaccharides, proteins or lipid on the cell wall surface containing functional groups such as amino, hydroxyl, carboxyl and sulphate groups, which can act as binding sites for metals (Ramelow *et al.,*1992). The binding sites always contain a lone pair of electrons or anions which can bind a metal ion via dative or ionic bond. Replaceable hydrogen ions are also important binding sites.

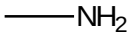
1.2.1 Mechanisms of metal adsorption on green algae

Ion-exchange is an important adsorption mode in biosorption because it explains many of the observations made during heavy metal uptake experiments. It has been shown that ion-exchange takes place between metals when binding to alginate **(**Myklestad, 1968). Kuyucak and Volesky reported an enhanced release of ions (Ca^{2+}, K^{+}, Mg^{2+}, and Na^{+}) from *Ascophyllum nodosum* (a type of algae) when reacted with cobalt bearing aqueous solution rather than observed with

cobalt-free solution (Kuyucak and Volesky, 1995). Untreated biomass generally contains light metal ions such as K^+, Na^+, Ca^{2+} and Mg^{2+}. These are originally bound to the acidic functional groups of the algae and are acquired from saline water.







(a) (b) (c) (d)

Figure 2: Structures of some of the functional groups found in algae (a) L – fucose 4 – sulfate (b) Carboxylate ion, (c) amine group and (d) hydroxyl ion.

Treatment of biomass generally implies protonation of the biomass with a strong acid such as hydrochloric acid whereby the proton displaces the light metal ions from the binding sites like carboxylic and sulfonic sites. Alternatively, the biomass may be reacted with an aqueous solution of a given ion at high concentration so that the majority of sites are occupied by the ion. The term ion-exchange does not explicitly identify the binding mechanism, rather it is used here as an umbrella term to describe the experimental observations. The precise binding mechanism(s) may range from physical (electrostatic/ionic) to chemical binding (covalent). Furthermore, the term sorption refers to binding of a metal cation to a free site as opposed to one that was previously occupied by another cation. It is distinct from adsorption which defines binding in terms of physical or

chemical sorption. In the case of biosorption of heavy metals by algal biomass, the mechanisms can be viewed, in principle, as being extra cellular, or occurring discretely at the cell wall. Intracellular sorption would normally imply bioaccumulation by a viable organism, such as the case of metal uptake in streams, ponds and other environmental sources (Davis *et al.*, 2003).

1.2.2 Adsorption Isotherms

An adsorption isotherm is an equation that describes how the amount of a substance adsorbed onto a surface depends on its concentration (if in a solution), or its pressure (if a gas) at a constant temperature. Adsorption isotherms focus mainly on systems where the adsorbate particles are mostly concentrated on the surface of an adsorbent. The Langmuir isotherm describes the dependence of the surface coverage of an adsorbed species on the pressure/concentration of the species at a fixed temperature. The Freundlich describes physical adsorption in solution while the BET isotherm applies to multi-layer adsorption.

1.2.2.1 Langmuir Isotherm

The Langmuir isotherm has been widely used to describe and determine the adsorption capacity q_{max} of metal ions during biosorption processes. The Langmuir adsorption isotherm is useful in quantifying and contrasting the performance of different biosorbents. In its formulation, binding to the surface is primarily by physical forces mainly electrostatic and implicit in its derivation is the assumption that all sites possess equal affinity for the adsorbate. It has been used to empirically describe equilibrium relationships between a bulk liquid phase and a solid phase. One of the simplest representations of the adsorption

phenomenon calls for the migration to and the occupation of a surface site, S, on the adsorbent by an adsorbate, A.

This can be represented by an equilibrium reaction as follows:

$$S + A \rightleftharpoons SA \quad \text{..... (1)}$$

where SA is the adsorbed complex. Surface species concentration may be expressed in molarity of solution, per gram of solid, per unit area of solid surface or per mole of solid. Assuming that all surface sites have the same affinity for the solute A, a mass action law can be written as;

$$K_{ads} = \frac{[SA]}{[S][A]} \quad \text{..... (2)}$$

where K_{ads} is the equilibrium constant for the adsorption process, [S], [SA] and [A] refer to molar concentrations. The total concentration of surface sites S_T, is given by;

$$[S_T] = [S] + [SA] \quad \text{..... (3)}$$

Combining equations 2 and 3 gives

$$[SA] = [S_T]\left(\frac{K_{ads}[A]}{1 + K_{ads}[A]}\right) \quad \text{..... (4)}$$

Defining the surface concentration as Γ, we have

$$\Gamma = \frac{[SA]}{mass\ of\ adsorbent} \quad \text{..... (5)}$$

where Γ is the surface concentration of the adsorbate whose limiting value Γ_{max}, is given by $\Gamma_{\max} = \dfrac{[S_T]}{mass\ of\ adsorbent}$ ..... (6)

The surface concentration of an adsorbate can be expressed as,

$$\Gamma = \Gamma_{\max}\left(\frac{K_{ads}[A]}{1+K_{ads}[A]}\right) \quad \text{..... (7)}$$

Equation (7) is the general form of the Langmuir equation. Compliance to the Langmuir isotherm theory requires that (1) adsorption is limited to the formation of a monolayer, or the number of adsorbed species, [SA], does not exceed the total surface sites [ST]; and (2) all surface sites have equal affinity for the adsorbate. This means that the [SA]: [ST] ratio does not affect the energy of adsorption (Davis *et al.*, 2003). At least one of these conditions is implicitly not met in the case of biosorption (Stumm and Morgan, 1996). We have previously seen that there is more than one type of functional group contributing to the biosorption process, each of which has a different affinity for a sorbing heavy metal. Furthermore, the one-to-one stoichiometry is also not complied with, since ion-exchange has been shown to be a dominant mechanism, and typically approximately two protons are released upon the binding of one divalent heavy metal ion. Despite this fact, the Langmuir equation is frequently used to fit experimental data. In this case, the following form of the Langmuir equation (7) above is traditionally applied:

$$q = q_{\max}\left(\frac{bC_e}{1+bC_e}\right), \quad \text{..... (8)}$$

where q, q_{max}, C_e and b is the metal uptake at any time (in milligrams of heavy metal per gram of biosorbent), the maximum metal uptake, the final equilibrium concentration of the heavy metal in solution and the Langmuir empirical constant, respectively.

Experimental results can most easily be compared with the Langmuir theory if equation (8) above is expressed in its linear form as follows;

$$\frac{C_e}{q} = \frac{1}{q_{\max} b} + \frac{C_e}{q_{\max}} \quad \text{.....} \quad (9)$$

If the experimental data agrees with the theory, a plot of C_e/q versus C_e yields a straight line. From this curve the adsorption capacity q_{max} and the Langmuir constant b can be obtained.

1.2.2.2 Freundlich Isotherm

The Freundlich isotherm (Freundlich, 1907) was originally of an empirical nature, but was later interpreted as sorption to heterogeneous surfaces or surfaces supporting sites of varied affinities. It is assumed that the stronger binding sites are occupied first and that the binding strength decreases with increasing degree of site occupation. In this model, the energy of a metal ion binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. The Freundlich equation takes the form:

$$q_e = K_F \left(C_e\right)^{1/n} \quad \text{.....} \quad (10)$$

where q_e and C_e is the mass of solute adsorbed per gram of the adsorbent and the solute concentration at equilibrium respectively. K_F and n are empirical constants characteristic of the system and are indicators of the adsorption capacity and intensity, respectively. Large values of K_F and n indicate high adsorption capacity

and intensity, respectively. This equation is most conveniently used in its linearized form namely;

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e \quad \text{..... (11)}$$

Experimental results agree with the Freundlich isotherm if a plot of lnq_e versus lnC_e yields a straight line. The Freundlich constants K_F and n can be calculated from the linear plot.

1.2.2.3 Brunaur, Emmet and Teller, (BET) Isotherm

Other adsorption isotherms have been developed to explain the more complete adsorption that leads to multilayer formation. The BET isotherm accounts for multi-layer adsorption by assuming that the area of the first layer of adsorbate is also available for the second layer adsorption and the second layer area is available for third layer adsorption and so on. The concept of the theory is an extension of the Langmuir theory to multilayer adsorption with the following hypotheses: (a) gas molecules physically adsorb on a solid in layers infinitely; (b) there is no interaction between each adsorption layer; and (c) the Langmuir theory can be applied to each layer. The formulation of the BET is as follows;

$$\frac{1}{v\left[\left(P_O/P\right)-1\right]} = \frac{c-1}{v_m c}\left(P/P_O\right) + \frac{1}{v_m c} \quad \text{..... (12)}$$

where P and P_0 are the equilibrium and the saturation pressure (or concentration) of adsorbates at the temperature of adsorption, v is quantity of the adsorbed species (for example, in volume or mass units), and v_m is the monolayer adsorbed gas quantity. c is the BET constant. The bet isotherm deals with both physical and chemical sorption and assumes that adsorptions occur only on well-defined sites of the sample

surface (one per molecule), and the only considered molecular interaction is that a molecule can act as a single adsorption site for a molecule of the upper layer. The uppermost molecule layer is deemed to be in dynamic equilibrium with the gas phase that is, its adsorption and desorption rates are similar. The desorption is a kinetically-limited process for which a heat of adsorption must be provided. The heat of adsorption for this phenomenon is homogeneous for a given molecule layer. At the saturation pressure, the molecule layer number tends to infinity (equivalent to the sample being surrounded by a liquid phase)

1.3 Kinetics of metal adsorption

The dependence of a chemical reaction on initial reactant concentration can be shown by a rate equation. The rate equation once integrated gives expressions for the variation of concentration of a reactant with time. The order of reaction is deduced from the integrated rate equation. For instance, the first order rate equation for metal adsorption on algae is;

$$\frac{\partial q_t}{\partial t} = k_1(q_e - q_t) \quad \text{.....} \quad (1)$$

where q_t and q_e are the masses of metal adsorbed by the green algae in (mg/g) at any time t and at equilibrium, respectively k_1 is the rate constant for the adsorption.

On integration, equation 1 gives the solution;

$$k_1 t = \ln q_e - \ln(q_e - q_t) \quad \text{.....} \quad (2)$$

indicating that a plot of time t against $ln(q_e - q_t)$ should give a straight line.

If plotting t against $ln(q_e - q_t)$ gives a straight line with a correlation coefficient (R^2 value) tending to unity then the experimental data agrees with first order kinetics and the reaction is first order.

The second order rate equation can be represented as;

$$\frac{\partial q_t}{\partial t} = k_2(q_e - q_t)^2 \quad \text{.....} \quad (3)$$

where k_2 is the second order rate constant, q_e and q_t as defined above. Upon integration, equation 3 gives the solution;

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^{\,2}} \quad \text{.....} \quad (4)$$

Equation 4 predicts that for a second order process plotting t/q_t against t should give a straight line from which the rate constant k_2 and the metal uptake at equilibrium q_e, can be calculated.

From the R^2 values for the first and second order plots it is possible to deduce the order of reaction. The curve with the higher value of linear correlation coefficient corresponds to the order of the reaction.

1.4 Biomonitoring

Biomonitoring is the science of inferring the ecological condition of an area by examining the organisms that live there. Although biomonitoring may be applied to any ecosystem, it is most often used to assess water quality in rivers, lakes, streams, and wetlands (Rosenberg and Resh, 1993). It involves the quantitative measurement of an organism's exposure to toxic substances in the environment by determining the substances or their metabolites in specified parts

of the organism. Biomonitoring measurements are the most health-relevant assessments of exposure because they indicate the amount of the chemical that actually gets into the organism from all environmental sources such as air, soil, water, dust and food. Biological monitoring integrates all the physico-chemical processes that affect the transport and bioavailability of a pollutant. Traditional heavy metal monitoring practice, which merely involves the measurement of heavy metal levels in water and sediments, does not take these dynamics into consideration. In recent years a great deal has been done regarding the use of organisms as pollution bioindicators because they have the ability to concentrate the metals (sometimes more than a thousand-fold), besides integrating pollution over time thus making detection easier and cost effective (Moller *et al.*,1993, Rosenberg and Resh, 1993, Conti and Cecchetti, 2002 and Ramadan, 2003).

1.4.1 Bioaccumulation

Bioaccumulation is the active, gradual build up of a chemical in a living organism over time. This occurs when either the chemical is taken up faster than it can be used, or because the chemical is non-biodegradable. Once a toxic pollutant is in the water or soil, it can easily enter the food chain. For example, in the water, a pollutant may adsorb to a small particle such as a tiny living organism like phytoplankton. Because there is so little pollutant adsorbed on each phytoplankton, it does not cause significant damage at this level of the food chain. However several of the phytoplankton may be consumed by a small animal such as a zooplankton. One zooplankton that has eaten ten phytoplankton would have ten times the pollutant level as the phytoplankton. As the zooplankton may be slow to metabolize or excrete the pollutant, the pollutant may build up or

bioaccumulate within the organism. A small fish might then eat ten zooplanktons. The fish would have 100 times the level of toxic pollutant as the phytoplankton. This multiplication would continue throughout the food chain until high levels of contaminants have accumulated in the top predator, usually man. While the amount of pollutant might have been small enough not to cause any damage in the lowest levels of the food chain, the accumulated amount can cause serious damage to the top predators. This phenomenon is known as biomagnification and is a consequence of bioaccumulation. Bioaccumulation is indeed an important analytical property in bioindicators used for biomonitoring since it pre-concentrates an analyte making it easy to detect.

1.4.2 Bioindicator

A bioindicator is an organism that reveals the presence of a substance in its surroundings with observable and measurable changes, such as accumulation of pollutants (Smodis, 2008). The basic requirements of bioindicators are that they should be sedentary, of suitable dimensions, easy to identify and collect, widely distributed, and be able to accumulate the pollutant to a satisfactory degree (Conti *et al.,* 2002). Species that accumulate pollutants in their tissues from the surrounding environment or from food are important biomonitoring devices (Phillips and Rainbow, 1993). The use of marine organisms as bioindicators for trace metal pollution is on the increase. Macro invertebrates, especially fish and mollusks have been used frequently (Rosenberg and Resh, 1993). The use of plants to monitor metal content in the environment is currently on the increase. The plant species *Blepharis diversipinia* and *Helichrysum condelleanum* have been successfully used

in the bio-prospecting of nickel and copper in the soil (Nkoane and Sawula, 2003). Copper and nickel concentration in the plants corresponded well with their concentration in the soil where the plants grow. Mosses and lichens which lack root systems depend on surface adsorption of nutrients and hence reflect pollutants adsorbed from the atmosphere rather than from soil (Conti and Cecchetti, 2001).

Table 2. Some of the bioindicators of heavy metal pollution

Bioindicator	Pollutant	Reference
Raphanus sativa (Garden radish)	Pb	Ramadan, 2003
Ulva lactuca (green algae)	Cd, Cr, Cu, Pb	Conti and Checchetti, 2003
Cultured algae	Cd, Pb, Ni, Zn	Moller *et al.*,1993
Wolffia globosa (Asian watermeal)	Cd	Chandra and Sinha, 2000
Enteromorpha intestinalis (green macroalga)	Cd, Pb, Ni, Zn and Cu	Moller *et al., 1993*
Padina pavonica (brown algae)	Cd, Cr, Cu, Pb and Zn	Conti *et al.*, 2007
Monodonta turbinate (gastropod molluscs)	Cd, Cr, Cu, Pb and Zn	Conti *et al.*, 2007
Atriplex portulacoides (sea purslane)	Zn, Pb, Cd and Hg	Ramadan, 2003
Cyperus laevigatus (Smooth flatsedge)	Zn, Pb, Cd and Hg	Ramadan, 2003
Trifolium alexandrinum (Egyptian clover)	Zn, Pb, Cd and Hg	Ramadan, 2003
Typha domingensis (southern cattail)	Zn, Pb, Cd and Hg	Ramadan, 2003
Juncus rigidus (sea rush)	Zn, Pb, Cd and Hg	Ramadan, 2003
Spirogyra sp. (green algae)	Cd, Cr, Cu and Pb	This work

Marine weeds are by far the most commonly used plants in heavy metal biomonitoring. Wild plants such as *Typha domingensis, Juncus rigidus* and others

have been used in lake Manzala, Egypt (Ramadan, 2003). Algae and mollusks have also been used in heavy metal biomonitoring in the Tyrrhenian coastal area, Italy and found to have concentration factors in excess of 10,000 with respect to the concentration (soluble fraction) in the marine water (Conti and Cecchetti, 2003). Tolerance or accumulation in some plants apparently involves binding of potentially toxic metals at cell walls of roots and leaves away from sensitive sites within cells. Binding is probably to cell wall proteins.

1.4.2.1 Algae as a bioindicator of heavy metal pollution

Algae satisfy all the basic requirements for a bioindicator of heavy metal pollution because they are sedentary, easy to identify and collect, their dimensions are suitable, and they are widely distributed and accumulate metals to a satisfactory degree (Conti *et al.,* 2002). They can be found in macroscopic forms that are easily visible to the naked eye, as well as in microscopic forms that live freely-floating in the water column and on rocks, wood, sand, and aquatic plants. They form an important component of the ecosystem in rivers, streams, and wetlands, making them a valuable indicator of water quality. Since algae have rapid growth rates and respond quickly to changes in their habitat, they often provide an early warning of changing environmental conditions which may not be detected by other methods. They can also provide information about the historical condition of water bodies (http://www.state.me.us/dep/blwg, 09/08/2007). Algae are at the bottom of the food chain hence heavy metal concentration in them is a fair estimate of the heavy metal levels in the environment without the effects of

biomagnification. Algae have been successfully used as heavy metal pollution monitors in the Mediterranean Sea (Conti and Cecchetti, 2002).

1.5 Justification of the study

The presence of heavy metals in water systems is a threat to biota as they accumulate in various organisms often reaching lethal concentrations. Thus there is need to control pollution not only in water but also in sediment, suspended solids and organisms most of which are important in food chains with man at the top of the food chain.

The use of algae for the removal of heavy metals from polluted water is a healthy and cost-effective venture due to the biodegradability and lower cost of the algae. Biosorbents cost about US$ 4-7/kg while ion exchange resins cost about ten times more (US$ 30-50/kg). Reverse osmosis is even more costly. Precipitation is inefficient at low ion concentrations and generates toxic sludge which is difficult to dispose off. In addition, metal recovery from the sludge is difficult. Algae are abundant in water systems, can be regenerated for subsequent use and are biodegradable. Hence algae are safer and more cost effective than their competitors. The adsorption capacity for algae for all the selected metal ions is generally high and takes place at pH values which are near that of pure water hence easy to achieve. The biosorption process is fairly rapid, with contact times below one hour for most biosorbents. Biosorption is therefore a potential solution to heavy metal pollution.

Transport and bioavailability of heavy metals in water is strongly influenced by the prevailing physico-chemical conditions. Direct determination of heavy metal from water samples or sediment measures the total metal in the water or sediment without regard to bioavailability. It has been shown that the bioavailability of heavy

metals can not be calculated from the heavy metal concentration in the water (Moller *et al.*, 1993, Conti and Cecchetti, 2003). Biological indicators take up only the bioavailable metal fraction and hence provide a direct measurement of the actual health risk to the environment. In addition, they integrate the bioavailable metal fraction over time thus pre-concentrating it. This makes the determination of the metal possible using less costly techniques like flame atomic absorption spectrometry. A third advantage of algal biomass as a bioindicator is that they are at the base of the food chain and so the heavy metal content is from environmental pollution and not biomagnified along a food chain. Algae are known to accumulate heavy metals both intra- and extra-cellularly (Conti and Cecchetti, 2002). For this reason, biomonitoring of heavy metal pollution using algae is important. In this work the filamentous fresh water green algae (*spirogyra)* has been used as a bioindicator in water systems in Juja and Thika.

1.6 Statement of the problem

Heavy metals pose a threat to the environment due to their toxicity and non-biodegradable nature. They easily accumulate along food chains. The common effects of trace metal toxicity to living organisms include liver and kidney damage, brain disorder, carcinogenic effects and generally a disruption of biological processes leading to deformities in plant and animal development. Mercury, lead and arsenic are carcinogenic and affect the central nervous system while lead, copper and cadmium affect the liver and kidneys (Zevenhoven and Kilpinen, 2001). The existing technologies such as precipitation and the membrane technologies (ion exchange and reverse osmosis) for heavy metal removal and recovery from

wastewater are either costly or inefficient especially at trace metal concentration. For precipitation, the sludge produced is particularly difficult to dispose of.

Secondly, the use of water samples to determine heavy metals in water is not complete. It gives the total metal in the water system at a particular time and does not say how much of the metal is available to plants and animals living in the water. The heavy metal available to biota depends not on the total concentration but on the physico-chemical processes that affect the transport and availability of the heavy metal pollutants to organisms living in the water. Furthermore, only the bioavailable fraction of the heavy metal is a threat to the ecosystem, the rest being safely bound within the biota in various forms. Thirdly, in water the concentration of heavy metals is lower during the rains due to dilution by storm water and higher in the drier months due to evaporation.

Biosorption is a new technology that provides an effective, low cost and environmentally friendly means of removing heavy metal pollutants from water. Green algae is a promising biosorbent for this purpose. Bioindicators have been used to estimate the pollution level in an environment because they integrate the contaminant over the entire lifespan of the organism (Ramadan 2003, Conti and Checchetti, 2003). A bioindicator is a better indicator of pollution because it accumulates only the bioavailable metal fraction from the water thus giving an exact measure of the actual environmental risk. It also has the analytical advantage of pre-concentrating the analyte hence making detection easier. In the event of storms bioindicators do not lose any accumulated metal hence no dilution occurs.

1.7 Hypothesis

Green algae is not a bioaccumulator of the selected heavy metals from natural water.

1.8 Objectives

1.8.1 General objective

The general objective of this work was to investigate the metal adsorption parameters of green algae and the possibility of using algae for biomonitoring of heavy metal pollution in Juja and Thika town.

1.8.2 Specific objectives

i) To optimize the sorption parameters (pH, contact time and adsorption capacity) for cadmium, chromium, copper and lead on green algae (*spirogyra sp.*).

ii) To determine the concentration of heavy metals adsorbed on the surface of green algae sampled in Thika and Juja.

iii) To determine the total concentration of heavy metal in green algae sampled in Thika and Juja.

iv) To determine the heavy metal concentration in water collected from ponds and streams in Thika and Juja from which the algae was sampled.

CHAPTER TWO

2.0 METHODOLOGY

2.1.0 Materials

2.1.1 Apparatus and equipments

A Millipore filter funnel equipped with a 0.45 µm cellulose acetate filter membrane and attached to a vacuum pump was used for the filtration processes. The ground algae were sieved through a 0.5 mm sieve. A Flame Atomic Absorption Spectrophotometer (210 VGP, UK) equipped with hollow cathode lamps as the light source and air - acetylene fuel system and a Perkin Elmer Optima 3000X ICP – OES spectrophotometer (USA) operating in axial mode were used for metal determination. pH measurements were done using a digital pH meter fitted with a temperature probe (pH 211, HANNA Instruments, UK). A Fourier Transform IR Spectrophotometer 8400CE (Shimadzu, Japan) fitted with a pellet cell was used for characterization of the green algae.

2.1.2 Reagents

Analytical grade concentrated nitric acid (65%), hydrochloric acid (37%) and perchloric acid (96%) made by Reagent Chemical Services (UK) were used to digest the algae samples. Buffer solutions of pH 2 and 7 (from Sigma-Aldrich, USA) were used to calibrate the pH meter. The acetate buffer was prepared using sodium acetate and acetic acid from Sigma – Aldrich (USA). The FT-IR pellet was made of green algae powder in analytical grade potassium bromide crystals (May and Baker, England). The pH values were adjusted using sodium hydroxide pellets (purity 98%) and nitric acid (65%) both from Reagent Chemical Services (UK).

2.2.3 Sampling

The fresh green algae samples used for the investigation of adsorption parameters were collected from a fresh water pond in Juja, washed with tap water several times and rinsed with distilled water. They were sun-dried in the open for twenty-four hours then oven-dried at 60 ^{0}C for eight hours. Finally the sample was ground, sieved to 0.5 mm particle size and stored in a plastic bottle at room temperature until use.

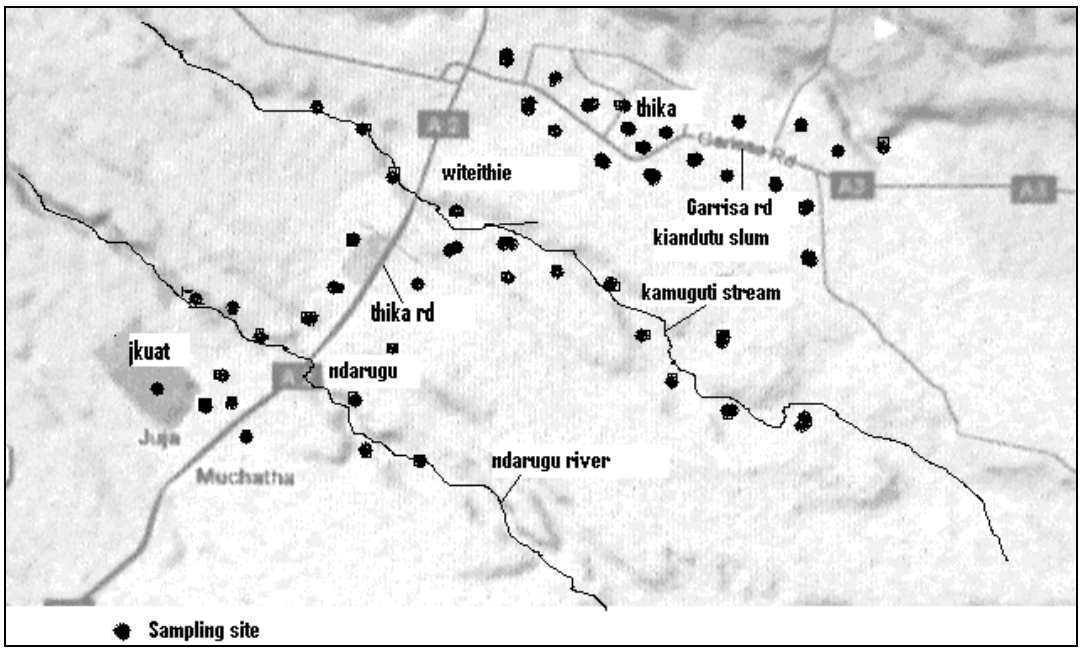


Figure 3: Sampling sites for environmental algae and water samples

A second batch of environmental algae samples was collected *in vivo* from various water ponds and streams along Thika road, Juja and in Thika town. Water samples were also collected from each site. The sampling sites are shown in Figure 3. This was done during the short rains (January and February), when there is a lot of surface water and algae is abundant. The algae were treated as described above,

while the water was acidified to pH 2 by addition of 0.1 mL of concentrated nitric acid. The samples were stored in a refrigerator at 4 ^{0}C in plastic bottles until use.

2.3.0 Experimental

2.3.1 Preparation of stock solutions

All solutions were prepared using distilled water. Stock solutions were prepared by dissolving appropriate amounts (as shown in Table 3) of analytical grade salts in 250 mL distilled water, acidifying with 0.5 mL of concentrated nitric acid (65 %) and making the solution to a litre using distilled water. This gave stock solutions with a concentration of 1000 µg/mL for each selected metal. All the solutions were refrigerated in plastic bottles at 4 ^{0}C.

Table 3. Preparation of 1000 µg/mL stock solutions

Metal salt	Amount weighed (g)
$Pb(NO_3)_2$ (99%, Sigma-Aldrich, USA)	1.5990
$Cd(NO_3)_2$ (98.0% Sigma-Aldrich, USA)	2.1032
$Cr(NO_3)_3.9H_2O$ (98.0%, Sigma-Aldrich, USA)	7.6960
$CuSO_4$ (99% May and Baker, England)	3.7890

2.3.2 Preparation of acetate buffer

0.1 M acetate buffer was prepared by mixing equal volumes of 0.2 M sodium acetate and 0.2 M acetic acid solutions, prepared from fresh hydrated sodium acetate and glacial acetic acid, respectively.

2.3.3 Cleaning of plastic/glass containers

All containers were cleaned well with distilled water then soaked in dilute hydrochloric acid bath before finally rinsing with distilled water. The glassware was dried in the oven at 100 ^{o}C and the plastics dried at room temperature.

2.3.4 Fourier Transform Infra-Red (FTIR) characterization of green algae

A sample of green algae was collected, washed with distilled water and dried first in the sun for two days, then in the oven at 100 ^{0}C for eight hours to remove all traces of water. The sample was divided into two portions. One portion was batch-equilibrated with a solution of selected heavy metal ions for two hours to adsorb metal ions, filtered and dried in the sun for a day and in the oven for eight hours at 100 ^{o}C. Fourier-Transform Infrared spectra of the free algae and the metal-loaded algae were obtained in the range 500–4000 cm^{-1} to identify the active functional groups. The pH values and initial concentrations used for each metal ion are shown in Table 4. The FTIR pellet was made by mixing equal masses of KBr salt and the algal biomass.

Table 4. Parameters used for metal ion loading onto green algae.

Metal ion	pH used	Initial concentration, µg/mL
Cd^{2+}	5.5	500
Cr^{3+}	5.8	600
Cu^{2+}	5.9	600
Pb^{2+}	5.0	500

2.3.5 Biosorption studies

Biosorption studies involved the optimization of pH, contact time, initial metal concentration and determination of adsorption capacity of the selected metals by algae.

2.3.5.1 Optimization of pH

Batch biosorption experiments were conducted on model solutions of cadmium, chromium, copper and lead to determine the optimum pH for metal uptake by algae. For each element, the stock solution was diluted to 200 µg/mL using 0.1 M acetate buffer solution and divided into two 50 mL batches. Both batches were adjusted to pH values of 2.0, 3.0, 4.0, 4.5, 5.0, 5.5, 6.0 and 7 using sodium hydroxide and nitric acid. One batch was equilibrated with 0.20 g of ground algae for two hours. The solution was filtered through a 0.45 µm filter membrane and the metal ion concentration in the filtrate determined by flame atomic absorption spectrometry (FAAS). The second batch (control) was treated like the first one but no algae was added. This was used to determine the amount of metal lost due to precipitation. All experiments were done in triplicate.

2.3.5.2 Optimization of contact time

Stock solutions, (1000 µg/mL) of cadmium, chromium, copper and lead were each diluted with acetate buffer to obtain 500 mL solutions of 100 and 200 µg/mL of cadmium, chromium, copper and lead. The pH of the solutions was adjusted to the optimum values of 5.0, 5.5, 5.8, and 5.9 for lead, cadmium, chromium, and copper, respectively. 2.00 g of dried and ground algae was added to 500 mL of each solution and stirred continuously with a magnetic stirrer at 300 revolutions per minute (rpm). 10 mL portions of this solution were withdrawn at 0, 2, 6, 10, 15, 20, 25, 30, 40, 50,

60, 75, 90, 105, 120 and 140 minutes. Each portion was immediately filtered through a 0.45 µm membrane filter after being withdrawn and the residual metal ion concentration in the filtrate determined by FAAS. All experiments were done in triplicate. A plot of percentage metal ion removal against time was used to determine the contact time for the adsorption process.

2.3.5.3 Initial metal ion concentration and adsorption capacity

The initial concentration which gives rise to the highest metal uptake was investigated. 50 mL of standard metal ion solution at concentrations between 50 – 1000 µg/mL were equilibrated with 0.2 g of dried and ground algae at their respective optimum pH for two hours with stirring at 300 rpm. After equilibration, each solution was filtered through a 0.45 µm membrane filter and the residual metal ion concentration in the filtrate determined by FAAS. The data was fitted to both Freundlich and Langmuir adsorption isotherms and the adsorption capacity calculated from the linearized Langmuir isotherm. A plot of equilibrium metal uptake (mg/g) against initial metal ion concentration was done to determine the optimum initial metal concentration for all metals.

2.8 Biomonitoring studies

The concentration of the selected metals in environmental samples was done. This involved determination of the selected metal concentrations in acid-leached algae, digested algae and in the water samples where the algae was collected from. Leaching was done by shaking 1 g of ground algae sample with 20 mL of 0.1M hydrochloric acid for five minutes followed by filtering through a 0.45 µm membrane and rinsing the residue with enough distilled water to make 50 mL of

filtrate. The dried algal biomass was digested in a 3.1.1 mixture of concentrated perchloric, nitric and hydrochloric acids, respectively. The digested samples were washed with distilled water, filtered through a 0.45 µm membrane filter and the filtrate made to 50 mL before analysis by FAAS alongside the parent water and the acid-leached filtrate. Correlation between metal ion concentrations in digested algae, acid-leached algae and the parent water samples was determined. The total metal concentration in algae and the parent water was also determined by ICP – OES and the results compared with those obtained by FAAS.

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 Fourier Transform Infra-Red (FTIR) characterization of algae

The functional groups responsible for heavy metal biosorption on green algae were investigated by FTIR analysis. The FTIR spectra of free algae and algae loaded with chromium metal is shown in Figure 4.

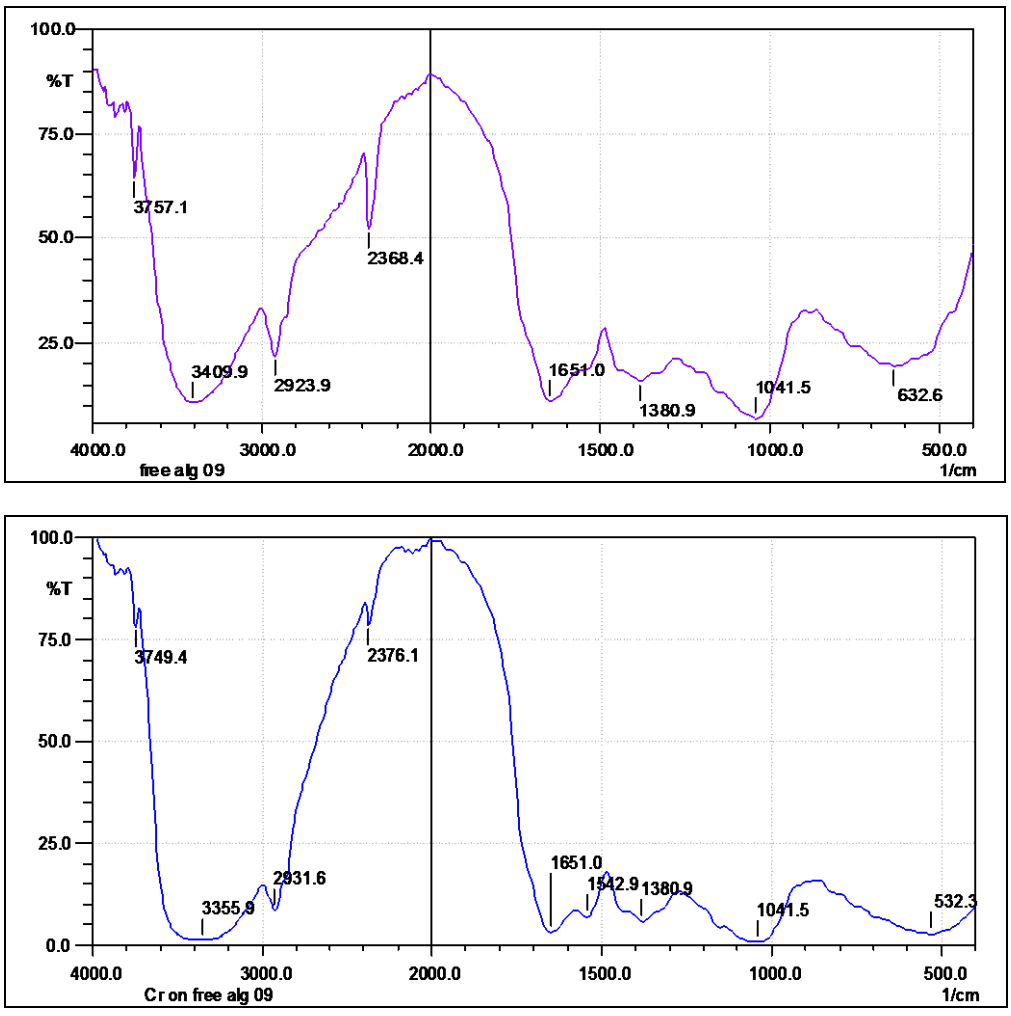


Figure 4. FTIR spectra of free algae and chromium loaded algae

The position of absorption bands and corresponding functional groups able to interact with metal ions are presented in Table 5. After adsorption of chromium, slight changes were observed in the absorption peak frequencies between 2000 and 4000 cm^{-1}. There was a slight shift of peaks to lower frequencies. For instance, the peak at 2376.1 cm^{-1} attributed to a cyanide group shifts slightly to 2368.4 cm^{-1}. This was probably due to the attachment of the heavier metal atom to an active functional group resulting in lower vibration frequency.The peaks observed confirm the presence of carboxylic and amino acids, hydroxyl and carbonyl groups among others on the algal surface as suggested in literature (Davis *et al.*, 2003, Gupta and Rastogi, 2009).

Table 5. Functional groups found on green algae

Peak position before loading (cm^{-1})	Peak position after loading (cm^{-1})	Possible assignment
3409.9	3355.9	$O\text{-}H_{str}$, $N\text{-}H_{str}$,
2931.6	2923.9	C-O, N-H, $C\text{-}H_{str}$
2376.1	2368.4	C≡N
1651.0	1651.0	C=N, $C{=}O_{vib}$, C=C, $N\text{-}H_{bend}$
1542.9	--	-NH, N=O
1380.9	1380.9	$O\text{-}H_{bend}$, $S{=}O_{str}$, $CH_{3\ bend\ and\ ss}$, N-O
1041.5	1041.5	C-O
532.5	632.3	S-S

3.2 Effect of pH on metal uptake

The residual metal ion concentrations after batch equilibration with algae at various pH values and those found in the control experiment were determined.

Table 6. Percentage metal removal by precipitation and biosorption at different pH values

Metal removal (%)		pH							
		2.0	3.0	4.0	4.5	5.0	5.5	6.0	7.0
	Total removal	13.47 ± 0.98	19.39 ± 1.11	24.20 ± 1.90	28.38 ± 2.30	31.56 ± 2.61	38.87 ± 1.99	39.23 ± 0.88	43.61 ± 3.13
Cd	Precipitation	0.02 ± 0.00	0.10 ± 0.05	0.62 ± 0.04	1.03 ± 0.07	2.12 ± 0.13	2.63 ± 0.16	7.11 ± 0.08	30.22 ± 2.71
	Biosorption	13.45	19.28	23.59	27.35	29.44	36.24	32.11	13.40
	Total removal	8.30 ± 0.60	10.93 ± 0.57	14.53 ± 0.67	19.23 ± 1.51	22.47 ± 1.90	33.26 ± 2.20	14.53 ± 2.04	19.23 ± 1.47
Cr	Precipitation	0.63 ± 0.68	8.33 ± 0.40	7.00 ± 1.54	9.17 ± 0.72	6.47 ± 0.32	9.60 ± 2.26	7.00 ± 0.33	9.17 ± 0.29
	Biosorption	7.67	2.60	7.53	10.07	16.00	23.66	7.53	10.07
	Total removal	0.03 ± 0.53	0.10 ± 0.64	18.02 ± 0.92	23.32 ± 1.17	34.90 ± 0.85	42.71 ± 0.59	61.39 ± 1.12	62.93 ± 1.32
Cu	Precipitation	0.02 ± 0.03	0.10 ± 0.03	0.62 ± 0.11	1.03 ± 0.36	2.12 ± 0.17	2.63 ± 0.19	3.42 ± 0.06	60.60 ± 0.76
	Biosorption	0.01	0.00	17.40	22.29	32.78	40.07	57.97	2.33
	Total removal	13.40 ± 0.59	33.03 ± 0.84	55.67 ± 0.43	61.33 ± 0.26	67.80 ± 0.48	70.67 ± 0.47	76.19 ± 0.18	78.33 ± 0.03
Pb	Precipitation	0.64 ± 0.10	1.84 ± 0.62	5.63 ± 0.94	8.22 ± 0.71	12.02 ± 0.78	20.58 ± 0.24	32.08 ± 2.16	70.02 ± 0.20
	Biosorption	0.23	31.19	50.04	53.11	55.77	50.09	44.10	8.32

The lower concentration of metal ions observed in the filtrates (Table 6) in both cases were due to removal of metal ions by biosorption and further loss through the discarded precipitate residues. For all the metals considered, both percentage precipitation (Figure 5) and biosorption (Figure 6) were low at low pH. Metal ion removal by de-sorption from biosorption sites increases to a peak between pH 5 and 6 then starts to decline while precipitation remains low up to pH 6 and then rises steeply. This is probably because at low pH there is high competition for sorption sites between metal ions and protons and this leads to isomorphous substitution from the sites. Since algal biomass has a high content of carboxyl groups on its cell walls, biosorption process can be affected by changes in the solution pH (Matheickal and Yu, 1999).

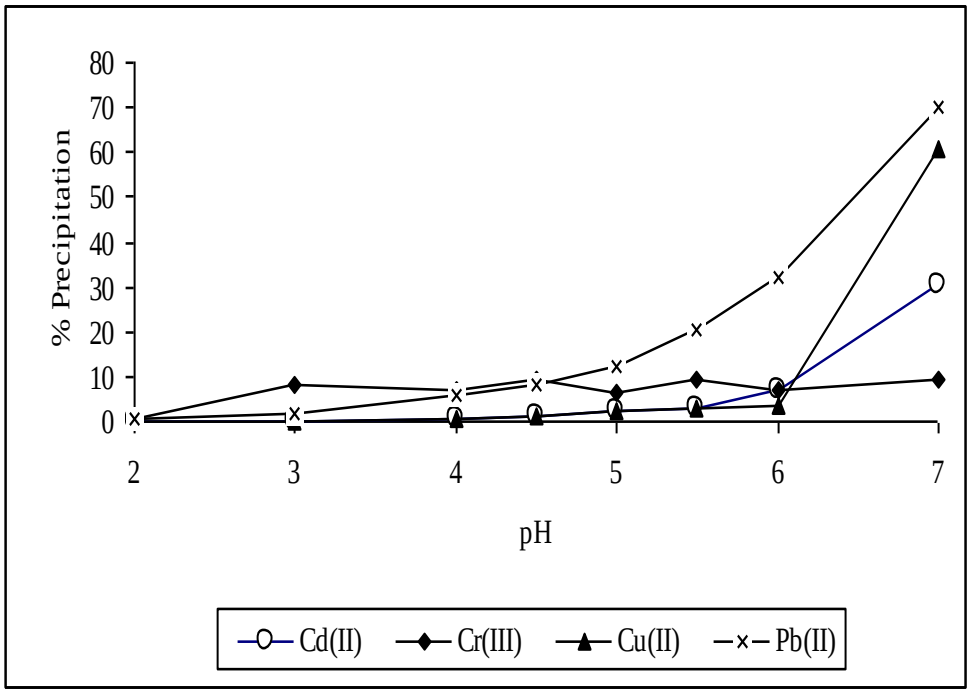


Figure 5. Percentage precipitation of Cd, Cr, Cu and Pb at pH values 2 – 7.

Change in pH affects the protonation-deprotonation equilibria of the functional groups as well as the metal chemistry. As the pH rises, the hydrogen ion concentration falls leading to less competition for the sorption sites therefore resulting in an increase in biosorption of heavy metals. The high pH also leads to precipitation of low solubility metal hydroxides as shown in Figure 5. Precipitation interferes with the biosorption process because it immobilizes the metal ions thus making them unavailable for biosorption. Precipitation is due to the formation of low solubility metal hydroxides such as $Cr(OH)_3$, $Cu(OH)_2$, $Cd(OH)_2$, $Pb(OH)_2$ at higher pH (Cotton and Wilkinson 2004, Chen *et al* 2006, Gupta and Rastogi, 2007, Gupta *et al.*, 2005). The optimum pH for biosorption is a compromise between interference from precipitation at high pH and competition with hydrogen ions for sorption sites at low pH. To obtain the optimum pH, a graph of percentage metal removal by biosorption against pH was plotted. The curves obtained for the selected metal ions are shown in Figure 6. From these curves the optimum pH values for the selected metals were found to be 5.0, 5.5, 5.8, and 5.9 for lead, cadmium, chromium and copper, respectively.

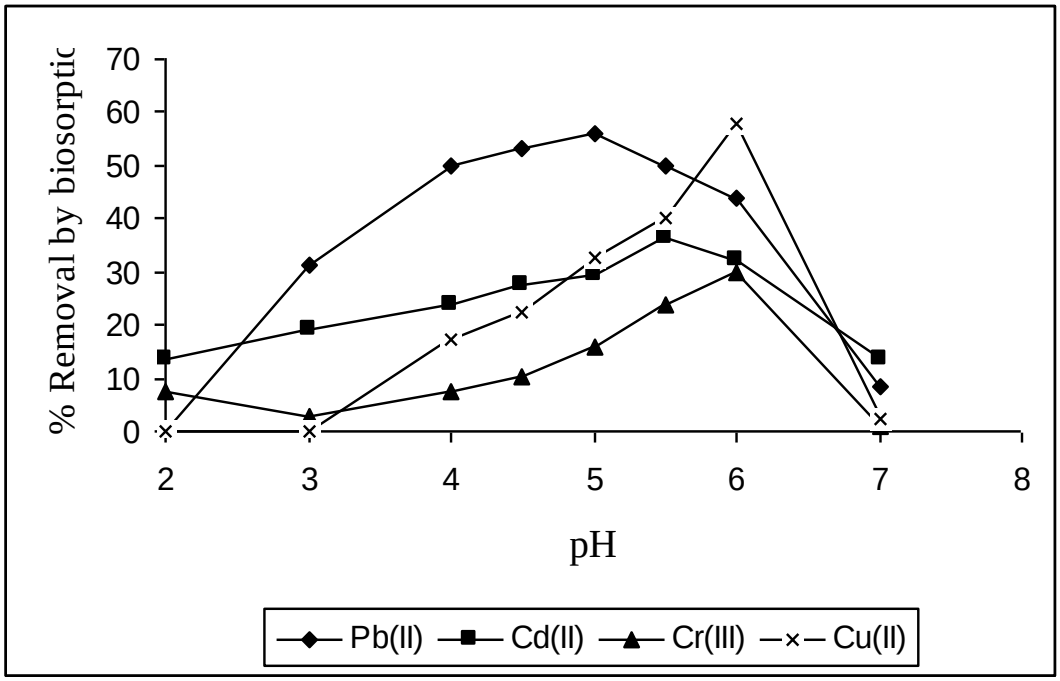


Figure 6. Percentage removal of metal ions from model aqueous solution by biosorption

3.3 Effect of contact time on metal uptake

The minimum time required for quantitative uptake of metal ions from solution was determined and the results recorded in Table 7. Effective contact times were obtained by plotting the mean percentage metal ion uptake against time as shown in Figures 7a - 7d. When the curve levels off, equilibrium has been established and there is no further uptake. The corresponding time is the effective contact time for the respective metal ions. Cadmium adsorption was the fastest with the process attaining equilibrium in fifteen minutes while chromium and copper took forty minutes. For lead, equilibrium was achieved in fifty minutes. Hence the effective contact times for the selected metals were found to be 15 minutes for cadmium, 40 minutes for both chromium and copper and 50 minutes for lead.

Table 7. Variation of concentration (µg/mL) of Cd, Cr, Cu and Pb in solution with contact time.

Contact time,(min)	Cd (II)		Cr (III)		Cu (II)		Pb (II)	
0	200.00	100.00	200.00	100.00	200.00	100.00	200.00	100.00
2	149.71 ± 0.55	63.89 ± 0.02	198.89 ± 0.04	94.50 ± 1.40	193.60 ± 1.52	99.93 ± 0.02	199.85 ± 0.03	94.24 ± 0.65
6	135.75 ± 0.73	51.97 ± 0.12	162.90 ± 1.66	71.94 ± 0.19	175.89 ± 1.13	69.27 ± 0.67	164.23 ± 0.45	83.18 ± 2.15
10	134.13 ± 0.62	50.23 ± 0.02	161.32 ± 1.02	61.72 ± 1.13	163.63 ± 1.27	62.87 ± 0.74	159.15 ± 0.40	71.17 ± 0.18
15	133.54 ± 0.92	48.85 ± 0.07	159.92 ± 1.34	58.76 ± 1.23	157.46 ± 0.94	61.40 ± 0.25	154.12 ± 0.87	66.57 ± 0.27
20	129.72 ± 0.55	49.32 ± 0.02	158.40 ± 0.43	57.44 ± 0.32	152.59 ± 0.83	56.31 ± 0.40	146.10 ± 0.14	57.75 ± 0.12
25	128.56 ± 1.22	48.67 ± 0.05	155.02 ± 0.51	56.10 ± 0.60	152.46 ± 2.29	56.14 ± 0.45	139.37 ± 0.44	54.69 ± 0.17
30	127.97 ± 0.87	48.66 ± 0.03	158.98 ± 0.80	53.34 ± 0.92	152.24 ± 0.82	55.07 ± 0.80	138.61 ± 0.43	52.43 ± 0.49
40	127.79 ± 0.95	48.81 ± 0.05	158.68 ± 0.85	50.32 ± 1.59	151.70 ± 1.22	54.32 ± 0.37	132.93 ± 0.39	49.15 ± 0.64
50	128.15 ± 1.17	48.32 ± 0.07	158.72 ± 1.02	48.68 ± 0.31	151.22 ± 1.17	52.35 ± 0.74	134.39 ± 0.46	48.35 ± 1.67
60	127.58 ± 0.66	48.79 ± 0.03	158.32 ± 0.89	48.14 ± 0.56	150.84 ± 0.38	52.68 ± 0.24	135.16 ± 0.32	47.65 ± 0.05
75	128.32 ± 0.3	48.76 ± 0.04	154.26 ± 0.61	46.54 ± 0.33	150.17 ± 1.63	52.10 ± 0.42	132.50 ± 0.53	47.46 ± 0.27
90	129.33 ± 0.44	50.48 ± 0.04	156.22 ± 0.84	48.06 ± 0.48	150.09 ± 0.28	52.39 ± 0.02	132.21 ± 0.47	46.42 ± 0.49
105	127.91 ± 0.73	49.04 ± 0.04	159.16 ± 0.84	47.10 ± 0.29	150.44 ± 0.55	51.60 ± 0.30	128.76 ± 1.14	45.27 ± 0.39
120	127.12 ± 0.28	48.93 ± 0.04	156.96 ± 0.69	47.52 ± 0.73	149.05 ± 1.90	51.73 ± 0.31	128.95 ± 0.94	45.01 ± 0.89
140	127.75 ± 0.91	48.63 ± 0.03	152.30 ± 0.89	45.70 ± 0.63	145.33 ± 0.27	51.60 ± 0.26	128.83 ± 1.18	44.65 ± 0.06

The short contact times taken for equilibrium to be established demonstrate the potential of algae as a suitable biosorbent for fast removal of heavy metals from contaminated waters as compared to slower processes like reverse osmosis.

It is worth noting that more mass is picked from the more concentrated solution in all cases but the percentages are higher for the lower concentrations (Figures 7a-7d). This is consistent with the trend of uptake increasing with concentration. In all cases considered, metal adsorption was very rapid.

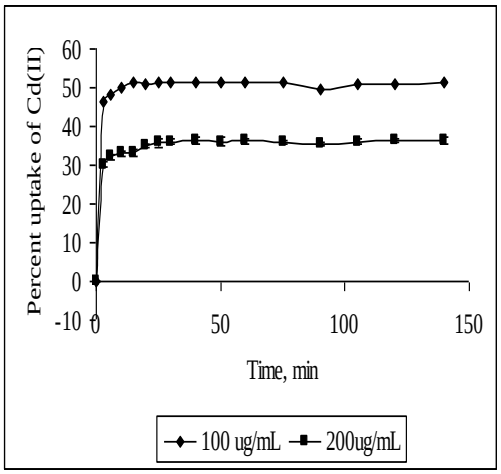


Fig 7a

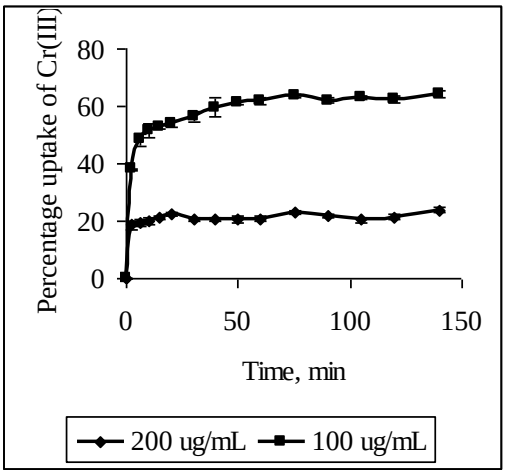


Fig 7b

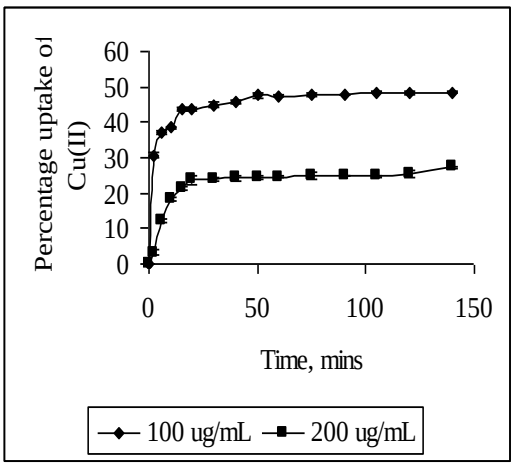


Fig 7c

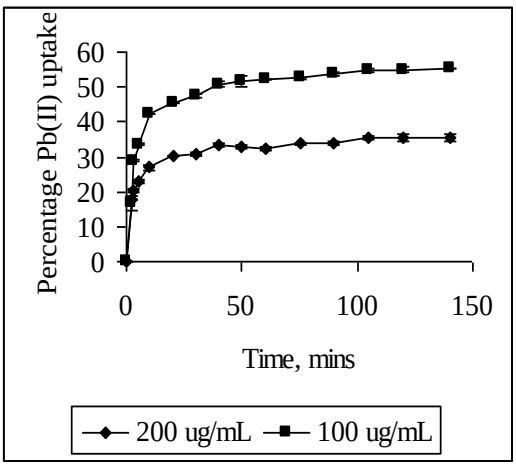


Fig 7d

Figure 7(a-d). Variation of metal uptake by green algae with time

3.4 Order of reaction

The variation of metal ion concentration with time during the adsorption process (Table 7) was used to follow the kinetics of the adsorption until equilibrium was achieved. The mass q_t of metal adsorbed after time t is related to the equilibrium metal uptake q_e by the integrated first and second order equations $k_1 t = \ln q_e - \ln(q_e - q_t)$ and $\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^{\,2}}$ respectively, where k_1 and k_2 are the first and second order rate constants. A plot of $\ln(q_e - q_t)$ against time (minutes) was used for the first order linearity test, while a plot of $\frac{t}{q_t}$ (min g/mg) against time (minutes) was used for the second order linearity test and the calculation of q_e, which is the metal uptake in milligrams per gram of biosorbent at equilibrium. The order of reaction for each metal was deduced from the linearity of the respective plots. Figures 8a – 8d gives the slopes and the R^2 values from which q_e and the linear correlation coefficients may be obtained. The second order plots for all metals have higher R^2 values than the corresponding first order plots as shown in Table 8. The process is therefore second order for all metals. This agrees with literature (Gupta and Rastogi 2007, Sari and Tuzen 2007, Patel and Suresh, 2008).

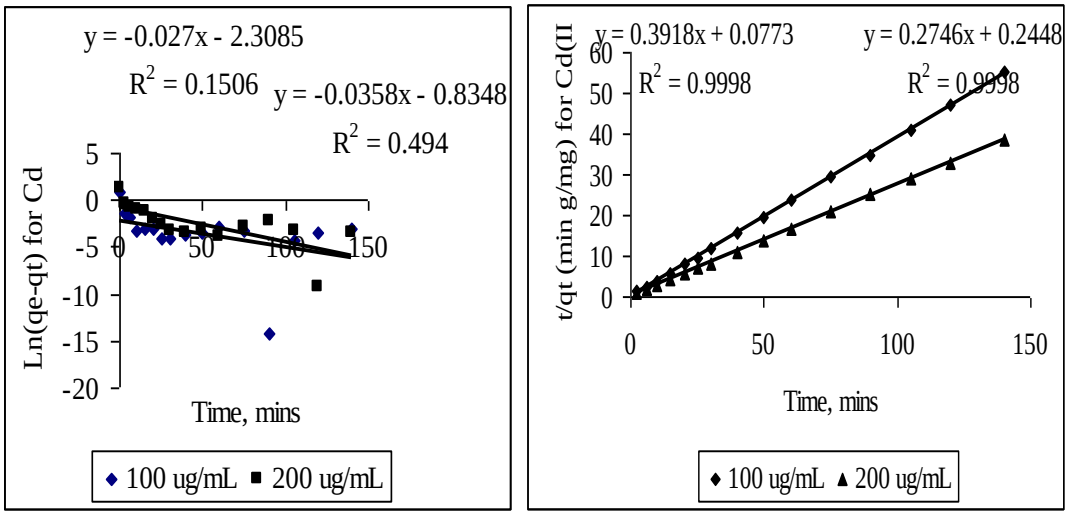


Fig 8a. First and second order linearity test for cadmium adsorption on green algae

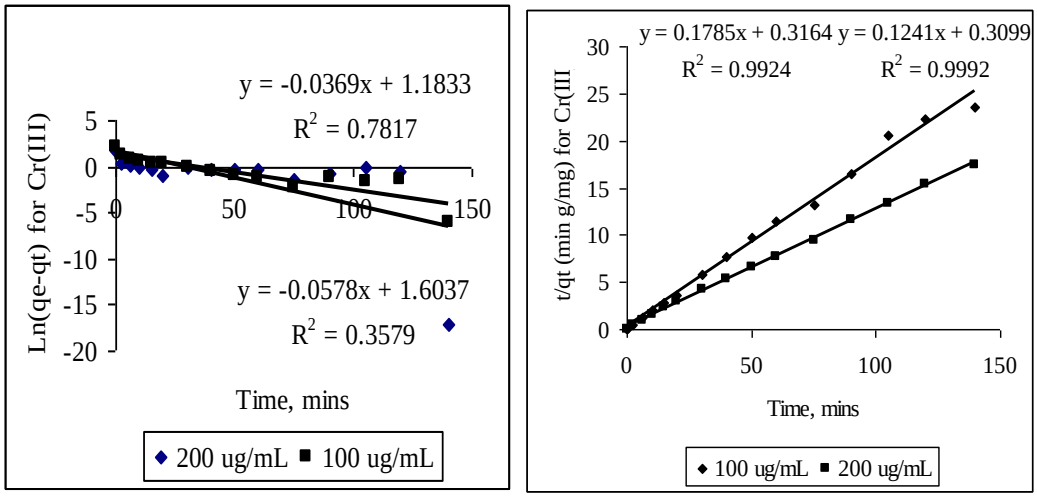


Fig 8b. First and second order linearity test for chromium adsorption on green algae

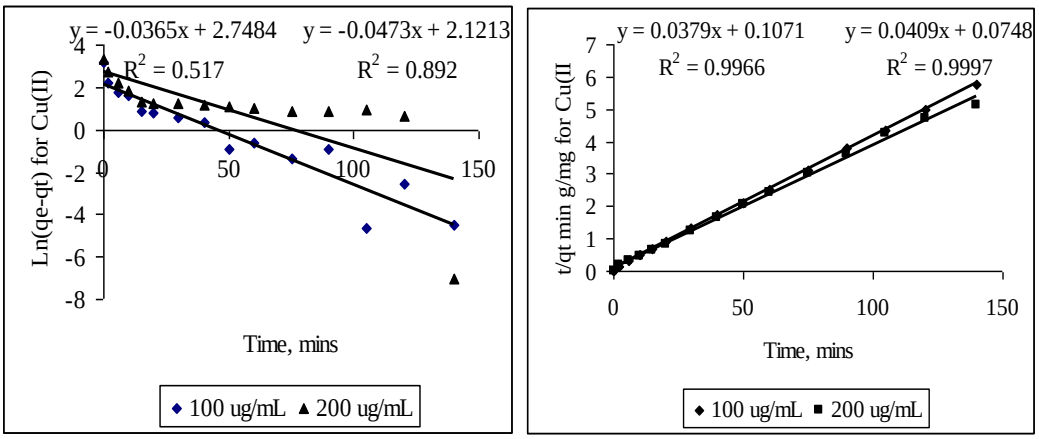


Fig 8c. First and second order linearity tests for copper adsorption on green algae

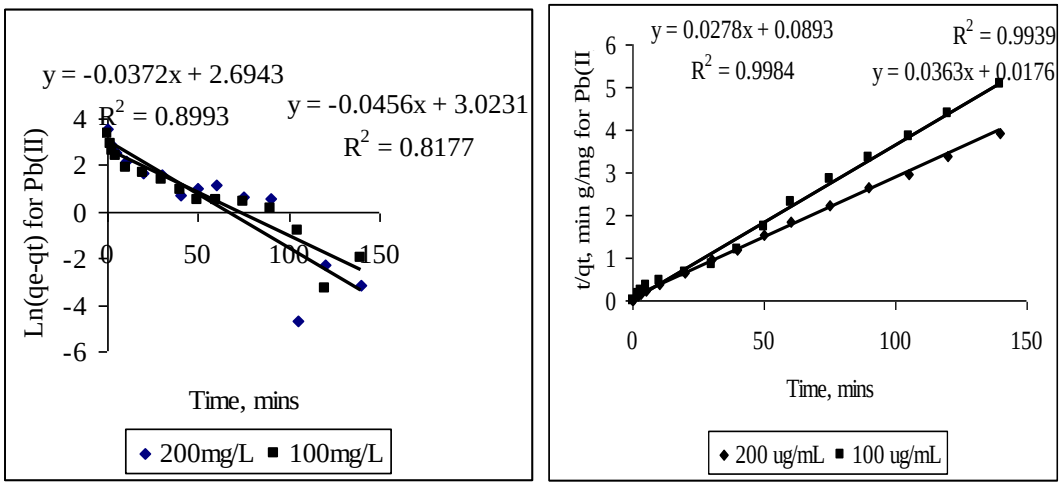


Fig 8d. First and second order linearity tests for lead adsorption on green algae.

Table 8. Kinetic parameters for Cd, Cr, Cu and Pb metal adsorption on green algae

Metal	Initial concentration, (µg/mL)	Calculated metal uptake at equilbrium q_e, (mg/g)	R value for first order linearity test	R value for second order linearity test
Cd (II)	200	3.64	0.338	1.000
	100	2.55	0.159	1.000
Cr (III)	200	8.06	0.598	0.999
	100	5.60	0.884	0.996
Cu (II)	200	26.39	0.719	0.998
	100	24.45	0.944	0.999
Pb (II)	200	35.97	0.904	0.999
	100	27.55	0.948	0.997

3.5 Adsorption capacity and optimum initial metal ion concentration

The equilibrium concentrations C_e of cadmium, chromium, copper and lead in the filtrates were determined by FAAS and reported in Table 9. The corresponding initial concentrations, C_i and the calculated metal uptakes q_e,. at equilibrium are listed in the same table. The data was fitted to both the linearized Freundlich and Langmuir isotherms (Figures 9 and 10), respectively. The linearized isotherm

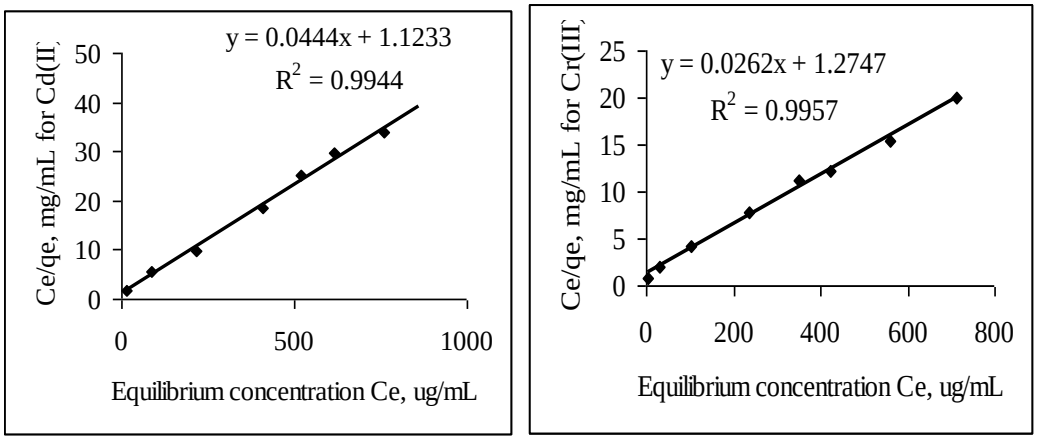
equations are $\ln q_e = \ln K_F + \frac{1}{n}\ln C_e$ and $\frac{C_e}{q_e} = \frac{1}{q_{\max} b} + \frac{C_e}{q_{\max}}$, respectively, where q_{max} is the adsorption capacity, b is a Langmuir constant, K_F and n are Freundlich constants. Linear regression coefficients (R^2) were used to deduce which isotherm best fitted the data.

Table 9. Equilibrium concentrations C_e (µg/mL) and metal uptake q_e (mg/g) at equilibrium

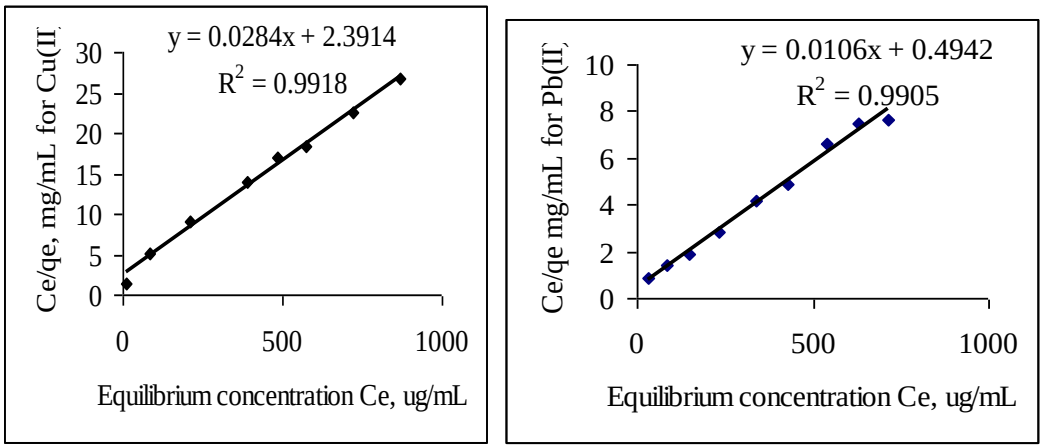
Metal ion	Cd (II)		Cr (III)		Cu (II)		Pb(II)	
$C_{i,}$ (µg/mL)	C_e	q_e	C_e	q_e	C_e	q_e	C_e	q_e
50	14.25 ± 0.59	8.94 ± 0.15	4.21 ± 0.51	5.72 ± 0.06	12.12 ± 0.79	9.47 ± 0.20	30.33 ± 0.50	34.83 ± 0.25
150	87.43 ± 0.48	15.64 ± 0.12	30.75 ± 1.93	14.91 ± 0.24	84.20 ± 0.48	16.45 ± 0.12	83.17 ± 8.50	58.42 ± 4.25
300	213.95 ± 0.38	21.51 ± 0.10	104.18 ± 1.73	24.48 ± 0.22	208.56 ± 0.80	22.86 ± 0.20	146.67 ± 12.35	76.67 ± 1.18
500	410.85 ± 1.03	22.29 ± 0.26	257.63 ± 4.56	30.30 ± 0.57	388.99 ± 2.57	27.75 ± 0.64	338.17 ± 2.35	80.92 ± 3.01
600	517.85 ± 0.53	20.54 ± 0.13	349.20 ± 1.64	31.35 ± 0.20	485.54 ± 0.61	28.62 ± 0.15	426.00 ± 6.03	87.00 ± 0.25
700	616.73 ± 0.28	20.82 ± 0.07	424.07 ± 7.75	34.49 ± 0.97	574.70 ± 13.41	31.32 ± 3.35.	537.61 ± 1.84	81.19 ± 0.92
850	760.21 ± 0.48	22.45 ± 0.12	559.71 ± 4.16	36.28 ± 1.77	721.58 ± 0.30	32.11 ± 0.08	631.50 ± 3.00	84.25 ± 1.50
1000	859.33 ± 0.59	35.17 ± 0.15	713.32 ± 5.70	35.84 ± 0.72	869.55 ± 4.56	32.62 ± 1.15	713.17 ± 11.18	93.42 ± 5.59

The adsorption capacity q_{max} was obtained as the reciprocal of slope of the linearized Langmuir plot $\frac{C_e}{q_e}$ against the equilibrium concentration C_e, and the

Langmuir constant *b* from the y – intercept. The adsorption capacities of green algae were found to be 22.52, 35.59, 38.19and 94.34 mg/g for cadmium, copper, chromium and lead, respectively (Table 10). These results show that the adsorption capacities of green algae for all selected metals compare well with those of other biosorbents in literature.



(a) (b)



(c) (d)

Figure 9. Linearized Langmuir plots for Cd, Cr, Cu and Pb

The difference in adsorption capacities of different metals to a biosorbent arise from differences in their ions. The ability of a metal ion to form a strong ligand with the

biosorbent is important in adsorption (Chen and Wang, 2007; Remacle, 1990; Brady and Tobin, 1995). The trend in biosorption capacity obtained in this work agrees with literature (Chen and Wang, 2007; Kogej and Pavko, 2001 and Reddad *et al*, 2002) who used *Saccharomyces cerevisiae*, *Rhizopus nigricans and* sugar beet pulp waste, respectively and found the adsorption capacity varied as; $Pb^{2+} > Cu^{2+} > Cd^{2+}$.

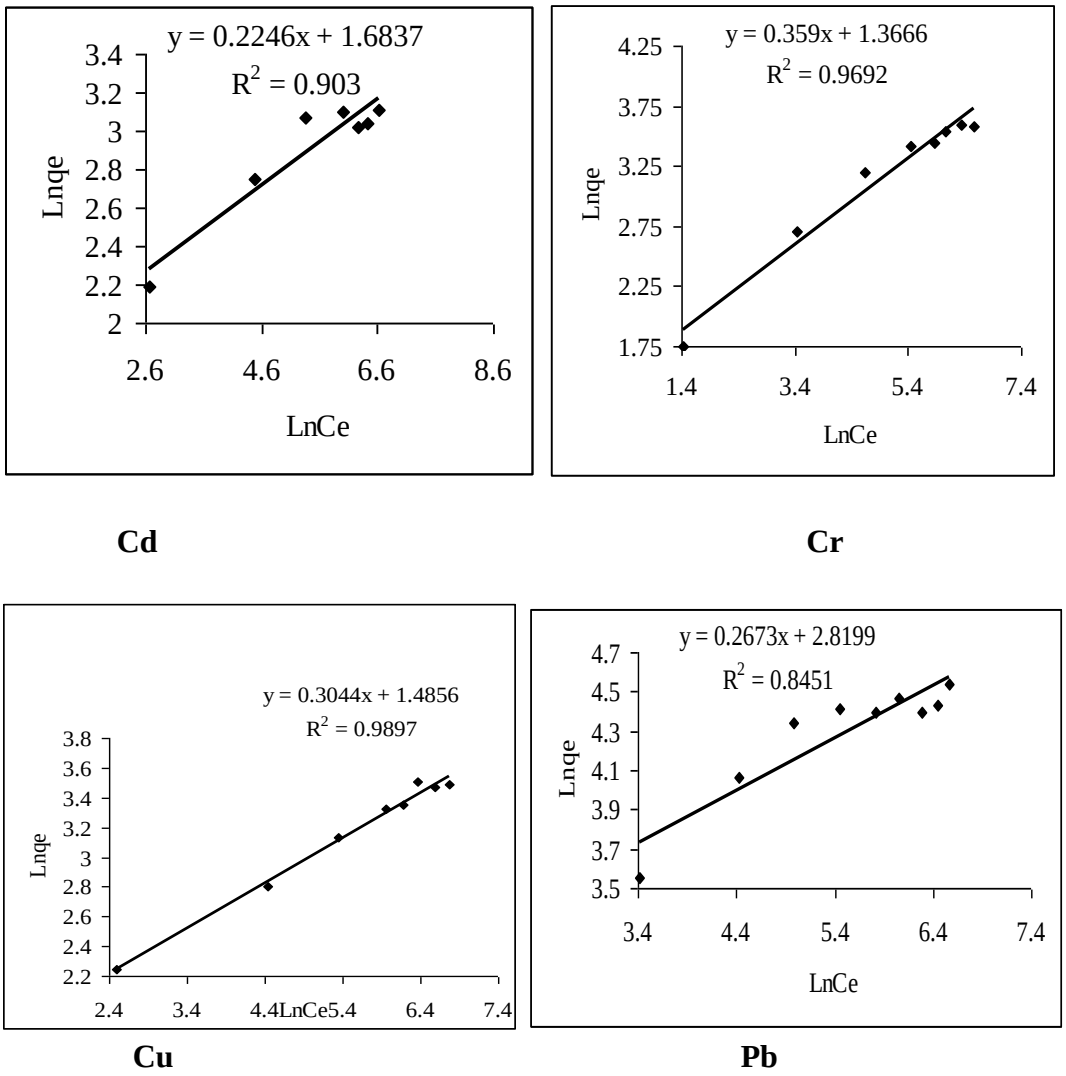


Figure 10. Linearized Freundlich plots for Cd, Cr, Cu and Pb, respectively

The magnitude of the Freundlich constants was used to asses the adsorption intensity. Freundlich constants were obtained from a plot of lnq_e against lnC_e in

which n is the reciprocal of the slope and lnK_F is the y – intercept. The large values (greater than unity), of the Freundlich constants K_F and n (Table 10), indicate a high affinity of the metal ions for the sorbent sites hence a good surface coverage at equilibrium. Figure 9 shows the linearized Freundlich plots for cadmium, chromium, copper and lead. The Langmuir and Freundlich constants obtained from Figures 9 and 10 are reported in Table 10. The higher linear correlation coefficients for the Langmuir plots (Table 10), suggest that the experimental data fits better to the Langmuir isotherm than the Freundlich isotherm.

Table 10. Calculated adsorption isotherm parameters for metal adsorption

	Langmuir parameters			Freundlich parameters		
Metal	R	b (L/mg)	q_{max} (mg/g)	R	K_F	n
Cd (II)	0.997	0.035	22.52	0.950	5.40	4.45
Cr (III)	0.998	0.021	38.19	0.984	3.93	2.79
Cu (II)	0.990	0.011	35.59	0.996	4.47	3.32
Pb (II)	0.995	0.021	94.34	0.919	16.78	3.74

The trend in metal uptake q_e (mg/g) by green algae at various initial concentrations was determined. The uptake increased with increasing initial metal concentration and levelled off at initial concentrations between 500 – 700 mg/L (Figure 11). At concentrations above 800 mg/L the uptake started to rise again perhaps as a result the onset of precipitation. Thus the optimum initial metal ion concentrations ranges from 500 to 700 mg/L for all the metals considered.

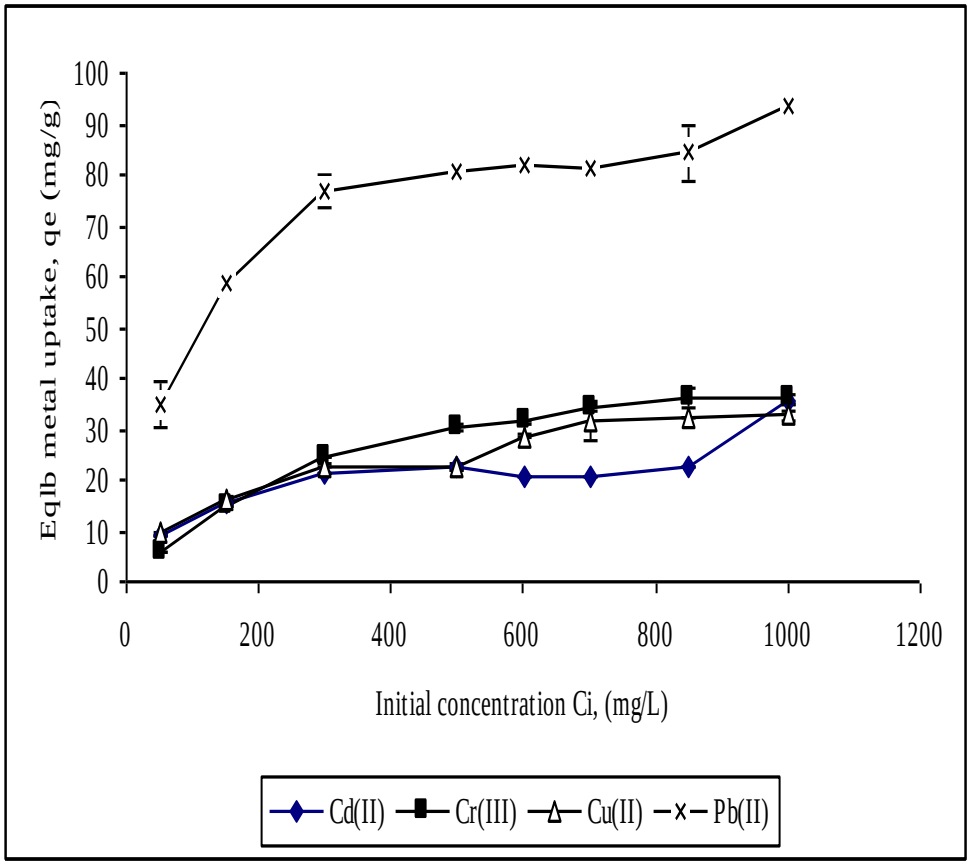


Figure 11. Variation of metal ion uptake with initial concentration of Cd, Cr, Cu and Pb ions.

3.6 Biomonitoring studies

3.6.1 Determination of metal concentrations in water, acid-leached and digested algae

The concentration of the heavy metals in environmental water samples and in algae collected from the water were determined. In algae, both the total and surface adsorbed metals (leachable fractions) were determined by FAAS. Concentrations of cadmium, chromium, copper and lead were 1.36 ± 0.10, 12.42 ± 1.74, 14.88 ± 0.99 and 14.98 ± 1.01 µg/g, respectively in the digested algae (Appendix 9) and 0.74 ±0.09, 4.95 ± 1.86, 8.23 ± 0.59 and 9.41 ± 0.74 µg/g, in the acid-leached fraction for

cadmium, chromium, copper and lead, respectively (Appendix 10). In the parent water samples, cadmium was not detected by FAAS. The concentrations of, copper lead and chromium in the water were, 5.58 ± 0.68, 19.03 ± 0.43 and 86.87 ± 0.42 ng/mL, respectively. The average ICP – OES results for the total metal in the same algae samples were 2.30 ± 0.09, 12.17 ± 0.20, 25.61 ± 0.74 and 60.50 ± 1.57 µg/g for cadmium, chromium, copper and lead, respectively. The concentration of the leachable (surface adsorbed) metal on the algae was 0.1 ± 0.09, 0.19 ± 0.01, 4.43 ± 1.86, and 8.23 ± 0.59 µg/g for cadmium, lead, chromium and copper, respectively.

Table 11. Mean concentrations by ICP-OES of Cd, Cr, Cu and Pb in environmental samples

	Water, (ng/mL)	Algae, (µg/g)	
Metal	Total concentration	Leachable concentration	Total concentration
Cd	1.82 ± 0.11	0.1 ± 0.09	2.30 ± 0.09
Cr	64.33 ± 0.35	4.43 ± 1.86	12.17 ± 0.20
Cu	17.14 ± 0.15	8.23 ± 0.59	25.61 ± 0.74
Pb	12.08 ± 1.80	0.19 ± 0.01	60.50 ± 1.57

As expected the leachable fraction is less than the total metal in all cases. This is so because leaching with acid removes only the metal adsorbed on the surface of the algae. Within the parent water the average concentrations of cadmium, lead, copper and chromium by ICP-OES were 1.82 ± 0.11, 12.08 ± 1.80, 17.14 ± 0.15 and 64.33 ± 0.35 ng/mL, respectively, (Appendix 10). The summary of these concentrations is reported in Table 11. These results point to the dominance of sorption process vis-à-vis diffusion. This is seen in the fact that the algae can maintain a high internal

concentration of metal against the large concentration gradient between it and the water. This indicates a high possibility of active transport assisted sorption.

Within the algae and the parent water, cadmium metal was the least abundant. This could be due to the fact that cadmium is not required for the growth or metabolism of the algae. Secondly, cadmium is not as widely used as the other metals and so dumping of cadmium based products in the environment is not very widespread hence its lower concentration in environmental samples. The relatively lower adsorption capacity of algae for cadmium may also account for the lower values of the total metal in algae.

The heavy metal concentrations (obtained by FAAS) in digested algae, acid-leached algae and the water from which the algae was collected were correlated. Table 12 gives the correlation coefficients for the three pairs of data. The results show a high correlation between the total metal in algae and the adsorbed metal on the algal surface, yet there was no correlation between the metal concentration in algae and in the parent water. The lack of correlation between metal concentration in algae and the water disproves the possibility of a diffusion-controlled transport in favor of active sorption, namely bioaccumulation.

Table 12. Correlation between the concentrations of various metal fractions

FAAS DATA			
Metal	Digested: Acid-leached	Acid-leached : Water	Digested : Water
Cd	0.88	ND	ND
Cr	0.55	0.32	-0.1
Cu	0.95	-0.03	0.11
Pb	0.94	0.10	0.04

These results suggest that while direct analysis of water samples gives us the total metal concentration, in any a water body it does not tell us how much of the pollutant is available to biota living in the water. Use of a bioindicator such as green algae would be more informative.

3.6.2 Concentration factors

Concentration factors were calculated as the ratio of metal concentration in green algae to that in the parent water. ICP – OES data was used due to its higher reliability. The results are reported in Figure 12.

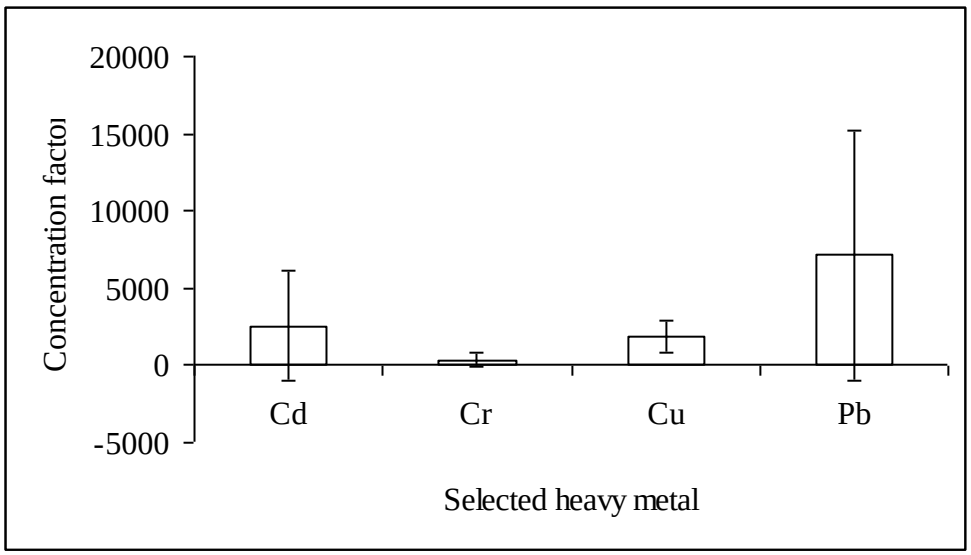


Figure 12. Average concentration factors for Cd, Cr, Cu and Pb from water by green algae

From the data, lead had the highest concentration factors and chromium had the lowest. The trend is Pb > Cd > Cu > Cr, the mean concentration factor for each metal being, Cr (367.02), Cu (1843.59) Cd (2547.01), and Pb (7154.95). The high concentration factors confirm that algae is a good bioaccumulator for the selected

metals. These values possibly indicate also the trend in the strengths of active metal transport by the algae.

The ICP-OES values were generally higher than those obtained by FAAS. The higher ICP-OES values are expected due to the superior analytical features of the ICP torch. The torch operates at a higher temperature than the FAAS flame. This produces a larger degree of sample ionization hence higher sensitivity. It is also more precise than FAAS because the plasma torch is more stable than the flame.

A paired t-test was performed to determine whether the ICP – OES and FAAS data for metal concentration in both algae and the parent water were significantly different. The results indicated no significant difference between the two results at the 95% confidence level. In all cases the calculated t values are less than the tabulated values (Table 13).

Table 13. Paired t - test for ICP-OES and FAAS data for digested algae and the parent water**.**

95% confidence level, n = 48		Digested algae ICP – OES : FAAS	Parent water ICP – OES : FAAS
Metal	$t_{tabulated}$	$t_{calculated}$	$t_{calculated}$
Cd	2.021	5.0×10^{-6}	N/A
Cr	2.021	0.97	0.42
Cu	2.021	1.1×10^{-5}	7.8×10^{-7}
Pb	2.021	6.0×10^{-12}	0.02

N/A = Cadmium was not detected in water by FAAS.

3.7 Conclusion and recommendations

3.7.1 Conclusion

The biosorption and biomonitoring study conducted in this work provides significant information regarding suitability of green algae as a biosorbent and a biomonitor for the selected heavy metal pollution. Adsorption parameters were determined. The best pH for adsorption of the selected metals was found to be 5.0, 5.5, 5.8, and 5.9 for lead, chromium, copper and cadmium, respectively and the times required for equilibrium to be established for metal adsorption from model solutions by green algae were 15 minutes for cadmium, 40 minutes for both chromium and copper, and 50 minutes for lead. The adsorption process was found to be second order and the data fitted better to the Langmuir isotherm than the Freundlich. The adsorption capacities were found to be 22.52, 35.59, 38.19 and 94.34 mg/g for cadmium, copper, chromium and lead, respectively. The initial metal concentrations which resulted in highest metal adsorption onto green algae were between 500 - 700 mg/L for all the metals considered.

The average concentrations of cadmium, lead, copper and chromium in the parent water were 1.82 ± 0.11, 12.08 ± 1.80, 17.14 ± 0.15 and 64.33 ± 0.35 ng/mL, respectively while in the digested algae, cadmium, chromium, copper and lead concentrations were 1.36 ± 0.10, 12.42 ± 1.74, 14.88 ± 0.99 and 14.98 ± 1.01 µg/g, respectively. The adsorbed metal (leachable) fraction concentrations were found to be 0.74 ±0.09, 4.95 ± 1.86, 8.23 ± 0.59 and 9.41 ± 0.74 µg/g, for cadmium, chromium, copper and lead, respectively. There was correlation between the total and leachable metal concentrations, (R = 0.88, 0.55, 0.95 and 0.94 for cadmium,

chromium, copper and lead respectively). As expected, no correlation was found between the heavy metal concentration in algae and the parent water ($R \leq 0.11$). this observation points towards an active transport assisted sorption process as opposed to a diffusion mediated one. Furthermore average concentration factors in the range of 2547.01 for cadmium, 367.02 for chromium, 1843.59 for copper and 7154.95 for lead were observed.

From this work, green algae was found to be a biosorbent which can be used for effectively removing heavy metals from polluted water. The algae is also suitable as a bioindicator because it is able to accumulate metals to a satisfactory degree. While the metal concentration in the water samples was negligible for all metals considered, the algae was much richer in heavy metal content. This is evidence for pre-concentration of heavy metals from water. The research hypothesis that green algae is not a bioaccumulator of heavy metals from water systems is therefore rejected.

3.7.2 Recommendations

The kinetics and the adsorption parameters in algal biosorption favor green algae as a promising biosorbent and bioindicator. This makes the algae suitable for water purification both in municipal supplies and in irrigation water. Thus the following is recommended:

1. Algae being such a promising biosorbent able to grow in most climatic zones be deliberately cultivated in all water bodies and in water treatment units as a pollution control.
2. Algae be grown deliberately in all irrigation waters as a natural water purification regime.

3. Other possible biosorbent materials be investigated with a view to making biosorption the technology of the future in heavy metal pollution control due to its environmental friendliness.

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APPENDICES

Appendix 1: FTIR spectrum of free and cadmium loaded algae

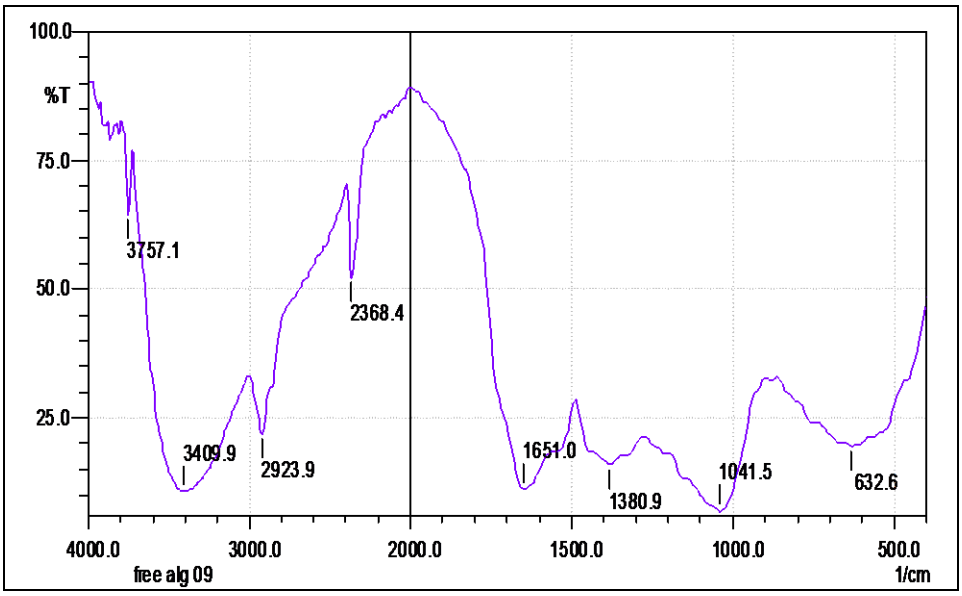


Figure 8. FTIR spectra for cadmium

Appendix 2. FTIR spectrum of free and copper loaded algae

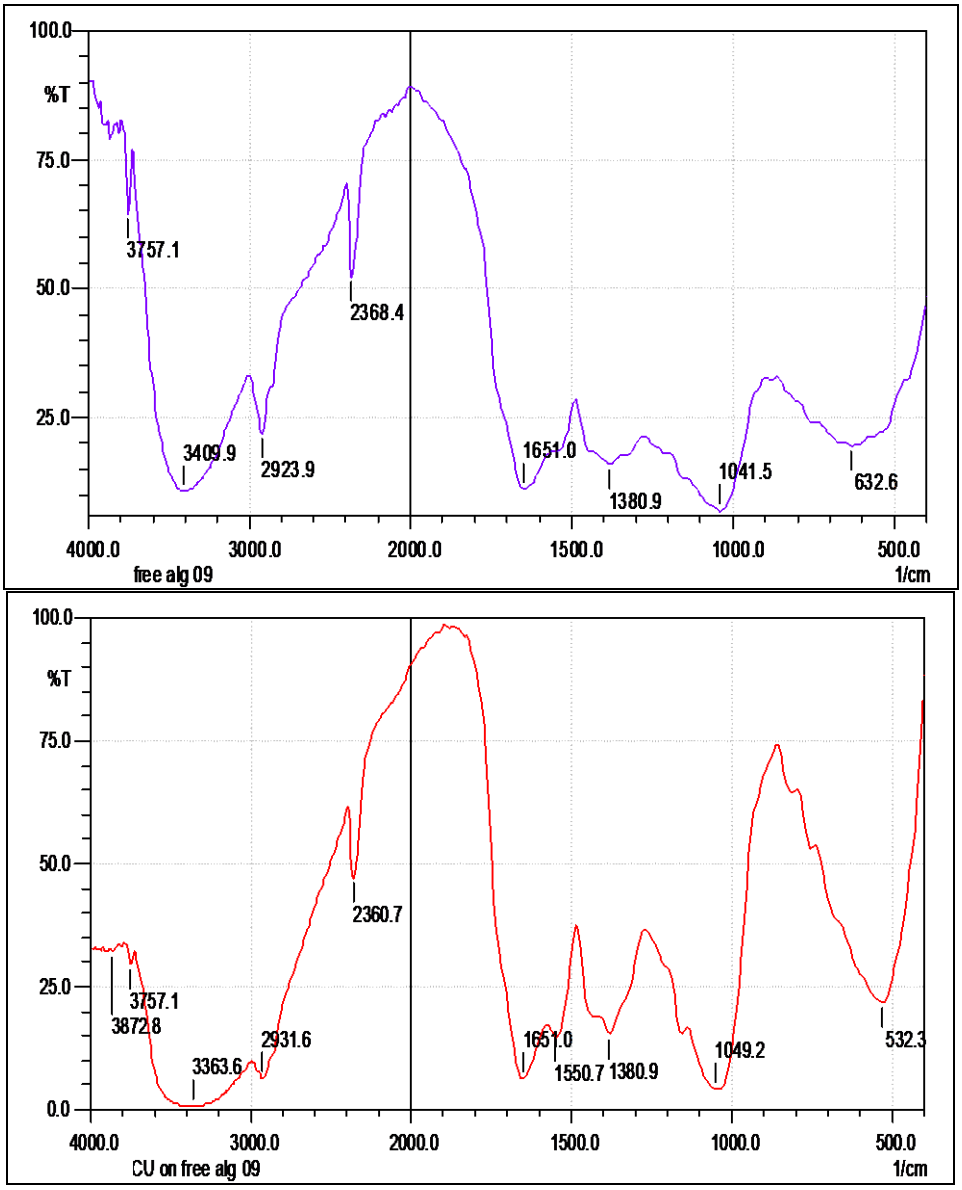
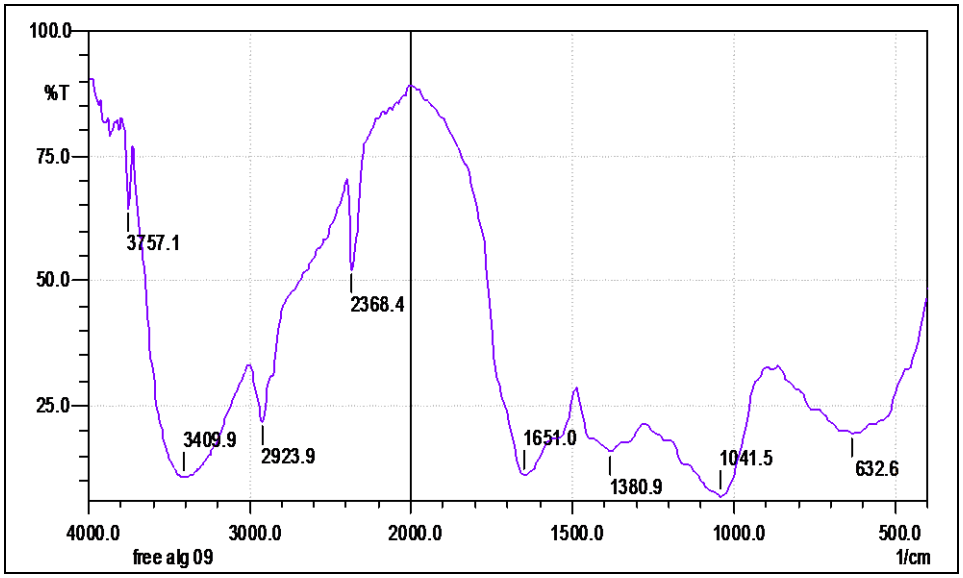


Figure 9. FTIR spectra for copper

Appendix 3. FTIR spectra for free and lead loaded algae



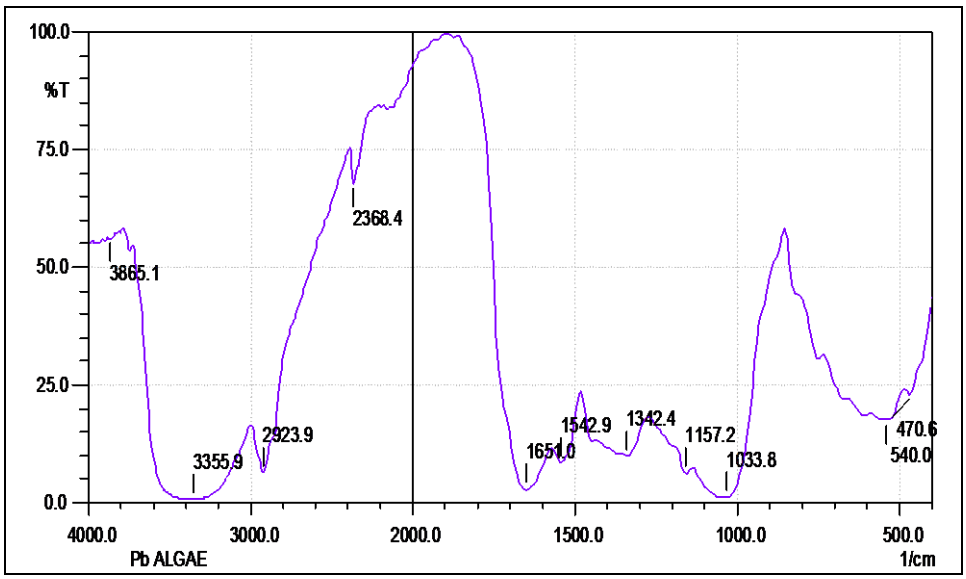


Figure 10. FTIR spectra for lead

Appendix 4. Calibration curve for cadmium

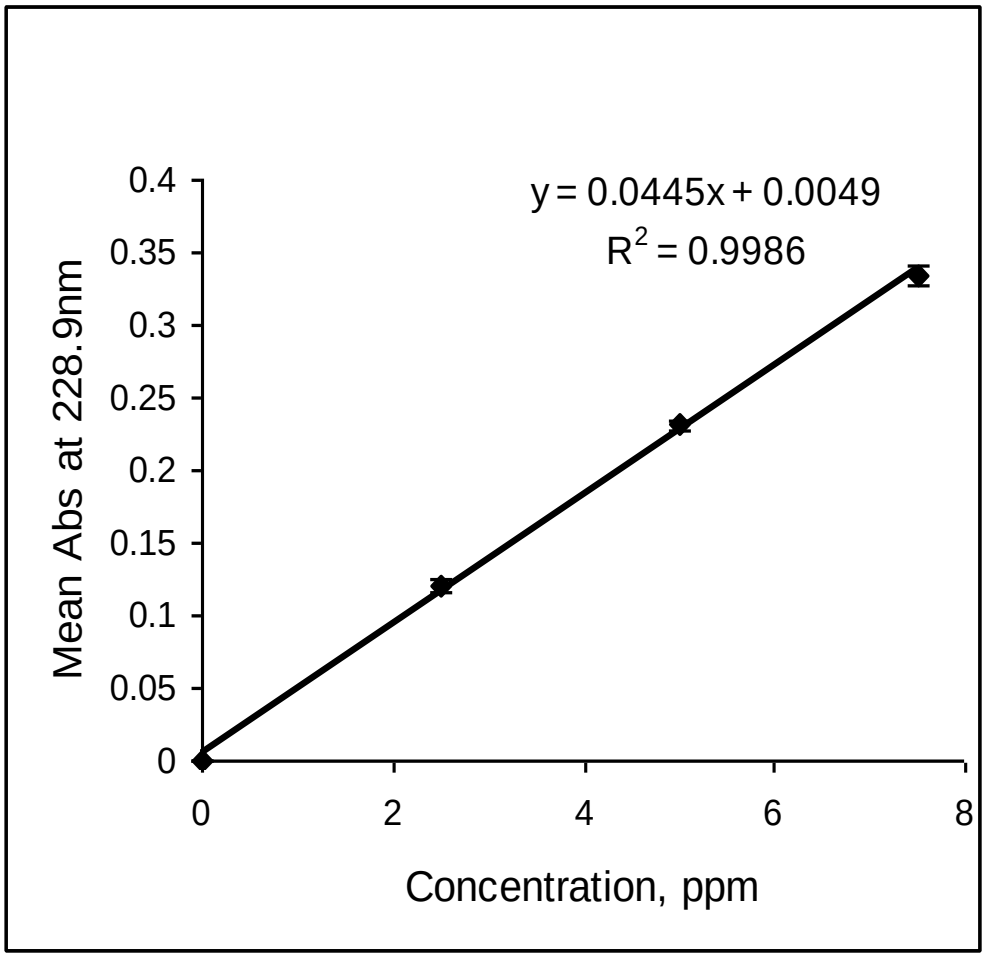


Figure 11. Calibration curve for cadmium

Appendix 5. Calibration curve for chromium

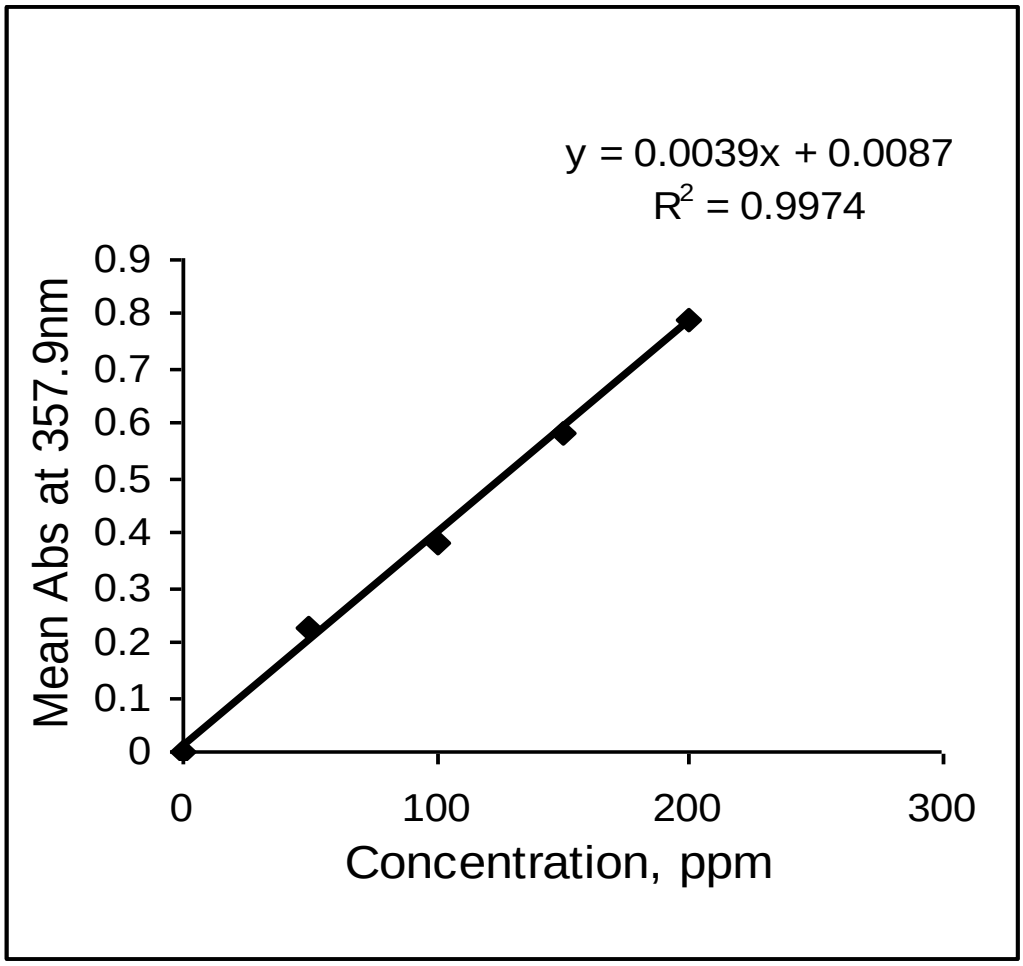


Figure 12. Calibration curve for chromium

Appendix 6. Calibration curve for copper

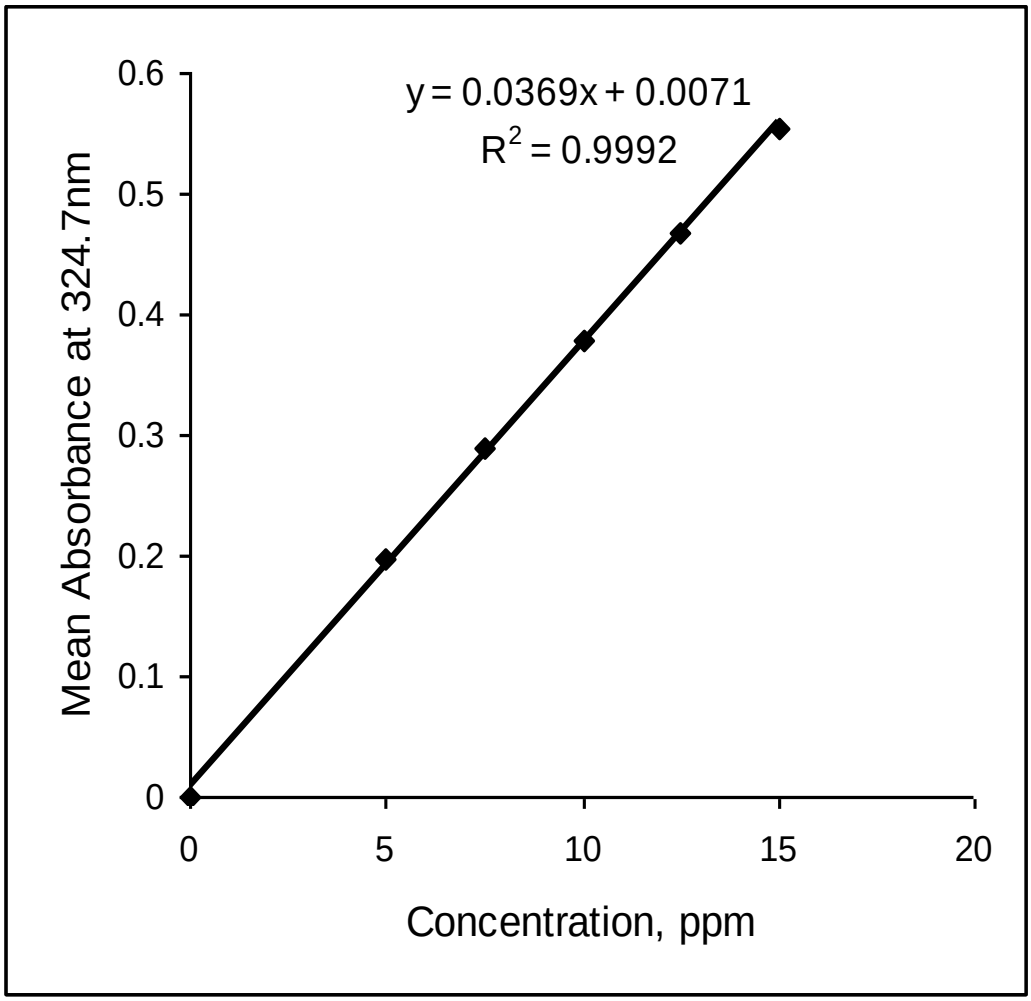


Figure 13. Calibration curve for copper

Appendix 7. Calibration curve for lead

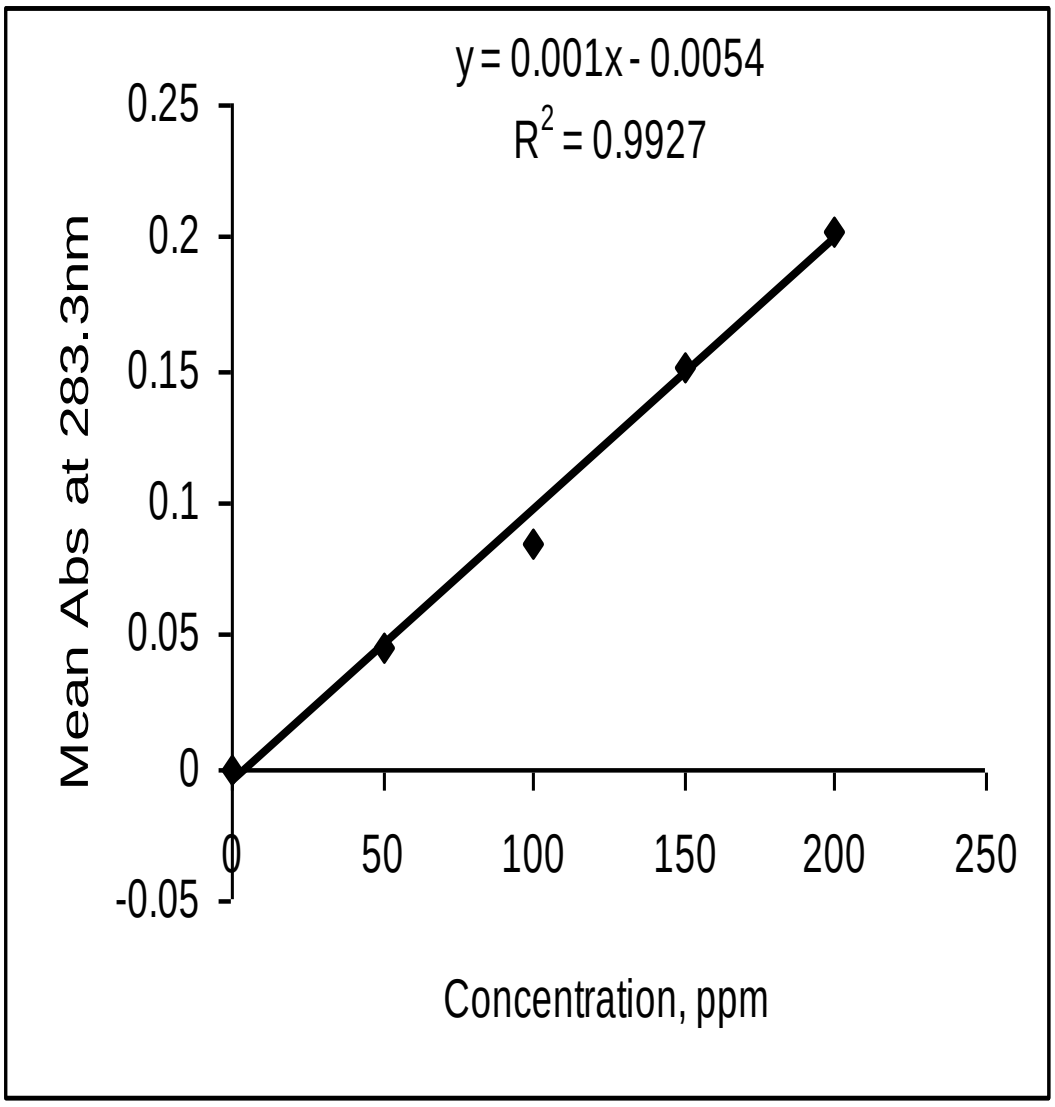


Figure 14. Calibration curve for lead

Appendix 8. Solubility of selected metal hydroxides

Metal hydroxide	Solubility product, K_{sp}	Solubility, $moldm^{-3}$
$Cd(OH)_2$	2.5×10^{-14}	1.84×10^{-5}
$Cr(OH)_3$	6.3×10^{-31}	5.43×10^{-9}
$Cu(OH)_2$	2.2×10^{-20}	1.77×10^{-7}
$Pb(OH)_2$	1.2×10^{-15}	6.69×10^{-6}

Appendix 9. Concentration (µg/g) of Cd, Cr, Cu and Pb in digested algae samples $K_1 – K_{48}$

DIGESTED ALGAE					
Samples		Cd	Cr	Cu	Pb
K1	ICP-OES	5.99±0.08	22.23± 0.12	33.97± 0.82	18.39±3.88
	FAAS	5.02±0.22	30.56±2.62	5.28± 0.17	4.56± 0.51
K2	ICP-OES	9.15±0.13	13.13±0.03	18.29±0.35	113.27±1.22
	FAAS	8.91±0.18	24.31±2.17	6.20± 0.22	13.11±0.69
K3	ICP-OES	1.76± 0.04	33.95±0.23	31.44±0.15	68.91±1.17
	FAAS	1.46±0.44	19.79±1.04	5.93± 0.35	7.44± 0.69
K4	ICP-OES	4.52± 0.06	18.09±0.12	27.36±1.07	97.69±1.80
	FAAS	5.60±0.24	22.92±1.20	7.17± 0.21	13.00±0.88
K5	ICP-OES	1.78± 0.09	13.42±0.05	12.23±0.17	55.84±1.87
	FAAS	1.09±0.04	18.86±1.52	13.18±0.59	12.34±0.72
K6	ICP-OES	ND	ND	ND	ND
	FAAS	ND	ND	ND	ND
K7	ICP-OES	0.48± 0.05	12.01±0.14	18.73±0.67	48.51±1.05
	FAAS	0.88±0.06	15.33± 1.16	11.71±0.59	7.39± 0.70
K8	ICP-OES	5.87± 0.05	29.58±0.10	49.39±0.75	180.44±2.34
	FAAS	2.13±0.07	43.36± 1.91	62.55±1.80	98.08±5.52
K9	ICP-OES	3.47± 0.11	19.06±0.04	36.14±0.28	117.08±1.84
	FAAS	ND	20.11± 0.57	35.98±1.88	54.01±4.96
K10	ICP-OES	1.59± 0.12	21.53±0.13	45.45±1.12	83.33±2.71
	FAAS	0.03±0.06	29.69± 1.76	23.92±1.94	31.43±0.37
K11	ICP-OES	1.24± 0.02	10.93±0.05	28.32±0.22	57.87±0.54
	FAAS	0.16 ±0.02	29.50± 0.88	20.75±1.54	21.94±0.56
K12	ICP-OES	0.51± 0.03	6.33±0.10	16.19±0.41	29.86±1.42
	FAAS	ND	20.31± 2.39	9.51± 0.60	7.95± 1.60
K13	ICP-OES	0.74± 0.08	11.67±0.17	20.11±0.36	41.96±1.16
	FAAS	0.55±0.04	18.55± 1.91	11.20±0.84	7.33± 0.61
K14	ICP-OES	0.50± 0.09	5.99±0.05	15.73±0.35	29.97±2.62
	FAAS	0.53 ±0.10	8.23±0.62	6.16± 0.63	6.20± 0.50
K15	ICP-OES	1.87± 1.37	11.68±4.	25.47±12.72	16.82±0.38
	FAAS	0.62±0.03	4.86±0.17	17.66±0.69	8.37± 0.50
K16	ICP-OES	0.74± 0.09	12.33±0.07	28.86±0.62	43.91±0.52
	FAAS	0.59±0.04	4.46±0.52	9.25± 0.60	7.57± 0.37
K17	ICP-OES	1.99± 0.06	16.64±0.12	43.47±1.38	80.97±1.92
	FAAS	0.52±0.12	5.65±0.30	9.18± 0.23	9.42± 0.48
K18	ICP-OES	0.73± 0.10	13.72±0.23	19.84±0.17	46.30±3.15
	FAAS	0.57±0.05	5.06±0.30	9.56± 0.53	8.53± 1.33
K19	ICP-OES	3.71± 0.05	24.73±0.10	81.85±1.60	151.34±2.32
	FAAS	0.87±0.06	19.73± 1.02	47.21±3.24	45.37±0.87
K20	ICP-OES	1.46± 0.06	10.49±0.10	24.88±0.34	44.63±0.33
	FAAS	1.26±0.08	20.54± 1.00	66.61±5.72	47.69±1.09
K21	ICP-OES	4.27± 0.06	28.71±0.07	79.97±0.57	187.47±1.74
	FAAS	1.19±	29.96± 2.36	61.98±3.77	51.54±3.06
K22	ICP-OES	16.5±0.04	16.35±0.04	21.80±0.23	55.25±2.98
	FAAS	1.07±0.12	7.80±0.85	18.19±1.44	17.90±0.93
K23	ICP-OES	0.50± 0.06	6.03±0.07	40.18±1.04	30.64±2.08
	FAAS	0.37±0.03	7.40±0.82	8.11± 0.40	10.19±0.31
K24	ICP-OES	2.69± 0.12	15.16±0.13	35.21±0.93	84.60±0.94
	FAAS	0.65±0.10	7.13±0.96	15.50±1.64	19.14±2.16
K25	ICP-OES	1.99± 0.06	11.19±0.07	16.92±0.09	62.19±0.94
	FAAS	1.35±0.18	17.45± 0.81	10.85±0.64	8.90± 0.82

K26	ICP-OES	4.23± 0.04	14.92±0.14	28.59±0.95	101.94±3.59
	FAAS	1.47±0.17	16.85± 0.76	11.55±0.80	11.64±1.60
K27	ICP-OES	10.7± 0.12	10.67±0.12	19.77±0.35	47.39±2.36
	FAAS	0.98±0.14	15.13± 1.41	8.90± 0.88	7.31± 0.82
K28	ICP-OES	0.72± 0.04	8.24±0.05	42.29±1.68	37.99±0.76
	FAAS	1.15±0.09	15.94± 1.26	6.94± 0.49	5.94± 0.65
K29	ICP-OES	1.68± 0.02	16.83±0.05	16.11±0.15	70.19±0.87
	FAAS	1.50±0.10	15.13± 1.54	10.01±1.04	19.25±1.30
K30	ICP-OES	1.47±0.20	13.51±0.22	19.16±0.44	62.41±3.60
	FAAS	1.09±0.09	14.69± 1.35	9.98± 0.88	8.52± 0.95
K31	ICP-OES	3.22±0.08	18.82±0.08	25.76±0.42	113.42±1.94
	FAAS	1.30±0.22	2.06±0.84	17.31±1.06	9.96± 0.48
K32	ICP-OES	0.76± 0.04	6.32±0.15	16.94±0.25	41.56±1.77
	FAAS	0.90±0.21	ND	6.79± 0.60	4.19± 0.48
K33	ICP-OES	0.73± 0.03	10.48±0.06	14.62±0.21	40.69±1.73
	FAAS	0.74±0.21	1.43±0.37	2.70± 0.25	8.70± 0.79
K34	ICP-OES	1.10± 0.05	5.30±0.15	6.39±0.10	21.92±0.09
	FAAS	0.82±0.10	ND	2.57± 0.20	11.43±0.48
K35	ICP-OES	0.25± 0.05	3.48±0.06	15.92±0.20	22.14±1.08
	FAAS	0.71±0.09	ND	6.70± 0.54	10.80±0.96
K36	ICP-OES	0.97± 0.06	6.30±0.17	12.35±0.20	37.79±0.81
	FAAS	1.27±0.03	6.13±0.19	10.02±1.12	7.04± 0.61
K37	ICP-OES	1.72± 0.02	10.55±0.08	37.54±0.28	68.20±2.87
	FAAS	1.23±0.08	8.20±0.38	12.04±0.09	3.20± 0.23
K38	ICP-OES	0.25± 0.07	6.28±0.01	18.58±0.24	26.12±0.77
	FAAS	0.53±0.10	7.00±0.33	11.53±0.62	5.05± 0.83
K39	ICP-OES	1.26± 0.10	9.79±0.06	22.60±0.37	48.47±1.00
	FAAS	1.04±0.20	11.25±0.86	11.31±2.48	8.89± 1.19
K40	ICP-OES	1.98± 0.02	13.84±0.03	20.27±0.32	76.62±2.18
	FAAS	0.72±0.03	11.36± 0.75	11.43±1.07	21.85±1.28
K41	ICP-OES	1.24±0.01	9.68±0.07	20.36±0.28	51.14±1.26
	FAAS	1.30±0.08	6.46±0.94	17.46±0.34	7.50± 0.28
K42	ICP-OES	1.76±0.02	5.04±0.19	19.15±0.32	39.82±0.82
	FAAS	1.79±0.01	ND	10.30±0.35	8.08± 0.95
K43	ICP-OES	0.51±0.04	4.82±0.08	16.98±0.19	29.14±1.22
	FAAS	1.78±0.02	6.98±2.42	10.41±0.72	6.19± 0.95
K44	ICP-OES	ND	3.18±0.06	16.38±0.37	19.80±1.00
	FAAS	ND	1.26±0.49	10.01±0.35	7.95± 1.00
K45	ICP-OES	0.25±0.03	3.52±0.02	15.09±0.31	21.38±0.12
	FAAS	1.78±0.01	ND	9.92± 0.63	7.95± 0.38
K46	ICP-OES	0.5± 0.06	6.01±0.15	18.77±0.39	33.53±1.04
	FAAS	1.78±0.01	14.21± 0.81	11.95±0.95	9.85± 0.76
K47	ICP-OES	1.00±0.02	7.99±0.09	19.72±0.59	38.69±1.54
	FAAS	1.77±0.00	8.66±0.59	12.33±1.45	12.37±0.95
K48	ICP-OES	ND	3.47±0.07	13.88±0.32	19.82±2.84
	FAAS	ND	5.43±0.39	7.62± 0.86	4.42± 0.22

ND = Not detected

Appendix 10. Concentration (µg/L) of Cd, Cr, Cu and Pb in parent water samples $K_1 - K_{48}$

PARENT WATER SAMPLES					
Samples		Cd	Cr	Cu	Pb
K1	ICP-OES	2.00±0.09	202.20±0.33	28.26±0.14	27.28±0.45
	FAAS	ND	198.33±0.35	ND	6.67±0.91
K2	ICP-OES	1.68±0.23	76.91±0.19	11.63±0.10	14.19±1.12
	FAAS	ND	63.33±0.40	ND	6.67±0.97
K3	ICP-OES	1.20± 0.09	75.18±0.29	10.99±0.10	2.54± 4.50
	FAAS	ND	175.18±0.20	ND	1.21±0.07
K4	ICP-OES	1.10± 0.15	66.75±0.35	9.46± 0.05	8.81± 2.14
	FAAS	ND	166.75±0.30	ND	6.67±0.72
K5	ICP-OES	1.83± 0.22	104.40±0.22	34.88±0.17	14.82±2.11
	FAAS	ND	184.40±0.25	ND	11.70±0.47
K6	ICP-OES	0.87± 0.26	92.69±0.41	17.21±0.06	4.74± 1.88
	FAAS	ND	92.69±0.25	ND	3.85±0.56
K7	ICP-OES	0.91± 0.20	57.79±0.18	10.63±0.19	3.59± 1.63
	FAAS	ND	107.79±0.40	ND	2.60±0.55
K8	ICP-OES	1.00± 0.13	33.98±0.50	19.68±0.10	14.48±1.30
	FAAS	ND	29.09±0.10	ND	12.09±0.35
K9	ICP-OES	1.61± 0.09	41.30±0.14	20.09±0.07	19.89±3.48
	FAAS	ND	37.47±0.12	ND	17.43±0.21
K10	ICP-OES	0.99± 0.30	13.10±0.19	9.83± 0.12	12.81±1.01
	FAAS	ND	11.49±0.21	ND	10.96±0.61
K11	ICP-OES	0.93± 0.26	11.41±0.20	13.00±0.05	10.89±0.86
	FAAS	ND	10.99±0.06	ND	8.08±0.38
K12	ICP-OES	1.14± 0.12	11.87±0.15	32.18±0.09	17.12±0.79
	FAAS	ND	11.49±0.25	ND	18.06±0.40
K13	ICP-OES	1.16± 0.01	18.42±0.17	35.08±0.08	24.66±1.35
	FAAS	ND	ND	ND	ND
K14	ICP-OES	1.37± 0.05	29.92±0.42	17.82±0.14	21.28±3.08
	FAAS	ND	ND	33.69±1.46	ND
K15	ICP-OES	1.16± 0.10	12.50±0.43	22.58±0.23	15.76±1.33
	FAAS	ND	ND	27.48±0.40	ND
K16	ICP-OES	1.10± 0.25	8.45± 0.25	24.13±0.12	15.42±0.14
	FAAS	ND	ND	26.60±0.70	ND
K17	ICP-OES	1.03± 0.13	8.41± 0.24	22.47±0.24	15.84±1.54
	FAAS	ND	ND	10.64±0.36	ND
K18	ICP-OES	1.51± 0.18	15.52±0.15	32.28±0.12	27.87±4.50
	FAAS	ND	ND	33.69±0.23	ND
K19	ICP-OES	0.70± 0.14	118.50±0.52	15.01±0.10	12.87±2.03
	FAAS	ND	28.50±0.26	13.61±1.01	9.17±0.06
K20	ICP-OES	0.95± 0.19	45.43±0.33	22.76±0.41	19.92±1.73
	FAAS	ND	45.43±0.47	ND	17.07±0.53
K21	ICP-OES	0.89± 0.15	77.15±0.35	16.83±0.22	16.57±2.67
	FAAS	ND	77.15±0.74	ND	19.38±0.40
K22	ICP-OES	1.07± 0.11	201.40±0.78	11.50±0.10	4.99± 1.05
	FAAS	ND	201.40±0.75	ND	3.17±0.21
K23	ICP-OES	0.83± 0.25	91.12±0.33	15.98±0.16	8.36± 1.87
	FAAS	ND	144.61±1.78	0.10±0.01	ND
K24	ICP-OES	0.85± 0.23	138.70±0.46	13.76±0.15	6.06± 1.41
	FAAS	ND	106.80±0.61	0.97±0.06	4.57±0.23
K25	ICP-OES	0.71± 0.18	119.30±0.71	12.04±0.15	7.99± 2.18

	FAAS	ND	84.14±0.81	0.67±0.32	ND
K26	ICP-OES	0.91± 0.11	86.07±0.74	4.97± 0.20	7.96± 0.74
	FAAS	ND	93.85±0.51	0.83±0.51	4.57±0.15
K27	ICP-OES	0.90± 0.07	133.10±0.44	12.65±0.06	5.02± 1.62
	FAAS	ND	66.21±0.50	0.17±0.21	ND
K28	ICP-OES	1.36± 0.03	29.28±0.28	19.65±0.11	22.99±0.11
	FAAS	ND	74.76±0.70	ND	ND
K29	ICP-OES	1.16± 0.11	84.93±0.54	9.81± 0.06	7.73± 1.74
	FAAS	ND	ND	7.02±0.26	4.57±0.12
K30	ICP-OES	1.04± 0.13	27.79±0.27	10.21±0.16	8.59± 1.54
	FAAS	ND	ND	8.95±1.12	4.57±0.10
K31	ICP-OES	1.16± 0.12	55.19±0.14	14.96±0.09	6.08± 4.55
	FAAS	ND	ND	ND	13.90±0.20
K32	ICP-OES	1.07± 0.07	68.76±0.53	9.73± 0.15	5.04± 1.42
	FAAS	ND	ND	ND	3.09±0.20
K33	ICP-OES	1.17± 0.18	80.27±0.72	55.71±0.32	19.59±0.54
	FAAS	ND	59.52±0.55	ND	4.06±0.12
K34	ICP-OES	1.01± 0.17	51.42±0.40	32.18±0.09	4.82± 1.29
	FAAS	ND	ND	ND	4.06±0.29
K35	ICP-OES	1.29± 0.19	93.68±0.42	39.39±0.26	7.43± 0.97
	FAAS	ND	117.65±0.70	ND	3.90±0.17
K36	ICP-OES	0.95± 0.06	37.54±0.31	14.60±0.22	8.57± 0.84
	FAAS	ND	254.90±0.78	ND	12.77±0.12
K37	ICP-OES	1.00± 0.18	44.34±0.38	13.57±0.07	6.20± 2.60
	FAAS	ND	385.62±1.62	ND	19.05±0.06
K38	ICP-OES	0.84± 0.10	25.55±0.14	9.38± 0.10	10.88±2.10
	FAAS	ND	143.79±0.58	ND	6.98±0.10
K39	ICP-OES	1.00± 0.05	30.81±0.14	12.57±0.08	7.26± 1.75
	FAAS	ND	143.79±0.15	ND	3.17±0.29
K40	ICP-OES	0.82± 0.11	58.53±0.36	14.52±0.32	10.24±2.18
	FAAS	ND	183.01±0.15	ND	19.05±0.17
K41	ICP-OES	1.01± 0.26	90.89±0.68	14.34±0.13	10.38±2.53
	FAAS	ND	ND	ND	4.92±0.10
K42	ICP-OES	0.98± 0.16	69.35±0.32	9.68± 0.16	6.05± 2.03
	FAAS	ND	ND	ND	9.05±0.25
K43	ICP-OES	0.67± 0.15	72.36±0.50	7.53± 0.14	8.86± 2.13
	FAAS	ND	23.26±0.26	ND	2.73±0.25
K44	ICP-OES	1.13± 0.09	72.67±0.42	19.23±0.09	18.69±1.07
	FAAS	ND	46.51±0.46	2.65±0.50	ND
K45	ICP-OES	0.87± 0.11	70.38±0.37	6.75± 0.20	6.10± 1.52
	FAAS	ND	23.26±1.40	3.24±0.70	7.58±0.40
K46	ICP-OES	1.05± 0.13	47.99± 0.11	8.96± 0.30	7.66± 1.63
	FAAS	ND	15.50±0.72	14.02±3.70	12.73±0.52
K47	ICP-OES	0.87±0.11	70.38±0.37	6.75±0.20	6.10±1.52
	FAAS	ND	ND	5.7±0.20	7.58±0.25
K48	ICP-OES	1.05±0.13	47.99±0.11	8.96±0.30	7.66±1.63
	FAAS	ND	69.77±0.00	ND	7.58±0.40

ND = Not detected

Appendix 11. Leachable concentration (µg/g) of Cd, Cr, Cu and Pb found on the surface of green algae by washing the algae with dilute HCl

	FAAS DATA FOR ACID-LEACHED ALGAE			
Sample	Cd	Cr	Cu	Pb
K1	ND	7.29±0.03	4.21±0.13	0.07±0.01
K2	5.78±0.45	16.32±0.60	4.27±0.11	0.17±0.02
K3	ND	8.33±1.04	2.73±0.04	0.05±0.00
K4	1.60±0.04	21.53±2.08	4.03±0.16	0.10±0.01
K5	0.48±0.05	5.23±0.76	10.89±1.03	0.22±0.03
K6	0.49±0.04	6.49±1.58	4.06±0.07	0.11±0.02
K7	0.29±0.05	12.05±0.76	7.21±0.15	0.15±0.02
K8	1.13±0.04	14.07±0.44	38.32±0.81	1.24±0.01
K9	ND	1.34±0.33	31.05±0.77	1.03±0.03
K10	ND	1.92±0.33	20.40±0.82	0.43±0.05
K11	ND	9.77±0.57	17.90±1.09	0.38±0.02
K12	ND	6.13±0.88	7.09±0.47	0.15±0.02
K13	0.17±0.03	0.69±0.17	6.60±0.77	0.14±0.01
K14	0.44±0.12	3.47±0.17	5.20±1.11	0.08±0.01
K15	0.40±0.06	2.98±0.30	6.69±1.06	0.15±0.00
K16	0.41±0.02	2.58±0.17	8.36±0.07	0.09±0.01
K17	0.20±0.01	1.49±0.30	6.31±0.43	0.07±0.01
K18	0.27±0.03	1.69±0.17	6.66±0.50	0.07±0.02
K19	0.51±0.01	4.09±0.49	39.52±3.57	0.51±0.05
K20	0.54±0.01	4.38±0.31	43.50±4.33	0.51±0.07
K21	0.53±0.09	18.20±0.82	38.38±2.91	0.48±0.05
K22	0.59±0.01	4.18±0.71	0.94±0.07	0.20±0.01
K23	0.17±0.01	4.11±0.71	4.39±0.62	0.12±0.01
K24	0.58±0.02	0.59±0.20	4.58±0.64	0.13±0.01
K25	0.72±0.06	6.21±0.38	3.75±0.44	0.14±0.01
K26	1.38±0.21	6.06±0.31	3.80±0.37	0.15±0.00
K27	0.77±0.18	4.29±0.24	3.83±0.28	0.07±0.01
K28	0.37±0.12	8.06±0.69	3.67±0.17	0.09±0.00
K29	0.54±0.14	7.80±0.80	4.51±0.46	0.16±0.01
K30	0.58±0.04	8.86±0.91	6.33±0.43	0.13±0.01
K31	0.16±0.02	5.69±0.02	2.31±0.17	0.19±0.01
K32	0.42±0.04	4.82±0.19	1.47±0.20	0.06±0.01
K33	0.04±0.02	5.69±0.86	0.82±0.13	0.11±0.01
K34	0.06±0.02	4.60±0.19	1.64±0.14	0.13±0.01
K35	0.11±0.03	5.58±0.50	0.26±0.03	0.23±0.03
K36	0.15±0.03	7.44±0.19	2.42±0.09	0.17±0.01
K37	0.77±0.14	5.69±0.01	1.62±0.20	0.07±0.01
K38	0.70±0.04	4.82±0.19	2.54±0.29	0.05±0.01
K39	0.56±0.11	5.69±0.86	3.11±0.31	0.09±0.01
K40	0.52±0.06	4.60±0.19	2.07±0.22	0.14±0.01
K41	0.74±0.13	5.58±0.50	5.68±0.34	0.20±0.03
K42	0.34±0.13	7.44±0.19	5.93±0.22	0.13±0.01
K43	1.72±0.01	ND	5.26±0.55	0.13±0.03
K44	1.69±0.01	ND	4.31±0.27	0.02±0.00
K45	1.69±0.02	ND	4.52±0.85	0.03±0.00
K46	1.69±0.01	ND	3.66±0.05	0.05±0.01
K47	1.70±0.01	1.81±2.58	3.86±0.67	0.04±0.01

K48	1.72±0.01	ND	4.65±0.70	0.01±0.00
K49	1.69±0.02	ND	2.58±0.28	0.06±0.01
K50	1.69±0.01	ND	3.52±0.14	0.09±0.01

ND = Not detected

Appendix 12. The t–distribution table

t Table

cum. prob	$t_{.50}$	$t_{.75}$	$t_{.80}$	$t_{.85}$	$t_{.90}$	$t_{.95}$	$t_{.975}$	$t_{.99}$	$t_{.995}$	$t_{.999}$	$t_{.9995}$
one-tail	0.50	0.25	0.20	0.15	0.10	0.05	0.025	0.01	0.005	0.001	0.0005
two-tails	1.00	0.50	0.40	0.30	0.20	0.10	0.05	0.02	0.01	0.002	0.001
df											
1	0.000	1.000	1.376	1.963	3.078	6.314	12.71	31.82	63.66	318.31	636.62
2	0.000	0.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925	22.327	31.599
3	0.000	0.765	0.978	1.250	1.638	2.353	3.182	4.541	5.841	10.215	12.924
4	0.000	0.741	0.941	1.190	1.533	2.132	2.776	3.747	4.604	7.173	8.610
5	0.000	0.727	0.920	1.156	1.476	2.015	2.571	3.365	4.032	5.893	6.869
6	0.000	0.718	0.906	1.134	1.440	1.943	2.447	3.143	3.707	5.208	5.959
7	0.000	0.711	0.896	1.119	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	0.000	0.706	0.889	1.108	1.397	1.860	2.306	2.896	3.355	4.501	5.041
9	0.000	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	0.000	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	0.000	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	0.000	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	0.000	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	0.000	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	0.000	0.691	0.866	1.074	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	0.000	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	0.000	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	0.000	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	0.000	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	0.000	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	0.000	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	0.000	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	0.000	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807	3.485	3.768
24	0.000	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	0.000	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	0.000	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	0.000	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	0.000	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	0.000	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756	3.396	3.659
30	0.000	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750	3.385	3.646
40	0.000	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704	3.307	3.551
60	0.000	0.679	0.848	1.045	1.296	1.671	2.000	2.390	2.660	3.232	3.460
80	0.000	0.678	0.846	1.043	1.292	1.664	1.990	2.374	2.639	3.195	3.416
100	0.000	0.677	0.845	1.042	1.290	1.660	1.984	2.364	2.626	3.174	3.390
1000	0.000	0.675	0.842	1.037	1.282	1.646	1.962	2.330	2.581	3.098	3.300
Z	0.000	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576	3.090	3.291
	0%	50%	60%	70%	80%	90%	95%	98%	99%	99.8%	99.9%
	Confidence Level										

Appendix 13. Concentration factors for Cd, Cr, Cu and Pb by green algae

Sample id	Cd	Cr	Cu	Pb
K1	3001.51	109.93	1201.91	3973.30
K2	5434.55	170.76	1572.89	7982.66
K3	1473.27	451.63	2860.66	27174.09
K4	4119.16	271.03	2893.43	11087.66
K5	1187.06	128.52	350.73	3767.92
K6	625.40	129.55	1088.44	10236.56
K7	6427.13	511.93	4646.17	50303.90
K8	5044.17	560.90	1836.31	8085.58
K9	991.23	521.33	2262.55	4189.71
K10	2330.07	834.27	2879.98	4517.86
K11	542.41	554.42	1245.72	2741.81
K12	654.47	983.01	624.90	2450.72
K13	430.98	325.41	448.53	1215.33
K14	1366.34	390.45	1429.14	984.34
K15	639.04	986.68	1278.14	2785.99
K16	2905.09	1970.38	1801.39	5251.21
K17	1035.12	1630.66	883.10	2923.03
K18	2458.05	1593.30	2535.62	5430.04
K19	2081.67	88.50	1657.43	3468.08
K20	5576.34	632.05	3513.69	9411.16
K21	18477.77	211.96	1295.53	3334.50
K22	469.40	29.93	3493.98	6136.16
K23	3595.57	339.81	2396.72	14239.45
K24	3085.35	122.85	1058.54	7442.44
K25	8144.03	107.56	2077.96	16832.78
K26	14946.34	89.45	1642.36	5930.29
K27	790.35	95.78	5062.71	4771.77
K28	1867.58	126.42	1273.18	13976.96
K29	1087.97	461.53	975.30	4026.31
K30	2775.36	221.61	2624.61	14665.44
K31	732.36	227.52	1659.63	4446.29
K32	683.17	152.38	1502.71	8078.62
K33	953.95	66.00	114.77	1119.03
K34	254.35	67.73	2059.83	4596.08
K35	1031.94	67.23	846.21	5086.92
K36	1815.40	281.02	3403.16	7957.55
K37	6.27	157.39	471.79	638.73
K38	1656.53	220.89	1665.56	7819.96
K39	2362.32	541.72	2160.65	7042.18
K40	1236.37	314.26	1619.53	7049.21
K41	2887.26	86.12	1319.09	3888.53
K42	552.72	52.98	1184.05	2807.65
K43	0.00	45.83	2279.63	3274.54
K44	375.95	48.66	2003.26	2413.17
K45	443.71	82.65	976.02	1794.20
K46	1154.34	113.50	2922.84	6346.07
K47	0.00	72.28	1548.56	2586.66