

**INVESTIGATION OF POLLUTANT LEVELS AND EFFICIENCY OF HEAVY
METAL REMOVAL USING BROWN ALGAE (*Sargassum* sp) BIOSORBENT:
A CASE STUDY OF MIGORI RIVER, KENYA**

BY

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JULY, 2013

DECLARATION

DECLARATION BY THE CANDIDATE

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DEDICATION

This work is dedicated to my beloved wife Pamela, for her support, and my children.

Special dedications also go to my late mother Siprosa.

ABSTRACT

An increase in population initiating rapid industrialization has been found to consequently increase the effluents and domestic wastewater into the aquatic system. This has resulted in heavy metal pollution which has become one of the most serious environmental problems today. Similarly, excessive nutrients have an effect of increasing the accumulation of organic matter through biosynthesis. Therefore, there is need to assess the impact of these agricultural activities and urban runoff on heavy metal pollution and nutrient levels of the river. Since conventional methods for the removal of heavy metals from wastewater are often cost prohibitive, a search for cheap methods of removal of heavy metals has been on the increase and one such method is use of algae. The ability of algae to adsorb heavy metal ions (Ni^{2+} , Pb^{2+} , Zn^{2+} and Cu^{2+}) by biosorption has been investigated in river Migori. Factors influencing the biosorption of the heavy metals, such as contact time at constant pH and temperature, were studied. Six sampling points, during wet and dry seasons, were established along the river, upstream and downstream of Migori town. The metal ion concentrations were determined at 15 minutes intervals using spectrophotometer. The results showed that Cu^{2+} varied between 0.02 and 0.29 mg/L in wet season and between 0.07 and 0.26 mg/L in dry season. Zn^{2+} ranged between 0.10 and 0.28 mg/L in wet season and between 0.09 and 0.22 mg/L in dry season. Lead ions and Ni^{2+} ranged between 0.00 to 0.07 mg/L and 0.02 to 0.08 mg/L, respectively in wet season, while in dry season their respective range was between 0.00 to 0.04 mg/L and 0.02 and 0.06 mg/L. The results obtained after biosorption showed that Cu^{2+} ions were most biosorbed (89%) while Pb^{2+} were least biosorbed (70%), and brown algae achieved the percent removal in the order of $\text{Cu}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Pb}^{2+}$. The equilibrium biosorption data were analyzed using two isotherm models (Langmuir and Freundlich). The results indicate that Langmuir model provide best correlation of experimental data. From the results, brown algae have a potential for being used for decontamination of waters containing heavy metal ions. Samples were also analysed for nutrients (nitrates and phosphates), chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Phosphates and NO_3^- were both determined by spectrophotometer while BOD was analyzed using Winkler method and COD by dichromate digestion method. Phosphates and NO_3^- ranged between 0.6 to 2.6 mg/L and 0.6 to 2.3, respectively. The respective BOD and COD varied between 7-25 mg/L and 14-50 mg/L. Although the nutrient levels were within WHO standards, the river seemed contaminated by runoff from the town since the levels were higher downstream than upstream of town. The COD and BOD levels were higher than the WHO standards, but the levels were higher downstream of the town.

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LIST OF ABBREVIATIONS

APHA	-American Public Health Association
AWWA	-American Water Works Association
ELDOWAS	-Eldoret Water and Sanitation Company Ltd.
BOD	-Biochemical Oxygen Demand
COD	-Chemical Oxygen Demand
DO	-Dissolved Oxygen
mg/L	-milligrams per liter
SD	-Standard deviation
WEF	-Water Environment Federation
WHO	-World Health Organization

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Throughout world history, water sources have been the centre of life, providing habitat and sustenance for animals and plants alike. Fresh water is a finite resource, essential for agriculture, industry and human existence. Without fresh water of adequate quantity and quality, sustainable development will not be possible. Water pollution and wasteful use of fresh water threaten development projects and make water treatment difficult in order to produce safe drinking water.

The increasing concern about pollution has been accompanied by more sensitive means of detecting pollutants and measuring their concentrations (Troeh *et al.*, 1991). This increased sensitivity identifies pollutants that would have escaped unnoticed and raises the question of how much hazard such tiny amounts can cause.

Agricultural land use without environmental safeguard to prevent over-application of agrochemicals is causing widespread deterioration of the water ecosystem. Large amounts of fertilizers (N, P, and K) are being applied in the world as mineral fertilizers which are misused. Agronomic crops such as corn, cotton, and tobacco receive more N, P and K fertilizers than in other crops (Troeh *et al.*, 1991).

The pollution of the environment with toxic metals is a result of many human activities, such as mining and metallurgy, and the effects of these metals on the ecosystems are of large economic and public health significance. The inadequacy of our conventional

methods of river dumping is further exposed by the death of fishes and even deforestation of nearby trees on the shore, affecting human and animal lives (Oboh *et al.*, 2009). Therefore the study of the existing disposal methods, facilities, and attitudes on waste management is essential in order to make a positive impact on our environmental hygiene.

1.2 Study area

Migori River passes through Migori town, which is situated in Western part of Kenya, Nyanza Province (Fig. 1.1). The town is 63 km south of Kisii town and 22 km north of the Tanzanian border. The region, with a population of 82,000 people, is not served with adequate piped water. Most of the residents depend on the raw water from the river. The town is also poorly served with sewerage system, making most of the wastes from residential areas to find their way into the river through run off.

Migori town is a major agricultural growing area and is situated on both sides of the river, downstream the main agricultural region. The main agricultural activities include tobacco and sugarcane growing besides other crops such as maize. Rainfall is in two seasons, like most parts of Kenya, and the highest rainfall is between the months of March and May. The second season is between the months of September and November. The average rainfall is approximately 1200 mm, with an altitude of about 1500 metres above sea level. The soils are well drained and tending to loamy. This favours the cultivation of tobacco, sugarcane, maize, beans, coffee, groundnuts and vegetables.

Figure 1.1 Site Map of Migori Town showing sampling points along the Migori River

1.3 Statement of the problem

Water pollution is a major problem in the global context. It is the leading worldwide cause of deaths and diseases. Quality of water is of paramount importance because of its role to human health, aquatic life, ecological integrity and sustainable economic growth (Ajibade, 2004). Many sources of pollution, including sewage and fertilizers contain nutrients such as nitrates and phosphates, and heavy metals. The rapid development of urban economy, human settlement and industrial development has created severe pollution loads in inland surface water bodies in urban localities. Most of the rivers in urban areas in the developing world are the end point of effluents discharged from industries and untreated or partially treated sewage (Phiri *et al.*, 2005).

The control of water pollution is important not only for amenity and public health reasons, but also because clean water for domestic, industrial and agricultural use is in short supply, even in comparatively wet countries like United Kingdom (Mason, 1991).

Migori River discharges its water into Lake Victoria. Its transboundary importance lies on its volumetric water contribution into Lake Victoria as an agent for carrying pollutants into the lake. The river system supports major farming activities, the system experiences both non-point and point sources like runoff from Migori town as reported by the National Nile basin water quality monitoring baseline report for Kenya (NBI, 2005). Moreover, St. Joseph's Mission Hospital Ombo and Prinsals Limited fish factory are some of the culprits discharging raw or half treated wastes into the river.

1.4 Justification

Clean water is valuable for many reasons. Man depends on streams and rivers for drinking water and recreation. Streams, rivers and springs are the main sources of water for both rural and urban abstraction. The quality of the water from these sources is therefore of major concern.

Excessive nutrients have an effect of increasing the accumulation of organic matter through biosynthesis (UNEP and GEMS, 2006). The increased amount of organic matter reduces the dissolved oxygen (DO) content of the water body, and increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values. Therefore, there is need to assess the impact of these agricultural activities and urban runoff on the heavy metal and nutrient levels and hence pollution level of the river. It is also important to investigate how the heavy metals can be removed from polluted water. Conventional methods for the removal of heavy metals from polluted waters are often cost prohibitive hence there is need for cheap methods for effluent treatment.

Rivers are important natural assets which can become a liability and a source of nuisance and health hazard if use of the river is misused. Many rivers in the world have been subjected to this fate (IAWPRC, 1988). Examples of such rivers are; Thames River in UK (cleaned by 1974), Toyohira River, in Saporu Japan (restored in 1978) and Singapore's urban rivers which were finally restored by 1987, after 10 years of cleaning up (IAWPRC, 1988). Our own example is the Nairobi River where millions of shillings is being used to try and restore it.

A statement by environmental experts, and reported by the Daily Nation of Tuesday 13th July, 2010, read “Environmentalists have warned that River Migori will die if no action is taken to save it. The experts are worried about the falling water volume and pollution in the river that cuts across Migori town”.

1.5 Objectives

1.5.1 General objective

The general objective of the study was to determine the pollutant levels of nutrients, physicochemical parameters and the removal of heavy metals by biosorption using algae.

Specific objectives

The specific objectives were:

- 1) To determine levels of heavy metals (Ni^{2+} , Pb^{2+} , Zn^{2+} , Cu^{2+}) in River Migori.
- 2) To evaluate the removal of heavy metals by adsorption using brown algae.
- 3) To investigate the effect of pH on biosorption of heavy metals.
- 4) To investigate the effect of contact time on biosorption of heavy metals.
- 5) To determine concentrations of the nutrients (nitrates and phosphates) in River Migori.
- 6) To determine levels of pH, BOD, COD in River Migori.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General

Rapid population growths, land development along river basins, urbanization and industrialization have subjected rivers to increased stress, giving rise to water pollution and environmental deterioration (Sumok, 2001; Dobson and Burgess, 2007). Surface water pollution issue has been one of the most serious problems in developing countries. In addition, other pressures faced by developing countries have led to pollution of water resource (Dobson and Burgess, 2007) causing serious environmental pollution and even threatening human life (Wang *et al.*, 2000; Nilanjana *et al.*, 2008).

Comprising over 70% of the earth's surface, water is the most valuable natural resource existing on our planet. Although this fact is widely recognized, pollution of water resources is a common occurrence. In particular, potable water has become greatly affected, and in many instances has lost its original purpose. There are many sources of water pollution, but two main general categories exist: direct and indirect contaminant sources. Direct sources include effluent outfalls from industries, refineries and waste treatment plants; whereas, indirect sources include contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water. In general, contaminants come under two broad classes, viz. organic and inorganic. Some organic water pollutants include industrial solvents, volatile organic compounds, insecticides, pesticides and food processing wastes, etc. Inorganic water pollutants

include metals, fertilizers and acidity caused by industrial discharges, etc. The scope of this study takes into consideration heavy metal and nutrients, which come under inorganic, and BOD and COD, which come under organic pollutants. They are common contaminants and are known to be toxic and carcinogenic.

Pollution is contamination that makes water unclean or impure. Water is polluted physically, chemically or biologically. Water pollutants squander the resources that support life, and causes aesthetic, health and productivity problems. Water pollution and wasteful use of fresh water threaten development projects and make water treatment difficult in order to produce safe drinking water (Troeh *et al.*, 1991).

Water pollution is a major problem in the global context. It is the leading worldwide cause of deaths and diseases (Pink, 2006; West, 2006). Quality of water is of paramount importance because of its role to human health, aquatic life, ecological integrity and sustainable economic growth (Ajibade, 2004). A wide range of contaminants can reach the river either via ground water or through drainage ditches, including artificial fertilizer residues, insecticides, herbicides and pesticides and farmyard waste, all of which are potentially very harmful. The control of water pollution is important not only for amenity and public health reasons but also because clean water for domestic, industrial and agricultural use is in short supply, even in comparatively wet countries such as United Kingdom (Mason, 1991).

Many metals and their compounds have been found to be toxic, while others are also subjected to biomagnifications (Karvelas *et al.*, 2003). The presence of these heavy

metals in wastewater poses a problem for the environment as well as for the treatment process, because the disposal for both the treated water and activated sludge contaminated with heavy metals give rise to detrimental impacts on the environment. Metal ions present in the wastewater are characterized by their mobility in the liquid phase of the eco-system and by their toxicity to higher forms even at low concentrations (Addagalla *et al.*, 2009).

In addition, these metal ions are non-biodegradable and thus persistent, leading to both ecological and health problems. In view of the toxicity and in order to meet regulatory safe discharge standards, it is essential to remove heavy metals from wastewater both to decrease the amount of wastewater produced and to improve the quality of treated effluent, before it is released to the environment. Conventional in the field of wastewater treatment can be divided into two main phases (Ajaykumar *et al.*, 2008): i) generation of suspended solids from colloidal and dissolved solids by physical, chemical and biological means in addition to the already existing suspended solids; ii) separation of suspended solids by chemical and mechanical methods including sedimentation, floatation and filtration. A number of physico-chemical methods have been developed over the years to remove toxic metal ions from aqueous solution which include chelation extraction, chemical coagulation, evaporation, adsorption, chemical precipitation, ion exchange, electrochemical processes, flocculation and membrane processes (Gulnaz *et al.*, 2005; Ajaykumar., *et al.*, 2008)

2.2 Sources of Heavy Metals

River water is used to an increasing extent as drinking and irrigation water particularly in highly urbanized and industrialized areas. In addition to water pollution by nutrients or organic compounds, the heavy metal content in flowing waters has become one of the most important problems because of its toxic effect even in minor concentrations (Symder, 2001). The occurrence of heavy metals in aquatic system has also become a global phenomenon due to their carcinogenic and mutagenic nature (Mahiva *et al.*, 2008). A heavy metal is a metal with a specific gravity greater than about 5.0 g/m³, and is mostly toxic or poisonous.

Heavy metal pollution is one of the most important environmental problems today. Various industries produce and discharge wastes containing different heavy metals into the environment, such as mining and smelting of metal, surface finishing industry, energy and fuel production, fertilizer and pesticide industry and application, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace and atomic energy installation etc. Thus, metal as a kind of resource is also becoming a serious environmental pollution, threatening human health and ecosystem. Three kinds of heavy metals are of concern, including toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.) (Wang and Chen, 2006).

Heavy metals can be present in industrial, municipal, and urban runoff, which can be harmful to humans and aquatic life. Increased urbanization and industrialization are to

blame for an increased level of trace metals, especially heavy metals, in our waterways. Heavy metal contamination appears in aqueous waste streams of many industries such as metal plating facilities, mining operations and tanneries. Heavy metals are not biodegradable and tend to accumulate in living organisms, causing various diseases and disorders.

To many people, heavy metal pollution is a problem associated with areas of intensive industry. However, roadways and automobiles now are considered to be one of the largest sources of heavy metals (*Conservation Currents, 2005*). Zinc, copper, and lead are three of the most common heavy metals released from road travel, accounting for at least 90% of the total metals in road runoff. Smaller amounts of many other metals, such as nickel and cadmium, are also found in road runoff and exhaust.

About half of the zinc and copper contribution to the environment from urbanization is from automobiles. Brakes release copper, while tyre wear releases zinc. Motor oil also tends to accumulate metals as it comes into contact with surrounding parts as the engine runs, so oil leaks become another pathway by which metals enter the environment (*Conservation Currents, 2005*). On the road surface, most heavy metals become bound to the surfaces of road dust or other particulates. During precipitation, the bound metals will either become soluble (dissolved) or be swept off the roadway with the dust. In either case, the metals enter the soil or are channeled into a storm drain. Lead is present in petrol-based materials and many other industrial facilities. Other sources are auto exhaust, battery manufacturing, car batteries, cigarette smoke, coal combustion, cosmetics, glass production, lead pipes, lead-glazed earthenware pottery, paint, pencils,

pesticides, and lubricating oil and grease. Zinc is widely used in industry to make paint, rubber, dye, wood preservatives, and ointments and electroplating industries. It is found in motor oil, corrosion of galvanized parts and tyre wear.

Nickel comes from paint and powder batteries processing units, fertilizers, food processing, fuel oil combustion, industrial waste, stainless steel cookware, tea and tobacco smoke.

The source of copper is copper cookware, engine parts, brake emissions, copper pipes, fungicides, industrial emissions, insecticides and welding.

2.3 Effects of Heavy Metals

It has been established beyond any doubt that dissolved heavy metals into the environment pose a serious health hazard (Volesky, 1999). Heavy metals are dangerous because they tend to bioaccumulate (Volesky, 1999). Compounds accumulate in living things when they are taken up and stored faster than they are metabolized or excreted. Heavy metals are toxic to aquatic organisms at very low concentrations. The contamination of water by toxic heavy metal ions is a worldwide environmental problem. The most common and harmful heavy metals are lead, copper, nickel, chromium and zinc. They are stable elements that cannot be metabolized by the body and get passed up in the food chain to human beings (Oboh *et al.*, 2009). When waste is disposed into the environment, a further long-term hazard is encountered. There are possibly more problems from these metals, which interfere with normal body function, than have been considered in most medical circles. Since even low concentrations of heavy metals can

cause serious harm to aquatic systems, there is a need for controlling their emissions into the environment (Volesky, 1999). It is therefore important to very precisely measure the concentrations of these substances in water samples.

In humans, exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Various effects occur over a broad range of doses, with the developing foetus and infant being more sensitive than the adult. High levels of exposure may result in toxic biochemical effects in humans which in turn cause problems in the synthesis of haemoglobin, effects on the kidneys, gastrointestinal tract, joints and reproductive system, and acute or chronic damage to the nervous system (<http://www.tip2000.com/health/waterpollution.asp>, 2000).

Lead poisoning, which is so severe as to cause evident illness, is now very rare. At intermediate concentrations, however, there is persuasive evidence that lead can have small, subtle, subclinical effects, particularly on neuropsychological developments in children. Some studies suggest that there may be a loss of up to 2 IQ points for a rise in blood lead levels from 10 to 20µg/dl in young children (http://en.wikipedia.org/wiki/Lead_poisoning). Long term exposure to nickel can cause decreased body weight, heart and liver damage, and skin irritation (Bishop, 2002).

Copper is an essential element to human life, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation. People with Wilson's disease are at greater risk for health effects from overexposure to copper. Copper normally occurs

in drinking water from copper pipes, as well as from additives designed to control algal growth.

Heavy metals are major pollutants in marine, lake, river and ground waters as well as treated effluents and they can create serious environmental pollution and threaten biolife (Yu *et al.*, 1999; Bishop, 2002; Francois *et al.*, 2007). Heavy metals can be extremely toxic as they damage nerves, liver, kidney and bones. They also block functional groups of vital enzymes affecting the biochemical and physiological processes (Travieso *et al.*, 1999). For a number of years, researchers have been looking for cheaper and more effective methods to remediate heavy metal-contaminated water and reduce the growing public health risk (Farah and Sneddon, 1991).

2.4 Biosorption

An increase in population initiating rapid industrialization and agricultural activities was found to consequently increase the effluents and domestic wastewater into the aquatic ecosystem. Heavy metals are major toxicants found in such effluents and run offs which may adversely affect the biological treatment of wastewater and even conventional treatment of river water. Several methods are being used for the removal of heavy metal ions from aqueous wastes like chemical precipitation, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon, among others (Matheickal and Yu, 1999; Volesky, 1999; Nilanjana *et al.*, 2008). Each of these methods has its own merits and demerits. Chemical precipitation and electrochemical treatments are ineffective, especially when metal ion concentration in aqueous solution is lower than 50mg/L. Such treatments also produce large amounts of sludge to be treated

with great difficulties. Ion exchange, membrane technologies and activated carbon absorption processes are extremely expensive (Volesky, 1999; Oboh *et al.*, 2009). Therefore, the search for new cost-effective technologies for the removal of heavy metals from wastewaters has been directed towards biosorption, which is known for the last few decades (Hammaini *et al.*, 2006; Addagalla *et al.*, 2009). Algae, bacteria, fungi and yeasts have proved to be potential metal biosorbents, due to metal sequestering properties and can decrease the concentration of heavy metal ions in solution. Most of these methods involve high capital and regeneration costs of the materials.

Developments in the field of environmental biotechnology indicate that bacteria, actinomycetes, fungi, yeasts, algae and seaweeds can remove heavy metals from aqueous solutions by biosorption and hence have proved to be potential metal biosorbents (Volesky, 1999). The term biosorption is used to describe the accumulation of metal ions by material of biological origin. It is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals. Biosorption has also been defined as the property of certain biomolecules (or types of biomass) to bind and concentrate selected ions or other molecules from aqueous solutions (Volesky, 2007). Ahalya *et al.*, (2003) have defined biosorption as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake, while Gadd, (1992) has defined it as the removal of metal or metalloid species, compounds and particulates from solution by biological materials.

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolve species to be

sorbed (sorbate, metal ions) (Yasemin and Tez, 2007). Due to higher affinity of the sorbent for the sorbate species, the later is attracted and removed by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases.

The major advantages of biosorption over conventional treatment methods include low cost, high efficiency, minimization of chemical and biological sludge, and regeneration of bisorbent and possibility of metal recovery (Ajaykumar *et al.*, 2008). The disadvantages of biosorption are: early saturation, that is, when metal interactive sites are occupied, metal desorption is necessary prior to further use; the potential for biological process improvement (for example, through genetic engineering of cells) is limited because cells are not metabolizing; and there is no potential for biologically altering the metal valency state (Ahluwalia and Goyal, 2007).

The metal biosorption process by living cells is a two-step process. In the first step, metal ions are absorbed to the surface of cells by interactions between metals and functional groups displayed on the surface of cells by interactions between metals and functional groups displayed on the surface of cells. All the metal ions before gaining access to the cell membrane and cell cytoplasm come across the cell wall. The cell wall consists of a variety of polysaccharides and proteins and hence offers a number of active sites capable of binding metal ions. Difference in the cell wall composition among the different groups of micro organism, viz, algae, bacteria, cyan bacteria and fungi, cause

significant differences in the type and amount of metal ion binding to them. Algal cell walls are mainly cellulosic. The potential metal binding groups in this class of microbes are carboxylates, amines, imidazoles, phosphates, sulfhydryls, sulfates and hydroxyl. Of these, amines and imidazoles are positively charged when protonated and may build negatively charged when protonated and may build negatively charged metal complexes (Nilanjana *et al.*, 2008).

The first step, passive biosorption, is metabolism independent and proceeds rapidly by any one or a combination of the following metal binding mechanisms: coordination, complexation, ion exchange, physical absorption (for instance, electrostatic) or inorganic microprecipitation. Passive biosorption is a dynamic equilibrium of reversible absorption–desorption. Metal ions bound on the surface can be eluted by other ions, chelating agents or acids. In the second step, due to active biosorption, metal ions penetrate the cell membrane and enter into the cells (Nilanjana *et al.*, 2008).

Metal uptake by non-living cells is mainly in passive mode (Wang *et al.*, 2000). By investigating the biosorption of Cr (VI) and Fe (III) on *Streptococcus equisimilis*, *S. Cerevisiae* and *Aspergillus niger*, Goyal *et al.*, (2003) confirmed that the metal uptake by microorganisms occurs in two stages: passive uptake which takes immediately, and active uptake which takes place slowly. The first stage is thought to be physical absorption or ion exchange ion exchange at the cell surface, reaching the absorption equilibrium within 30-40 min. Therefore, passive mode is independent of energy, mainly through chemical functional groups of the material, comprising the cell and particularly cell wall.

The major factors that affect the biosorption processes are (i) initial metal ion concentration, (ii) temperature, (iii) pH, and (iv) biomass concentration in solution. pH seems to be the most important parameter in the biosorption processes. It affects the solution chemistry of the metal, the activity of the functional groups in the biomass and the competition of the metallic ions (Saeed *et al.*, 2005; Vijayaraghavan *et al.*, 2006). Biomass concentration in the solution seems influence the specific uptake; for lower values of biomass concentration leads to interference between the binding sites.

2.4.1 Adsorption isotherms

Preliminary testing of solid-liquid absorption system is based on two types of investigations: a) equilibrium batch sorption tests and b) dynamic continuous flow sorption studies (Nilanjana *et al.*, 2008). The equilibrium of the biosorption process is often described by fitting the experimental points with models usually used for the representation of isotherm adsorption equilibrium. The two widely accepted and linearised equilibrium adsorption isotherm models are given by the Langmuir and Freundlich isotherms. Each of these isotherms has two constants in their general forms (Singh and Srivastava, 2001).

The models can be applied at a constant pH, and used for modeling of biosorption equilibrium in the presence of one metal. Studies using biosorbents have shown that both living and dead microbial cells are able to uptake metal ions and offer potential inexpensive alternative to conventional absorbents (Khoo and Ting, 2001). However, living cells are subject to toxic effect of the heavy metals, resulting in cell death. Moreover, living cells often require the addition of nutrients and hence increase the BOD

and COD in the effluent. For these reasons, the use of non-living biomaterials or dead cells as metal binding compounds has been gaining advantage because toxic ions do not affect them.

2.4.1.1 Langmuir isotherm

The Langmuir isotherm is used to determine the amount of a contaminant which can be removed from water using adsorbent. It estimates the maximum adsorption capacity on the adsorbent surface (Igwe and Abia, 2007). Adsorption will never remove every contaminant from water. Once a certain amount of contaminant molecules have been adsorbed on the surface of adsorbent, the process reaches equilibrium. At this time, a longer contact time will not result in more adsorption.

The Langmuir isotherm (equation) is in the form:

$$\frac{Y}{M} = \frac{abC}{1+aC} \quad (2.1)$$

Where,

Y = concentration of contaminant adsorbed

M = adsorbent concentration

C = equilibrium concentration of contaminant

a = constant related to energy of adsorption

b = constant, adsorption capacity (amount of adsorbate adsorbed per weight of adsorbent)

The constant, b denotes the maximum value of adsorbate (metal ion) that can be obtained at equilibrium concentration. The other constant, a represents the energy associated with

adsorption, and increases as the strength of the adsorption bond increases (Babu and Ramakrishna, 2003). When the Langmuir equation is linearized as in equation 2.2, the constant values can be determined from the graph plotted from the linear equation, Figure 2.1.

The equation rearranged to a linear form,

$$\frac{M}{Y} = \frac{1}{ab} \times \left(\frac{1}{C}\right) + \frac{1}{b} \quad (2.2)$$

A graph of M/Y against $1/C$ gives a straight line.

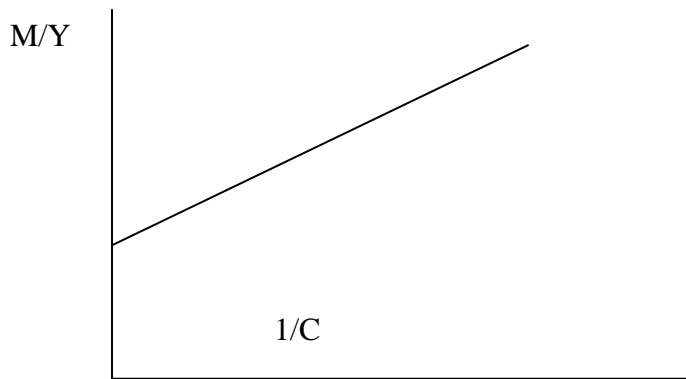


Fig. 2.1: Linearized Langmuir plot.

$$\text{slope} = \frac{1}{ab} \text{ and}$$

$$y - \text{intercept} = \frac{1}{b}$$

2.4.1.2 Freundlich isotherm

Freundlich adsorption isotherm has been widely used for determining the adsorption capacity of adsorbents (Babu and Ramakrisna, 2003). It estimates the adsorption intensity of sorbent towards adsorbent. Freundlich isotherm (equation) is in the form:

$$\frac{x}{m} = kC^{1/n} \quad (2.3)$$

Where,

x = mass of adsorbate

m = mass of adsorbent

C = equilibrium concentration of adsorbate

n = constant, degree of favourability of adsorption

k = constant, adsorption capacity

The equation can be arranged to a linear form;

$$\log\left(\frac{x}{m}\right) = \log k + \frac{1}{n} \log C \quad (2.4)$$

A plot of $\log x/m$ against $\log C$ gives a straight line, Figure 2.2.

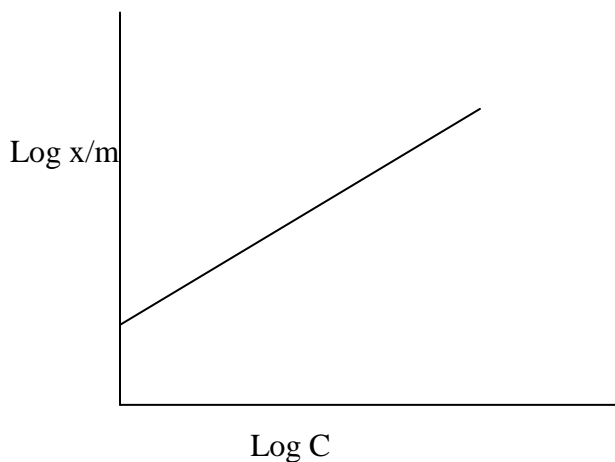


Fig. 2.2: Linearized Freundlich plot.

$$\text{Slope} = \frac{1}{n} \quad \text{and}$$

y-intercept = $\log k$

A smaller $1/n$ indicates stronger bond between adsorbate and adsorbent, while higher k indicates the rate of adsorbate removal is high or adsorbate adsorbed increases with k (Ramu and Srivathsan, 1992; Ajmal *et al.*, 1998). It has been reported in literature that the value of n is in the range of 1 to 10 indicates favourable adsorption (Raji *et al.*, 1997; Rao and Bhole, 2001). But for majority of adsorbents, n lies in the range of 0.9 to 10.

2.5 Biosorbents

Biosorption is an effective and versatile method and can be easily adopted in low cost to remove heavy metals from large amount of industrial wastewaters (Oboh *et al.*, 2009). Studies have shown that heavy metals can be removed using plant materials such as palm pressed fibres and coconut husks (Tan *et al.*, 1993), water hyacinth, *Azolla filiculoids* (Zhao and Duncan, 1997), duck weed, *Wolffia globosa* (Upatham *et al.*, 2002), lignocellulosic substrate extracted from wheat bran (Dupont *et al.*, 2003), *Rhizopus nigricans* (Bai and Abraham, 2001), cork and yohimbe bark wastes (Villaescusa *et al.*, 2002) and leaves of indigenous biomaterials. A part from the plant based material, chemical modification of various adsorbents, phenol formaldehyde cationic matrices (Singanan *et al.*, 2006), polyethylenamide modified wood (Swamiappan and Krishnamoorthy, 1984), sulphur containing modified silica gels and commercial activated charcoals (Verwilghen *et al.*, 2004) are also employed.

Biosorbents for the removal of metals/dyes come under the following categories: bacteria, fungi, algae, industrial wastes, agricultural wastes and other polysaccharide materials. In general, all types of biomaterials have shown good biosorption capacities towards all types of metal ions.

In general, the major advantages of biosorption over conventional treatment methods include low cost, high efficiency of metal removal from dilute solutions, minimization of chemical and/or biological sludge, no additional nutrient requirement, regeneration of biosorbent and the possibility of metal recovery (Radway *et al.*, 2001; Omar, 2002). The use of biomass for accumulation and concentration of toxic heavy metals from industrial metal-bearing effluents has been studied for some time (Davis *et al.*, 2003). Low costs and relatively high efficiency of heavy metal removal from diluted solutions are among the leading advantages of biosorption.

According to Schiewer and Volesky, (2000), among the relevant heavy metals, cadmium is considered to be one of the more easily removed metals from waste streams, primarily because of its ability to form stable complexes with different ligands. The nickel complexes are much less stable and, hence, nickel is considered to be one of the least removable heavy metals. According to the “hard–soft–acid–base” theory, cadmium is a soft metal that tends to form stable complexes with different ligands by covalent and ionic bonding, while nickel is an “intermediate metal that forms less stable complexes, mostly by weaker ionic bonding (Schiewer and Volesky, 2000).

Involvement of physico-chemical mechanisms including biosorption, precipitation and microbial reductive processes has been established to play important roles in metal immobilization (Tsezos *et al.*, 1996). Over the past two decades an increased interest in the phenomenon of metal ions sequestering by living or inactive microbial biomass has been seen in the scientific and engineering community. This phenomenon has potential application in environmental pollution control as a biochemical process (Gadd, 1992; Tsezos *et al.*, 1996). Biosorptive processes are excellent candidates for use for water pollution control applications (Tsezos *et al.*, 1996).

2.5.1 Algae

Use of algal biomass as a biosorbent is emerging as an attractive, economical and effective proposition because of certain added advantages of algae over others (Singh *et al.*, 2005). Algae have low nutrient requirements; being autotrophic they produce a large biomass, and unlike other biomass and microbes, such as bacteria and fungi, they generally do not produce toxic substances. Binding of metal ions on algal surface depends on different conditions like ionic charge of metal ion. Algal species and chemical composition of the metal ion solution (Sheng *et al.*, 2004; Freire-nordi *et al.*, 2005).

The metal uptake capacities exhibited by non-living biomass of micro-algal (green algae or fresh water algae) and macro-algal species (brown algae or marine algae) change from 0.066 to 1.20 mmol/g and 0.65 to 1.21 mmol/g, respectively (Freire-nordi *et al.*, 2005). Microalgae can sequester heavy metal ions by the same biosorption mechanisms as other microbial biomass as well as by the formation of phytochelatin which they synthesize in

response to toxic heavy metal stress. Microalgae use light as an energy source, facilitating the maintenance of metabolism in the absence of organic carbon sources and electron acceptor required by bacteria or fungi. Thus, the use of metabolically active microalgal systems may be more readily achieved. Also, microalgae can be cultivated in open ponds or in large-scale laboratory culture, providing a reliable and consistent supply of biomass for eventual scale-up work.

The two principal mechanisms involved in biosorption by microalgae are:

i) ion exchange wherein ions such as Na, Mg, and Ca become displaced by heavy metal ions, and ii) complexation between metal ions and various functional groups such as carboxyl, amino, thiol, hydroxy, phosphate, and hydroxy-carboxyl, that can interact in a coordinated way with heavy metal ions. Although thousands of micro-algal species have been identified, very few have been investigated for their biosorption potential, and the vast majority of studies have been conducted using unicellular green algae, principally *Chlorella vulgaris* and *Chlamydomonas reinhardtii* (Wilde and Benemann, 1993).

Another important biosorbent, which has gained momentum in recent years, is seaweeds. Marine algae, popularly known as seaweeds, are biological resources, which are available in many parts of the world. Algal divisions include red, green and brown seaweed; of which brown seaweeds are found to be excellent biosorbents (Davis *et al.*, 2003). This is due to the presence of alginate, which is present in gel form in their cell walls. Also, their macroscopic structure offers a convenient basis for the production of biosorbent particles that are suitable for sorption process applications (Vieira and Volesky, 2000). Biomass of brown marine macroalgae is a biological resource that is available in large quantities and

can form a good base for the development of biosorbent material. Studies by Holan and Volesky (1993), Fourest and Volesky (1996) and Chu *et al.*, (1997), have focused on the use of this biomass for heavy metal removal from water and wastewater.

2.5.2 Fungi

Biomaterials like fungi have been proved efficient and economical for the removal of toxic metals from dilute aqueous solutions by biosorption because fungal biomass offers the advantage of having a high percentage of cell wall material, which shows excellent metal binding properties (Nilanjana *et al.*, 2008). Moreover, large quantity of fungal biomass is available from the antibiotic- and food-industries. Some types of industrial fermentation waste biomass are really excellent metal sorbers. For that reason, fungi, including yeasts, have received increased attention. Fungi can also be grown using unsophisticated fermentation techniques and inexpensive growth media or even waste carbohydrate-containing growth media based on for example, molasses or cheese whey. The eukaryotic microorganisms are always of unicellular nature; however, the vegetative phases of fungi and algae are frequently multicellular (Shuler and Kargi, 1992).

The sequestering of metallic species by fungal biomass has mainly been traced to the cell wall. Various polysaccharides are the main constituents of the fungal cell wall. They are often complexed with proteins, lipids, and other substances (for example, pigments). Large quantities of phosphate and glucouronic acid and chitin-chitosan complex existing in these cell walls offer extensive possibilities for binding metals through ion exchange and coordination. In the fungal cell wall, several types of ionizable sites affect the metal uptake capacity: phosphate groups, carboxyl groups on uranic acids and proteins, and

nitrogen-containing ligands on protein as well as on chitin or chitosan. Important fungal biosorbents include *Aspergillus* (Kapoor and Viraraghavan, 1997), *Rhizopus* (Bai and Abraham, 2002) and *Penicillium* (Tan and Cheng, 2003), etc. Since these microorganisms are used widely in different food/pharmaceutical industries, they are generated as waste, which can be attained free or at low cost from these industries.

2.5.3 Waste Materials

Agricultural by-products have been widely studied for metal removal from water. Peat, wood, pine bark, banana pith, soybean and cotton bulls, rice bran, saw dust, wool, orange peel have been demonstrated to remove heavy metals from wastewater (Ozer and Ozer, 2004). These agricultural waste biosorbents are inexhaustible, cheap and non-hazardous materials, which are specifically selective for heavy metals and can be easily disposed by incineration. The feasibility of using bio-waste obtained from fruit juice industry for the removal of toxic heavy metals, Hg(II), Pb(II), Cd (II), and Ni (II), from wastewater was explored by (Senthilkumaar *et al.*, 2000).

2.5.4 Bacteria

Bacteria have polysaccharide slime layers and readily provide amino, carboxyl, phosphate and sulphate groups for metals biosorption. Bacterial biomass is generally produced as a waste by-product of industrial operations or can be specifically propagated in large scale. The uptake capacities of bacteria generally range between 0.23 to 0.90 mmol/g (Chang and Hong, 1994). Potent metal biosorbents under the class of bacteria include genre of *Bacillus* (Nakajima and Tsuruta, 2004), *Pseudomonas* (Uslu and Tanyol, 2006) and *Streptomyces* (Mameri *et al.*, 1999), to name a few.

2.6 Methods of heavy metal analysis

Extensive studies on the methods for the determination of metals in various matrices with satisfactory sensitivity, selectivity and reliability have been carried out (Balcerzak, 2002). Spectroscopic techniques like UV-Vis spectrometry, AAS, ICP are widely applied in the analysis of a variety of samples containing metals over a large range of concentrations. Balcerzak (2002) analysed data obtained by using different spectroscopic methods such as UV-Vis, AAS, AES and ICPS in the determination of metal ions in water samples and found the results to be closely comparable especially in the mg/L range. Samia *et al.* (2011) used differential pulse anodic stripping voltammetry (DPASV) method for the determination of heavy metal ion concentrations in aqueous solutions. Addagalla *et al.* (2009) also analyzed metal ions using inductively coupled plasma optical emission spectroscopy (ICP-OES).

2.7 Sources of nutrients

During the last two centuries, human activities have resulted in large scale changes in the nutrient cycle, with an increase since about 1950 (UNEP, 2005). The global annual nitrogen and phosphorus input to ecosystems has more than doubled during this time. This increase is caused by the higher demand for food, fuel and fibre (UNEP, 2005).

A wide range of contaminants can reach the river via a variety of ways, including through drainage ditches by artificial fertilizer residues, insecticides, herbicides and pesticides. Majority of polluting nutrients enter water sources in effluents from untreated sewage or from farming activities. Nutrients from urban sources may be derived from domestic

sewage and industrial wastes. Rural sources of nutrients include those from agriculture and forest management.

Nutrients can be from point sources or nonpoint sources. Point sources refer to contaminants that enter a waterway through a discrete conveyance such as a pipe. In these sources the nutrients travel directly from the source water. Point source nutrients come from municipal and industrial effluents, runoff and infiltration from animal feedlots and untreated sewage (Mason, 1991). Nonpoint sources refer to diffuse contamination that does not originate from a single discrete source. These sources are difficult to regulate and usually vary spatially and temporally. Nonpoint source nutrients come from runoff from agricultural fields, urban runoff from unsewered areas and septic tank leachate (Carpenter *et al.*, 1998).

Nutrients are lost from farmland in three ways, namely:

- i) Drainage water percolating through the soil leaching soluble plant nutrients
- ii) Inefficient return to the land of the excreta of stock
- iii) The erosion of surface soils or by the movement of the fine soil particles into subsoil drainage systems.

Modern agriculture often involves the application of nutrients onto fields in order to maximize production. However, farmers frequently apply more nutrients than taken up by crops (Buol, 1995). Regulations aimed at minimizing nutrient exports from agriculture are typically far less than those placed on sewage treatment plants and other point source pollutants (Carpenter *et al.*, 1998). The use of fertilizers has greatly increased. Nitrogen

and phosphorus behave differently in soils. The nitrate ion is fairly soluble, hence, it is readily leached if it is not taken up by plants. By contrast, phosphorus is precipitated as phosphates and released only slowly. The solubility of nitrate means agriculture is a major contributor to nitrate loading in fresh waters.

2.7.1 Nitrates

According to research, less than half of the nitrogen applied as fertilizer is taken up by plants; the rest is lost or dissolved in surface waters (UNEP, 2005). Nitrites react directly with haemoglobin in human blood and warm blooded animals to produce methaemoglobin (Kim-Sapiro *et al.*, 2005). Methaemoglobin destroys the ability of red blood cells to transport oxygen. This condition is especially serious in babies under three months of age. It causes a condition known as methaemoglobinaemia or “blue baby” disease.

In most cases of excess nitrate concentrations in aquatic systems, the primary source is surface runoff from agricultural or landscaped areas, which have received excess nitrate fertilizer. Such levels of nitrate can lead to algal blooms and then eutrophication. Presence of nitrates can also lead to water anoxia, in which such blooming may cause other changes to ecosystem function, favouring some species of organisms over others. Consequently, as nitrates form a component of total dissolved solids, they are widely used as an indicator of water quality. Maximum contaminant level for NO_3^- and NO_2^- are 10 ppm and 1ppm respectively (Kim-Shapiro *et al.*, 2005).

2.7.2 Phosphates

Phosphorus is often regarded as the main culprit in cases of eutrophication in rivers and streams subjected to point source pollution. The concentration of algae and the trophic state of rivers/lakes correspond well to phosphorus levels in water. Studies conducted have shown a relationship between the addition of phosphorus and the rate of eutrophication. Humankind has increased the rate of phosphorus cycle on earth by four times, mainly due to agricultural fertilizer production and application (Carpenter *et al.*, 1998).

Phosphates enter water ways from human and animal waste, industrial effluent and fertilizer runoff from agricultural sites. Non-point sources of phosphates include storm water runoff, agricultural runoff, erosion and sedimentation. Point sources include wastewater treatment plants and industrial discharges. Plants may not be able to utilize all the phosphate fertilizers applied. As a consequence, much of the PO_4^{3-} is lost from the land through erosion because it has a stronger affinity to binding with the soil than NO_3^- (Daniel *et al.*, 1994; Sharpley *et al.*, 1994). Phosphates will stimulate the growth of plankton and aquatic plants. If an excess of it enters the waterway (river/stream), algae and aquatic plants will grow wildly, choke up the water course and use large amounts of oxygen. This condition is known as eutrophication, which is discussed in section 2.7, or over fertilization of receiving waters, which will decrease dissolved oxygen (DO) levels (Sharpley *et al.*, 1994).

2.8 Eutrophication

Eutrophication is the fertilization of surface water by nutrients. It also refers to the progressive deterioration of water quality from over stimulation by nutrients. Thus, it is a result of nutrient pollution. It promotes plant growth and decay and can cause a severe reduction in water quality. Eutrophication decreases the resource value of rivers or streams such that recreation, fishing and aesthetic enjoyment are hindered. Health related problems can also occur where eutrophic conditions interfere with drinking water treatment (Daniel *et al.*, 1994; West, 2006).

Eutrophication is thus depriving the river of oxygen (called oxygen debt). As algae dominate and turn water green, the growth of water plants is suppressed; these die first, disrupting the food chain. Death of invertebrates and fish follow on, and their remains in turn lead to excess bacterial activity during decomposition, thereby reducing further oxygen levels (Womach, 2005).

2.8.1 Effects of Eutrophication

The following characterizes surface water quality with increasing nutrient (Mason, 1991): Increased biomass and algal growth; increased blooms of gelatinous zooplankton; water treatment problems (odour, bad taste, and increased filtration costs, disinfection byproducts-trihalomethanes with potential health effects); dissolved oxygen depletion in water; decreased water quality (increased turbidity); and decrease in perceived aesthetic value of water body.

The effects of an increase in the rate of nutrient income on water bodies lead to:

Decrease in species diversity and dominant biota change; increase of plant and animal biomass; ncrease in turbidity; increase in the rate of sedimentation; and development of anoxic conditions.

The above could cause the following problems:

Treatment of potable water may be difficult and the supply may have an unacceptable taste or odour; the water may be injurious to health; the amenity value of the water may decrease; increased vegetation may impede water flow and navigation and commercially important species -such as salmonids and coregonids (water fish) -may disappear.

2.8.2 Problems for man

Problems for man can be summarized in three main areas that are associated with:

- i) Water purification, supply and consumption
- ii) Aesthetic and recreational activities
- iii) The management of water courses.

2.8.3 Water purification problems

The increase in phytoplankton with eutrophication frequently causes severe problems in water purification. Residual large algae may block rapid sand filters which reduces the throughput of water at treatment works. The smallest algae cells often pass through the filters, producing turbid final water. Water supply with high nitrate levels presents potential health risk. Infants under six months of age may develop methaemoglobinaemia by drinking water high in nitrates (Kim-Shapiro *et al.*, 2005).

2.9 Dissolved Oxygen (DO)

In aquatic environments dissolved oxygen (DO) is a relative measure of the amount of oxygen dissolved in water. In fresh water under atmospheric pressure at 25 °C, oxygen saturation is 9.1 mg/L (CIESE, 2009). Adequate DO is necessary for good quality water and a sufficient supply of it is essential for the survival of aquatic organisms. A deficiency in this parameter is a sign of an unhealthy river (Dohner *et al.*, 1997). Oxygen is a necessary element to all forms of life. Natural stream purification processes require adequate oxygen levels in order to provide for aerobic life forms. As DO in water drops below 5.0 mg/L, aquatic life is put under stress, and the lower the concentration, the greater the stress (CIESE, 2009).

Nonpoint-source pollution can decrease the amount of DO in water. The decomposition of leaf litter, grass clippings, sewage, and runoff from feedlots decreases DO readings. Decreased DO can be harmful to fish and other aquatic organisms (Dohner *et al.*, 1997). The abundant planktonic algae (green algae) are generally related to the amount of nutrients present. When phytoplankton becomes so abundant that water visibility is limited to 30 cm there is a danger of oxygen depletion. This problem is often a consequence of over fertilization of fields or nutrients from livestock.

2.10 pH

pH is a measure of the amount of free hydrogen ions in water. Specifically, it is the negative logarithm of molar concentration of hydrogen ions ($\text{pH} = -\log [\text{H}^+]$). Generally, it is an indicator, of acidic and alkaline condition of water status. Most natural waters are buffered by a carbon dioxide-bicarbonate system, since the carbon dioxide in the

atmosphere serves as a source of carbonic acid. Buffering capacity is water's ability to resist changes in pH, and is critical to the survival of aquatic life. This reaction tends to keep pH of most waters around 7.0 to 7.5, unless large amounts of acid or base are added to the water. pH of water affects the solubility of many toxic and nutritive chemicals, therefore the availability of these substances to aquatic organisms is affected. As acidity increases, most metals become more water soluble and more toxic.

A pH test measures the alkalinity or acidity concentration in water. A pH of 7.0 is neutral, below 7.0 is acidic, and above 7.0 is basic or alkaline. Pollution from accidental spills, agricultural runoff and sewer overflows can change the pH. While young fish and insect larvae are sensitive to a low pH (acidic), extreme values on either end of the scale can be lethal to most organisms.

When the pH is more than 8.5, it interferes with chlorination during water treatment. pH affects many chemical and biological processes in the water. For example, different organisms flourish within different ranges of pH. The largest variety of aquatic animals prefers a range of 6.5-8.0. pH outside this range reduces the diversity in the stream because it stresses the physiological systems of most organisms and can reduce reproduction.

2.11 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is a measure of the total quantity of oxygen required to oxidize all organic materials into carbon dioxide and water. COD test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of

COD determine the amount of organic pollutants found in surface water, such as lakes and rivers, making COD a useful measurement of water quality. It indicates the mass of oxygen consumed per litre of solution.

2.12 Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand, also known as biological oxygen demand (BOD), is a measure of the quantity of oxygen used by microorganisms, such as aerobic bacteria in the oxidation of organic matter. Natural sources of organic matter include plant decay and leaf fall. However, plant growth and decay may be unnaturally accelerated when nutrients and sunlight are overly abundant due to human influence.

If effluent with high BOD levels is discharged into a stream or river, it will accelerate bacterial growth in the river and consume the oxygen levels in the river. Urban runoff wastes from streets and sidewalks; nutrients from lawn fertilizers; leaves, grass clippings, and paper from residential areas, increase oxygen demand of water. Oxygen consumed in the decomposition process robs other aquatic organisms of the oxygen they need to live. The oxygen may diminish to levels that are lethal for most and many aquatic insects. Organisms that are more tolerant of lower dissolved oxygen levels may replace a diversity of more sensitive organisms (Chapman, 1996; Clair *et al.*, 2003). Biochemical oxygen demand directly affects the amount of DO in rivers and streams. The greater the BOD, the more rapidly oxygen is depleted in the stream. This means less oxygen is available to higher forms of aquatic life. If BOD exceeds 3 mg/L, it affects coagulation and rapid sand-filtration processes in conventional water treatment plants, requiring expensive advanced water treatment (Clair *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Apparatus/Equipment

The sampling bottles and glassware were cleaned according to the recommended methods (APHA, 2005). All other apparatus like beakers, graduated cylinders, volumetric flasks, pipettes and conical flasks were cleaned with detergent and thoroughly rinsed with distilled water. The equipment used included UV-Vis spectrophotometer –DR/4000, pH meter –sension1, oven, BOD incubator and COD block digester.

3.2 Reagents

The reagents used included the following:

Citrate buffer, potassium cyanide, Pb standard solution for Pb^{2+} ions; cuver Cu reagent, standard Cu solution for Cu^{2+} ions; chloroform, Ni reagent powder, Ni standard solution for Ni^{2+} ions and cyclohexanone, Zn reagent, Zn standard solution for Zn^{2+} ions. $(NH_4)_2Fe(SO_4)_2$, H_2SO_4 and $K_2Cr_2O_7$ were used for the determination of COD, while LiOH, $MnSO_4$, alkaline iodide were for the determination of BOD. pH buffer solutions were used for pH determination. All the reagents used were of analytical grade. The reagents were bought from local laboratory chemical suppliers, Aquatreat Laboratory supplies and Chemoquick laboratory and equipment suppliers.

3.3 Sampling

Six sampling stations were selected, three upstream and three downstream of Migori town. The stations were designated S1, S2, S3, S4, S5 and S6 starting from the farthest

upstream to the lowest downstream, at intervals of 2 km apart. The sampling sites are shown in Figure 1.1.

Six water samples were collected from each station in six months, during the dry and wet seasons, spread from May to December 2010. The samples were collected midstream, in duplicates, using plastic sampling bottles. The samples were transported to the laboratory in ice boxes containing ice and were analyzed within 48 hours of sampling.

3.4 Biomass

Brown macroalgae, *Sargassum sp*, were collected from Department of chemistry laboratory, University of Plymouth, England. The biomass particles were washed using distilled de-ionised water, dried in the oven overnight at 30 °C and then sieved through stainless steel sieves of 1mm pore size. Solutions of 0.1 M NaOH and 0.1 M HNO₃ were used to adjust the pH to give the required value.

3.5 Biosorption of Heavy Metals

To the contents in each plastic bottle, and at a particular pH, 0.2 g of algae per 100 ml river water was added followed by periodic hand shaking and allowed to settle before taking heavy metal measurements. pH was adjusted at intervals of 1, up to pH of 9, and residual metal ion concentrations measured at the intervals. The percentage reduction of metal concentrations was then calculated. One set of measurement was taken for water without algae, and another with algae added. From the results, optimum pH was determined.

The same procedure was repeated, but with residual metal ion concentrations measured at constant optimum pH and time intervals of 15 minutes after addition of algae. Concentrations were recorded against time and the percentage reduction of metal concentrations was then calculated.

Metal ion measurements were done at the determined optimum pH value of 6.0 using UV-visible spectrophotometer, Hach DR/4000. The spectrophotometer was calibrated according to the operation manual (Hach Procedure Handbook, 2005). All the metal ions were complexed by their respective reagents to give coloured complexes at their corresponding wavelengths, for the spectrophotometer detection. The wavelengths used were: 560 nm for Cu^{2+} ; 430 nm for Ni^{2+} ; 515 nm for Pb^{2+} and 620 nm for Zn^{2+} . The rest of the experimental procedure was done as per the Hach Procedures handbook.

Samples were drawn at different time intervals (0, 15, 30, 45 ... 120 minutes), pH controlled using dilute HCl or NaOH and filtered using Whatman filter paper No. 1. The metal content in the filtrate was then analyzed.

Percentage absorption was calculated by evaluating changes in concentrations metal ions at various times with respect to changes based on maximum biosorption, using the expression;

$$R = \frac{C_o - C_t}{C_o} \times 100 \quad (3.1)$$

Where,

R = biosorption efficiency (%),

C_0 = original concentration and

C_t = concentration at time t.

The algal biomass metal cation uptake capacities after different experiments were calculated from the relevant metal cation concentration in water according to the following equation:

$$Q = \frac{V(C_i - C_f)}{S} \quad (3.2)$$

Where,

Q = metal cation uptake capacity (mg metal/gr dry algae)

S = dry weight of algal sample (g)

C_f = final metal cation concentration in the solution, after the adsorption test (mg/L)

C_i = initial metal cation concentration in the solution, before the adsorption test (mg/L)

V = solution volume (mL)

Langmuir and Freundlich adsorption models were adopted to describe the adsorption isotherms. Langmuir adsorption isotherm equation:

$$q_{max} = \frac{bC_f}{1 + bC_f} \quad (3.3)$$

Freundlich adsorption isotherm equation:

$$q = kC_f^{1/n_f} \quad (3.4)$$

Where q = metal cation uptake capacity (mg metal/gr dry algae)

C_f = final metal cation concentration in the solution, after the adsorption test (mg/L)

q_{max} = Langmuir maximum metal uptake capacity (mg/gr)

b = Langmuir adsorption affinity constant (mg/L)

k = Freundlich maximum metal uptake capacity

n_f = Freundlich adsorption affinity constant

3.6 Determination of Phosphates

Phosphate analysis was achieved using spectrophotometer, where phosphorus is oxidized to phosphate ion as described by APHA, (2005). Spectronic 20 spectrophotometer was employed in the measurement of colour intensity at a wavelength of 650nm.

3.7 Determination of Nitrates

Total nitrates were determined by Hach spectrophotometer DR/4000. The detailed experimental procedure used was from the Hach Procedures Handbook, (2003).

3.8 Determination of pH

The pH was determined by potentiometric method, using digital pH meter model session1-51935. Calibration of the electrode was done with 2 buffer solutions of pH 4 and pH 7 prior to its use. The results were displayed in pH units.

3.9 Determination of biochemical oxygen demand (BOD)

The BOD in the samples was determined using standard method (APHA, 2005). Twenty millilitres of sample was measured and transferred into the BOD bottle. Distilled water was added until it was filled to overflow. The initial oxygen reading was read using the DO meter. The solution was stoppered carefully not to trap in any air bubbles and then incubated in the water bath in an inverted manner. The temperature was maintained at 20 °C. The solution was incubated for 5 days. At the end of the five days, the final oxygen

reading was recorded. The BOD is given by the difference between the initial and the final readings.

3.10 Determination of chemical oxygen demand (COD)

The COD in the samples was determined using standard method (APHA, 2005). About 2.5 ml sample was measured and transferred into borosilicate culture tubes then 1.5 ml of potassium dichromate digestion solution added. Approximately 3.7 ml H_2SO_4 was carefully run down inside the vessel so that an acid layer formed under the sample digestion solution layer. Contents were mixed thoroughly before applying heat to prevent local heating of vessel bottom and possible explosive reaction. The borosilicate culture tubes were then placed in block digester and refluxed for 2 hrs then cooled to room temperature. The contents were then transferred into an Erlenmeyer flask and 3 drops of ferroin indicator solution added and placed on a magnetic stirrer and stirred while titrating with 0.01M ferrous ammonium sulphate. The end point was marked by a sharp colour change from blue-green to reddish brown. Distilled water blank was made and treated in the same manner.

3.11 Data Analysis

The results were corrected according to recovery rates and presented in graphs, tables and figures in terms of mean values and standard deviation. The data were subjected to linear analysis using Microsoft Excel, EuraChem and SAS software packages. Quality assurance and quality control procedures for the laboratory included analysis of duplicates, and blanks.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Table 4.1 shows the mean concentration levels of Ni^{2+} , Pb^{2+} , Zn^{2+} and Cu^{2+} during wet and dry seasons, with their standard deviations (SD). The concentrations are presented graphically in Figures 4.1 and 4.2 for wet and dry season, respectively. Metal ion concentrations varied among the six sampling sites down the river and in different seasons as explained here below.

During the wet season, Cu^{2+} at all sampling stations S1, S2, S3, S4, S5 and S6 varied between 0.02 and 0.29 mg/L with the mean values of 0.05 ± 0.030 , 0.11 ± 0.010 , 0.26 ± 0.020 , 0.28 ± 0.020 , 0.27 ± 0.010 and 0.25 ± 0.010 , respectively. During the dry season, Cu^{2+} at all sampling stations varied between 0.07 and 0.26 mg/L with the corresponding mean values of 0.08 ± 0.010 , 0.15 ± 0.006 , 0.19 ± 0.010 , 0.25 ± 0.010 , 0.25 ± 0.006 and 0.23 ± 0.010 mg/L.

Table 4.1: Concentrations of metal ions in river (mg/L) during the wet and dry seasons

Site	Cu ²⁺		Zn ²⁺		Pb ²⁺		Ni ²⁺	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
S1	0.050 ±0.030	0.080 ±0.010	0.120 ±0.020	0.090 ±0.006	0.005 ±0.001	0.005 ±0.001	0.030 ±0.005	0.030 ±0.006
S2	0.110 ±0.010	0.150 ±0.006	0.180 ±0.010	0.160 ±0.005	0.010 ±0.007	0.010 ±0.00	0.060 ±0.006	0.030 ±0.005
S3	0.260 ±0.020	0.190 ±0.010	0.230 ±0.009	0.200 ±0.006	0.050 ±0.010	0.040 ±0.010	0.070 ±0.015	0.040 ±0.011
S4	0.280 ±0.020	0.250 ±0.010	0.270 ±0.017	0.210 ±0.011	0.060 ±0.006	0.050 ±0.006	0.070 ±0.006	0.050 ±0.010
S5	0.270 ±0.010	0.250 ±0.006	0.240 ±0.010	0.200 ±0.050	0.060 ±0.005	0.04 ±0.005	0.060 ±0.015	0.050 ±0.006
S6	0.250 ±0.010	0.230 ±0.010	0.230 ±0.006	0.180 ±0.050	0.050 ±0.006	0.030 ±0.006	0.050 ±0.011	0.040 ±0.010

During the wet season, Zn²⁺ at sampling stations S1, S2, S3, S4, S5 and S6 varied between 0.10 and 0.28 mg/L with the mean values of 0.12 ± 0.020 , 0.18 ± 0.010 , 0.23 ± 0.009 , 0.27 ± 0.017 , 0.24 ± 0.010 and 0.23 ± 0.006 , respectively. During the dry season, Zn²⁺ at all sampling stations varied between 0.09 and 0.22 mg/L with the corresponding

mean values of 0.09 ± 0.006 , 0.16 ± 0.005 , 0.20 ± 0.006 , 0.21 ± 0.011 , 0.20 ± 0.150 and 0.18 ± 0.150 .

The Pb^{2+} concentration during wet season at sampling stations S1, S2, S3, S4, S5 and S6 varied between 0.00 and 0.07 mg/L with the mean values of 0.005 ± 0.004 , 0.01 ± 0.007 , 0.05 ± 0.010 , 0.06 ± 0.006 , 0.06 ± 0.005 and 0.05 ± 0.006 , respectively. During the dry season, Pb^{2+} at all sampling stations varied between 0.00 and 0.04 mg/L with the respective mean values of 0.005 ± 0.004 , 0.01 ± 0.00 , 0.04 ± 0.015 , 0.05 ± 0.006 , 0.04 ± 0.005 and 0.03 ± 0.006 . Ni^{2+} during wet season at all sampling stations S1, S2, S3, S4, S5 and S6 varied between 0.02 and 0.08 mg/L with the mean values of 0.03 ± 0.005 , 0.06 ± 0.006 , 0.07 ± 0.015 , 0.07 ± 0.006 , 0.06 ± 0.015 and 0.05 ± 0.011 , respectively. During the dry season, Ni^{2+} at all sampling stations varied between 0.02 and 0.06 mg/L with the respective mean values of 0.03 ± 0.006 , 0.03 ± 0.005 , 0.04 ± 0.011 , 0.05 ± 0.010 , 0.05 ± 0.006 and 0.04 ± 0.010 mg/L.

Figure 4.1 shows the variations of metal ions in the six sampling sites during wet season. Generally, in wet season, the metal ions had the lowest concentrations in site S1 and highest in site S4. Since S1 and S2 are upstream of the town, heavy metal pollution is minimal. On reaching S4, which is in the middle of the town, pollution is evident and metal concentrations were highest. There was a slight decrease of metal ion concentrations downstream towards S6. This could be associated with self purification of the river and the dilution factor.

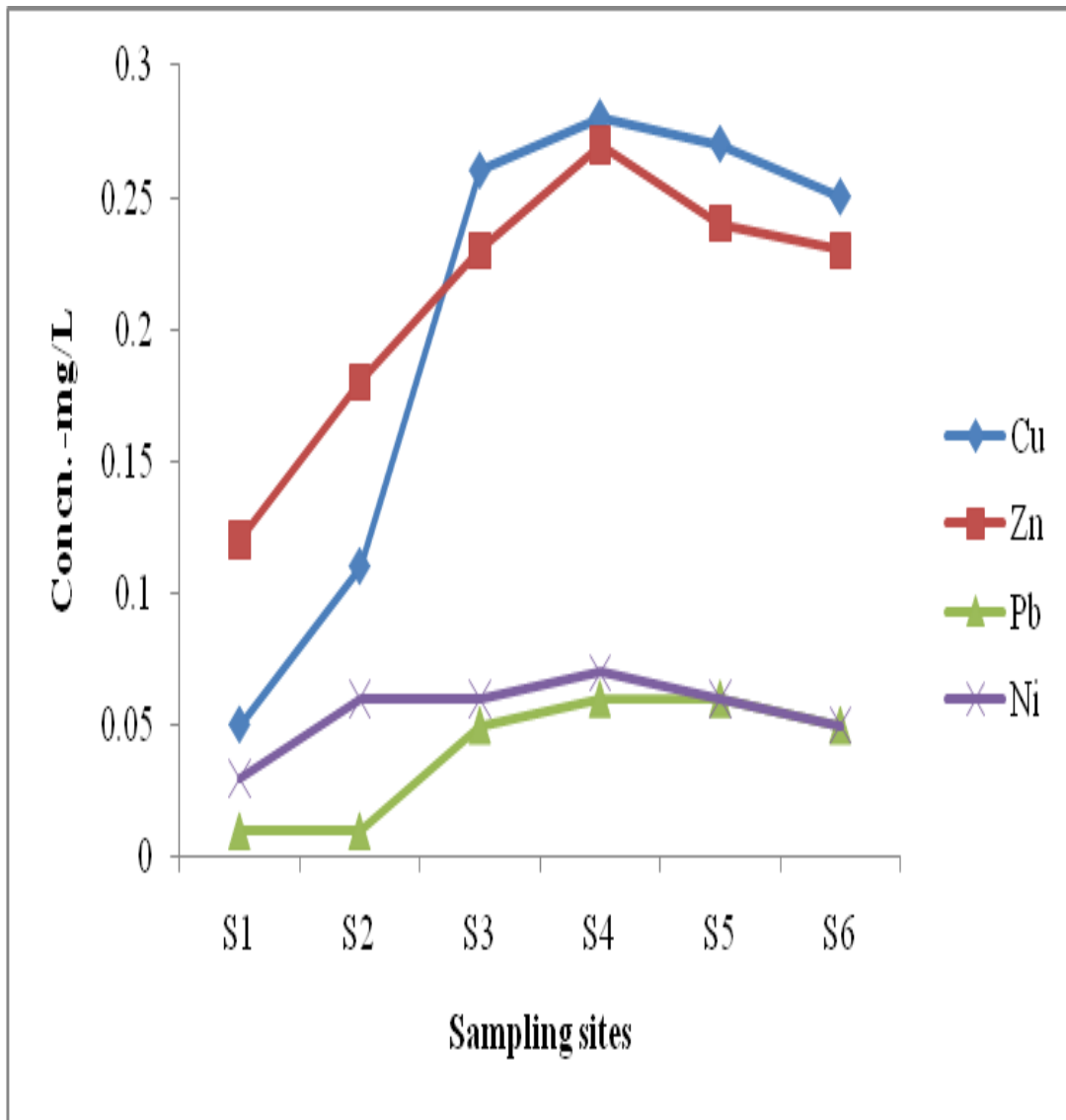


Fig. 4.1: Concentration of metal ions during wet season.

Figure 4.2 shows the variations of metal ions in the six sampling sites during dry season. Like in wet season, the general trend, was that site S1 had the lowest concentrations of metal ions and highest in site S4. The reason for this could be due to variation as has been explained above. However, generally the metal concentrations were higher in wet season than in dry season which could be due to runoff, bringing in more loads from town.

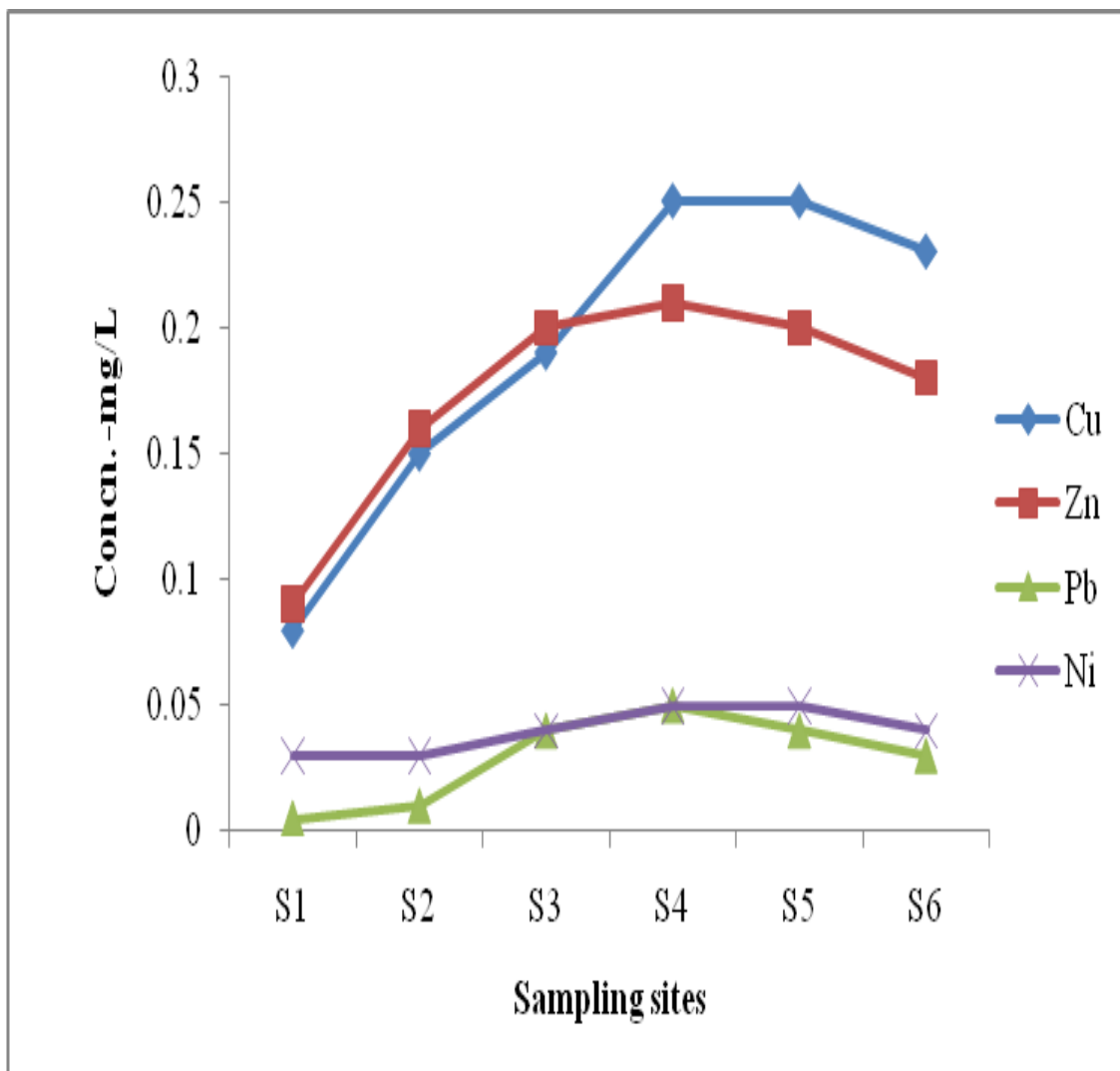


Fig. 4.2: Concentration of metal ions during dry season.

Figure 4.3 shows the variations of residual metal ion concentrations with pH. The efficiency of removal of metal ions by biosorption increased with increase in pH from 3 to around 6 then started decreasing. The metal ion uptake increased with an increase in pH, but decreased as the solution became more basic. This is because at low pH, metal ions exist in solution and are easily adsorbed to the binding sites while at high pH metals precipitate as insoluble oxides and hydroxides and less adsorbed hence higher concentration remaining (Radovic *et al.*, 1997). Similarly according to Aksu (2001), at

lower pH, cell wall ligands are closely associated with H^+ ions and hence access of the metal ions are restricted. At higher pH values there was increased biosorption of for all the metals because carboxyl groups would be exposed leading to attraction between these negative charges and the metals and hence increase in biosorption onto the cell. Carboxyl is generally the most abundant acidic functional group in the brown algae (Davis *et al.*, 2003). The pH of 6 was therefore taken to be the optimum pH value for the biosorption of the metals since it recorded the lowest remaining concentrations.

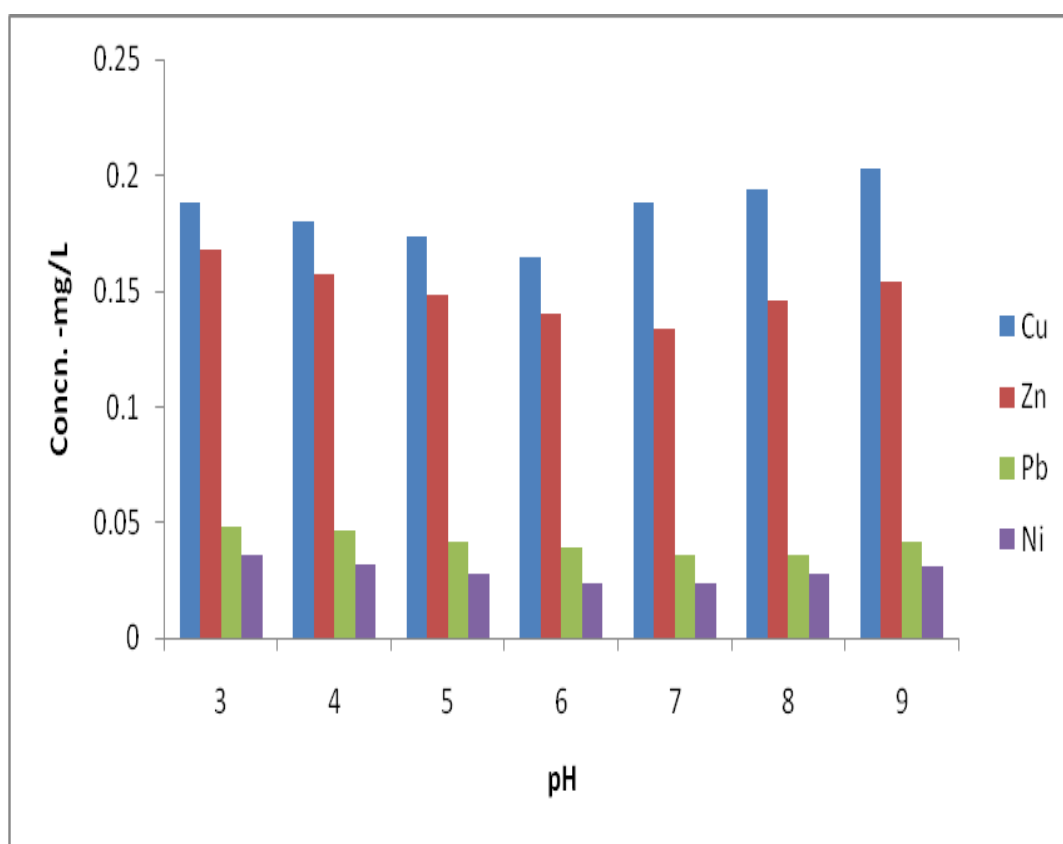


Fig. 4.3: Variations of residual metal concentration with pH.

Table 4.2 shows the percentage removal of the various metal ions by the algae adsorbent at different pH values. The influence of initial pH on the adsorption characteristics of the

four metals using biomass is evident. The solution pH played a critical role in the biosorption process and the metal uptake increased with an increase in pH, but decreased as the solution became more basic. The optimum pH for the adsorption was 6.0. When alkalinity increased, there was a decrease in the rate of adsorption by algae for the metal in water (Leon *et al.*, 1992; Radovic *et al.*, 1997).

Table 4.2: Percentage removal efficiency of metal ion with pH

pH	Cu ²⁺	Zn ²⁺	Pb ²⁺	Ni ²⁺
3	35	40	20	55
4	38	44	22	60
5	40	47	30	65
6	43	50	35	70
7	35	52	40	70
8	33	48	40	65
9	30	45	30	61

A trend of increasing metal ion binding with increasing pH was observed for the adsorbent for pH values above 4.0, up to about 6.0. This was consistent with the results obtained from other studies (Liu *et al.*, 2009).

Figure 4.4 shows how the concentration of residual metal concentrations varied with contact time with the adsorbent. There was a decrease of the metal ions remaining with time. With increase in contact time there was less and less metal ions remaining in solution. However, the decrease almost leveled towards 120 minutes.

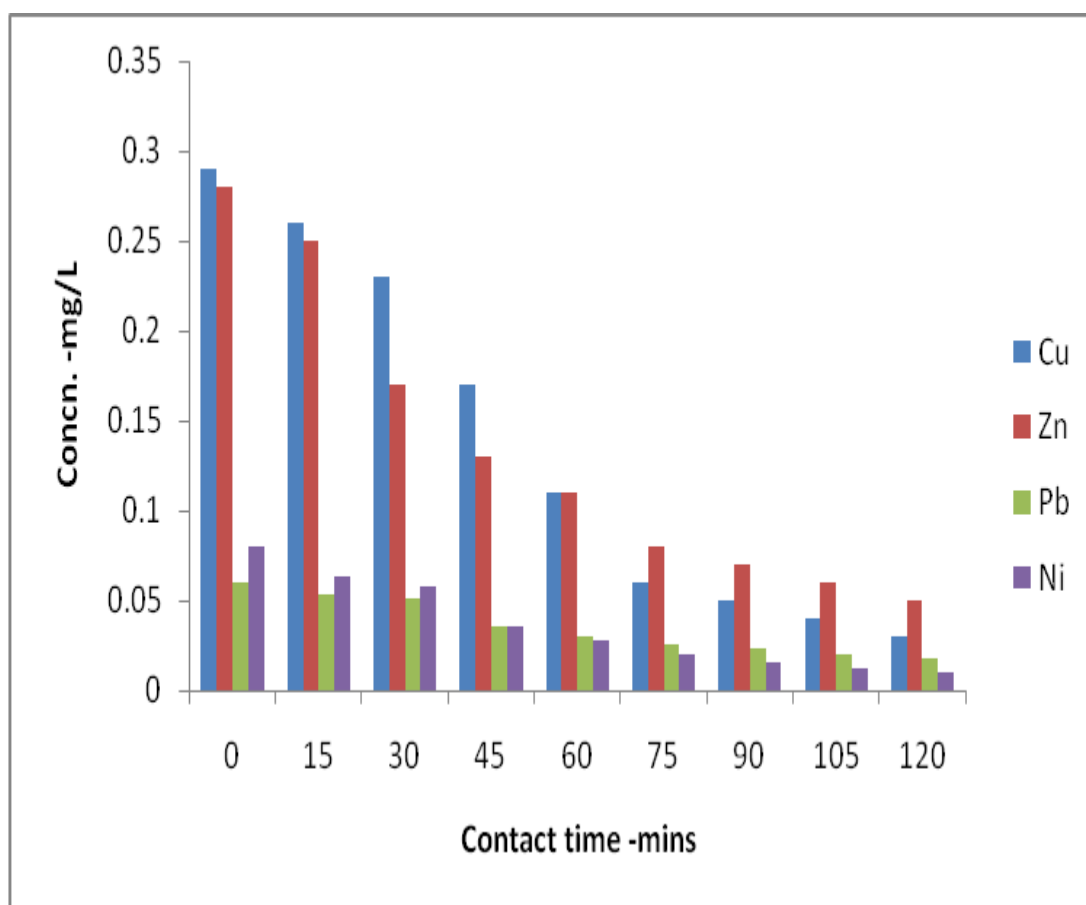


Fig. 4.4: Variations of residual metal ion concentration (mg/L) with contact time (minutes)

The metal sorption rate was very high at initial stages followed by a lower sorption rate. This behaviour has been reported by other researchers (Crist *et al.*, 1990).

Table 4.3 and Figure 4.5 show the percentage removal of the metal ions by algae with contact time. There was a progression in the percentage removal of metal ion in water. From the results, Cu^{2+} ions had the highest percent removal of 89% at the end of 120 minutes, followed by Ni^{2+} , Zn^{2+} and Pb^{2+} values of 87%, 80% and 70%, respectively .

Table 4.3: Percentage removal efficiency of metal ion with contact time.

Mins.	Cu^{2+}	Zn^{2+}	Pb^{2+}	Ni^{2+}
15	12	10	10	20
30	25	40	15	28
45	40	55	40	40
60	68	60	50	65
75	78	70	56	75
90	84	75	61	80
105	87	78	66	85
120	89	80	70	87

The affinity of metals for algae appeared to decrease in the order: $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Pb}^{2+}$. Ionic sizes decrease in the order: $\text{Pb}^{2+} > \text{Zn}^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+}$. The large ions such as Pb^{2+} has largest naked radius and approach active sites more closely and so are strongly adsorbed than small ones. The trend was followed between Zn^{2+} and Ni^{2+} but was broken by Pb^{2+} . This could be due to very small amounts of Pb^{2+} ions which were present in the water sample because one of the factors affecting biosorption is the initial metal concentration in the solution (Nilanjana *et al.*, 2008).

It was also observed that with increase in contact time, there was sharp increase in adsorption rate before increasing gradually after 75 minutes of contact time. Contact time plays an important role in the efficient removal of heavy metals using biomass.

The influence of contact time on the biosorption capacity for different metal ions is shown in Figure 4.5. It reveals that the rate of adsorption is higher at the beginning and this is due to availability of large number active sites on the adsorbent. As these sites get exhausted, the uptake rate is controlled by the rate at which the adsorbate is transported from the exterior to the interior sites of the adsorbent particles (Dorris *et al.*, 2000). The kinetics of metal ion sorption is an important parameter for designing sorption systems and is required for selecting the optimum operating conditions for full scale batch metal removal process (Liu *et al.*, 2009). Figure 4.5 resembles Langmuir isotherm, which relates the coverage or adsorption of molecules on a solid surface to gas pressure or concentration of medium above the solid surface at a fixed temperature.

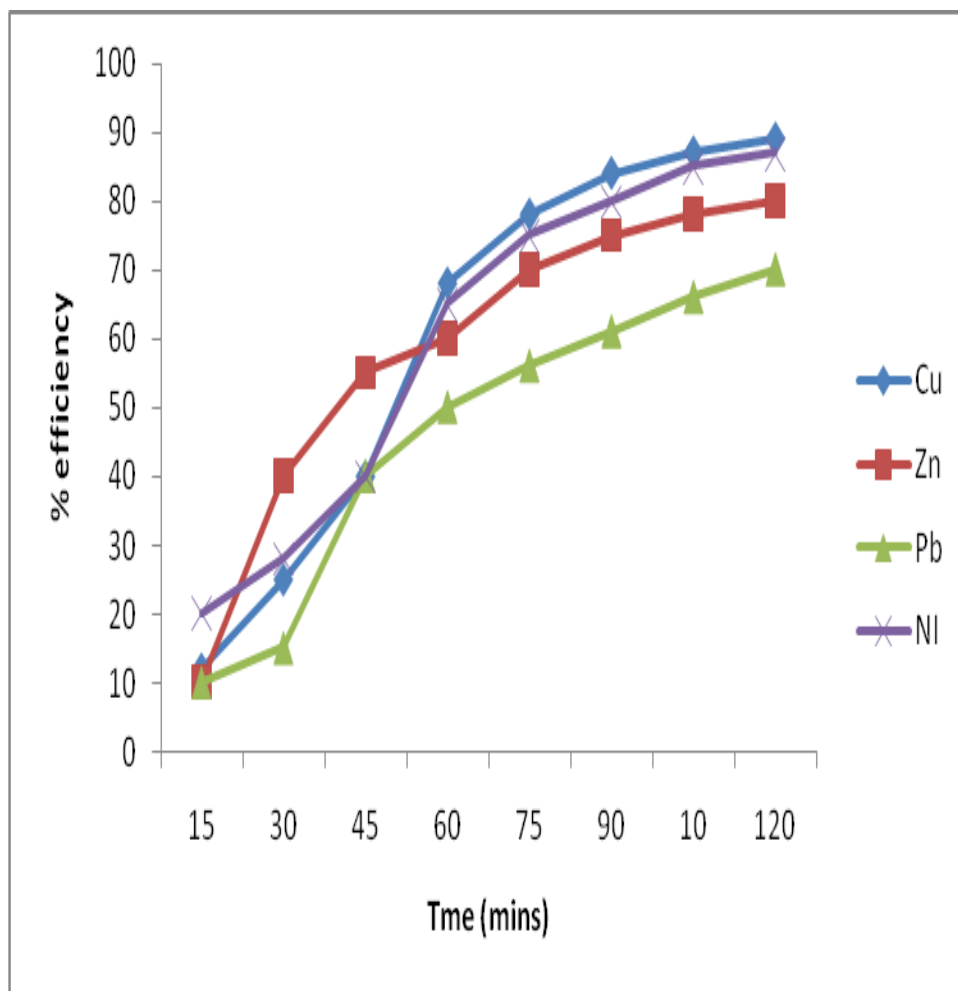


Fig. 4.5: Percentage removal efficiency of metal ion with contact time

Adsorption process is usually studied through graphs known as adsorption isotherms. The amount of adsorbate on the adsorbent is considered to be a function of its concentration at constant temperature. The quantity adsorbed is nearly always normalized by the mass of the adsorbent. From Figure 4.5 it can be predicted that after saturation, adsorption does not occur anymore, since there are limited numbers of vacancies on the surface of the adsorbent. A stage was reached when all the sites were occupied and no further significant difference in adsorption process occurred.

Figure 4.5 can be easily compared to Langmuir adsorption isotherm shown Figure 4.6 and closely resembles the Langmuir Isotherm.

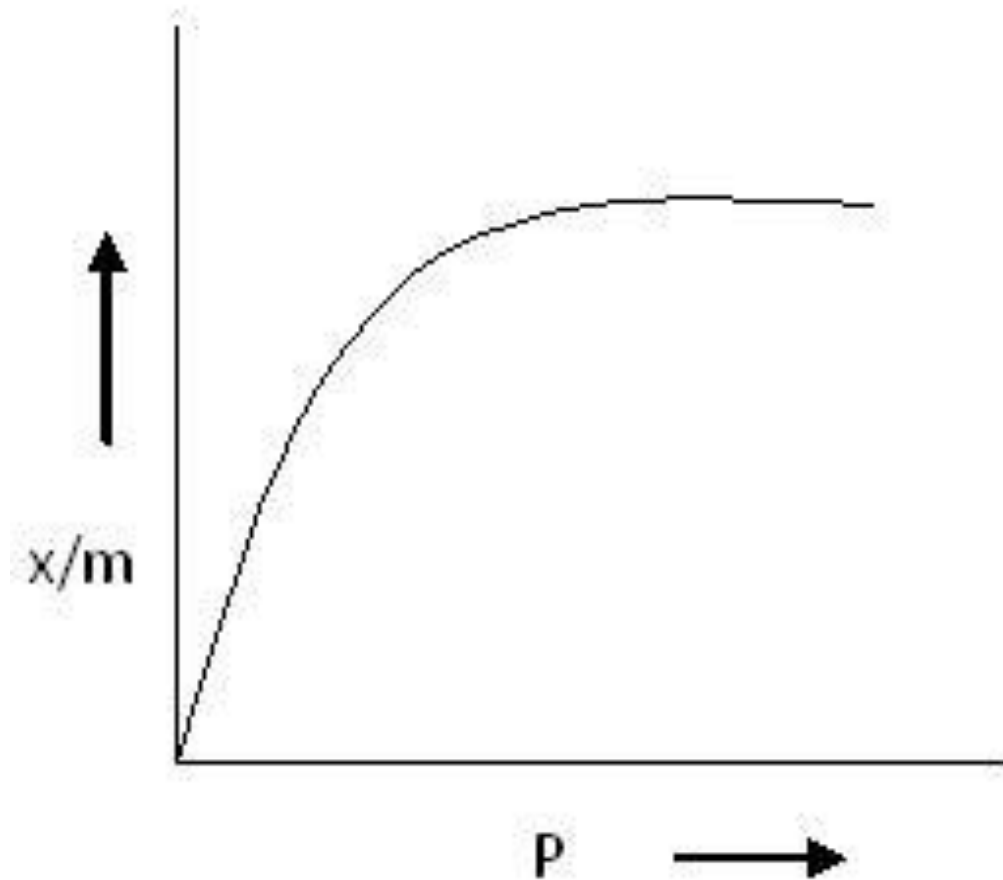


Fig. 4.6: Langmuir Adsorption Isotherm (<http://en.wikipedia.org/wiki/Adsorption>)

x/m = adsorption per gram of adsorbent

P = concentration or contact time

This isotherm describes adsorbate-adsorbent systems in which the extent of adsorbate coverage is limited to the surface of the adsorbent available.

Fitting the data from biosorption experiment in the linear form of Langmuir isotherm (equation 2.2), different values of the terms in the equation were obtained and tabulated in Table 4.4, for Zn^{2+} .

Table 4.4: Values of Langmuir equation terms for Zn^{2+}

M	Y	C	M/Y	1/C
0.20	0.04	0.25	0.70	4.00
0.20	0.11	0.17	0.85	5.90
0.20	0.15	0.13	0.92	7.69
0.20	0.17	0.11	1.11	9.50
0.20	0.20	0.08	1.52	12.50
0.20	0.21	0.07	1.58	14.30
0.20	0.22	0.06	1.80	16.70
0.20	0.23	0.05	2.10	19.00

Y = concentration of contaminant adsorbed

M = adsorbent concentration

C = equilibrium concentration of contaminant

The data shown in Table 4.4 was plotted in a graph and best fit line drawn. The equation of the best fit line is shown in Figure 4.7

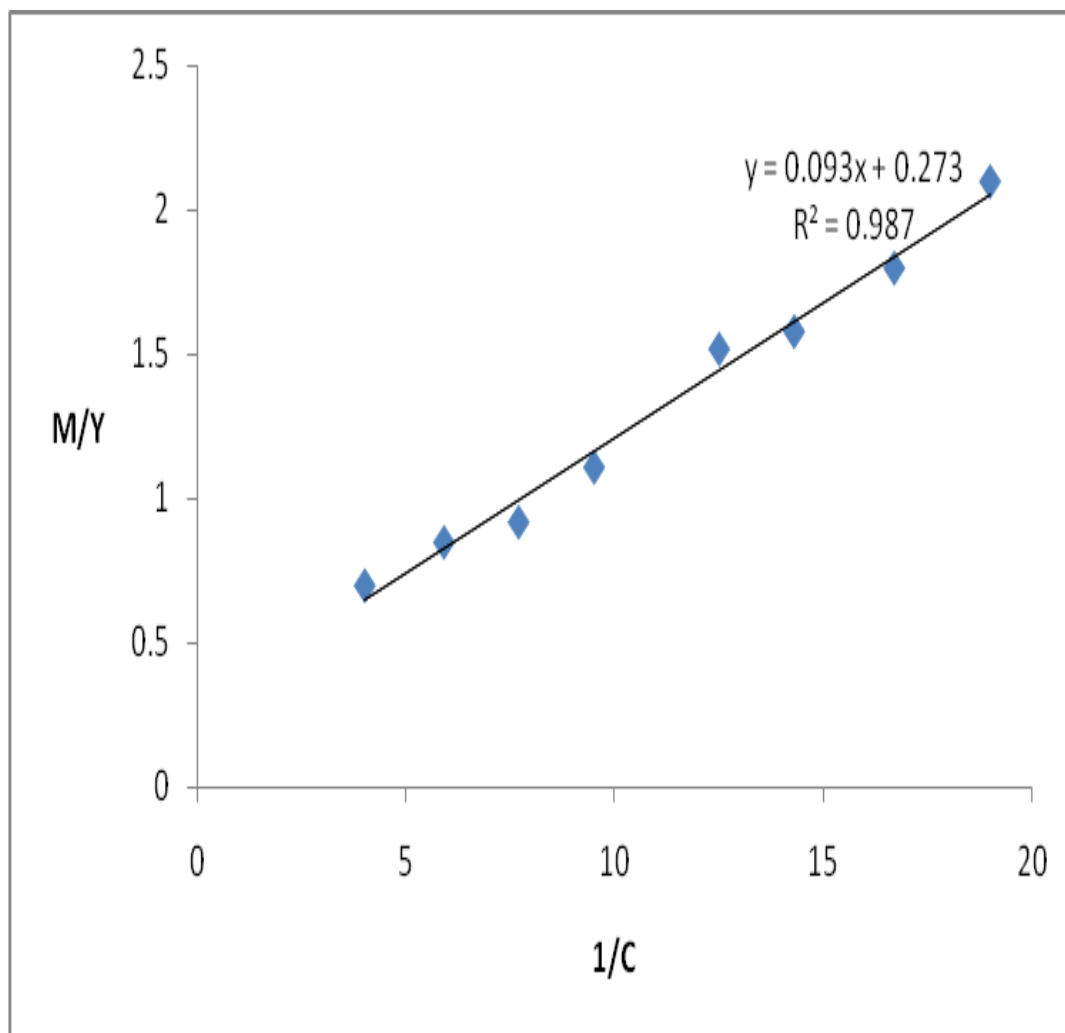


Fig. 4.7: Linearized Langmuir plot for Zn^{2+}

From the equation, y-intercept = 0.273, by extrapolation, the value of b is 3.66.

Similarly, the slope is 0.093, and by applying this and the value of b calculated above, the value of a is determined as 2.94.

The linearized Langmuir plots for the other metal ions are given in the Appendix 11 to 14. From the plots, the Langmuir constants were determined for all the metal ions and given in Table 4.5.

Table 4.5: Langmuir constants of metal ions in River Migori samples

	a	b	R ²
Cu ²⁺	13.1	7.75	0.817
Ni ²⁺	4.0	2.70	0.884
Zn ²⁺	3.0	3.66	0.988
Pb ²⁺	19.2	0.43	0.879

The b term denotes the adsorption capacity, and therefore from Table 4.5, it shows that the adsorption capacity of brown algae towards Cu²⁺ ions was the highest while for Pb²⁺ ions was the least, and biosorption was in the order of Cu²⁺ > Zn²⁺ > Ni²⁺ > Pb²⁺. Although Pb²⁺ ions are the largest, they were the least biosorbed. This irregularity could be due to the very low concentration of Pb²⁺ ions as compared to Cu²⁺ ions in the river water. Further more river water was used in its natural state and there could have been other underlying factors such as competition of ions towards biosorption to the biosorbent. This order agrees with the results found earlier in Figure 4.1. It also follows closely the results of other works done earlier (Babu and Ramakrishna, 2003).

The Freundlich isotherm is given by equations 2.3 and 2.4. Fitting the data from biosorption experiment in the linear form of Freundlich isotherm (equation 2.4), different

values of the terms in the equation were obtained and tabulated in Table 4.6 below, for Zn. On plotting $\log x/m$ versus $\log C$, Figure 4.8 was obtained.

Table 4.6: Values of Freundlich equation terms for Zn^{2+}

C	x/m	log C	log x/m
0.05	0.4	-1.28	-0.38
0.06	0.6	-1.22	-0.22
0.07	0.9	-1.15	-0.05
0.08	1	-1	0
0.11	1.2	-0.98	0.08
0.13	1.25	-0.88	0.1
0.17	1.3	-0.77	0.11

x = mass of adsorbate

m = mass of adsorbent

C = equilibrium concentration of adsorbate

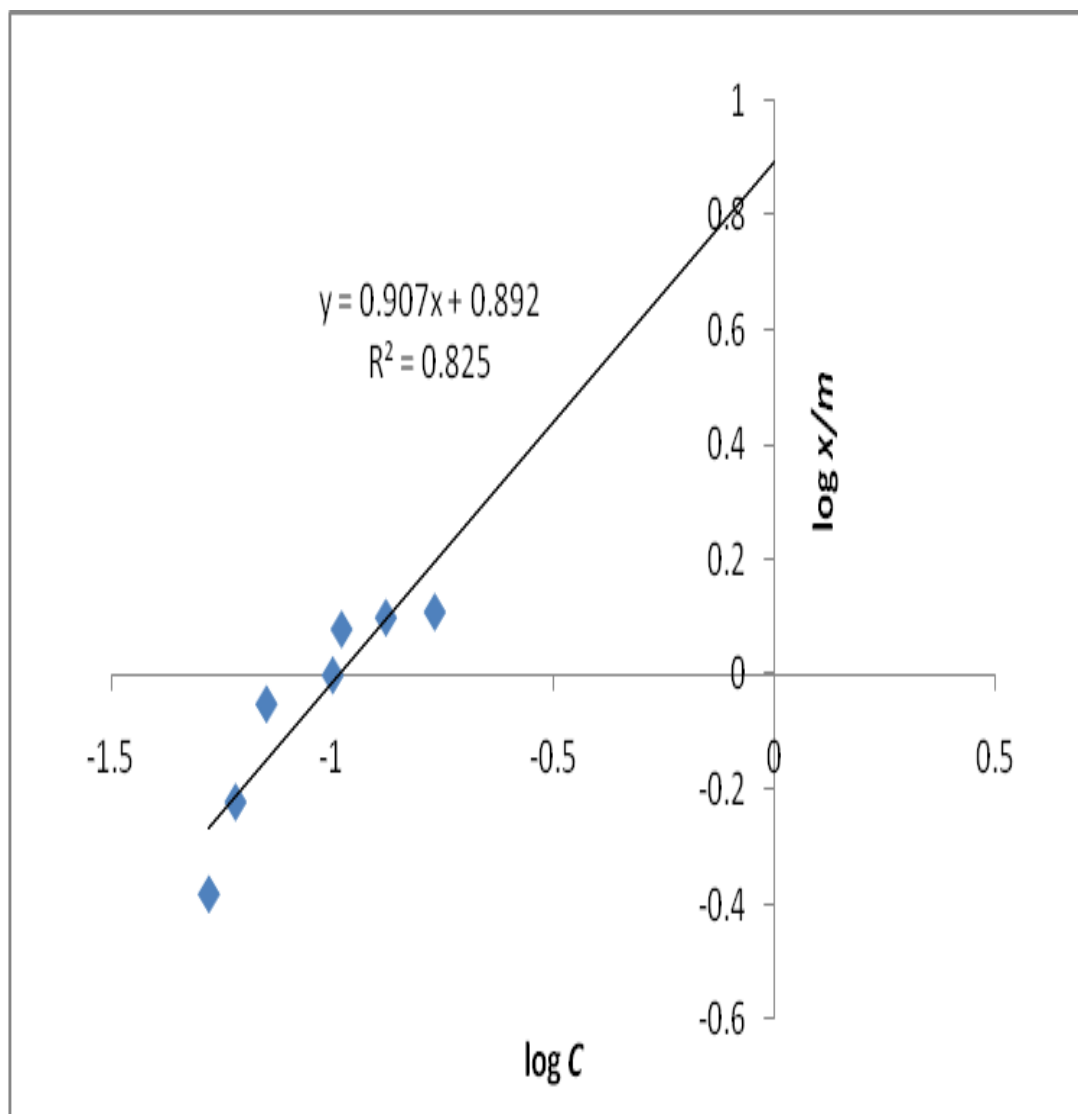


Fig. 4.8: Linearized Freundlich plot for Zn^{2+}

The equation of the line is shown on the graph where slope = 0.907 and y-intercept = 0.892. The value of n was calculated to be 1.17.8 and k determined as 7.8.

The linearized Freundlich plots for the other metal ions are given in the Appendix 15 to 18. From the plots, the Freundlich constants were determined for all the metal ions and presented in Table 4.7.

Table 4.7: Freundlich constants of metal ions in River Migori samples

	n	k	R ²
Cu ²⁺	1.70	3.5	0.870
Ni ²⁺	1.53	2.5	0.846
Zn ²⁺	1.10	7.8	0.826
Pb ²⁺	0.80	10.2	0.719

Since the value of n indicates favourability of adsorption, it is evident from Table 4.7 that adsorption of Cu²⁺ ion was most favourable while that of Pb²⁺ ion least favourable and biosorption was, therefore, in the order of Cu²⁺ > Ni²⁺ > Zn²⁺ > Pb²⁺. This closely relates to Langmuir data but deviates slightly in order of Ni²⁺ and Zn²⁺ ions. The slight deviation may be attributed to the fact that the two isotherms behave differently with pressure. Langmuir isotherm is valid at low pressures while Freundlich isotherm varies directly with pressure till saturation is reached (<http://www.chemistrylearning.com/freundlich-adsorption-isotherm/> and <http://www.transtutors.com/chemistry-homework-help/surface-chemistry/adsorption-isotherm>).

4.1 Order of reaction

The order of reaction explains the relationship between the concentration of reactants and the rate of reaction. It is given by the sum of the exponent of the concentration terms of the rate law: $\text{Rate} = k[A]^x[B]^y$. The data was subjected to reaction kinetics to deduce whether the process followed pseudo first order or pseudo second order. The rate equation gives an expression of the variation in concentration of metal ions with time. The order of reaction was deduced from the integrated rate equation given as;

$$\frac{dq_t}{dt} = k(q_e - q_t) \quad (4.1)$$

where, q_t and q_e are masses of metal biosorbed by the biomass at time t and at equilibrium, respectively. The k is the rate constant of biosorption. The values of k_1 , k_2 (for first and second order) and q_e were calculated and tabulated in Figure 4.8. From the R^2 values, it is possible to deduce the order of reaction.

Table 4.8: Data for first and second order kinetics for metal ions

	Pseudo first order			Pseudo second order		
	k_1	q_e	R^2	k_2	q_e	R^2
Cu^{2+}	0.021	24.34	0.870	1.725	0.101	0.817
Ni^{2+}	0.081	46.34	0.846	1.812	0.124	0.884
Zn^{2+}	0.023	39.45	0.826	1.900	0.120	0.988
Pb^{2+}	0.031	55.46	0.719	2.010	0.110	0.879

A comparison of R^2 values between first and second order shows the values of second order higher than first order. Therefore the biosorption of metal ions by brown algae followed pseudo second order.

From Table 4.9, it can be shown that during the wet season, nitrates at all sampling stations S1, S2, S3, S4, S5 and S6 varied between 0.8 and 2.3mg/L with the mean values of 2.1 ± 0.153 , 1.8 ± 0.252 , 1.3 ± 0.503 , 1.9 ± 0.173 , 1.8 ± 0.153 and 1.6 ± 0.208 mg/L, respectively. During the dry season, nitrates at all the sampling stations varied between 0.6 and 1.3 mg/L with the respective mean values of 1.1 ± 0.100 , 1.1 ± 0.058 , 0.9 ± 0.153 , 1.2 ± 0.100 , 1.0 ± 0.153 and 0.9 ± 0.305 mg/L. Phosphates varied between 1.9 and 2.6mg/L during the wet season, at the six sampling sites, with the corresponding mean values of 2.4 ± 0.200 , 2.1 ± 0.071 , 1.9 ± 0.153 , 2.4 ± 0.115 , 2.3 ± 0.100 and 2.0 ± 0.100 mg/L. During the dry season, phosphates at all the sampling stations varied between 0.6 and 1.3 mg/L with the mean values of 0.38 ± 0.020 , 0.40 ± 0.006 , 0.37 ± 0.150 , 0.41 ± 0.100 , 0.33 ± 0.152 and 0.34 ± 0.010 mg/L, respectively.

Table 4.9: pH values and concentration of nutrients (mg/L) for all sampling points

		S1	S2	S3	S4	S5	S6
pH	Wet	6.8 ±0.153	6.7 ±0.053	6.8 ±0.153	6.8 ±0.252	6.8 ±0.058	6.7 ±0.153
	Dry	7.1 ±0.100	7.2 ±0.115	7.2 ±0.058	6.9 ±0.058	6.9 ±0.100	6.9 ±0.058
NO₃⁻	Wet	2.1 ±0.153	1.8 ±0.252	1.3 ±0.503	1.9 ±0.173	1.8 ±0.153	1.6 ±0.208
	Dry	1.1 ±0.100	1.1 ±0.058	0.9 ±0.153	1.2 ±0.100	1.0 ±0.153	0.9 ±0.305
PO₄³⁻	Wet	2.4 ±0.200	2.1 ±0.071	1.9 ±0.153	2.4 ±0.115	2.3 ±0.100	2.0 ±0.100
	Dry	0.38 ±0.200	0.40 ±0.006	0.37 ±0.015	0.41 ±0.010	0.33 ±0.015	0.34 ±0.010

From Table 4.10, BOD ranged from 7 to 17 mg/L during the wet season, at the six sampling stations with the mean values of 8 ± 1.00 , 11 ± 1.00 , 13 ± 1.73 , 14 ± 1.00 , 16 ± 0.577 and 14 ± 1.53 mg/L, respectively. During the dry season, BOD at all the sampling stations varied between 10 and 25 mg/L with the corresponding mean values of 13 ± 2.52 , 15 ± 0.00 , 18 ± 0.577 , 20 ± 0.577 , 24 ± 1.00 and 22 ± 1.73 mg/L. COD varied between 14 and 32mg/L during the wet season, at the six sampling sites, with the mean values of 17 ± 3.00 , 23 ± 1.53 , 27 ± 3.78 , 29 ± 3.00 , 32 ± 2.08 and 28 ± 1.52 mg/L, respectively. During the dry season, COD varied between 21 and 50 mg/L with the

respective mean values of 26 ± 5.03 , 31 ± 1.15 , 37 ± 1.15 , 40 ± 3.00 , 47 ± 0.577 and 44 ± 3.05 mg/L.

Table 4.10: Mean values of COD and BOD (mg/L) along River Migori

		S1	S2	S3	S4	S5	S6
BOD	Wet	8 ± 1.00	11 ± 1.00	13 ± 1.73	14 ± 1.00	16 ± 0.577	14 ± 1.53
	Dry	13 ± 2.517	15 ± 0.00	18 ± 0.577	20 ± 0.577	24 ± 1.00	22 ± 1.73
COD	Wet	17 ± 3.00	23 ± 1.53	27 ± 3.78	29 ± 3.00	32 ± 2.08	28 ± 1.53
	Dry	26 ± 5.03	31 ± 1.15	37 ± 1.15	40 ± 3.00	47 ± 0.577	44 ± 3.055

The concentrations of nutrients during wet season are shown in Figure 4.9. Both nitrates and phosphates concentrations were maximum at S1 during wet season and minimum at S3, and then increased downstream. An increase in these nutrient values observed at S4 could be attributed to the runoff from town and residential areas. There was a decrease after S4 which shows the river's self-purification and dilution factor.

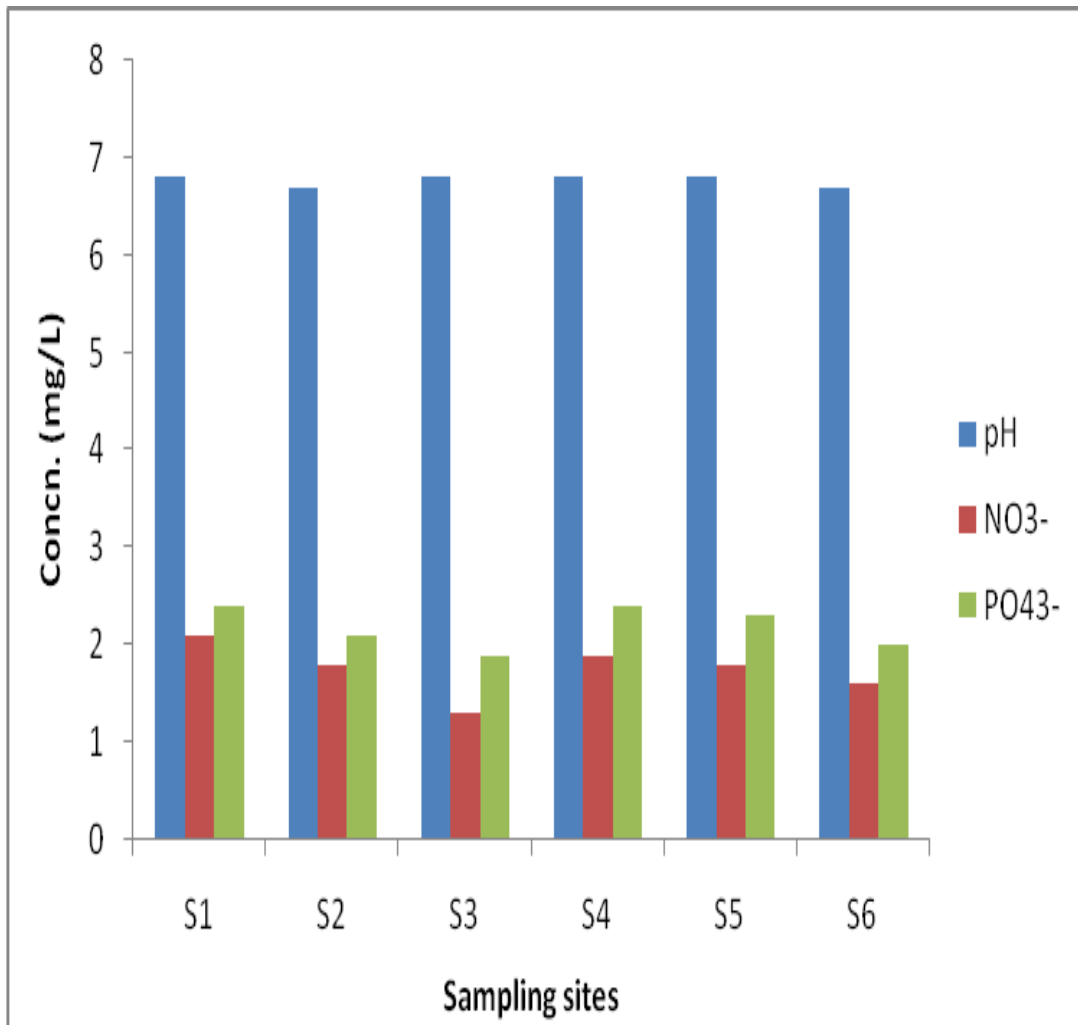


Fig. 4.9: Concentration of nutrients for the wet season

The concentrations of nutrients during dry season are shown in Figure 4.10. As in wet season, both nitrates and phosphates concentrations were maximum at S1. It followed the same trend as in the wet season.

The nitrate levels for all the sites sampled during both the dry and wet seasons were below the water quality standards (10 mg/L) set by WHO (1990). But generally values from the wet season were higher than dry season.

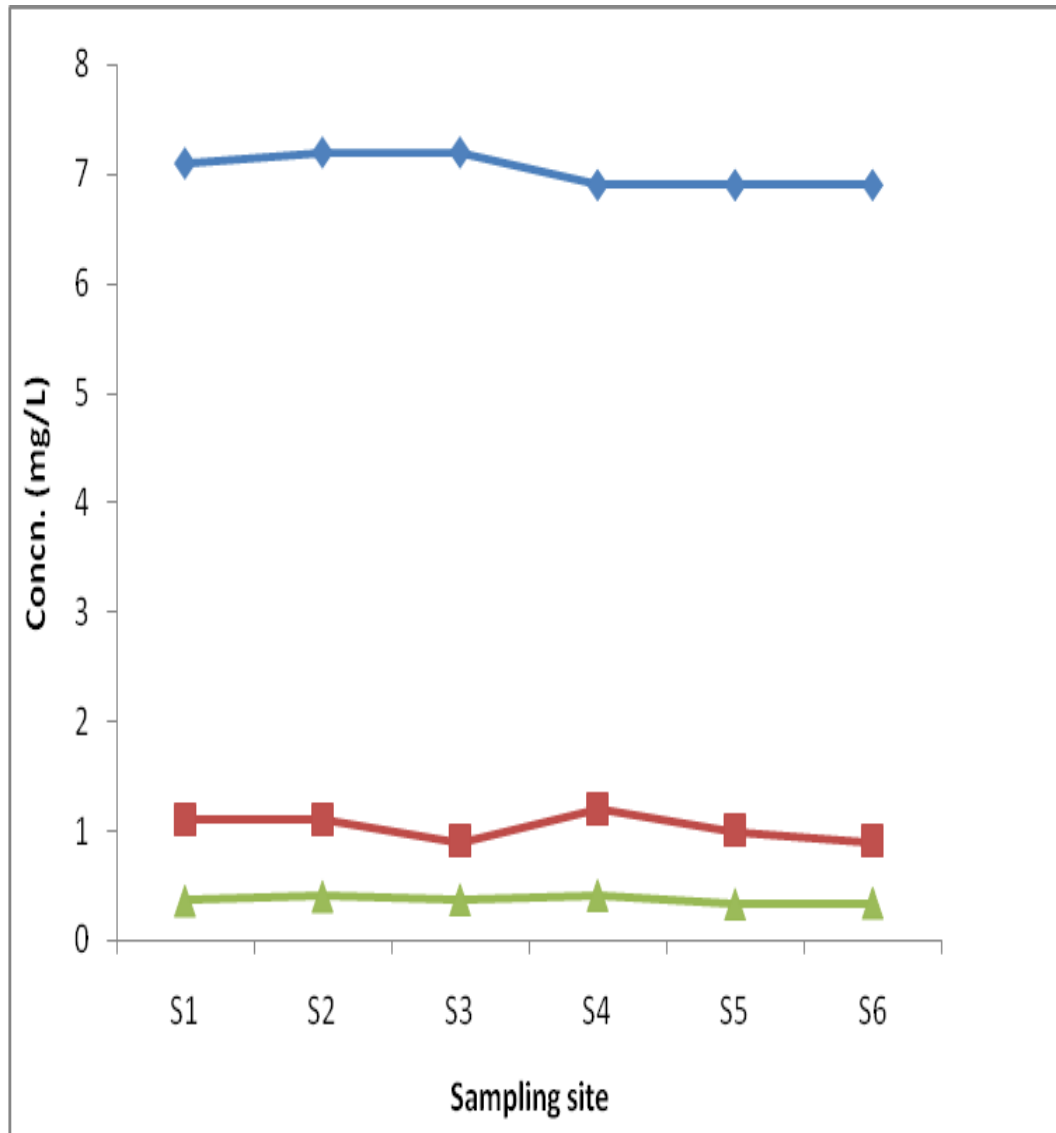
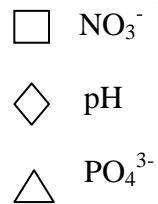


Fig. 4.10: Concentration of nutrients for the dry season



The concentration values of COD and BOD during wet season are shown in Figure 4.11.

The values were minimum at S1 during wet season, increased to maximum at S5 then decreased downstream towards S6. This decrease could be attributed to the river's self-

purification dilution factor. The low values at S1, upstream of the river, are where pollution from town's runoff had not been felt.

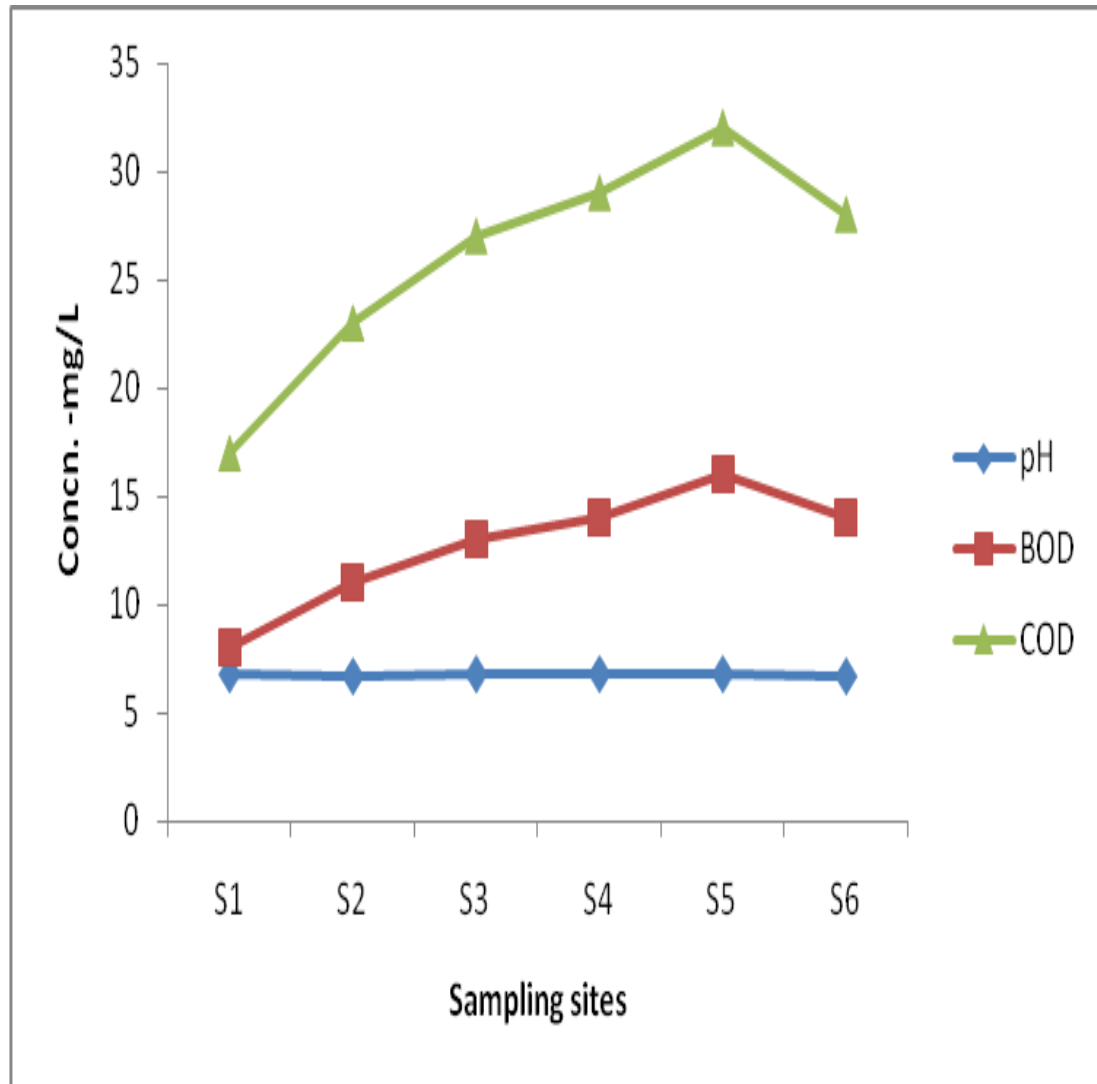


Fig.4.11: Mean values of COD and BOD during wet season

The values of COD and BOD during dry season are shown in Figure 4.12. Just like wet season, the values were minimum at S1 and maximum at S5 during the dry season then decreased downstream towards S6. This decrease could be attributed to the river's self-purification and dilution factor.

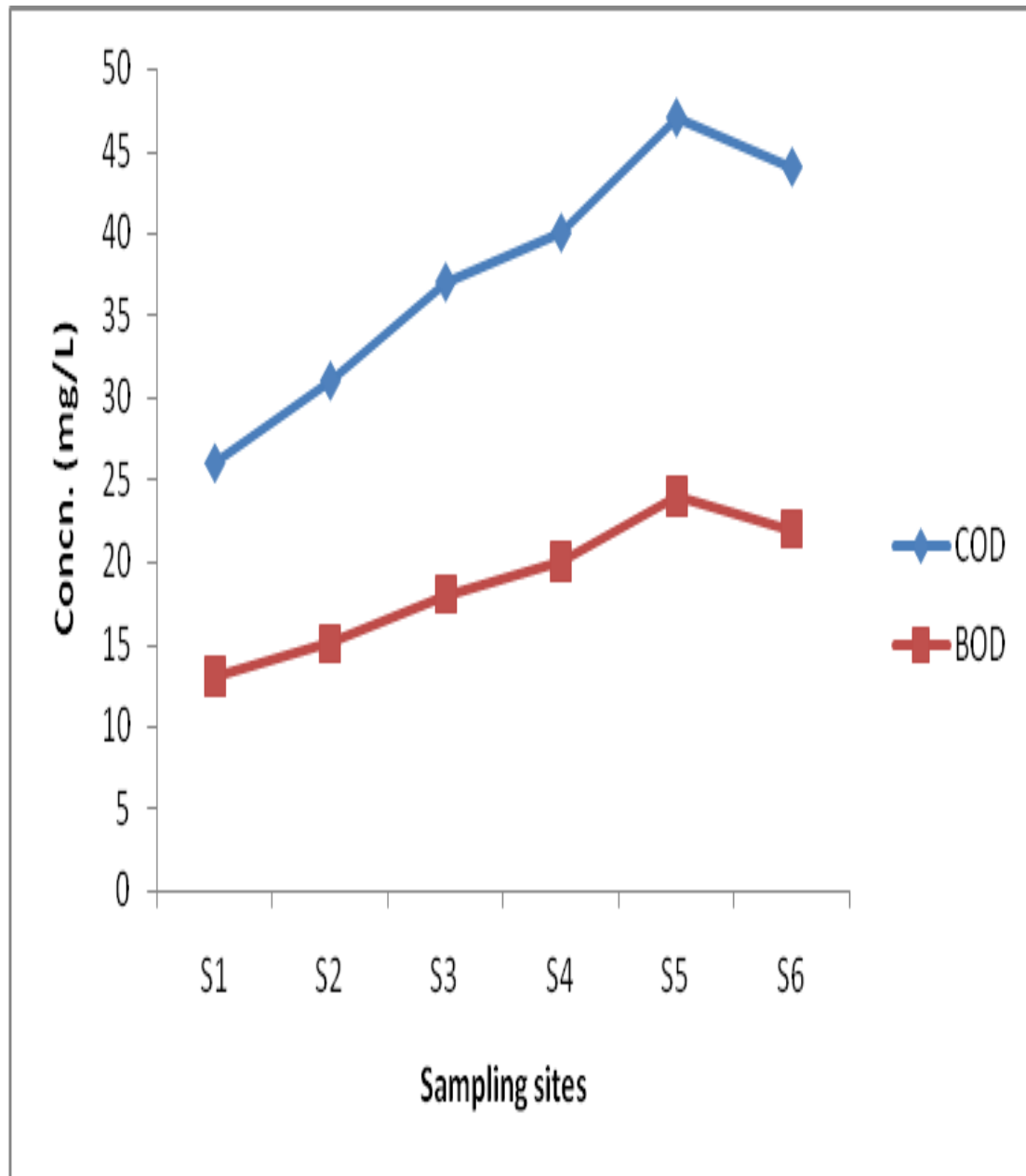


Fig.4.12: Mean values of COD and BOD for dry season

The levels of both COD and BOD in dry season were higher than in wet season. This is could be due to dilution of the river with storm water during rainy season. However, the concentrations in both wet and dry seasons were above WHO standards for surface waters. The recommended values are 10mg/L and 5mg/L, for COD and BOD (WHO, 1990), respectively.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The initial concentrations of Ni^{2+} , Pb^{2+} , Zn^{2+} , and Cu^{2+} ions in the river water were 0.07, 0.06, 0.27 and 0.28 mg/L, respectively before adsorption. Urban runoff contributed to heavy metal contamination of the river. The ability of algal biomass to adsorb metal ions as shown from the results can be used for the development of an efficient, clean and cheap technology for effluent treatment. From the results of this study, it was found that algal biomass can easily be applied as a cheap adsorbent for heavy metal removal such as Ni^{2+} , Pb^{2+} , Zn^{2+} , Cu^{2+} . It was also found that pH had a clear influence on the sorption capacity of algal biomass for removal of heavy metals.

From the study it was found that the optimum pH value was between 6.0 and 7.0 at which the adsorption capacity of algae was highest. The Cu^{2+} ion had the highest percent removal of 89% after 120 minutes of adsorption, followed by Ni^{2+} , Zn^{2+} and Pb^{2+} with 87%, 80% and 70%, respectively. Maximum removal was attained within 120 minutes of contact time. The group of cheap biosorbent materials, like brown algae (*Sargassum sp*), based on natural and waste biomasses seems to constitute the basis for a new cost-effective technology that can find its largest application in the removal of metal contaminated industrial effluents. Although the levels for nutrients were within the accepted WHO standards, runoff through town could have contributed to the river

contamination. Nutrient levels downstream after the town were higher than those upstream before. The same trend was observed with COD and BOD.

5.2 Recommendations

- 1) The development of biosorption processes requires further investigation in the direction of modeling, of regeneration of biosorbent material and of testing immobilized raw biomasses with industrial effluents.
- 2) Since there is still low degree of understanding of its metal-binding mechanisms, it poses a scientific challenge, which calls for continued research and development (R&D) efforts.
- 3) To attract more usage of biosorbent technology, some strategies have to be developed where further processing of biosorbent can be done to regenerate the biomass and then convert the recovered metal into usable form.
- 4) The pollution in the river should be continuously monitored in order to follow up properly the pollution parameters to enable the right action to be taken at the right time.
- 5) There is need for a study cataloguing the different chemicals used by different factories within Migori town. This will hopefully establish the definite sources of the heavy metals and nutrients and control their concentrations before they become too high and harmful to the ecosystem.
- 6) Riparian buffer zone should also be used to prevent river pollution by checking runoff into the river.
- 7) There should be strict enforcement of statutory and legal regulations which are in place to safeguard environment. These are, The Water Act (2002); Environment Management and coordination Act (EMCA) (1999), among others.

REFERENCES

- Addagalla, K.**, Naif, D. and Nidal, N. (2009). Study of various parameters in the biosorption of heavy metals by activated sludge. *World Applied Sci Jour.*, **5**:32-40.
- Ahalya, N.**, Ramachandra, T. and Kanamadi, R. (2003). Biosorption of heavy metals, *Res. J. Chem. Environ.* **7**(4):66-75.
- Ahluwalia, S.**, and Goyal, D. (2007). Microbial and plant derived biomass for removal of heavy metals from wastewater. *Biosour Technol.*, **98**:2243-2257.
- Ajaykumar, A.**, Saleh, A. and Nidal, H. (2008). Investigation of kinetics and mechanisms involved in the biosorption of heavy metals on activated sludge. *Intl. Jour. Green Energy*, **4**(5):313-321.
- Ajibade, L.** (2004). Assessment of water quality along River Asa, Nigeria; *The Environmentalist*, **24**:11-18.
- Ajmal, M.**, Ali, M., Rahan, Y. and Anus, A. (1998). Adsorption behavior of Cadmium, Zinc, Nickel and Lead from aqueous solutions by *Mangifera indica* seed shell. *Indian J. Environ Hlth* **40**(1):15-26.
- Aksu, Z.** (2001). Equilibrium and kinetic modeling of cadmium (II) biosorption by *C. vulgaris* in a batch system: effect of temperature. *Sep. purify. Technol.* **21**:285-294.

APHA, (2005). *Standard methods for the examination of water and wastewater*. 19th ed. American Public Health Association, Washington, DC.

Babu, B. and Ramakrishna, V. (2003). Modeling of adsorption isotherm constants using regression analysis and neural networks. Proceedings of 2nd international conference on Water Quality Management, organized by Central Board of Irrigation and Power, India, New Delhi, Feb. 13-15, 2003. Paper No. II pp 1-11.

Bai, R. and Abraham, T. (2001). Biosorption of Cr (VI) from aqueous solution by *Rhizopus nigricans*. *Biores. Technol.* **79**:73-81.

Bai, R. and Abraham T. (2002). Studies on enhancement of Cr(VI) biosorption by chemically modified biomass of *Rhizopus nigricans*. *Water Res.* **36**:1224–36.

Balcerzak, M. (2002). Sample digestion methods for the determination of traces of metals by spectroscopic techniques. *Anal Sci.* **18**:737-750

Bishop, P. (2002). *Pollution prevention: Fundamentals and practice*. Beijing: Tsinghua University Press.

Buol, S. (1995). Sustainability of soil use: *Annual Review of Ecol and Systematics* **26**:25-44.

Carpenter, S., Caraco, N. and Smith, V. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen: *Ecological applns* **8**:559-568.

Chang, J. and Hong, J. (1994) Biosorption of mercury by the inactivated cells of *Pseudomonas aeruinos*a. *Biotechnol. Bioeng.* **44**: 999-1006.

Chapman, D. (1996). Water quality Assessment: A guide to the use of biota, sediments and water in environmental monitoring, 2nd Ed. Routledge, UK. pp.72-73.

Chu, K., Hashim, M., Phong, S., and Samuel, V. (1997). Biosorption of Cadmium by Algal Biomass Adsorption and Desorption Characteristics. *Water Sci. Technol.*, **35** (7), 15.

CIESE (2009). Dissolved oxygen Worksheet;

<http://www.ciese.org/curriculum/dipproj2/en/fieldbook/oxygen.shtml> -accessed3/8/2011.

Clair, N., Perry, L., and Gene, F. (2003). Chemistry for Environmental Engineering and Science (5th ed.). New York: McGraw-Hill.

Conservation Currents, (2005). Northern Virginia Soil and Water Conservation District, March 2005. <http://www.fairfaxcounty.gov/nvswcd/newsletter> -accessed 3/8/2011.

Crist, R., Martin, H., Robert, J., Guptill, P., Eslinger, J. and Crist, D. (1990). Interaction of metals and protons with algae. Ion exchange in adsorption and metal displacement by protons. *Environment and Sci and Technol*, **24**(3): 337-342.

Daily Nation, Tuesday 13th July, 2010.

Daniel, T., Sharpley, A., Wedepold, R. and Lemunyon, J. (1994). Minimizing surface water eutrophication from agriculture, *Jour of soil and water conservation*, **49**:30-38.

Davis, T., Volesky, B. and Musci, A. (2003). A review of biochemistry of heavy metal biosorption by brown algae. *Water Resour*, **37**(18):4311-4330.

Dobson, R. and Burgess, J. (2007). Biological treatment of precious metal from refinery wastewater: A review. *Minerals Eng.*, **20**:519-532.

Dohner, E., Markowitz, A., Barbour, M. and Simpson, J. (1997). Volunteer Stream Monitoring: A Methods Manual, USEPA, EPA 841-B-97-003.

Dorris, K., Yu, B., Zhang, Y., Shukla, A. and Shukla, S. (2000). The removal of heavy metals from aqueous solution by sawdust adsorption-removal of copper. *J. Hazardous Mat.* B80:33-42.

Dupont, L., Bouanda, J., Dumoneau, J. and Applincourt, M. (2003). Metal ions binding onto a lignocelulosic substrate extracted from wheat bran: a nica donnan approach. *J. Colloid Int. Sci.* **263**:35-41.

Farah, S. and Sneddon, J. (1991). Selective removal of silver in the presence of copper, manganese and zinc in solution by an algal biomass. *J. Environ. Sci. Health.* **A27(7):**1579-11587.

Fourest, E. and Volesky, B. (1996). Contribution of Sulfonate Groups and Alginate to Heavy Metal Biosorption by the Dry Biomass of *Sargassum Fluitans*. *Environ. Sci. Technol.*, 30, 277.

Francois, L., Fortin, C. and Campbell, P. (2007). pH modulates transport rates of manganese and cadmium in the green algae *Chlmydomonas reihardtii* through non-competitive interaction for an algal BLM. *Aquat. Toxicol.* **84:**123-132.

Freire.nordi, C., Vieira, A. and Nascimento (2005). The metal binding capacity of *Anabaena spirides* extracellular polysaccharide: An EPR study. *Process Biochem*, **40:**2215-2224.

Gadd, G. (1992). Biosorption. *Jour of Chemical Technol and Biotechnol*, **55:**302–304.

Goyal, N., Jain, S. and Qian, Y. (2003). Comparative studies on the biosorption of heavy metals. *Adv Environ Res*, **7:**311-319.

Gulnaz, O., Sayagideder, S. and Kusvuran, E. (2005). Study of copper (II) biosorption by dried activated sludge: Effect of physic-chemical environment and kinetics study. *Jour Harzadous mat*, **B120:**193-200.

Hach Procedures Handbook, (2003).

Hammaini, A., Gonzalez, F., Ballester, A., Blazquez, M and Munoz, J. (2006). Biosorption of heavy metals by activated sludge and their desorption characteristics. *Jour Environ. Mangnt*, **84**(4):419-426.

Holan, Z. and Volesky, B. (1993). Biosorption of Cadmium by Biomass of Marine Algae. *Biotechnol. Bioeng.*, 41, 819.

<http://www.tip2000.com/health/waterpollution.asp> (2000). Effects of heavy metals to human health –accessed 6/10/2011.

<http://en.wikipedia.org/wiki/Adsorption>. -accessed 7/1/2012

<http://www.chemistrylearning.com/freundlich-adsorption-isotherm/> -accessed 7/1/2012

<http://www.transtutors.com/chemistry-homework-help/surface-chemistry/adsorption-isotherm>. -accessed 7/1/2012

http://en.wikipedia.org/wiki/Lead_poisoning accessed 2/2/2012

http://www.trendsimwatching.com/2006/04/investigating_in_to.html -accessed 5/3/2011.

IAWPRC (1988). Advances in Water pollution control, p 45.

Igwe, J. and Abia A. (2007). Equilibrium sorption isotherm studies of Cd, Pb and Zn ions detoxification from wastewater using unmodified and EDTA-modified maize husk. *Electronic Jour of Biotechnol.* Vol. 10 No. 4, 33-42.

Kapoor, A. and Viraraghavan T. (1997). Heavy metal biosorption sites in *Aspergillus niger*. *Biores Technol.* **61**:221–7.

Karvelas, M., Katsoyiannis, A. and Samar, C. (2003). Occurrence and fate of heavy metals in wastewater treatment process. *Chemosphere*, **53**:1201-1210.

Khoo, M. and Ting, Y. (2001). Biosorption of gold by immobilized fungal biomass. *Biochem Eng Jour*, **8**:51-59.

Kim-Shapiro, D., Gladwin, M., Patel, R. and Hog, N. (2005). The role of nitrite in haemoglobin-mediated hypoxic vasodilation; *Jour. of inorganic biochem*, **99**:(1):237-246.

Leon, Y., Leon, C., Solar, J., Calemma, V. and Radovic, L. (1992). Evidence for the protonation of basal plane sites on carbon. *Carbon.* **30**:797-811.

Liu, Y., Cao, Q., Luo, F. and Chen, J. (2009). Biosorption of Cd²⁺, Cu²⁺, Ni²⁺ and Zn²⁺ ions from aqueous solutions by pretreated biomass of brown algae.

Mahiva, A., Gholami, F. and Nazmara, S. (2008). Cadmium adsorption from wastewater by almas leaves and their ash. *Euro Sci. Res.* **23**:197-203.

Mamari N., Boudries N., Addour L., Belhocine D., Lounici H., Grib H., and Pauss, A. (1999). Batch zinc biosorption by a bacterial nonliving *Streptomyces rimosus* biomass. *Water Res.* **33**:1347-54.

Mason, C. (1991). Biology of fresh water pollution 2nd Ed., Longman Scientific and Technical, N.Y. pp. 94-100.

Matheicka, J. and Yu, Q. (1999). Biosorption of lead (II) and copper (II) from aqueous solution by pretreated biomass of Australian marine algae. *Bioresour Technol*, **69**:223-229.

Nakajima, A. and Tsuruta, T. (2004). Competitive biosorption of thorium and uranium by *Micrococcus luteus*. *J Radioanal Nucl Chem*, **260**:13-8.

Nilanjana, D., Vimala, R. and Karthika, P. (2008). Biosorption of heavy metals –An overview. *Indian Jour of Biotechnol*, **7**:159-169.

Nile Basin Initiative –NBI (2005). National Nile basin water quality Quality Monitoring Baseline Report for Kenya: the state of water quality monitoring in the Kenya portion of the Nile basin (Lake Victoria) pp 29-32.

Nilanjana, D., Vimala, R. and Karthika, P. (2008). Biosorption of heavy metals –An overview. *Indian Jour of Biotechnol.* Vol. 7, 159-169.

Oboh, I., Aluyor, E. and Audu, T. (2009). Biosorption of heavy metal ions from aqueous solutions using a biomaterial. *Leonardo Jour of Sci*, **14**:58-65.

Omar, H. (2002). Bioremoval Zinc ions by *Scenedesmus obliquus* and *Scenedesmus quadricauda* and its effects on growth and metabolism. *Int. Biodeterior. Biodegrad.* **50**:95-100.

Ozer, A. and Ozer, D. (2004). The adsorption of copper ions onto dehydrated wheat bran (DWB): Determination of equilibrium and thermodynamic parameters. *Process Biochem.* **39**:2183-2191.

Phiri, O., Mumba, P., Moyo, B. and Kadewa, W. (2005). Assessment of the impact of industrial effluents on water quality of receiving rivers in urban areas of Malawi. *International Jour of Environmental Sci and Technol*, **2(3)**:237-244.

Pink, D. (2006). Investing in tomorrow's liquid gold: water.

Radovic, L., Silvia, Ume, J., Menendez, J., Leon, C., Leon, Y. and Scaroni, A. (1997). An experimental and theoretical study of the adsorption of aromatics possessing electron-withdrawing and electron-donating functional groups by chemically modified activated carbons. *Carbon*, **35**:1339-1348.

Radway, J., Wilde, E., Whitaker, M. and Wessmman, J. (2001). Screening for algal strains for metal removal capabilities. *J. Applied Phycol.* **13**:451-455.

Raji, C., Shubha, K. and Anirudhan, T. (1997). Use of chemically modified sawdust in the removal of Pb(II) ions from aqueous media. *Indian J. Environ. Hlth.* **39(3)**:230-238.

Ramu, N. and Srivathsan, S. (1992). Adsorption of carboxylic acids on fly ash and activated carbon. *Indian J. Environ. Hlth* Vol. 34 No. 3, 192-199.

Rao, M. and Bhole, A. (2001). Chromium removal by adsorption using fly ash and bagasse. *Jour of Indian Water Works Assoc.* XXXIII (1), 97-100.

Saeed, A., Akhtar, M. and Iqbal, M. (2005). Removal and recovery of heavy metals from aqueous solution using papaya wood as a new biosorbent. *Sep Purif Technol*, **45**:25-31.

Samia A., Ghalia, A., Mohamed A. (2011). Removal of heavy metals from aqueous solutions by multi-walled carbon nanotubes modified with 8-hydroxyquinoline. *Chemical Eng Jour.*, **181**:159-168.

Senthikumaar, S., Bharathi, S., Nithyanandhi, D. and Subburam, V, (2000). Biosorption of toxic heavy metals from aqueous solutions. *Bioresour Technol*, **75**:163-165.

Schiewer, S. and Volesky, B. (2000). Biosorption Processes for Heavy Metal Removal. In *Environmental Microbe-Metal Interactions*; Lovley, D. R., Ed.; ASM Press: Washington, D.C. pp 329–357.

Sharpley, A., Chapra, S., Wedepold, R., Sims, J., Daniel, T. and Reddy, K. (1994). Managing agricultural phosphorus for protection of surface waters: issues and options, *Jour of Environ Quality*. **23**:437-451.

Shuler, M. and Kargi, F. (1992). Bioprocess Engineering, *Biotechnol. Bioprocess Eng.* 2001, Vol. 6, No. 6 *Basic Concepts*. Prentice Hall, Englewood Cliffs, New Jersey, USA.

Singanan M., Vinodhini, S. and Alemayehu, A. (2006). Phytoremediation of heavy metals from industrial waste waters by using indigenous biomaterials. *Indian J. Environ. Prot.* **26(5)**:385-391.

Sheng, P., Ting, Y. and Hong, L. (2004). Sorption lead, copper, cadmium, zinc and nickel by marine algal biomass: Characterization of biosorptive capacity and investigation of mechanisms. *Jour. Colloid Interface Sci*, **275**:131-141.

Singh, S., Rai, B. and Rai, L. (2005). Ni (II) and Cr (IV) sorption kinetics by *Microcystis* in single and multimetallic system. *Process Biochem*, **36**:1205-1213.

Singh, D. and Srivastava, B. (2001). Basic dyes removal from wastewater by adsorption on rice husk carbon. *Indian Jour of Chemical Technol* **8**:133-139.

Sumok, P. (2001). River water quality monitoring: Sharing Sarawak experience, proceedings of 6th site research seminar, pp. 1-4.

Swamiappan, N. and Krishnamoorthy, S. (1984). Phenol-formaldehyde cationic matrices substitutes by bagasse. *Charcoal Res. Ind.* **29**:293-297.

Synder, W. (2001), Heavy metals in water and sediments,
<http://ahs.info/redbooks/a123/iahs-123-0339.pdf> -accessed 5/2/2012

Tan, T., and Cheng, P. (2003). Biosorption of metal ions with *Penicillium chrysogenum*.
Appl Biochem Biotechnol. **104**:119–28.

Tan, W., Ooi, S. and Lee, C. (1993). Removal of chromium (VI) from solution by coconut husk and palm pressed fibres. *Environ. Technol.* **14**:277-282.

Travieso, L., Canizares, R., Borja, R., Benitez, F. and Dominguez, A. (1999). Heavy metal removal by microalgae. *Bull. Environ. Contam. Toxicol.*, **62**:144-151.

Troeh, F., Hobbie, A., and Danahue, R. (1991). Soil and water conservation 2nd Ed., Peatice Hall, New Jersey. pp. 435-455.

Tsezos, M., Remoudaki, E. and Angelatou, V. (1996). A study of the competing ions effects in the biosorption of metals, *Intl Biodeterioration and Biodegrad.* **36**:19–28.

UNEP (2005). Ecosystems and Human well being: Current state and trends.

UNEP and Gems Water (2006). Water quality for ecosystem and human health, Ontario Canada.

Upatham, E., Boonyapookana, B., Kriatracjie, M., Pokethitiyook, P. and Parkpoomkamol, K. (2002). Biosorption of cadmium and chromium in duck weed, *Wolffia globosa*. *Inter. J. Phyto.* **4(2)**:73-86.

Uslu G., and Tanyol, M. (2006). Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead(II) and copper(II) ions onto *Pseudomonas putida*: effect of temperature. *J Hazard Mater.* **135**:87–93.

Verwilghen, C., Guilet, R., Deydier, E., Menu, M. and Dartiguenave, Y. (2004). Lead and cadmium uptake by sulphur-containing modified silica gels. *Environ. Chem. Lett.* **2**:15-19.

Vieira, R., and Volesky, B. (2000). Biosorption: a solution to pollution. *Int Microbiol.* **3**:17–24.

Villaescusa, I., Martinez, M. and Miralles, N. (2002). Heavy metal uptake from aqueous solution by cork and yohimbe bark wastes. *J. Chem. Technol. Biotechnol.* **74**:812-816.

Vijayaraghavan, K., Palanivelu, K. and Velan, M. (2006). Biosorption of copper II and cobalt II from aqueous solution by crab shell particles. *Bioresour Technol*, **97**:1411-1419.

Volesky, B. (2007), Biosorption and me; *Water Research* **41**:4017 – 4029).

Volesky, B. (1999). Biosorption for the next century, the International Biohydrometallurgy Symposium El Escorial, Spain.

Wang, J., Han, Y. and Qian, Y. (2000). Progress in metal biosorption by microorganisms, *Microbiol* **27**:449-452.

Wang, J. and Chen, C. (2006). Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. *Biotechnol.* **24**:427–51.

West, L. (2006). World water day: A billion people worldwide lack safe drinking water. <http://environment.about.com/od/environmentalevents/a/waterdayqa.htm> –accessed 3/3/2011.

WHO (1990). Global fresh water quality: A first assessment. Basil Blackwell Ltd., Oxford.

Wilde, E. and Benemann, J. (1993). Bioremoval of heavy metals by the use of microalgae. *Biotechnol.* **11**: 781-812.

Womach, J. (2005). Congressional Research Service document: Report for Congress, Agriculture.

Yasemin, B. and Tez, Z. (2007). Adsorption studies on ground shells of hazelnut and almond. *Jour. Hazardous Mat*, **149**(1):35-41.

Yu, Q., Matheickal, J., Yin, P. and Kaewsarn, P. (1999). Heavy metal uptake capacities of common marine macro algal biomass. *Water Res.* **33**:1534-1537.

Zhao, M. and Duncan, J. (1997). Batch removal of hexavalent chromium by *Azolla filiculoides*. *Biotech. Appl. Biochem.* **26**:179-182.

APPENDICES

Appendix 1: Sample 1 –wet season metal ions concn. (mg/L)

Station	Cu ²⁺	Zn ²⁺	Pb ²⁺	Ni ²⁺
S1	0.02	0.10	0.008	0.02
S2	0.10	0.19	0.02	0.06
S3	0.28	0.24	0.06	0.08
S4	0.29	0.28	0.06	0.08
S5	0.27	0.23	0.05	0.06
S6	0.24	0.22	0.05	0.06

Appendix 2: Sample 2 –wet season metal ions concn. (mg/L)

Station	Cu ²⁺	Zn ²⁺	Pb ²⁺	Ni ²⁺
S1	0.08	0.13	ND	0.03
S2	0.12	0.18	0.007	0.05
S3	0.26	0.23	0.05	0.07
S4	0.28	0.28	0.07	0.07
S5	0.28	0.24	0.06	0.05
S6	0.26	0.23	0.05	0.04

Appendix 3: Sample 3 –wet season metal ions concn. (mg/L)

Station	Cu²⁺	Zn²⁺	Pb²⁺	Ni²⁺
S1	0.06	0.14	0.006	0.03
S2	0.12	0.17	0.01	0.06
S3	0.24	0.22	0.04	0.05
S4	0.26	0.25	0.06	0.07
S5	0.27	0.25	0.06	0.08
S6	0.25	0.23	0.04	0.06

Appendix 4: Average metal ions concn. –wet season (mg/L)

Station	Cu²⁺	Zn²⁺	Pb²⁺	Ni²⁺
S1	0.05	0.12	0.01	0.03
S2	0.11	0.18	0.01	0.06
S3	0.26	0.23	0.05	0.06
S4	0.28	0.27	0.06	0.07
S5	0.27	0.24	0.06	0.06
S6	0.25	0.23	0.05	0.05

Appendix 5: Sample 1 –dry season metal ions concn. (mg/L).

Station	Cu²⁺	Zn²⁺	Pb²⁺	Ni²⁺
S1	0.08	0.09	ND	0.03
S2	0.15	0.17	0.01	0.03
S3	0.18	0.20	0.05	0.05
S4	0.26	0.22	0.05	0.06
S5	0.25	0.18	0.04	0.05
S6	0.23	0.18	0.03	0.05

Appendix 6: Sample 2 –dry season metal ions concn. (mg/L).

Station	Cu²⁺	Zn²⁺	Pb²⁺	Ni²⁺
S1	0.07	0.09	0.008	0.03
S2	0.14	0.16	0.01	0.04
S3	0.19	0.21	0.04	0.05
S4	0.25	0.20	0.05	0.05
S5	0.25	0.20	0.03	0.04
S6	0.22	0.17	0.03	0.04

Appendix 7: Sample 3 –dry season metal ions concn. (mg/L).

Station	Cu²⁺	Zn²⁺	Pb²⁺	Ni²⁺
S1	0.09	0.10	0.006	0.02
S2	0.15	0.16	0.01	0.03
S3	0.20	0.20	0.02	0.03
S4	0.24	0.22	0.04	0.04
S5	0.26	0.21	0.04	0.05
S6	0.24	0.20	0.02	0.03

Appendix 8: Average metal ions concn. –dry season (mg/L).

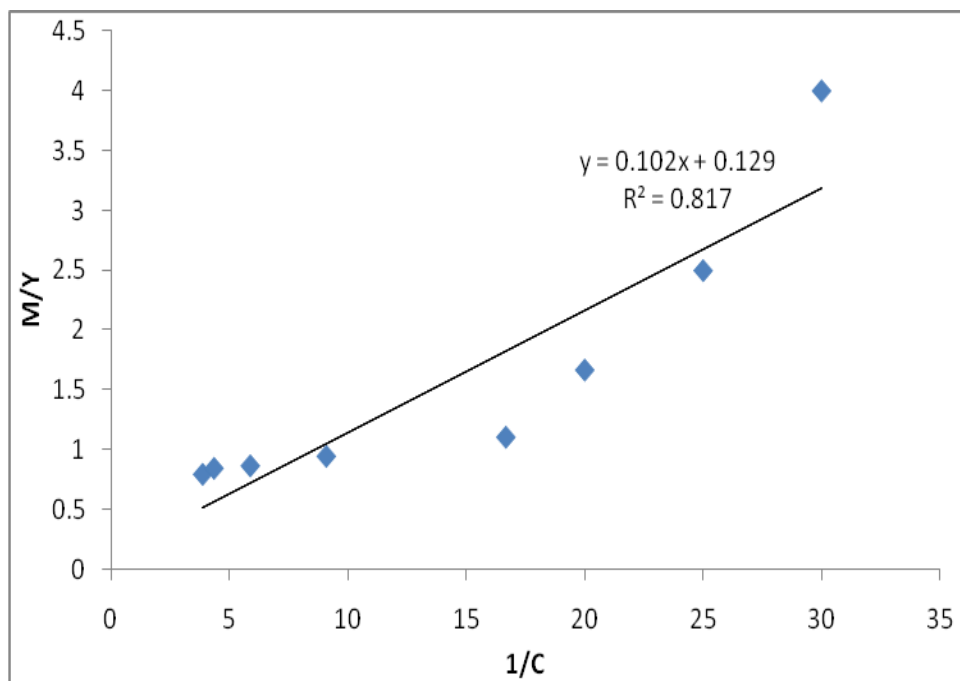
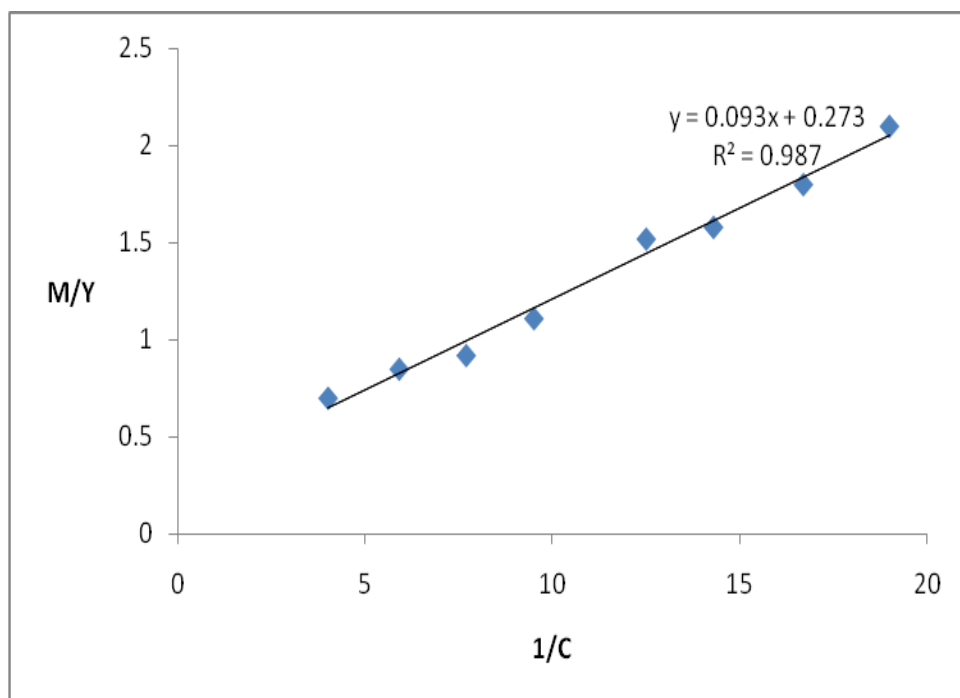
Station	Cu²⁺	Zn²⁺	Pb²⁺	Ni²⁺
S1	0.08	0.09	0.005	0.03
S2	0.15	0.16	0.01	0.03
S3	0.19	0.20	0.04	0.04
S4	0.25	0.21	0.05	0.05
S5	0.25	0.20	0.04	0.05
S6	0.23	0.18	0.03	0.04

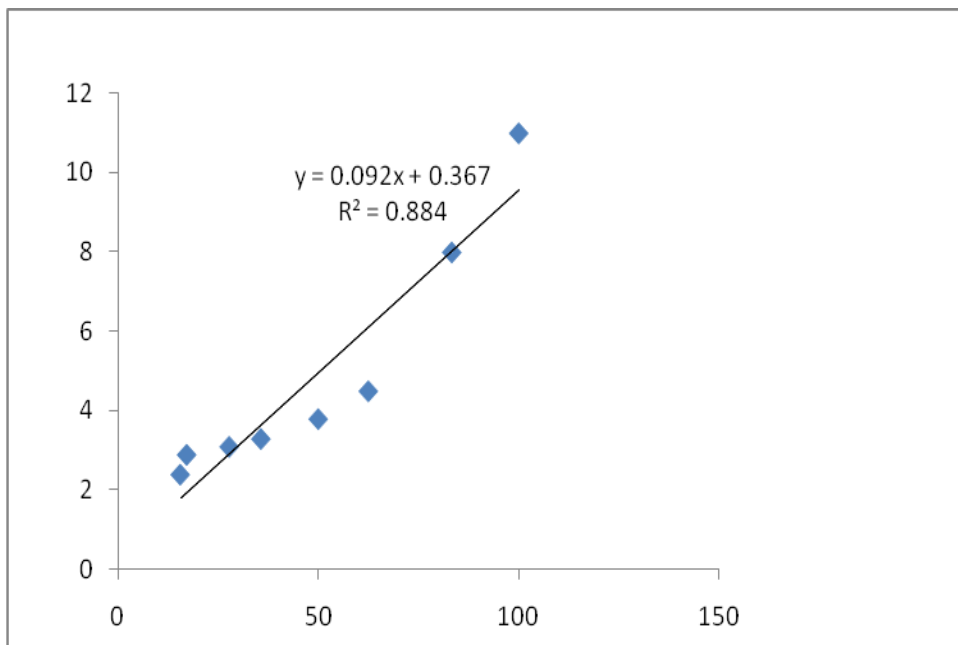
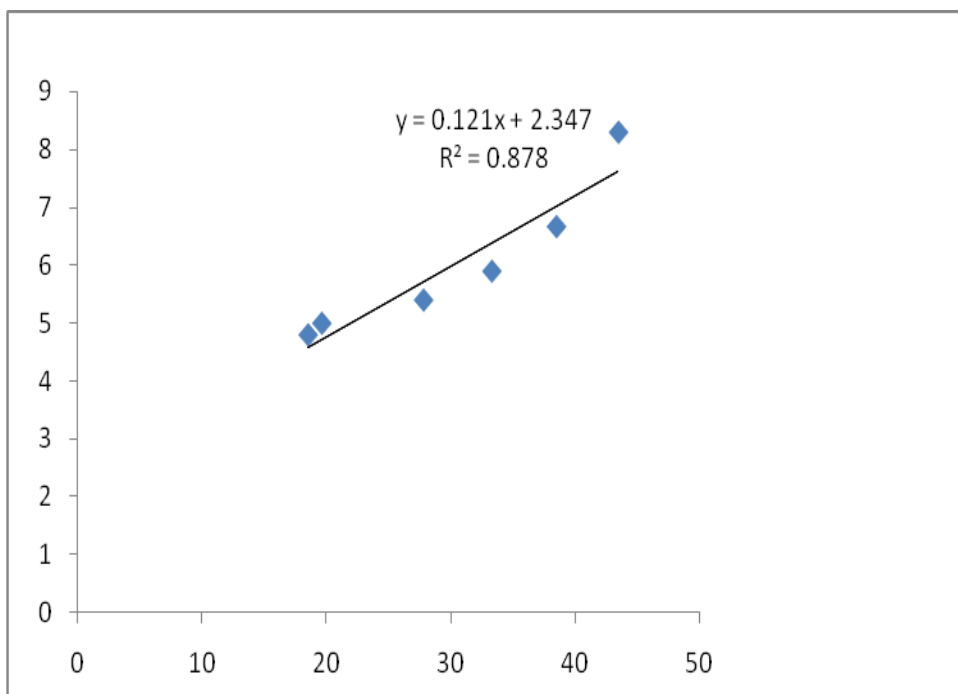
Appendix 9: Variations of metal ion concentration (mg/L) with pH

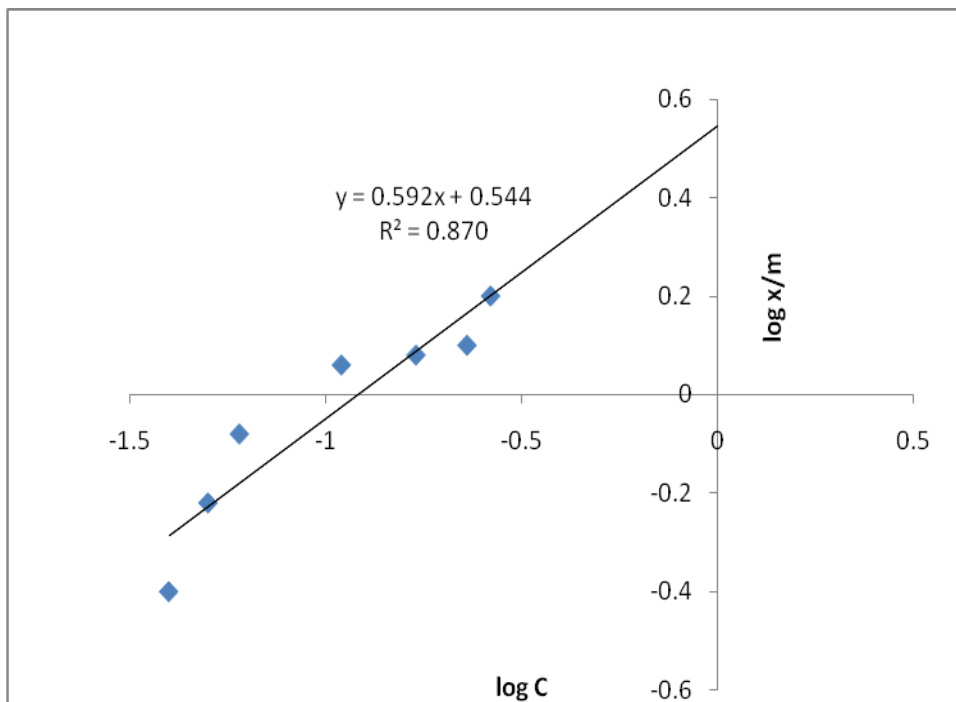
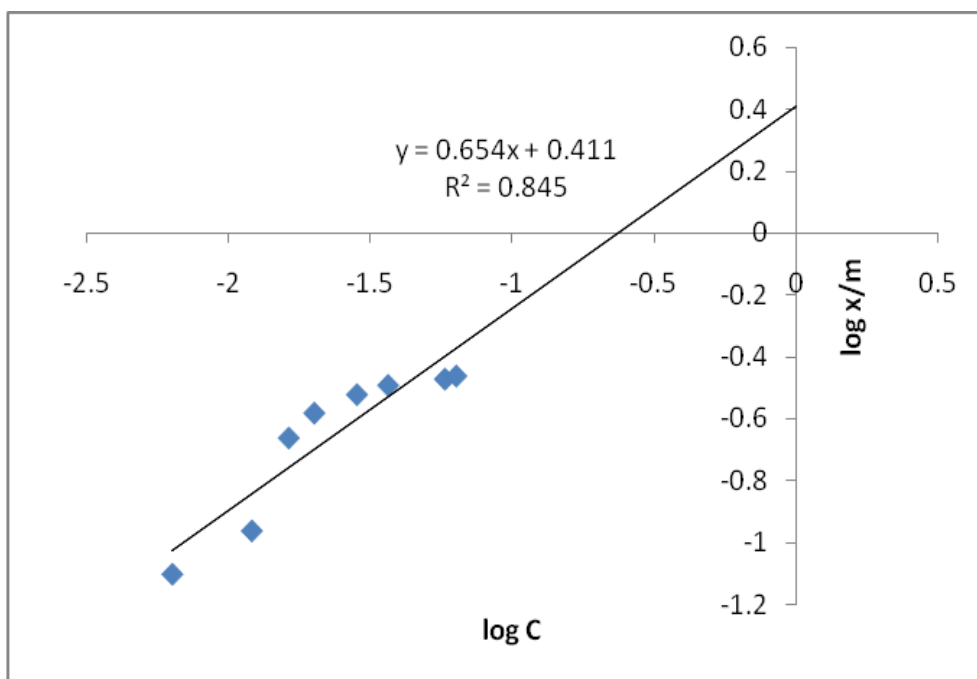
pH	Cu²⁺	Zn²⁺	Pb²⁺	Ni²⁺
3	0.188	0.168	0.048	0.036
4	0.180	0.157	0.047	0.032
5	0.174	0.148	0.042	0.028
6	0.165	0.140	0.039	0.024
7	0.188	0.134	0.036	0.024
8	0.194	0.146	0.036	0.028
9	0.203	0.154	0.042	0.031

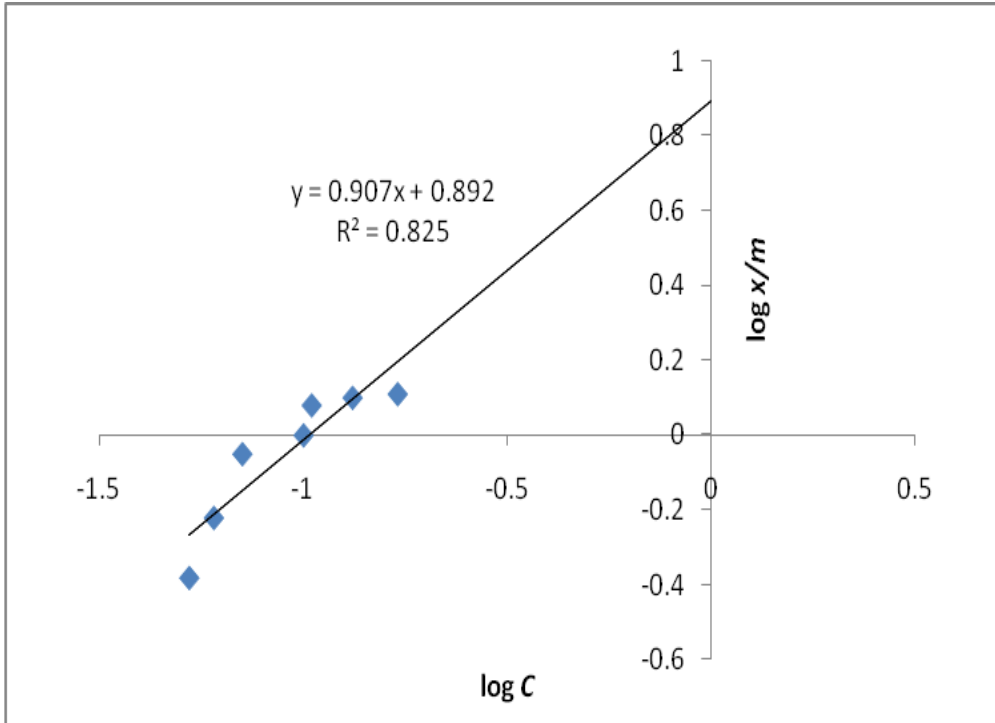
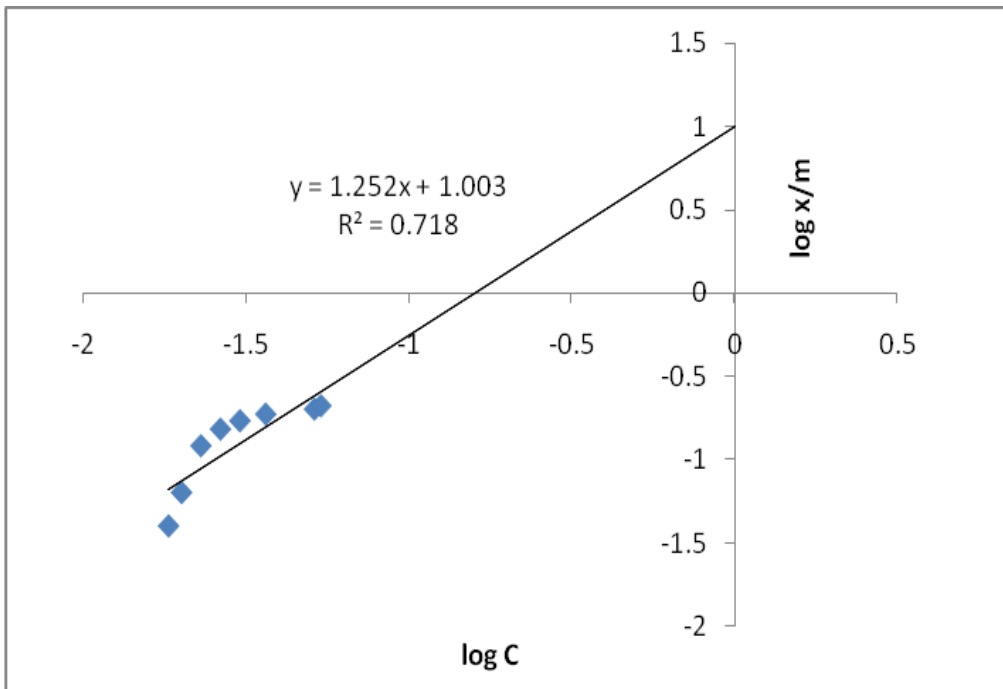
Appendix 10: Variations of residual metal ion concn. (mg/L)with contact time.

Mins.	Cu²⁺	Zn²⁺	Pb²⁺	Ni²⁺
0	0.29	0.28	0.060	0.080
15	0.26	0.25	0.054	0.064
30	0.23	0.17	0.051	0.058
45	0.17	0.13	0.036	0.036
60	0.11	0.11	0.030	0.028
75	0.06	0.08	0.026	0.020
90	0.05	0.07	0.023	0.016
105	0.04	0.06	0.020	0.012
120	0.03	0.05	0.018	0.010

Appendix 11: Langmuir linear plot for Cu^{2+} **Appendix 12: Langmuir linear plot for Zn^{2+}** 

Appendix 13: Langmuir linear plot for Ni²⁺**Appendix 14: Langmuir linear plot for Pb²⁺**

Appendix 15: Freundlich linear plot for Cu^{2+} **Appendix 16: Freundlich linear plot for Ni^{2+}** 

Appendix 17: Freundlich linear plot for Zn²⁺**Appendix 18: Freundlich linear plot for Pb²⁺**

Appendix 19: Sample 1 –wet season physiochemical parameters (mg/L).

Station	pH	Conduct.	NO₃⁻	PO₄³⁻	BOD	COD
S1	6.7	125	2.1	2.6	7	14
S2	6.7	127	1.6	2.1	10	21
S3	6.6	130	0.8	1.8	12	24
S4	6.5	140	2.0	2.5	13	26
S5	6.8	136	1.8	2.4	16	31
S6	6.9	130	1.4	1.9	14	27

Appendix 20: Sample 2 –wet season physiochemical parameters (mg/L).

Station	pH	Conduct.	NO₃⁻	PO₄³⁻	BOD	COD
S1	6.8	130	2.0	2.4	9	20
S2	6.7	131	1.8	2.0	11	23
S3	6.8	133	1.2	1.9	12	25
S4	7.0	141	1.7	2.3	14	29
S5	6.8	135	1.7	2.2	17	34
S6	6.7	132	1.5	2.0	16	30

Appendix 21: Sample 3 –wet season physiochemical parameters (mg/L).

Station	pH	Conduct.	NO₃⁻	PO₄³⁻	BOD	COD
S1	7.0	128	2.3	2.2	8	17
S2	6.8	130	2.1	2.1	12	24
S3	6.9	132	1.8	2.1	15	31
S4	6.8	138	2.0	2.3	15	32
S5	6.7	137	2.0	2.3	16	30
S6	6.6	137	1.8	2.1	13	28

Appendix 22: Average concn. –wet season physiochemical parameters (mg/L).

Station	pH	Conduct.	NO₃⁻	PO₄³⁻	BOD	COD
S1	6.8	128	2.1	2.4	8	17
S2	6.7	129	1.8	2.1	11	23
S3	6.8	132	1.3	1.9	13	27
S4	6.8	140	1.9	2.4	14	29
S5	6.8	136	1.8	2.3	16	32
S6	6.7	133	1.6	2.0	14	28

Appendix 23: Sample 1 –dry season physiochemical parameters (mg/L).

Station	pH	Conduct.	NO₃⁻	PO₄³⁻	BOD	COD
S1	7.2	190	1.0	0.38	10	21
S2	7.3	200	1.0	0.41	15	30
S3	7.3	215	0.8	0.39	18	38
S4	6.9	220	1.1	0.40	19	37
S5	6.8	210	0.9	0.32	23	45
S6	6.9	210	0.6	0.34	20	41

Appendix 24: Sample 2 –dry season physiochemical parameters mg/L).

Station	pH	Conduct.	NO₃⁻	PO₄³⁻	BOD	COD
S1	7.0	210	1.1	0.36	12	25
S2	7.1	205	1.1	0.40	15	32
S3	7.2	216	0.9	0.37	17	36
S4	7.0	222	1.2	0.41	20	40
S5	6.9	215	1.0	0.33	24	46
S6	6.9	215	0.8	0.33	23	43

Appendix 25: Sample 3 –dry season physiochemical parameters (mg/L).

Station	pH	Conduct.	NO₃⁻	PO₄³⁻	BOD	COD
S1	7.1	225	1.2	0.40	15	31
S2	7.1	230	1.1	0.40	15	32
S3	7.0	230	1.1	0.36	18	37
S4	6.9	250	1.3	0.42	20	43
S5	7.0	220	1.2	0.35	25	50
S6	6.8	225	1.2	0.35	23	47

Appendix 26: Variations of metal ion concentration (mg/L) with pH

pH	Cu	Zn	Pb	Ni
3	0.188	0.168	0.048	0.036
4	0.180	0.157	0.047	0.032
5	0.174	0.148	0.042	0.028
6	0.165	0.140	0.039	0.024
7	0.188	0.134	0.036	0.024
8	0.194	0.146	0.036	0.028
9	0.203	0.154	0.042	0.031

Appendix 27: Percentage removal efficiency of metal ion with pH

pH	Cu	Zn	Pb	Ni
3	35	40	20	55
4	38	44	22	60
5	40	47	30	65
6	43	50	35	70
7	35	52	40	70
8	33	48	40	65
9	30	45	30	61