ENVIRONMENTAL IMPACT ASSESMENT ON THE USE OF COPPER BASED FUNGICIDES IN COFFEE FARMS ALONG KAMITI RIVER

By

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DECLARATION

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

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This thesis is dedicated to the glory of God, my wife Naomi, our children Mwangi, Wangui and Wambui, and my parents Jeremiah and Esther.

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"I need Thee every hour, Most precious and Holy God Oh make me Thine indeed, Thou blessed son".

ABSTRACT

Copper-based fungicides are extensively used in the control of coffee pests and diseases because they are relatively cheap and effective. This practice has serious environmental implications owing to the toxic nature of copper. An assessment of the effects of the use of copper-based fungicides is carried out on River Kamiti, which flows along coffee growing areas of Kiambu District, Kenya. Water samples were collected at various points along the river from Thika Road Bridge (TRB) to Kamiti Road Bridge (KRB). The levels of copper in the river were determined with total copper being obtained using flame atomic absorption spectrophometry (FAAS) and the labile fraction by differential pulse anodic stripping voltammetry (DPASV) at a glassy carbon electrode. Also Dissolved Organic Carbon (DOC), Fluoride (F^-), Chloride (Cl^-), and pH were determined using the standard analytical methods. Total copper was found to range from 0.3 to 2.20 x 10^{-2} μg/ml with the labile fraction being 49%. The concentration of copper was found to depend on the rainfall, which carried copper to the river from the fields and coffee-harvesting season thus amount of factory discharge. The levels of DOC were found to range between 5.26 - 9.7 x 10 x 10^{-2} μg/ml, F^- 0.4-2.2 x 10^{-2} μg/ml and Cl^- 8.5-17.4 x 10^{-2} μg/ml therefore can be said to be generally low. Taking glycine, catechol and proline as typical ligands in a water system, speciation modeling showed that at pH<6.5, the fraction of "free" and labile copper species, were observed to increase with decrease in pH. The pH of this water system was found be between 7.4 and 8.14. This implies that copper in the river will be complexed by the host of ligands contributed by the DOC but there will be toxic traces contributed by simple complexes that could easily dissociate. Based on the results obtained in this work, it is concluded that the use of copper compounds in the coffee industry is of environmental concern.

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis Of Variation
BBC	Bacteria Blight of Coffee
CBD	Coffee Berry Disease
CRF	Coffee Research Foundation
CV	Cyclic Voltametry
DOC	Dissolved Organic Carbon
DME	Dropping Mercury Electrode
DOM	Dissolved Organic Matter
DPP	Differential Pulse Polarography
DPSV	Differential Pulse Striping Voltametry
EMF	Electro Motive Force
EPU	Environmental Protection Unit
EU	European Union
FAAS	Flame Atomic Absorption Spectrophotometry
GA	Gallic Acid
HCL	Hollow Cathode Lamp
HMDE	Hanging Mercury Drop Electrode
KRB	Kamiti Road Bridge
MAL	Maximum Allowable Limit
MCL	Maximum Contaminant Level
NTU	Nephelometric Units
SE	Support Electrolyte
TA	Tannic Acid
TISAB	Total Ionic Strength Adjustment Buffer
TRB	Thika Road Bridge
UV	Ultra Violet
Vis	Visible
WHO	World Health Organisation

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CHAPTER ONE

INTRODUCTION

1.1 Background

Human activities such as mining, industrial processes, municipal wastes and farming have contributed to elevated levels of certain elements in the environment. This has resulted to contamination of the environment leading to serious health problems facing the world today. Presence of heavy metals in the environmental matrices is of a particular concern because they may get incorporated into drinking water and various food chains (Florence, 1982).

Heavy metals tend to accumulate in various body organs and as a result of long-term exposure can lead to serious toxic effects. For this reason, an investigation of the extent of pollution in rivers passing through coffee growing areas due to widespread use of copper based fungicides is presented in this work with a view of giving recommendations on pollution control. Kamiti river is taken to present a case study.

1.2 Coffee farming

Coffee is among the most important perennial cash crop in Kenya where it is grown in large scale and small-scale farms. It is a shrub indigenous to Ethiopia but first cultivated in Saudi Arabia as a beverage (Smith 1985). It belongs to the family Rubiceae and the genus *Coffea*. The crop does well at altitudes between 1372-2073 m above sea level with an annual rainfall of between 900-1500 mm (Smith, 1985).

Coffee farming has been practiced in Kenya from as early as 1952. Records from Coffee Research Foundation (CRF) indicate that there is more than 159,000 hectares under the crop. However, this lucrative industry is not without problems. There are two major diseases namely Coffee Berry Disease (CBD) and Leaf Rust that attack the crop and in deed are the problems. Climatic conditions suitable for coffee growing are also very favorable for the growth of the bacteria, *Colletotrichum coffeanum* that causes CBD. The bacteria thrives on the bark of young twigs of the coffee tree and its growth is triggered by wet weather and therefore CBD is an epidemic during the rainy seasons (Masaba *et al.*, 1993). In 1956, the industry experienced severe problems as a result of coffee diseases. Numerous field trials were carried out to contain the vice (Rayner, 1956). During such field trials several copper based fungicides were used. For example, Perenox that contain 50% copper was applied with a rate of application being 11 Kg per hectare, implying an annual national requirement of 6010 tones. Consequently, a lot of the fungicides were released to the environment (Masaba *et al.*, 1993).

Leaf Rust (*Hemileia vastatrix*) usually called bacterial blight of coffee (BBC) is predominantly a disease of lower altitude and attacks the leaves resulting in bronzening and therefore inhibiting photosynthesis. This results in severe crop loss and remedial measures have to be taken. As is the case for CBD, BBC is favoured by wet weather condition, making the disease very severe on the onset of rain (Masaba *et al.*, 1993).

Coffee usually flowers at the same period when these diseases start to thrive and there is likelihood that the berries will be attacked at all stages of their growth. If this happens, the infected berries fall off before maturity. Effective controls of these diseases is achieved by use of a good fungicide cover on the plant from a period before flowering to a period before ripening especially if these periods coincide with wet weather. Fungicide application should therefore be done before and during the rainy weather. In central Kenya, where Kamiti river is found, heavy rains are between March and May and therefore application of the fungicides should be done between February and June. As rainfall shows a strong bimodal distribution, there are short rains between October and December; application of the fungicides should therefore be done between September and December as well (Masaba *et al.*, 1993).

Several organic and inorganic based fungicides have been tried and proven to be effective against these diseases. The organic fungicides do not offer an adequate cover against the vice because they undergo degradation. A study done at CRF revealed that copper based fungicides or a mixture of the same with organic fungicides gave better yields than straight organic fungicides (Masaba *et al.*, 1993). The workers attributed this to the low solubility of the copper products in the rainwater compared to the others, and the fact that copper has nutrition value to plants. As a result several formulations of copper-based fungicides have been developed to effectively control CBD and leaf rust.

Copper fungicidal property was first recognized in 1885 in France when Milladet reported that grapes along the road side sprayed with a mixture of copper sulphate and lime to make them less attractive to thieves were not defoliated by downy mildew (Hirst, 1970). This led to the development of a fungicide called Bordeaux. The effective compound was thought to be $Cu(OH)_2$ but latter it was realized to be a mixture of copper and calcium sulphates, whose composition depended on ratio, and age of the mixture (Van Alphan, 1957).

However, it was noticed that fixed copper fungicides were toxic to plants and this led to the development of Bordeaux mixture, which is made of copper (II) oxy chloride maintained at a basic pH with lime. This compound was widely used for crop protection (Van Alphan, 1957). In Kenya this product is widely used for protection of coffee as advised by Coffee Research Foundation (CRF). Table 1.1 shows the recommended spray programme of bordeaux mixture given to farmers.

Table 1.1: Recommended spray program to effectively control CBD and Leaf Rust.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
High altitude		X		X	X		X	X	X		X	X
Low altitude		X		X	X				X	X	X	X

Source Masaba *et al.*, 1993

Key: X- Application

From the recommended spray program in Table 1, a lot of copper based products end up in the coffee fields. It has always been assumed that when copper comes into contact with organic matter such as humic acid from decomposing vegetation, it forms stable complexes and is therefore rendered harmless. However, coffee farms are maintained at a pH range of 4.4-5.4 so that minerals in the soil can solubilize and be available for plants uptake (Maroko, 1993). At this pH, copper salts are soluble and with the high rainfall and inclination of the terrain, these dissolved species together with others are washed into the water systems by the runoff water and therefore a possible accumulation occurs. Indeed there is a significant decrease in the concentration of copper in the topsoil of coffee fields if spraying is discontinued and the pH maintained at between 4.4-5.4. This is attributed to leaching and it confirms that copper is dissolved by water at that environment (Maroko, 1993).

Application of fungicides extends to the period beyond the ripening of the coffee berries, therefore some of the chemicals applied find their way to the pulping factories. The pulping process involves the use of a lot of water to wash these berries, and this water will also wash off the chemicals. Effluent from these factories is usually discharged into the rivers untreated and therefore adding the load of chemicals to the rivers.

It has been established that toxicity, bioavailability, mobility, and other chemical properties of elements depend on their speciation rather than their concentrations. (Lores, 1998). Speciation studies and water quality parameters like pH and dissolved organic matter were therefore needed to be carried out to find out if the continued use of the fungicides will be a threat to life. Speciation studies on Kamiti river were recommended by Kariuki (1993) who carried out studies on river Mugutha and coffee fields adjacent to it for the determination of total concentration of copper.

1.3 Study area

The study area, as shown in Figure 3.1, is situated in Kiambu district Kenya which lies between 0^0 25´ and 1^0 20´ South of the equator and 36^0 31 , $37^0$15´ East (Kiambu DDP, 1996). Kamiti River from which the samples were collected runs through a topographic region of upper highland to lower midland zone whose altitude range from 1500-1800m above sea level (Kiambu DDP, 1996). The land is intensively utilised for agriculture because of the suitable soil and is located in a well-drained rugged terrain in the upper footridges and foot slopes of the Abadere ranges. The main economic activity therefore is a mixture of large coffee plantation, small-scale coffee farming, subsistence farming and small-scale tea farming.

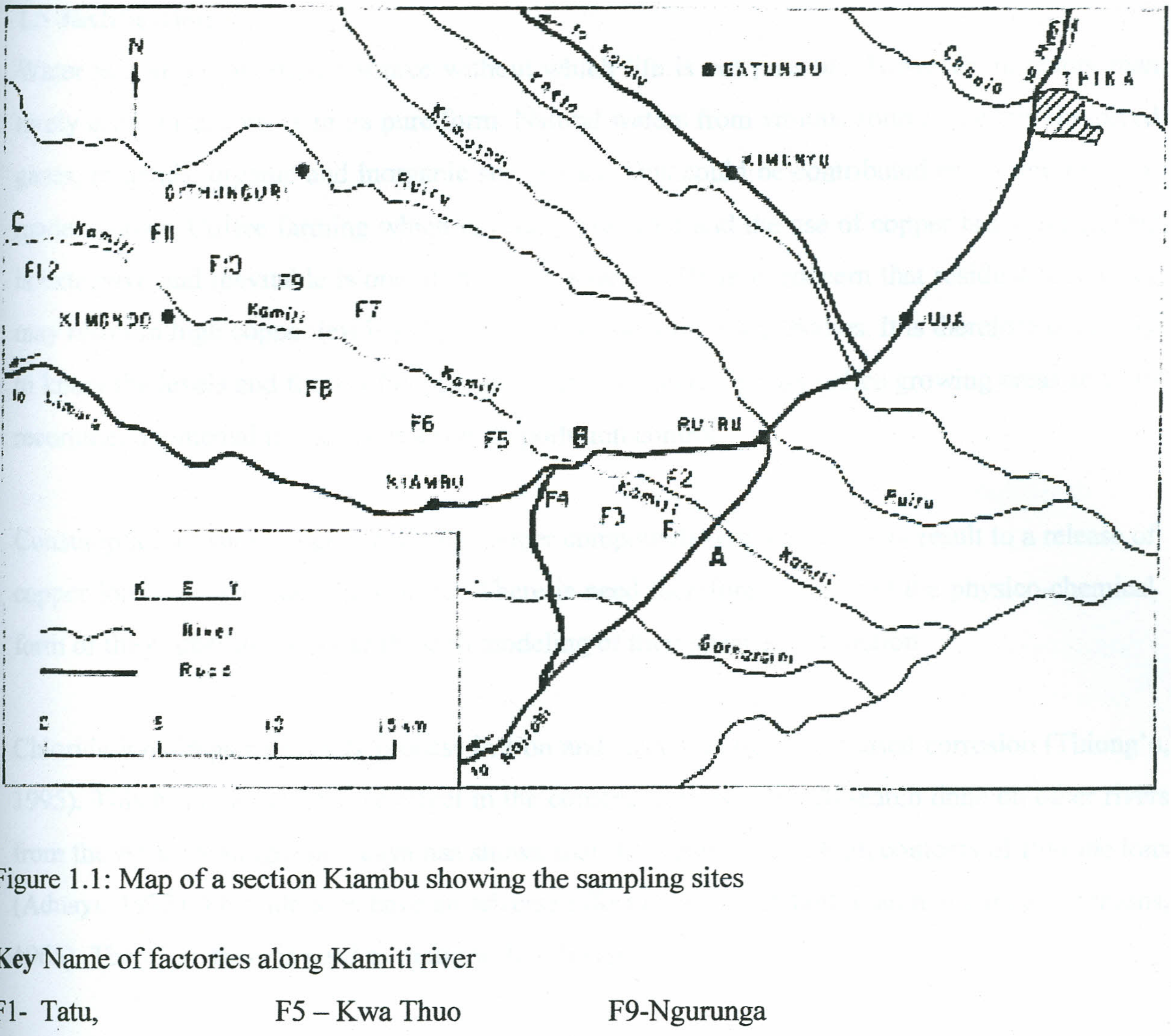


Figure 1.1: Map of a section Kiambu showing the sampling sites

Key Name of factories along Kamiti river

F1- Tatu,
F2- Munene
F3- Kiroeri
F4- Kwa Njoka

F5 – Kwa Thuo
F6 -Gatamaiyo
F7-Kamwaki
F8- Gwa kenda

F9-Ngurunga
F10- Lisura,
F11-Sasini
F12- Mbo-Kamiti

1.4. Problem statement

Kamiti river water is dirty brown in colour showing evidence of the topsoil carried to the river. As it meanders down-streams, it carries soluble ions and as a result the water's appreciation is compromised. Certainly, copper and chloride ions from the applied fungicides and fluoride ions from rock salts are among the lot carried down to the river. Indeed with reference to the order of toxicity of dissolved heavy metals given as Hg>Ag>Cu>Zn>Pb and Cr, copper is highly ranked (Beib, 1965).

1.5 Justification

Water is a very important resource without which life is not possible. However, in reality man rarely drink or use water in its pure form. Natural waters from various sources contain dissolved gases, minerals, organic and inorganic substances. This could be contributed by natural or man-made sources. Coffee farming which is widely practiced and the use of copper based fungicides is extensive and inevitable is one of the many sources. There is concern that residual fungicides may result in high copper levels in the soil and surrounding water bodies. It is therefore necessary to know the levels and forms of copper in the rivers flowing across coffee growing areas so as to recommend remedial measures as a way of pollution control.

Consumption of such water containing copper compounds (complexes) may result to a release of copper ions that will cause poisoning. There is need therefore to find out the physico-chemical form of the copper in the water through modeling of their species distribution.

Chloride ions impair passivity process in iron and steel leading to increased corrosion (Thiong'o, 1995). This has a direct adverse effect in the construction industry. Research done on other rivers from the Abadere ranges in Kenya has shown that the water contain high contents of fluoride ions (Aduayi, 1973). Fluoride ions have an adverse effect on bones in both man and animals (Simons, 1965). Therefore there is need to monitor their levels.

1.6 Hypothesis

There is dissolved copper in the river system even at elevated pH because of the presence of dissolved decayed matter. There should be no significant difference on the levels of copper between geographical regions but between seasons

1.7 Objectives

1.7.1 Broad Objectives

The broad objective of this study was to assess the effect of the use of copper based fungicides and to determine some water quality parameters in Kamiti river along the coffee growing areas.

1.7.2 Specific Objectives

The specific objectives were to:-

1. Determine:-

- Some physico- chemical parameters namely pH, dissolved organic carbon, fluoride and chloride levels.
- The total content of copper in water and sediments.
- The content of labile copper in the water.

2. To model copper speciation with some selected ligands.

Physico-chemical parameters of water, pH, DOC (Dissolved Organic Carbon), conductivity, anions and cations present are indicators of pollution. There is need to determine these parameters to asses the level of pollution. Modeling experiments are necessary to find out how specific cations and ions behave in the natural system. Otherwise there is little information available with respect to accumulation of copper in the soil and waters in the coffee growing areas.

CHAPTER TWO

LITERATURE REVIEW

2.1 Copper: Its compounds and toxicity

Copper is a $4s^1\ 3d^{10}$ metal whose principle oxidation states are (I) and (II). Oxidation state (I), $4s^1\ 3d^9$, is diamagnetic and forms colourless salts in solution. These solutions are unstable and undergo disproportionation to oxidation state (II) as shown in equation 1 below (Cotton, 1980).

$$2Cu^{+}{}_{(aq)} \rightleftharpoons Cu^{2+}{}_{(aq)} + Cu_{(s)} \tag{1}$$

Oxidation state (II) is the most common and forms simple salts with most anions except CN^- (cyanide) and I^- (iodide), which bond covalently to form Cu (I) compounds that are insoluble in water. Copper (II) salts, $3d^9$, are water-soluble coloured salts and crystallize as hydrates. The well-known salt is the pentahydrate (blue vitriol), which is widely used in electroplating, manufacture of algaecide, in purification of water and fungicide for crop protection.

Oxidation state (III) is rare but has been reported to have some involvement in biological processes in a complex form. Copper forms stable complexes with most organic ligands at a wide range of pH level because of its high coefficient of complexation (Sillen, 1964).

Copper is an essential trace element to both plants and animals. It acts as a catalyst in the formation of haemoglobin, the oxygen-carrying blood component. At low concentrations its compounds are non-toxic to man, but large quantities of soluble salts are toxic. A daily maximum requirement has been estimated at 30 μg/kg of body weight for an adult human (Sciencenet, 2005). Toxicity of a metal depends on several factors. These include the ability of that metal to combine and interact with vital enzymes and bind macromolecules, changes in pH of the body system and size of the metal. The potential of the metal to interact with such body systems depends on its electro-chemical property, oxidation state, size, solubility and its stability in biological systems. The electro-chemical character of the metal is directly involved in the capacity of that metal and its compounds to co-ordinate or chelate with electron donating compounds like oxygen, nitrogen and sulphur in biological fluids and the stability of these complexes within living tissue. Their stability therefore influences toxicity and there is the need to determine the speciation of copper in water containing ligands with some selected functional groups. The stability constants of these selected complexes may be calculated from pH-titrimetric titration data with the help of a suitable software such as SUPERQUARD (Gans *et al.*, 1985).

Copper is very toxic to marine vertebrates and invertebrates. It has been documented that a concentration of above 0.0015 μg/ml in water is enough to kill fish (Massey, 1973). Below these levels, copper, is non-toxic and indeed copper sulphate has been used as a medicine for fish against a disease called Inc (Niaragu, 1992).

Mance *et al.* (1984) reviewed data on the toxicity of copper to saltwater organisms. They found that invertebrates exhibited slightly greater sensitivity to divalent copper than the fish species tested. There were also indications that the moderation of toxicity in the presence of organic and inorganic ligands was 5μg/ml.

A study by Manhan (1992) has revealed some toxic effects of copper when ingested. These include vomiting, diarrhea, jaundice, and finally death. At first there is a chronic long-term accumulation of excess copper without any symptoms. When the concentration reaches 100 μg/ml in adult cattle, and 10 μg/ml in sheep, there is a sudden release of stored copper into the general circulation thus producing a haemolic crisis. This breakdown of red blood cells results in the release of hemoglobin in the urine resulting in jaundice because of liver injury, general weakness, anorexia, and sometimes death.

It has been established that there is a very important ratio between copper and molybdenum in ruminants (Manhan, 1992). Molybdenum intake alters the amount of copper toxicity by formation of an insoluble complex $CuMoO_4$ in the gastrointestinal tract thus reducing copper absorption and therefore ruminants are given molybdenum-containing supplements to avert the danger. The chronic accumulation of copper in man is rare and occurs in Wilson's disease caused by an inborn error of the copper metabolism characterised by a diminished capacity to eliminate copper via bile. High copper intake adversely effects zinc absorption, which is an essential element as well (Sciencenet, 2005).

2.2 Copper based fungicides

Copper based fungicides come in two forms. These are the fixed copper fungicide (tribasic copper sulfate, copper oxychloride sulfate, cupric hydroxide) and the bordeaux mixture. Fixed copper fungicides are ionic copper salts that were found to be highly toxic to plants. Bordeaux mixture contains copper sulfate, which is acidic, and therefore neutralized by lime (calcium hydroxide) causing a lower solubility. It is a fungicide with outstanding effect on many diseases of tree fruits and nuts, vine fruits, and was developed by Milandech and given the name bordeaux (Worthing, 1979). The mixture has been successfully used for over 150 years, on fruits, vegetables and ornamentals. Unlike other fungicides, bordeaux mixture has both fungicidal and bactericidal activity and as such, it can be effectively used against diseases such as leaf spots caused by bacteria or fungi, powdery mildew, downy mildew and various anthracnose pathogens. The bordeaux mixture can be applied either as dust or purchased as a liquid formulation and has the ability to persist through rains and to adhere to plants a reason for its effectiveness (Smith, 1984). This property has been attributed to its low solubility and can therefore be retained for long periods of time on the areas it has been applied (Worthing, 1979).

The bordeaux mixture comes in several formulations. One of the most popular, effective and least phytotoxic formulations for general home garden and orchard use is the 44-50 formulation which translates into four kg copper sulfate and four kg lime in 50 litres of water.

In a study carried out by Kariuki (1993) the total copper content in Mugutha river and the content of copper in the fields of Tatu farm in Kiambu district were determined. The observed mean levels of total copper in the coffee blocks ranged between 2.5-54.2 μg/ml. The mean levels of copper in Mugutha river was found to be 10.9 $\times 10^{-2}$ μg/ml and 130μg/g for water and sediments respectively. Further work was recommended to be carried out on Kamiti river which passes through a similar environment. AAS was used as an analytical tool in the research work.

2.3 Theoretical background of the analytical techniques

2.3.1. Flame Atomic Absorption Spectrophotometer (FAAS)

The atomic absorption spectroscopy in analytical chemistry is a technique for determining the concentration of a particular metal element within a sample. It can be used to analyse the concentration of over 62 different metals in a solution. It was first developed during the 1950s by a team of Australian chemists, lead by Alan Walsh, in Melbourne Australia (Walsh, 1955). The basic components of FAAS are Radiation source, nebulizer, excitation source (flame), monochromator and detector as shown in the Figure 2.1.

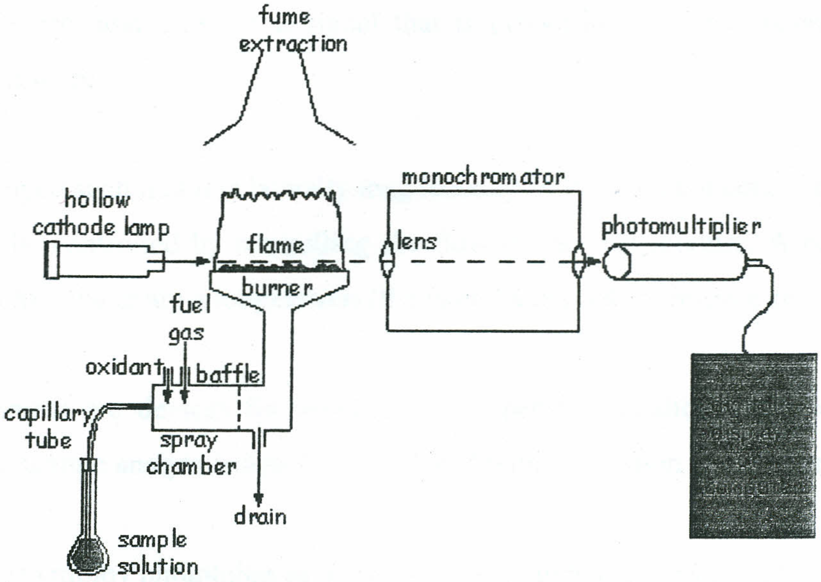


Figure 2.1 Schematic diagram of the flame atomic absorption spectrophotometer

The radiation source is a hollow cathode lamp (HCL), which emits a stable, intense radiation of the resonance wavelength of the element to be determined. Inside the tube is a cylindrical metal cathode containing the metal for excitation, and an anode. The type of hollow cathode tube depends on the metal being analysed. For analysing the concentration of copper in a sample, a copper cathode tube would be used, and likewise for any other metal being analysed. When a high voltage is applied across the anode and cathode, the metal atoms in the cathode are excited into producing light with a certain emission spectra which is focussed into the flame.

In the neubilizer, the liquid sample is converted into small droplets before the sample enters the atomizer. This is done using gas at very high velocity and metal ions in solution are turned into atomic vapour. Air/acetylene, nitrous oxide/acetylene or electrically heated graphite furnace are commonly used for analytical work in FAAS as excitation sources. The electrons of the atoms in the flame can be promoted to higher orbitals for an instant by absorbing a set quantity of energy (a quantum). This amount of energy is specific to a particular electron transition in a particular element. As the quantity of energy put into the flame is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured

The flame is arranged such that it is laterally long (usually 10 cm) and not deep. The height of the flame must also be controlled by controlling the flow of the fuel mixture. A beam of light is focussed through this flame at its longest axis (the lateral axis) onto a detector past the flame.

The Monochromators are devices for separating the spectral radiations with respect to their wavelengths. The sample analyte is therefore irradiated with a radiation of a chosen frequency.

Modern instrument employ phototubes as detector. A phototube contains a photocathode, a series of dynodes that amplify the optical signal and an anode. A photon of light from the monochromater strikes the photocathode and dislodges an electron, which is then accelerated to the dynode one. The electron ejects more electrons from dynode one and these electrons are accelerated to dynode two, three and eject more electrons in the process that finally reach the anode as an amplified electron current. This electron current is fed to an amplifier, which is read out on an analogue or digital display.

The basic principle in AAS analysis therefore is that when electromagnetic radiation is incidented on to a vapour of metallic atoms, the atoms will be receptive to light of their specific resonance wavelength (frequency). They are then transformed from low energy state E_1 to high-energy state E_2. This can be represented as shown in equation 2.

$$E_1 + h\nu \rightarrow E_2 \tag{2}$$

Where

h = Planck's constant

ν = frequency of the radiation

The excited atoms may revert back to the ground state by emitting radiation of the same frequency. The transitions are always stimulated by the absorption of radiation from an external source. The measurement of radiation absorbed in such a transition is the basis of atomic absorption spectrophotometry.

The AAS utilizes Beer and Lambert law which was independently discovered (in various forms) by Pierre Bouguer in 1729, Johann Heinrich Lambert in 1760 and August Beer in 1852. ("http://en.wikipedia.org/wiki/Beer-Lambert-law) The law states that, there is a relationship between the absorbance of light at a particular wavelength through a substance and the concentration of the substance, and also between the absorbance and the length of material that the light travels through as expressed in equation 3.

$$A = \varepsilon C l \tag{3}$$

Where.

- A =absorbance
- ε = molar absorptivity
- l = the distance that the light travels through the material (the path length)
- C = the concentration of absorbing species in the material

Thus if l and ε are known, the concentration of a substance can be deduced from the amount of light absorbed by it. The law tends to break down at very high concentrations, especially if the material is highly scattering. If the light is especially intense, nonlinear optical processes can also cause variances.

Fuel oxidant mixtures depend on the metal to be analysed and the flame temperature requred.

For a low temperature flame, acetylene and air is used. A hotter flame can be produced using acetylene and pure oxygen, and an even hotter flame can be attained using nitrous oxide and acetylene, although this mixture is explosive.

2.3.2 UV-Vis spectrophotometry

UV-Vis spectroscopy is the most popular analytical technique for the determination of molecular species. It involves measurement of the amount of radiation absorbed by the molecules or ions. It is preferred for its rapidity, high sensitivity, reasonably high detection limit and affordable price.

The principle behind this method of analysis involves the absorption of UV-Vis radiation by the ions and getting the difference from the total incidented radiation. Absorption of radiation also obeys the Beer-Lambert law given in equation 3. The relationship between absorption of incidented radiation and its absorption frequency is the basis behind the use of spectroscopy to identify substances as shown in equation 2 above.

2.3.3 Electroanalytical Methods

Electroanalytical methods were used to determine the labile fractions of copper in the water and study several copper-ligand systems. Labile metal fraction is defined as the fraction that is easily available (Scoullos, 1997). Since labilty is an operationally defined concept in that it depends on the analytical method used, in this study, it is the fraction that can dissociate fast enough at the surface of the electrode to give a signal (Florence, 1986).

2.3.3.1 Classical Polarography

Polarography was developed by a Czech scientist called Heyrovsky (1922). It is an electro-analytical technique based on measurement of current that develops at a dropping mercury microelectrode as a function of applied potential under conditions of complete concentration polarization (Albery, 1975).

2..3.3.2 Differential Pulse Anodic Stripping Voltametry (DPASV)

Although there are several types of polarography, the mode of choice for this study was differential pulse anodic stripping voltammetry. This involves superimposing a pulse of between 5-100 mV on a linear scan voltage ram. This pulse is added during the last milliseconds of the mercury drop. Current measurements are made 20 milliseconds before and after the pulse and the difference between the two currents is the signal. That amount of current is proportional to the electroactive species in the solution.

Water usually contains dissolved substances, which may act as electro active species and hence interfere with the analysis. It has been reported that in the reduction of copper in the presence of iron (III), the reduction current of copper is swamped. It is therefore recommended that hydroxylammonium chloride or sulphate be used to reduce the iron (III) to Iron (II), which does not interfere with the analysis of copper (Schronberger, 1980). Presence of Nickel (II) and Zinc (II) in the solution interferes by formation of Zn-Cu and Zn-Ni compounds in the Mercury film. Addition of gallanium ions in the ammonium acetate buffer prevents the formation of these cluster compounds (Schronberger, 1980).

The optimum pH range for the determination of copper is 4.5-6.5 (Tranfor, 1952). Above this pH, the peak current decreases and a second peak appears at 100 mV less negative than the original $E_{1/2}$, against a SHE. The sum of the two peak heights do not add up to the total reduction current of copper but less (Ligane, 1943). At pH below 4.5, the copper wave gets distorted but becomes reversible on addition of 0 .001% of sodium dodecylsulphate (Tranfor, 1952).

In our analysis, Standard addition method was used since it minimizes errors due to sample matrix and stirring variations from cell to cell (Parp EG&G Princeton app. Research, 1984). This involves recording a voltamogram of a known volume of an unknown solution followed by spiking a known volume of a standard solution and obtaining another voltamogram. The concentration of the unknown in the original voltamogram is evaluated using the equation 4.

$$C_u = \frac{i_1 v C_s}{i_2 v + (i_2 - i_1)V} \tag{4}$$

Where

C_u=Concentration of the unknown
C_s=Concentration of the standard
i_1 =Peak height of the sample
i_2 =Peak height mixture of sample and standard
V =Volume of the original sample
v=-volume spiked

When the added volume, v, is very small, then the equation is simplified to-

$$C_u = \frac{i_1 v C_s}{(i_2 - i_1)V} \tag{5}$$

2.3.3.3 Determination of stability constants by DPASV method

Stability constants of metal complexes can be determined using the polarographic analyser. This involves the determination of the shift of peak potential (or the half wave potential in DC mode). In case of labile metal-ligand system, a set of dynamic equilibrium exists between various complexes and aqua metal ion. The use of polarographic data in mathematical models to determine stability constants is based on the shift of peak potential upon the addition of a ligand. The shift depend on the size of the ion to be reduced; the larger the shift the bulky the metal ion. This is because bulky ions require more energy to undergo reduction. The shift has been used to study different metal ions to detect many types of amino acids and peptides. Therefore, peptides could be used as selective ligands for the detection of metal ions. The complexation of metal ions by even simple amino acids has produced remarkably low detection limits (Tanaka *et al.,* 1982). About 50 years ago Ligane derived an equation, as shown in equation 6, that related the shift and the excess concentration of the ligand at a fixed pH (Ligane, 1943).

$$\Delta E = E_M - E_C = \frac{0.0591}{n}\log_{10}\beta_j + \frac{0.0591}{n}\log_{10}[L] \qquad (6)$$

Where

E_M= Peak potential of the free metal

E_C= Peak potential of the complexed metal

β= stability constant of the complex

j= Coordination number

L=Concentration of free ligand

n= Charge on the species

The shift in potential is the most important experimental parameter for studying labile metal species because it has more influence on stability constants than the variation in peak height of the labile peak. Therefore the peak value must be established with the highest precision possible (Ngigi, 2001).

Oxygen is capable of dissolving in aqueous solution to form concentrations of as high as 10^{-3} M at room temperature and pressure. This oxygen interferes with polarographic analysis because it can be reduced at the electrode. Presence of oxygen in the analyte solution can also lead to oxidation of the metal analyte, or formation of hydroxyl ions that may precipitate some metal ions or change the pH of the test solution.

Oxygen should be removed by bubbling pure white spot nitrogen to the test solution, which has been passed through a solution of vanadium chloride.

2.3.3.4 Potentiometry

In this work potentiometry was used as analytical tool to determine the fluoride and chloride concentrations in the water samples. It is an electro-analytical method based on the measurement of variation of potential in a galvanic cell with respect to the activities of chemical species involved. The method was adopted as used by Maloba (1989) when he analysed the levels of fluoride and chloride ions in lake Elementaita. The concentration of the species were obtained by measuring the potentials of the respective galvanic cells using ion selective electrodes (ISE). Applying the Nernst expression, the potential of the cell is expressed as equation 7 (Alum, 1987).

$$E = E' \pm \frac{RT}{nF} \ln \alpha_i \qquad (7)$$

Where

E' =Cell constant

α_i = activity of an ion i

n = charge on the ion

In the equation 7, the sign ± is used to signify that it will be positive if *i* is a cation and negative for anions (Alum, 1987)

From the laws of mass action, the relation between activity and concentration of a species, X_i is given by the expression given in equation 22.

$$\alpha = \gamma . c_i \qquad (8)$$

Where

α = activity

γ = activity coefficient

c_i = concentration of X_i

Substituting equation 8 into equation 7 the latter can be re- written as: -

$$E(\text{cell}) = E' \pm \frac{RT}{nF} \ln c_i \gamma \tag{9}$$

Equation 9 can be expanded to: -

$$E(\text{cell}) = E' \pm \frac{RT}{nF} \ln c_i \pm \frac{RT}{nF} \ln \gamma_i \tag{10}$$

During the analysis, the ionic strength is kept constant by adding an excess of an ionic strength adjuster such as KNO_3 or KCl. Therefore equation 10 can be reduced to;

$$E(\text{cell}) = E^* \pm \frac{RT}{nF} \ln c_i \tag{11}$$

Where: -

$$E^* = E' \pm \frac{RT}{nF} \ln \gamma_i \tag{12}$$

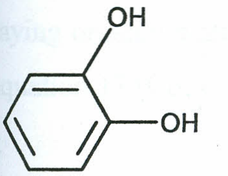
By keeping the ionic strength constant, the measured potential would be proportional to the concentration of the species involved.

2.4 Copper–ligand systems

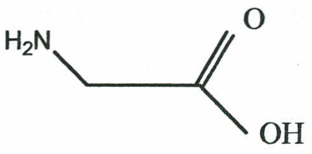
Copper ions cannot exist in solution above pH 5 due to precipitation. But they exist in natural water systems above this pH due to complexation with ligands from decayed organic matter (Onindo, 1999). Copper predominantly complexes with ligands containing nitrogen, oxygen and sulphur donor groups. At pH below 5, copper (II) salts readily dissolve in water to form aqua complex $[Cu\ (H_2O)]^+$. The water molecule can easily be replaced by another stronger ligand to form a different complex.

In order to model copper speciation, three ligands were considered for the study namely catechol, proline and glycine. Catechol was chosen to represent tannins, which are polyphenols (Kiptoon *et al.*, 1987). Glycine and proline are amino acids and proteins found in the fruit bean of coffee and therefore likely candidate ligands to complex copper in the natural waters. Glycine is a non-essential, neutral, genetically coded amino acid. It is the only protein-forming amino acid without a center of chirality. Proline is a non-essential, neutral, amino acid and is the only protein-forming amino acid with a secondary amino group (Kiptoo, 2001)

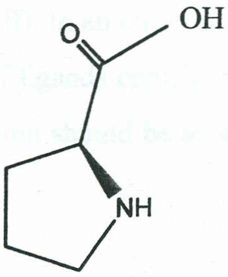
Catechol molecules have shown to be potential ligands for several metal ions by forming very stable chelate ring via the adjacent phenolate oxygen (Kozlowski *et al.*, 1989). Copper and the three ligands considered occur in coffee factory wastewater discharge and can therefore adequately represent possible ligand system found in natural waters. The structures of the ligands are represented below.



Catechol

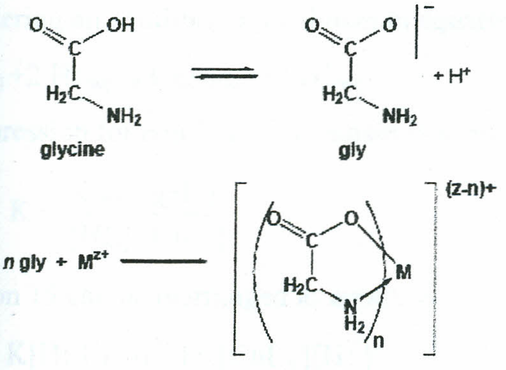


Glycine



Proline

The dissociation of glycine by loss of a proton to form the glycinate mono-anion (gly) and how it latter bonds with a transition metal by coordination is shown below.



2.5 Modeling of some selected copper-ligand systems

Modeling requires a data bank of all conceivable stability constants for the system. Stability or formation constants are equilibrium constants involved in the formation of metal complex from aqua-metal ion and the basic form of the ligand (Martell, 1996). Stability constants were first determined potentiometrically by Nernst, Ostwald and Arrhenius in 1923 as they studied metal ions and anions in aqueous solutions (Martell, 1996). A method of computing these stability constants was later developed by a Danish scientist, Bjerum (1957). He applied a mathematical treatment of equilibrium constants and mass balance equations for a number of complexes. Similarly in this research work the stability constants were calculated from data obtained from pH-metric titrations using SUPERQUAD program (Gans *et al.*, 1985).

Computer modeling of some selected copper-ligand systems was carried out to investigate the effect of simple or tenary complexes, if any, on the solubilisation of Cu (II). In an environmental sample, copper is not expected to exist as a free ion but as a complex of ligands contributed by decaying organic matter. If HL represents a typical ligand, then the reaction should be as shown in equation 13 (Cotton, 1980).

$$n\,Cu^{2+}{}_{(aq)} + n\,HL_{aq)} \rightleftharpoons CuL_{(aq)} + CuL_{2(aq)} + \ldots\ldots\ldots(n\text{-}i)CuL_{n(aq)} + n\,H^{+} \qquad (13)$$

Copper speciation was made with the selected ligands and was done by determining the stability constants values of these various possible complexes using potentiometry.

Considering an equilibrium as shown in equation 14

$$Cu^{2+}{}_{(aq)} + 2\,HL_{aq)} \rightleftharpoons CuL_{2(aq)} + 2\,H^{+}{}_{(aq)} \qquad (14)$$

An expression for equilibrium constant is shown in equation 15

$$K = \frac{[CuL_2][H^+]^2}{[HL]^2[Cu^{2+}]} \qquad (15)$$

Equation 15 can be rearranged as equation 16.

$$K[HL]^2[Cu^{2+}] = [CuL_2][H^+]^2 \qquad (16)$$

By making $[H^{+}]$ the subject, and taking logs,

$$pH = \frac{1}{2}(pK + 2pHL + pCu^{2+} - pCuL) \qquad (17)$$

If the pH of the reaction mixture was varied by titration with a solution of dilute sodium hydroxide and the concentration of various forms of copper definitely change and this is the basis of plotting of pH speciation diagrams.

Titration of a polyprotic ligand, H_nL, with a strong base generates a series of deprotonated species described by the following equilibrium and protonation constants arising from the equation 18.

$$H_nL \rightleftharpoons H_{n-1}L+H_{n-2}L.......H+L \tag{18}$$

For a diprotic ligand, the protonation equilibrium is given by equations 19 given below.

$$H_2L \rightleftharpoons H+HL \tag{19}$$

For a simple ligand HL

$$HL \rightleftharpoons H+L \tag{20}$$

Their respective protonation constants are given in equations 21 to 23 (Cotton, 1980).

$$K_1 = \frac{[HL]}{[H][L]} \tag{21}$$

$$K_2 = \frac{[H_2L]}{[HL][L]} \tag{22}$$

$$K_n = \frac{[H_nL]}{[H_{n-1}L][L]} \tag{23}$$

There exists a relation between the deprotonated ligand and the all the forms of ligand in solution. The relation is expressed as shown in equation 24 (Cotton, 1980).

$$\alpha_{L(H)} = \frac{T_L}{[L]} \tag{24}$$

Where

α = protonation coefficient

T_L =Total concentration of forms of metal in solution

[L] = concentration of complexed forms of metal in solution

The total ligand T_L and titrable proton T_H in solution can be expressed in terms of mass balance equations of all ligands containing species and all proton containing species

$$T_L = [L] + [HL] + .\ \ldots\ldots\ldots + [H_nL][H^+] \tag{25}$$

$$T_H = [H^+] + [HL] + .\ OH^- \ldots\ldots\ldots + n[H_nL] - [H^+] \tag{26}$$

The term $[OH^-]$ arises from hydrolysis of the type given in equation 27

$$H_{n-1}L + H_2O \rightleftharpoons H_n + OH^- + L \tag{27}$$

In terms of protonation constants, they can be represented as shown in equations 28 and 29 respectively.

$$T_H = [H] + K_1[H][L] + 2K_1K_2[H]^2[L] - [OH] \tag{28}$$

$$T_L = [L] + K_1[H][L] + 2K_1K_2.\ [H]^2[L] - [OH \tag{29}$$

Where T_L and T_H are the total concentration of ligand and titrable protons respectively.

Expressing equation 29 in terms of protonation coefficient,

$$\alpha_{L(H)} = (1 + K_1[H^+] + 2K_1K_2\ [H^+] \ldots\ldots K_N[H^+]) \tag{30}$$

This implies that $\alpha_{L(H)}$ is pH dependent

.5.1 Metal-ligand equilibrium

The interaction of metal M and ligand L can be expressed as: -

$$ML \rightleftharpoons M + L \tag{31}$$

$$ML_2 \rightleftharpoons M + 2L \tag{32}$$

$$ML_3 \rightleftharpoons M + 3L \tag{33}$$

$$ML_n \rightleftharpoons M + nL \tag{34}$$

Their respective thermodynamic equilibriums formation constants can be expressed as:-

$$K_1 = \frac{[ML]}{[M][L]} \tag{35}$$

$$K_2 = \frac{[M_nL]}{[M_{n-1}L][M]} \tag{36}$$

$$K_i = \frac{[M_iL]}{[M_{i-1}L][M]} \tag{37}$$

$$K_n = \frac{[M_nL]}{[M_{n-1}L][M]} \tag{38}$$

There exists a relation between the non-complexed metal and the all forms of metal in solution. The relation is expressed as shown in equation 39.

$$\alpha_{M(L)} = \frac{T_M}{[M]} \tag{39}$$

Where

α = complexation coefficient

T_M = Total concentration of forms of metal in solution

[M] = concentration of complexed forms of metal in solution

As protonation constants are expressed as shown in equation 28, T_M can be expressed in a similar manner as shown in equation 40.

$$T_M = [M] + [ML] + [ML_2] \ldots\ldots [ML_N] \tag{40}$$

Using stability constants

$$T_M = [M] + K_1 [M][L] + 2 K_1K_2 [M] [L_2] \ldots\ldots. K_N [M][L_N] \tag{41}$$

$$T_L = [M](1 + K_1[L] + 2 K_1K_2 [L_2] \ldots\ldots. K_N [L_N]) \tag{42}$$

Expressing equation 42 in terms of complexation coefficient,

$$_{M(L)} = (1 + K_1[L] + 2 K_1K_2 [L_2] \ldots\ldots. K_N [L_N]) \tag{43}$$

$$\alpha_{M(L)} = 1 + \sum_{i=N}^{V} \beta^{T}_{M(L)}[L] \qquad (44)$$

Since The variation of $\alpha_{L(H)}$ is pH dependent, it implies that $\alpha_{M(L)}$ will also depend on pH because [L] depends on the same. The variation of $\alpha_{M(L)}$ with respect to ligand concentration, can be in form of speciation diagrams as well The overall formation constant $ß^{T}_{ML}$ which is the product of the stepwise formation constant $ß^{s}_{i}$.

$ß^{T}_{ML} = ß^{S}_{1} \times ß^{T}_{2} \times \ldots\ldots ß^{S}_{n}$ (Cotton, 1974)

and

$$ß^{S}_{ML} = [ML_n]\,] \qquad (45)$$

therefore

$$ß^{T}_{ML} = ß^{s}_{ML} \times [ML_n]\,] \qquad (46)$$

The experiment is done under a constant ionic strength as earlier discussed in section 2.3.3.4.

Then

$$\log ß^{T}_{ML} \rightleftharpoons \log ß^{s}_{ML} + \text{constant} \qquad (47)$$

2.5.2 Computation of constants

Protonation constants ß are obtained by measuring the e.m.f of the solutions using a glass micro-electrode calibrated on a pH scale. Their successive pH values obtained with the help of a molspin pH-meter. The e.m.f at each point in the titration is a function of equation 48 as shown below.

$$E^{i} = f(T, c, v^{i}, v^{o}, E^{o}, ß_{HL} K_{w}) \qquad (48)$$

Where T= temperature

v^{i} = Volume added

v^{o} = Initial Volume

$ß_{HL}$ = protonation constant

Kw = Ionic product of water

All the other values are known except for E^{i}, which is measured, and $ß_{HL}$ (calculated). The resulting data is fed into a computer program, SUPERQUAD, which makes a series of guesses at

β_{HL} .For each K_i guess a value E^i_{calc}. is calculated for each of the n points in a titration curve and the β_{HL} chosen is the one which produces the minimum value of U in the least sum of squares.

$$U=\Sigma^n_{i=1}(E^i_{calc} - E^i)^2 \qquad (49)$$

Similarly, in the determination of stability constants of Metal-Ligand complexes, the equilibrium considered are:-

(1) Protonation of ligand $L + nH \rightleftharpoons H_nL$
(2) Metal-Ligand Equilibrium $pM + qH + rL \xrightarrow{\beta pqr} M_pH_qL_r$
(3) $xM + yOH = M_x(OH)_y$

A series of mass balance equations 50-52 are then written for each component.

Total metal $T_M = [M] + \varphi_p [M_pH_qL_r]$ (50)

$$T_L = [L] + \varphi_q [M_pH_qL_r] \qquad (51)$$

$$T_H = [H] + \varphi_r [M_pH_qL_r] - [OH] \qquad (52)$$

Where

$$[OH^-] = Kw\,[H]^{-1} \qquad (53)$$

And

$\varphi_p\ \varphi_q\ \varphi_r$= functions of stability constants

When the total metal T_M, total ligand T_L total ligand and the protonation constants are known, the mass balance equation are solved by SUPERQUAD to yield stability constants (Gans *et al.*, 1999)

2.6 Physico– chemical water quality parameters

2.6.1 pH measurements

The sensor for this parameter is a glass membrane, which has the test ions on both sides i.e. internal solution and external test solution. When this membrane is placed in a test solution, a potential difference between its two sides developes. This potential is proportional to the concentration of the ions in the external solution. This potential is calibrated to give pH values in a pH meter. Species of various metal complexes are affected by pH. Toxicity is dependent on the species and therefore influenced by pH and hence the need to determine the pH with a view of predicting the species distribution.

2.7 Dissolved Organic Carbon (DOC)

DOC is the organic materials from plant and animal sources broken down into small water-soluble fractions. Some DOC molecules have a well defined chemical structure such as fats, carbohydrates, and proteins. However most have no definite structure and are lumped under the term humic or tannic substances. Naturally occurring dissolved organic matter (DOM) are responsible for the mobility of metals in soils and aquatic systems (Nizhou *et al.,* 2002). Thus, DOM is expected to increase trace metal mobility in water systems through formation of complexes. The humic type of DOC which is the most abundant kind found in lakes and streams has a colour range of yellow to black, this has a great influence on water colour. Copper is strongly complexed by organic ligands with a binding capacity of between 1.86 to 14.3 mM Cu per mM DOC (Han *et al,* 1999).

Organic carbon plays a central role in aquatic systems chemistry because it complexes metals and nutrients, affects pH and alkalinity, and acts as a substrate for microbial production (Dillon *et al.,* 1997). DOC can protect as well as harm aquatic life depending on concentration, chemical composition and system characteristics. For example, the humic fraction of DOC can form particulates that serve as a food source for filter and deposit feeders, while at the same time increasing exposure of these organisms to higher levels of non-polar toxic chemicals (Björk, 1996).

Tannic acid (TA) and gallic acid (GA) are polyphenols that have more than ten 1,2–dihyroxy benzene units per molecule (Kiptoon, 1987). They originate from dead leaves, and are precursors of humic substances. The tannins are widely distributed in natural water and constitute a portion of lower molecular weight DOC, while humic substances make up the higher molecular weight fraction. A group of researchers found out that Cr (VI) is reduced efficiently by extracts of dead leaves (Nizhou *et al,* 2002). Therefore Cr (VI) can be used in determining low molecular weight compounds such as TA and GA (Arakawa *et al,* 1993). As a result these polyphenols are available to complex with many metals in the water system. The amount of DOC in water can be determined by reducing Cr (VI) and measuring the amount of reduced Cr (VI) by UV-Vis spectrophotometer. Since TA and GA have functional groups (carboxyl and phenolic groups) that are good model of humic substances, these may have the ability to reduce and complex with Cr (VI) as well (Collier, 1987).

2.8 Chlorides

Chloride ions are leached into the water out of land by rain and ground water to accumulate in water bodies. Solar evaporation of the water is responsible for underground deposits of rock salts found in many areas and as such almost all natural waters contain chloride ions. Their concentrations vary considerably according to the mineral content of the earth in any given area. In small amounts they are not significant but in large concentrations they present problems. Usually chloride concentrations are low and moderate concentrations of chloride ions add palatability to water. In fact, they are desirable for this reason but excessive concentrations can make water unpleasant to drink. Several secondary Drinking Water Regulations recommend a maximum concentration of chloride ions to be 250 µg/m1 (Maybeck *et al..,* 1990).

Chlorides give water a salty taste. At what concentrations this becomes noticeable again depends upon the individual. In large concentrations chlorides cause a brackish, briny taste that definitely s undesirable. Although chlorides are extremely soluble, they possess marked stability. This enables them to resist change and their levels to remain fairly constant in any given water unless he supply is altered by dilution or by industrial or human wastes (Maybeck *et al* 1990).

2.8.1 Chemical properties

There is no reported adverse effect of chloride ions on man, however on wet conditions, chloride eacts with metals making the metal available for further reaction. In such reactions, an electro-hemical cell is formed. Properties of the material involved in these reactions are definitely hanged. As a result, destruction degradation, or deterioration of the metal may occur. Pitting of a given material depends strongly upon the presence of an aggressive species in the environment and a sufficiently oxidizing potential like chloride ion in neutral, aerated aqueous solution on stainless steel (Thiongo, 1995).

This activity may take place on the surface forming a protective film that may prevent further reaction due to passivation. There are situations where a reaction may continue by passage of ions through the surface film. In the latter, reaction continues unabated hence a continuous degradation (Bockris, 1972).

2.9 Fluorides

Fluorides occur naturally in many water sources and are added in the drinking water treatment process by many public water systems. Fluorides in amounts between 1.0 and 1.5 µg/ml have beneficial effects in reducing tooth decay. Amounts greater than 2.0 µg/ml, however, can have harmful effects ranging from the discoloration or pitting of teeth to bone and skeletal damage. Because of these negative effects, the EPA (environmental Protection Agency) in the USA has established a Maximum Contaminant Level (MCL) for fluoride of 4.0 µg/ml, and requires all public water systems to ensure that the safe level is not exceeded (Björk, 1996).

Fluorides also occurs naturally in both sedimentary and ingenuous rocks in many places of the earths crust. It mainly occurs as fluorspar (CaF_2) or as rock phosphate ($Ca_5F(PO_4)_2$. In Kenya, there are large deposits of fluorspar in Gilgil situated on the western slopes of the Abaderes range, which is the source of Kamiti river.

2.9.1 Chemical properties and health effects

Fluorine is the most electronegative element in the halogen family and therefore chemically most energetic (reactive). Consequently, it enters into combination with most other chemical elements in the body system as is the case it is found in nature. Due to its size, the ionic bond formed between fluorine and calcium is very strong because it has a very high lattice energy (Cotton, 1980). Therefore fluoride salts are very fatal when ingested. They cause death in as little as 5 minutes from time of ingestion to not more than 24 hours. The acute lethal dose is 105 mg per body weight (Simons, 1965)

There is evidence that low concentrations of fluoride ions can have devastating effects on just about every organ in the body, and may even be partly responsible for behavioral problems like hyperactivity and many puzzling illnesses. However fluoride ions are at times found to be additives in toothpaste for dental health even if they are poisonous - more poisonous than lead and only slightly less poisonous than arsenic (Clin, 1984).

Fluoride has a strong affinity to calcium, but will also readily combine with magnesium and manganese ions and so can interfere with many enzyme systems that require these minerals. The interruption of these enzyme systems, in turn, may disturb carbohydrate metabolism, bone formation and muscle function. Indeed, all vital function in the body depends on enzymes; and since fluoride is very reactive, it easily reaches every organ resulting to many diverse toxic symptoms.

Enzyme systems react with fluoride in different ways; some are activated, others are inhibited. Lipase (essential for the digestion of fat) and phosphatases (needed to breakdown phosphates) are very sensitive to fluoride. For patients with skeletal fluorosis, succinate dehydrogenase activity is inhibited. In chronic fluoride poisoning, this diminished enzyme activity accounts for muscular weakness and even muscle wasting. Human salivary acid phosphatase is diminished by half when exposed to a concentration of 3.8 μg/ml fluoride. The blood enzyme cholinesterase is inhibited by 61 per cent (w/w) on exposure to 0.95 μg/ml fluoride-an amount within recommended levels adversely affecting functions of the nervous system. Nevertheless, fluorides are found in tissue of all plant and animals and hence ever present ingredient in diets of man and animals. No harmful effects have been documented in deficiency of fluorine but a relationship between reduced tooth health and increased fluorine intake has been established. A condition called crippling fluorosis has been observed in humans exposed to cryolite or fluorspar that is responsible to disabilities. Observed effects of fluorides on bones are increase in bone density resulting to the bones being brittle and very fragile, calcification of ligaments rendering reduced mobility and rusty or brown teeth. Fluorides disturb the normal calcium absorption in bones hence lead to a poor bone structure (Simons, 1965).

In this research work, the content of total copper was determined by FAAS. This method has the advantage that it is capable of analysing copper selectively in the presence of other metals without interference. The labile fraction was determined by using DPASV voltammetry. Voltamograms were plotted and current I_{max} at reduction potential of copper obtained from which the concentrations of copper in the sample were evaluated. DPASV was used to determine stability constant values by measuring the shift in peak potential of copper-copper ligand complexes. In speciation modeling, stability constant values, which are critical parameters, were determined by potentiometric titration using a molspin pH meter attached to a computer. The data obtained was fitted into an appropriate program to compute the stability constant values of various chemical species at changing pH environment. Dissolved organic carbon was determined by UV/Vis method by reduction of potassium dichromate solution at a strong acid environment. The other parameters Cl^-, F^- and pH were analysed potentiometrically.

CHAPTER THREE

EXPERIMENTAL

3.1 Introduction

The study was carried out double fold; the analysis of water samples from Kamiti river and speciation studies of copper with some selected ligands. Sampling was done on different seasons, which were dictated by the weather and divided into two geographical regions. The first region started from Thika Road bridge to Kamiti/Ruiru road bridge was labeled TRB and the other from Kamiti/Ruiru road bridge to Kamundu tea estate labeled KRB a span of 30 km. Sampling was done on three different seasons of the year. The seasons were 1 (May)- after the long rains, 2 (August)- during the dry season and 3 (November)- during the short rain season.

3.2 Sampling procedure and sample treatment

Water was sampled using an extendable water-sampling scoop from the river. One liter of water was taken after thoroughly agitating the water on the sampling site and the pH monitored instantly using a Jenway potable pH meter model number 3071. The samples were placed in 500 ml plastic containers that had previously been soaked in dilute nitric acid and later cleaned with de-ionized water and rinsed with the sample water five times. It was then transported to the laboratory. Sediments (about 250 g) were also collected from the bottom of the river at the same sampling site using a extendable long handle spade, special for that exercise.

Once in the laboratory, water samples were filtered using a whatman filter paper number 41 to remove any solid particles and algae and later stored in a deep freezer at 0° C to prevent bacterial action with no further treatment.

The sediments were placed in an oven and dried at a temperature of 110^{0} C and then allowed to cool in a desiccator awaiting the determination of copper.

3.3 Cleaning of glassware and plastic containers

All the glassware and plastics used in this work were cleaned with soap and water to remove any grease and particles first, and then soaked in nitric acid for a period of twelve hours. They were later washed and rinsed with distilled and de-ionized water. When not in use, all containers were soaked in 10% nitric acid solution. Immediately before use, they were rinsed in de-ionised water. Plastic containers and bottles were dried in oven but at 50° C.

3.4 Preparation of stock solutions and other reagents

All the chemicals used were of analytical grade quality unless otherwise stated. Distilled and de-ionized water was used for making all dilution and rinsing of glassware.

3.4.1 Standard solutions

Copper standard solutions were prepared by weighing accurately 1.0000 g of copper powder in a clean beaker and 25 ml of conc. nitric acid carefully added. The resulting solution was transferred into a 1-litre flask and made to the mark with distilled water. This solution contained 1000 μg/ml of copper and was stored in a plastic container as a stock solution for a period of three months. It was from this solution that subsequent dilutions were made to prepare a calibration curve.

A working standard stock solution 1000 μg /ml chloride ions was prepared by accurately weighing 1.2220 g of sodium chloride salt and dissolving in one liter of distilled/de-ionised water. This solution was stored in a plastic container as Cl^- stock solution. From this solution, successive dilutions were done to prepare a calibration curve for the determination of the chloride ion in the water samples.

A standard solution for fluoride determination was prepared by accurately weighing 2.3691 g of sodium fluoride salt and dissolving it in one liter of distilled/de-ionised water. It was labeled 1000 μg/ml F^- stock solution and stored in a plastic container as well. Successive dilutions were done to prepare working standards.

3.4.2 Other reagents

The following reagents used in this study were prepared by dissolving requisite amounts of appropriate salts in distilled water as given below:-

Tannic acid (TA) was prepared by accurately weighing 0.0200 g of TA and dissolving in 1 litre of water. The solution was labeled 0.0200 g TA.

Potassium dichromate solution was prepared by accurately weighing 1.4740 g of the salt and dissolving in 500 ml of water to obtain 10.0 mM $K_2Cr_2O_7$ The solution was labeled and stored in separate container.

Silver sulphate solution was prepared by accurately weighing (3.11800 g) and dissolved in 1 litre of water. The solution was labeled 0.1 M $(Ag)_2SO_4$ and placed in a dark bottle.

A 2 M solution of sulphuric acid was prepared by taking 112.0 ml of concentrated sulphuric acid nto a one-litre flask and the resulting mixture made to the mark with distilled water.

Mercuric sulphate solution was prepared by accurately weighing 29.6650 g and dissolved in one iter of water. The solution was labeled 1.0 M $HgSO_4$,

.4.3 Support electrolyte

The support electrolyte used for voltammetric determination of copper was sodium acetate olution. This was prepared by dissolving 13.6000 g of sodium acetate in 750 ml of water to give .156 M solution. The pH was adjusted to 5.5 with ammonia before dilution to one liter with water. To remove trace metal impurities in the support electrolyte, it was purified by controlled otential electrolysis. Standards solutions for voltammetric analysis had their pH adjusted to the ame value as well but this was not done for the FAAS standards analysis. The resulting olutions were stored in plastic containers from where required dilutions were made.

3.4.4 Total Ionic Strength Adjuster Buffers (TISAB)

For potentiometric titration, total ionic strength adjuster buffer was prepared by weighing accurately 20.2220 g of potassium nitrate and dissolving in two liters of distilled water.

3.4.5 pH buffers

Buffer solutions for pH 4, 7 and 9 for the calibration of the pH meter were prepared by dissolving espective commercially available buffer tablets in 100 ml of water. All these solutions were tored in labeled plastic bottles.

.4.6 Sodium hydroxide

Carbon dioxide free 0.1M sodium hydroxide (for potentiometric titration) was prepared by issolving 4.0000 g of sodium hydroxide in a hot solution of 0.1 M potassium nitrate and the olution allowed to cool in a stoppered airtight two-way round-bottomed flask.

.4.7 Ligands

lycine and catechol (0.1 M) solutions were prepared by weighing 0.7512 and 1.0570g of the espective ligand and dissolving in 100 ml of 0.1 M potassium nitrate. To prepare a solution of roline, 0.2776 g of proline were taken and dissolved in 20 ml solution of 0.1 M potassium itrate.

.4.8 Vanadium chloride

his solution was prepared by boiling 2 g of ammonium metavanadate in 25 ml of concentrated ydrochloric acid. The resulting solution was transferred into a 250 ml flask and diluted to the ark with water. This solution was later put into a glass-washing tower for scrubing nitrogen as.

3.5 Determination of pH

Before use, the pH electrode was conditioned with saturated potassium chloride solution over night to wet the membrane and improve its sensitivity. The pH meter was later calibrated with two buffers of pH 4 and pH 9 at the site for immediate pH measurements.

3.6 Determination of dissolved organic carbon in water

The procedure proposed by Bauman (1974), for determination of DOC in waste and saline waters was adopted in this study. 5 ml of standard solutions were placed into separate screw cap test tube. To this solution, 2.0 ml H_2SO_4, 1.0 ml 1.0 M $(Ag)_2SO_4$, and 1.0 M $HgSO_4$ were added followed by 2 ml 10.0 mM of $K_2Cr_2O_7$.The resulting volumes were adjusted to 12 ml with distilled water. They were then placed in an autoclave and heated for 2 hrs (Gonzalez, 1986).

Upon cooling, absorbance readings for the standard solutions were then taken at 606 nm using a UV-Vis spectrophotometer, model no 4040 Nova spec II. From this, a calibration curve was made

The water samples, 5 ml, were taken and treated in the same way as the standards above. The concentrations of the DOC in the water samples was then evaluated.

3.7 Determination of fluoride content in water

Fluoride ions were determined using an F1052 ion selective electrode attached to a pH meter Jenway electrochemical analyzer model no 3303. 25 ml of the standards and samples were placed in a plastic beaker and 25 ml TISAB added to maintain a constant ionic strength. The potentials for the standards were then measured from which a calibration curve of potentials against logarithms of concentration was plotted. The fluoride ions in the samples were then calculated.

3.8 Determination of chloride content in water

The content of chloride ion was measured using a 6560-10C ion selective electrode attached to a pH meter. A solution of 25 ml of the standards was pippetted into a plastic beaker and 25 ml TISAB added to maintain a constant ionic strength. The samples were treated in the same way and their potentials were measured using a Jenway electrochemical analyzer model no 3305, from which a calibration graph was made. The concentration of chloride ion in the samples could therefore be evaluated.

3.9.1 Determination of total copper

The analysis was done using Buck scientific 210VGP Flame Atomic Absorption Spectrophotometer. Its operating parameters were set according to the specifications given by the manufacturer as shown in Table 3.1.

Table 3.1 Operating parameters of the Buck scientific 210VGP FAAS

Parameter	Wavelength	Slit-width	Lamp current	Fuel system	Sensitivity
Value	324.8 nm	0.7 nm	1.2 A	Air/acetlyne	2.0 μg /ml

The analytes were asipirated into the machine and their absorbance readings were taken for both the standards and samples. A calibration curve was plotted from which the concentration of copper in water samples was then calculated.

The sediments were dried in the oven at 110 °C. They were allowed to cool in a desiccator after which 1.00 g of sample was accurately weighed into a conical flask. Concentrated hydrochloric acid (25 ml) was added and the mixture placed on a hot plate, then boiled for ten minutes. It was left to cool and filtered through a whattman No. 41 ashless filter paper into a 100 volumetric flask. The resulting solution was made to the mark with distilled water. These samples were then analysed for copper in a similar manner as the water samples above.

3.9.2 Determination of the labile fraction of copper

3.9.2.1 Preparation of the instrument

The Polarographic analyzer EG&G model 303 is usually equipped with a mercury capillary electrode for both DME and HMDE. However it was modified to accommodate a glassy carbon electrode as a working electrode. After this modification, the three electrode system measurements were made against a silver/silver chloride electrode with platinum wire as the counter electrode.

Prior to use, the glassy carbon electrode was cleaned by successive polishing on aqueous slurries of 1 µm, 0.3 µm and 0.05 µm alumina abrasive powder, followed by thorough rinsing in de-ionised water. The sample was placed in the voltammetric vessel and then purged with nitrogen for one minute. Cyclic voltammograms (CV) were then obtained at a scan rate of 50 mV s^{-1} under the diffusion-limited conditions starting from a range of –1.6 V to 2.0 V. The instrumental settings were as indicated in Table 4.

Table 3.2 Instrumental settings for the determination of labile copper

Initial potential	Final potential	Deposition potential	Deposition time	Sweep rate	Equil. time
-0.60 V	+0.10 V	-0.60 V	30 sec.	0.10 Vs^{-1}	10 sec.

Noise was observed on the initial Voltamograms acquired, which resulted from the glassy carbon leed wire and conductors from the clock and hammer for the mercury electrode. Proper insulation of the leed wire and discontinuation of power for the clock and hammer solved the problem and the machine was then ready for use.

3.9.2.2 Optimisation of the instrument

Several samples were tried with a view of establishing their reduction potentials. A mixture of copper and lead standards were analysed within a potential range between -0.3 V to + 2.00V. To confirm the equipment was selective to various metals, a sample containing a mixture of lead 5 µl of 1.00 µg/ml of lead and 5 µl of 1.00 µg/ml of copper solutions were ran. The concentration of lead was varied by spiking 5 µl of that solution two times whereas the concentration of copper was varied by addition of 5 µl each time until the reading went out of scale.

3.9.2.3 Analysis for labile copper in the sample by DPASV

The support electrolyte, 10 ml, was pippetted into the polarographic cell, followed by 5 ml of the sample. After purging the mixture with nitrogen, an initial voltamogram of sample alone was acquired followed by another which had the sample and a solution of copper spiked in the same cell (standard addition method) without changing the machine settings.

3.9.3 Voltammetric determination of stability constants

An electrochemical analyser comprising of an autolab recorder and a voltammeter (Metrohm 663 VA) was used and the settings were as shown in Table 3.3 below. Voltammetric measurements were done using 0.1 M potassium nitrate (pH 7.24) as background electrolyte. The solution was taken into the polarographic cell and continuously a voltammogram obtained to check the purity of the solution. This was followed by addition of 50 μl of 0.05 M copper solution and a voltammogram of this solution was obtained. 25 μl of the ligand under study, that gave a Cu : L ratio of 1 : 1 was introduced into the cell and a voltammogram obtained. This was followed by varying the concentration of the ligand to get other Cu : L ratios then obtaining their respective voltammograms. Stability constants were calculated using the shifts in peak potential.

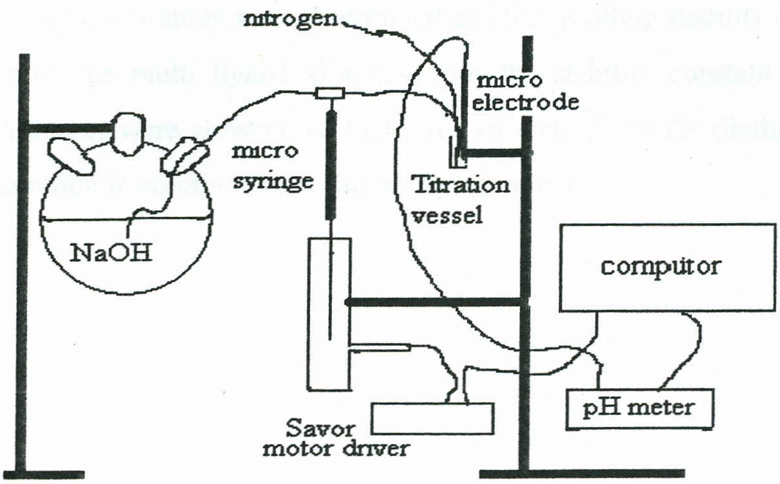
Table 3.3 Instrumental settings for the determination of stability constants

Initial potential	Final potential	Deposition potential	Deposition time	Sweep rate	Clean time	Clean potential	Equil. time
-3.00 V	+ 1.00 V	-3.00 V	30 sec.	0.05 Vs^{-1}	20 sec.	- 1.1 V	5 sec.

3.10 Potentiometric titration

A pH- titration study was carried out to measure stability constant of some selected ligands to nvestigate the effect of ternary complexes on the solubilisation of Cu (II), Cd (II) and Pb (II) systems by Onindo (1999). He observed that mono glycinato complex held copper in solution in physiologically important pH range. His procedure was adopted to study some selected ligand systems on copper.

The devised apparatus used to carry out Potentiometric titration was set up as shown in the Figure 3.1. It consisted of a computer, micro syringe, titration vessel, molspin pH meter and a ombined glass calomel microelectrode.



gure 3.1 An assembly of potentiometric titrations using a molspin pH meter

arbon dioxide free solution of sodium hydroxide was placed in a stoppered three way round ttomed flask and dispensed to the micro syringe burette by siphoning making sure that the stem was air tight to avoid reaction of carbon dioxide in the air with the titer as shown in gure 3.1 above. The pH- metric titrations were carried out using a combined glass icroelectrode with the titrant added from a Hamilton syringe auto burette controlled by a olspin titrator set on 'slow reaction rate (Molspin Ltd., Newcastle upon Tyne, UK). This icroelectrode was calibrated in hydrogen ion concentrations using two buffers pH 4 and 7 fore use. Several titrations were performed in duplicates and using a total volume of 1.0 ml of M sodium hydroxide, which was added by 1 ml micro syringe calibrated by both weight and titration with standard materials. Metal ion concentration was 0.5 $\times 10^{-3}$ mol l^{-1}, and the tal-to-ligand molar ratio was 1:1:1 and 1:2:2. Titrations using pH meter were performed at °C in 0.10 M KNO_3. During titration, the reaction mixture was stirred continuously by bbling nitrogen gas, which created an environment free of carbon dioxide atmosphere as well.

The resulting data were analysed using the SUPERQUAD computer program (Gans, 1985). Stability constants of the copper-ligand complexes were determined by pH-metric methods using ligand concentrations in the range 0.0125–0.05 mM. Prior to this, deprotonation constants of the individual ligands under study were determined and the resulting stability constants introduced in the modeling of the multi ligand system. From the stability constant values obtained, pH-concentration curves were constructed that gave an idea of the distribution (model) of copper-ligand concentration in solution at various pH environments.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

The physico-chemical parameters of the water samples that included pH, F^- and Cl^- (discussed in section 4.1.1-4.1.4) were determined by potentiometric method. During this analysis the ionic strength was kept constant by using KNO_3 or KCl, which were inert salts so that the effectiveness of a common ion on the position of equilibrium becomes dependent on the molar concentration and independent of other ions (Skoog, 1985). DOC was determined by UV–Vis spectrophotometric method. Total content of copper was determined by FAAS while the labile fraction was determined by voltammetric method on a glassy carbon electrode. Plots of calibration curves where applicable (from which the parameters were determined) for both Potentiometric analysis and UV-Vis are presented in their respective sections. Product moment correlation coefficient values to give a measure of linearity (Miller, 1998) and regression analysis equations were also provided in each curve. Modeling speciation of copper in aquatic media was done by potentiometric titration. All the raw data obtained in this study is presented in appendix A.

4.1.1 pH values

Although hydrogen ions are not themselves contaminants they are important parameters as they act as indicators to reveal contamination and hence need for correction. The average pH values of the water samples taken for all the seasons are as presented in Table 4.1.

Table 4.1 Average pH values of samples taken from KRB and TRB sampling sites

SEASON	pH values	
	KRB	TRB
May (After long rains)	7.87± 0.33	7.85 ± 0.14
August (The dry season)	7.52± 0.25	7.54± 0.09
November (The short rains)	7.54± 0.13	7.49± 0.24

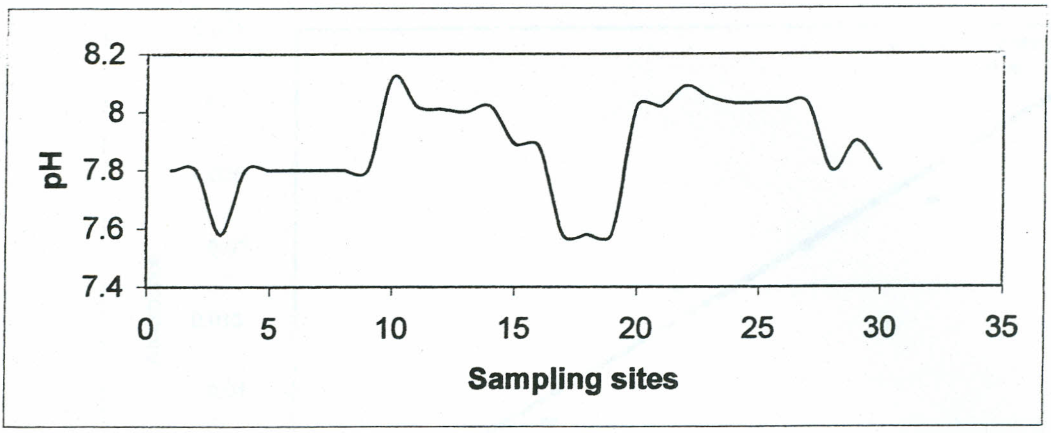


Figure 4.1 Variation of pH along sampling sites TRB after the long rains

As shown in table 4.1 it is observed that the average pH values were below 8. However, it was noticed that some sampling sites had a pH as high as 8.14 especially on areas where lime was applied in the coffee fields. Figure 4.1 represents the variation of pH where lime had just been applied in sites 10 to 13 and sites 20-27. This indicates that the liming process, which is normally done during the heavy rains, contributes to the raise in pH of the water. On other sampling sites, and during the short rain and dry seasons, the pH remained fairly below 8. It may be deduced that liming was the major source of alkalinity as indicated by an otherwise raised values. In general the observed pH values can be said to be normal and within the acceptable limits (Maybeck *et al.,* 1990).

4.1.2 Dissolved Organic Carbon

Absorbance readings at 606 nm were taken to determine the DOC for both the standards and the water samples. The results yielded data given in Table 4.2 below and from that data, a calibration curve was obtained as shown in Figure 4.2.

Table 4.2 Data for preparation of a calibration curve for DOC determination

Conc. x10^{-5}µg/ml	0.000	3.0769	6.1538	9.2307	12.3076	15.384
Absorbance	0.000	4.12x10^{-3}	6.12x10^{-3}	9.2x10^{-3}	12.3x10^{-3}	15.2x10^{-3}

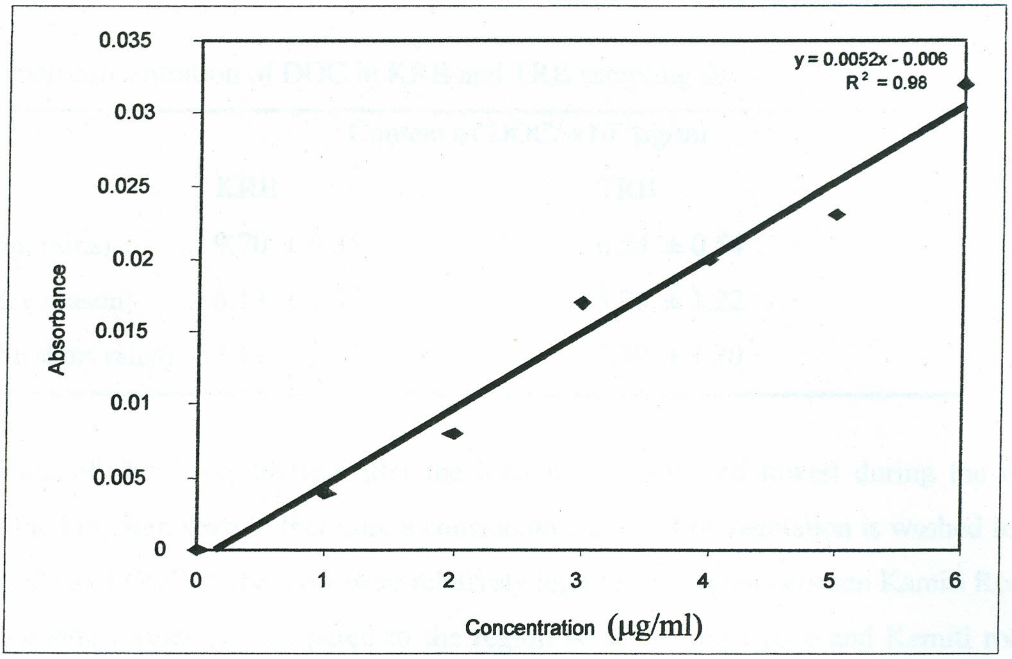


Figure 4.2 Calibration curve for determination of DOC

The regression equation describing the relationship obtained in this analysis was: Abs = 0.0052 x -0.006. (r^2=0.98). This regression equation corresponds very closely to one given by Lewis *et al,* (1977) when he used a 10 mm cell, similar to the one used in this research work, as they analysed some Venezuelans and southeastern USA waters. Their equation was Abs = 0.0053 x + 0.00124. (r^2=0.92). Grieve (1985) used Lewis equation to determine DOC concentrations in a small stream in Scotland and agreed that the equation has widespread validity. The correlation coefficient obtained in this research work indicated that experimental points fitted in a straight-line better than that obtained by Lewis and therefore the line is valid our analysis.

An average distribution of DOC from the water sampled in all the seasons is given in the Table 4.3 shown below.

Table 4.3 Average concentration of DOC in KRB and TRB sampling sites

SEASON	Content of DOC/ x10^{-3} μg/ml	
	KRB	TRB
May (After long rains)	9.70 ± 0.35	6.54 ± 0.54
August (The dry season)	6.13 ± 1.37	5.26 ± 1.22
November (The short rains)	7.39 ± 1.40	7.39 ± 1.20

The concentration of DOC was highest after the long rain season and lowest during the dry season During the long rain season, therefore a considerable amount of vegetation is washed into the river. The values of DOC in the river were relatively high on the region between Kamiti Road Bridge and Kamundu (upper) as compared to the region between Thika road and Kamiti road (lower). In this area, the river banks had a dense foliage cover in that area unlike the other sampling sites. A similar observation was made during the dry season. This was attributed to the fact that dead leaves from the dense foliage cover fell off and found their way to the river, contributing to the high DOC values. However, during the short rains the average values were the ame on both areas. During the short rains, foliage in the form of humus and dead leaves ransported to the river is low because run off water from land to river is low. The DOC in water upstream then equilibrates throughout the river flow giving this scenario. Dilution effects from mall tributaries do not affect these levels. A similar study was carried out by a group of esearchers from New Zealand who analysed rivers draining from forested catchment areas who ound DOC concentrations, which were within the range 4.64 –7.6 x10^{-3} μg/ml (Collier, 1987). Results obtained in this study revealed that the levels were significantly higher because they are ue to both natural and direct introduction of factory wastes as opposed to the natural inclusion nly in the New Zealand case. However, the levels observed in this study were below maximum ontaminant level (MCL) of 400 μg/ml (Maybeck *et al.,* 1990).

he DOC would present an array of possible ligands that would complex and solubilise copper nd any other metal in the river water, a reason for copper's presence at the observed pH.

4.13 Analysis for fluorides (F^-)

Potentiometric measurement of both standards and samples were done and the data on the Table 4.4 below was obtained. The Nernst equation applied to ion selective electrode in potentiometric analysis is given in equation 11 as shown below.

$$E(\text{cell}) = E^* \pm \frac{RT}{nF} \ln c_i \tag{11}$$

By substituting the values of $R = 8.314\ JK^{-1}\ mol^{-1}$. $T = 298K$ and $F = 96.500\ Cmol^{-1}$,.This equation can be written as

$$E(\text{cell}) = E^* \pm \frac{0.0591}{n} \log c_i \tag{54}$$

A plot of potential, E against concentration C from the data was plotted according to this equation to yield a calibration curve shown in Figure 4.3 below from which the concentrations of unknown samples were determined.

Table 4.4 Data for preparation of a calibration curve for fluoride ion determination

log C	0	-0.301	-0.698	-0.903	-1	-1.301	-1.477	-1.698
Potential V	0.3359	0.3503	0.3756	0.387	0.3947	0.4133	0.4271	0..301

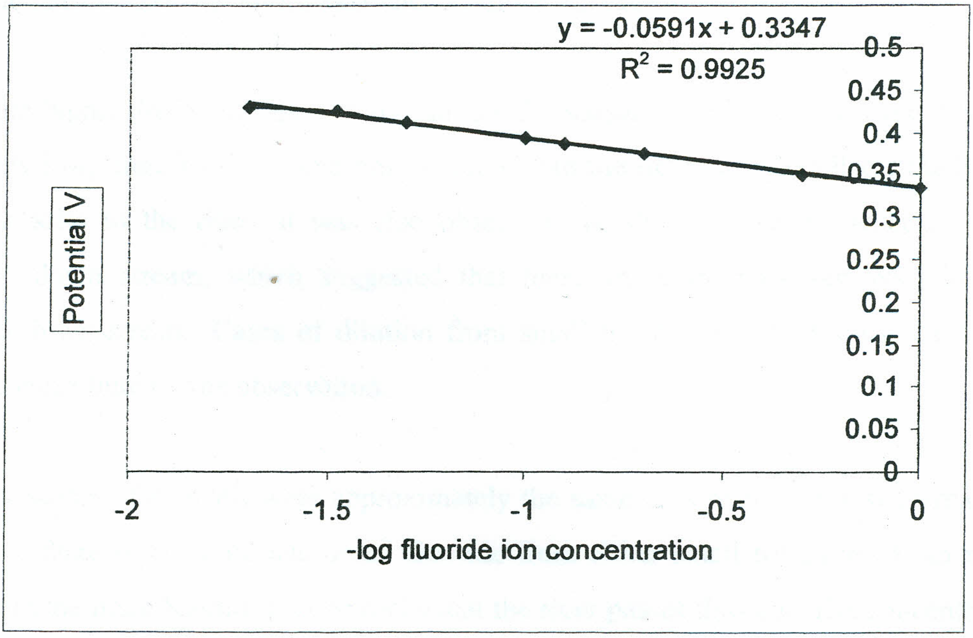


Figure 4.3 Calibration curve for determination of fluoride ions

A good plot was obtained as shown in Figure 4.3 with a gradient is 0.05913. In practice, values of proportionality constant (gradient) ranging from 0.0550 to 0.0591 are referred to as Nernstian (Alum, 1987). Therefore, electrodes with responses in such a range are acceptable for analytical purposes. The results of the average concentration of fluoride ions are presented in Table 4.5 below.

Table 4.5 Average distribution of fluorides in KRB and TRB sampling sites

SEASON	Content of fluoride ions μg/ml	
	KRB	TRB
May (After long rains)	1.049 ± 0.33	1.927 ± 0.57
August (The dry season)	1.589 ± 0.67	1.581 ± 0.33
November (The short rains)	1.602 ± 0.54	1.242 ± 0.55

It was observed that the levels of the fluoride ion gave no specific pattern. The levels were much higher on the lower region (1.927 μg/ml) as compared to the upper region (1.049 μg/ml) after the long rains unlike the other seasons where they are lower down stream. Liming activity, which was on during that season, an exercise that is usually done to minimize loss of minerals through leaching, is the reason likely to have contributed to this observation.

The levels were higher during the short rains than the dry season. This is because even if the rain fall is relatively low, there will be some runoff water from the fields which collects the fluoride from the rock salts to the river. It was also observed that the levels were generally higher upstream than down stream, which suggested that there are more rock salt with fluorides upstream than down stream. Cases of dilution from small tributaries, which do not introduce fluorides, also contribute to this observation.

During the dry season, the levels were approximately the same up stream and down stream. As the river flows, there is no more additional fluoride from either small tributaries from nearby sources that join the main Kamiti river or rocks that the river passes through. The concentrations of fluoride ions obtained in this study are therefore below maximum contaminant level (MCL), 1.5 µg /ml (Maybeck *et al.,* 1990), but a prolonged use of such untreated water can be responsible for discoloration or pitting of teeth to the bone structure, that can lead to skeletal damage. In order to use this water and avoid fluoride poisoning, consumers should be advised to introduce dry grounded bones in their water storage facilities so as to scrub most of the fluoride to a safe level and then filter the water before drinking. Bones have lime that facilitates forming fluoride compounds that precipitate and finally settle. This method has been adopted in several areas of India in raw water whose fluoride levels range from 2 to 20 µg/ml resulting to a high quality drinking water (Mariappan *et al.,* 2001).

4.14 Analysis for Chlorides (Cl^-)

The Nernst equation (equation 11) was again used for the analysis of chloride ions From the potentiometric data given in Table 4.6, a calibration curve for the determination of chloride ions was prepared as shown in Figure 4.4.

Table 4.6 Data for preparation of a calibration curve for chloride ion determination

og C	0	0.301	0.698	0.903	1	1.301	1.477	1.698	1.78	1.85	1.9	1.95	2
otential V	0.335	0.321	0.306	0.281	0.279	0.265	0.253	0.245	0.234	0.23	0.225	0.222	0.219

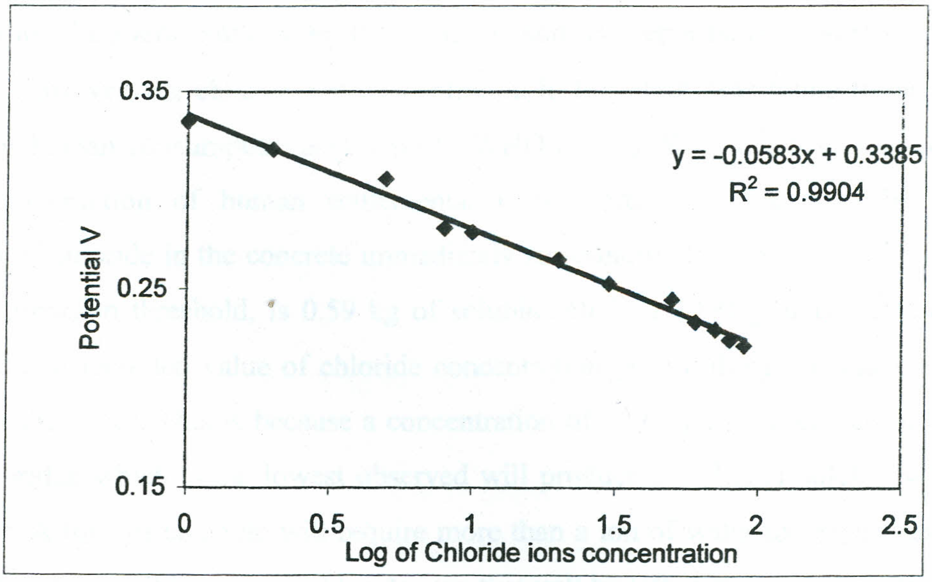


Figure 4.4 Calibration curve for determination of chloride ions

Results for this calibration curve for determination of chloride ions concentration gave a gradient of 0.0583 V per decade. As discussed in section 4.13, these results are Nernstian as well. A similar analysis was carried out by Back (1960) as he analysed underground waters. His calibration curve had a gradient of 0.058 V per decade and therefore results obtained from this calibration are acceptable and valid as well. The results obtained of the average concentration of chloride ions are presented in Table 4.7 below.

Table 4.7 Average concentration of chlorides in KRB and TRB sampling sites

SEASON	Content of chloride ions µg/ml	
	KRB	TRB
May (After long rains)	10.67 ± 1.80	12.47 ± 1.23
August (The dry season)	10.12 ± 1.25	15.87 ± 1.55
November (The short rains)	10.94 ± 1.06	13.48 ± 1.32

The chloride ion was high on the lower regions of the river, TRB, as compared to the upper regions KRB. In the lower region, the river flows through a rocky terrain with no vegetative cover. As it washes the natural rocks, soluble chloride ions are collected giving the observed phenomenon. A similar study was carried out by Maloba (1989) when he analysed the levels of chloride ions in lake Elementaita using the same method. He reported the chloride levels to be 213.0 µg/ml. However, the chloride ion concentration in this study was found to be within the safe limits for human consumption according to WHO (1990). With regards to habitation and subsequent construction of human settlements, it is worthy to note that the minimum concentration of chloride in the concrete immediately surrounding the steel to initiate corrosion, the chloride corrosion threshold, is 0.59 kg of soluble salt in a 1000 g of concrete (Thiongo, 1995). The lowest recorded value of chloride concentration pose a threat to concrete mixtures prepared with the water. This is because a concentration of 10.67 µg/ml observed in the region after Kamiti bridge which is the lowest observed will produce 10.67 g of soluble salt in 1000 liters of water. A tone of concrete will require more than a ton of water to prepare and to cure. This means that there will be enough chloride ions that will be left in the concrete after the water evaporates to initiate decay in the reinforcement bars.

4.2 Total copper

Total copper content was determined by FAAS. A plot of calibration curve for copper was obtained and is presented in the Figure 4.5 below. The data from which this calibration curve was made is given in Table 4.8.

Table 4.8 Data for preparation of a calibration curve for copper ion determination

Conc. µg/ml)	0	0.01	0.02	0.05	0.1	0.25	0.5
Absorbance	0.0003	0.0008	0.0019	0.004	0.008	0.0189	0.037

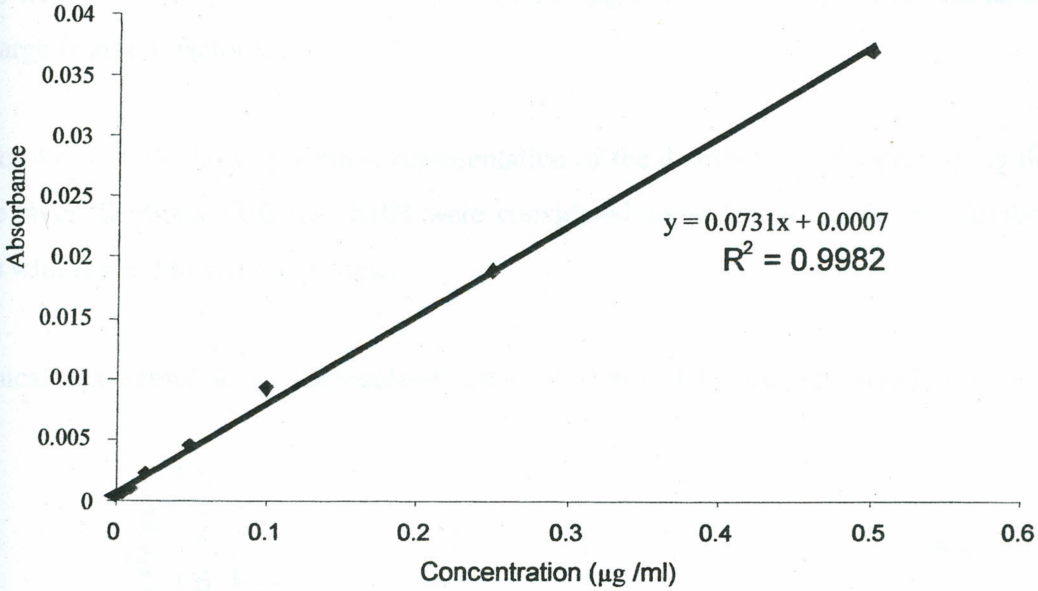


Figure 4.5 Calibration curve for determination of copper

A similar calibration curve was prepared by Hasan (2003) who studied both sea and river water. with eight standard solutions containing 1-100 µg/ml of each of metal when he determined Mn(II), Cu(II), Cd(II) and Pb(II) in river and seawater samples. For copper he obtained a regression equation Abs = 0.0747 x + 0.0 026, r^2=0.998 which was within the acceptable limits. n this study the regression equation was Abs = 0.0731 x +0.0007, r^2=0.9982. The calibration was therefore valid for determination of copper. The correlation coefficient obtained in this research work indicated that experimental points fitted in a straight. In his study, Hasan observed that the river water samples had 1.27 x 10^{-2} μg/l and seawater samples contained 2.11 x 10^{-2} μg/l of copper.

Analysis of the water sample is represented graphically in Figures 4.16 to 4.11 below. The concentration ranged from 0.8–1.38 x 10^{-2} μg/ml in KRB, 0.7-2.2 x 10^{-2} μg/ml TRB, 0-0.83 x 10^{-2} in 2KRB, 0.8-1.8 x 10^{-2} μg/ml in 2TRB, μg/m 0.75-.84 x 10^{-2} in KRB and 0.7-0.82 x 10^{-2} μg/ml in 3TRB. The section between Thika road bridge and Kamiti/Riuru road bridge has four factories namely Tatu, Munene, Kiroeri and Kwa Njoka as shown in Figure 3.1. It is observed that sites 12, 18, 23 and 27 had extremely high contents of copper than the other sampling sites. These levels were 1.1307, 1.6961, 1.7100, 0.9893 (x 10^{-2} μg/ml) respectively which was as a result of discharge from the factories.

Figures 4.6 to 4.11 shows graphical representation of the distribution of copper along the profile of the river. Sections TRB and KRB were considered separately to study the variation of the levels with respect to sampling sites.

Graphical representations of the levels of copper in section TRB are presented in Figure 4.6- 4.8.

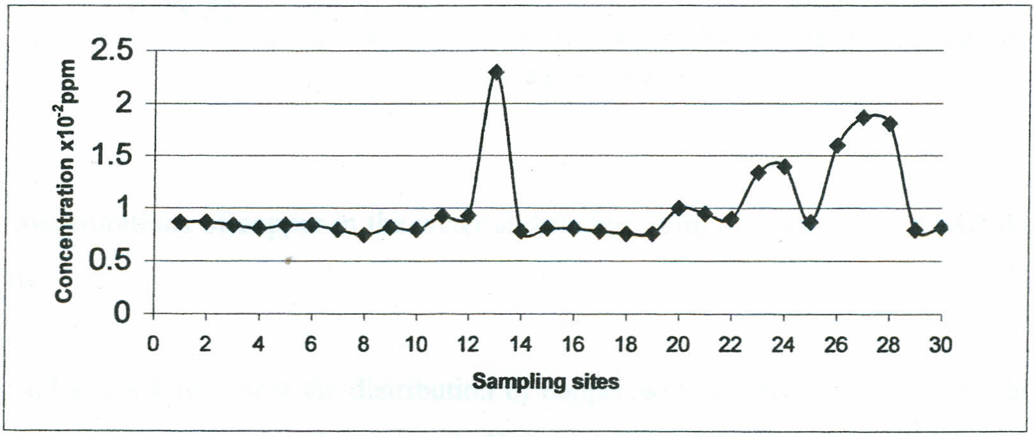


Figure 4.6 Concentrations of copper at different sampling sites along TRB after the long rains.

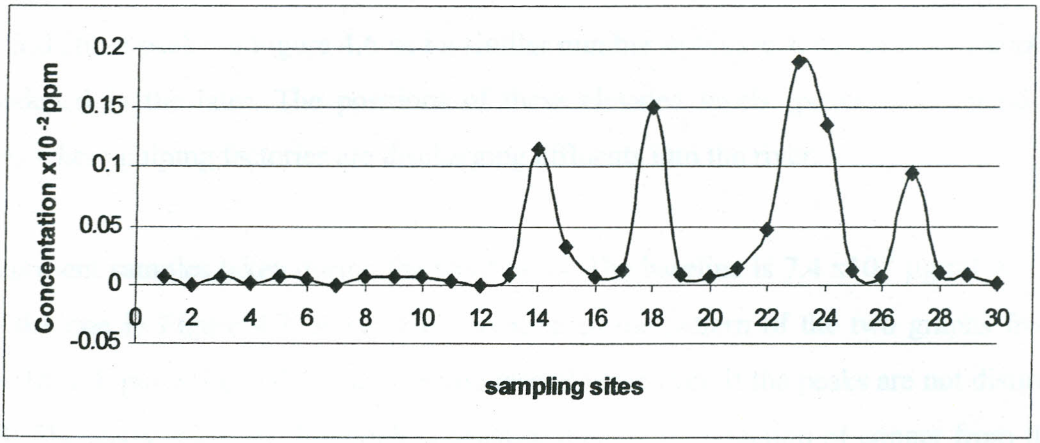


Figure 4.7 Concentrations of copper in the water at different sampling sites along 2TRB in the dry season

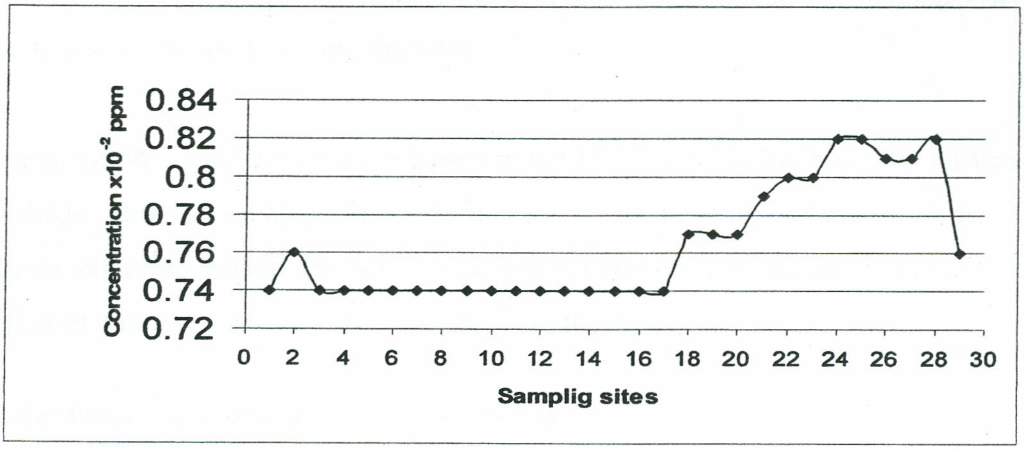


Figure 4.8 Concentrations of copper in the water at different sampling sites along 3TRB during the short rains

Figures 4.6, 4.7 and 4.8 represent the distribution of copper with respect to sample sites in the lower region for the three seasons. The pattern in Figures 4.6 and 4.7 appear to be similar except for the baseline, which is significantly lower in Figure 4.7 representing samples collected in the dry season. The elevated base in Figure 4.6 is as a result of introduction of copper by factory effluent, and the addition from the runoff water due to the rains.

There are four distinct peaks in Figure 4.6 and a similar number in Figure 4.7 even if they appear to have broadened in the later. The positions of these elevated levels (peaks) correspond to sampling sites where pulping factories are discharging effluents into the river.

Figure 4.8 represent samples taken during the short rains. The baseline is 7.4 x10^{-3} μg/ml which is similar to the one in Figure 4.7. A close observation in the pattern of the two graphs from sampling site 18 in Figures 4.6 and 4.7 show some resemblance even if the peaks are not distinct in Figure 4.8. The shape taken by the graph is because there is introduction of copper from the coffee fields, which start from that particular site upstream as well as treatment ponds. However a peak at sites 10-14 in Figure 4.8 was conspicuously absent. At that site, there is a small tributary called Kajibi joining the main Kamiti river. Along this tributary, there a pulping factory, Tatu, that discharges its effluent into it. The absence of a peak suggested that pulping activity was not on and this was confirmed during the visit.

From all the three graphs sampling sites 1-12 correspond to the area below Kenyatta university and Kiwanja village. This section has neither a factory nor coffee farms on either side of the river contributing to the observed results. The level of copper is constant at the baseline because there is no introduction of copper by any factory or the fields to the river.

Graphical representations of section KRB are given below

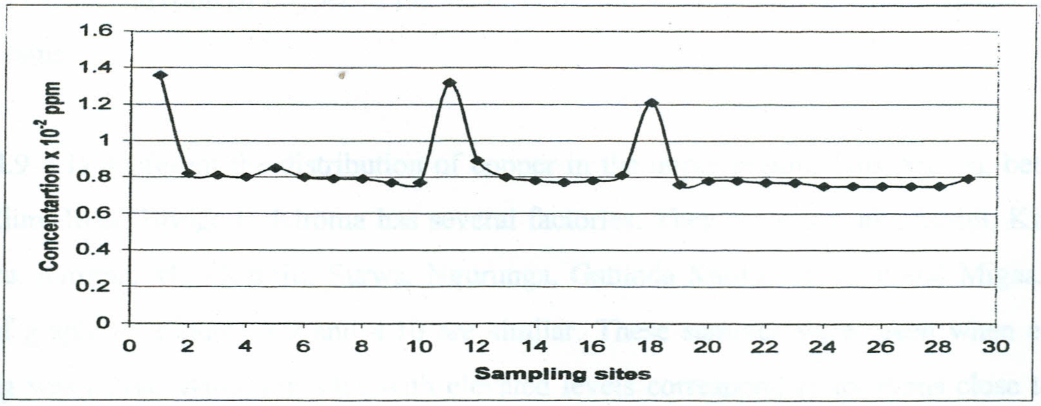
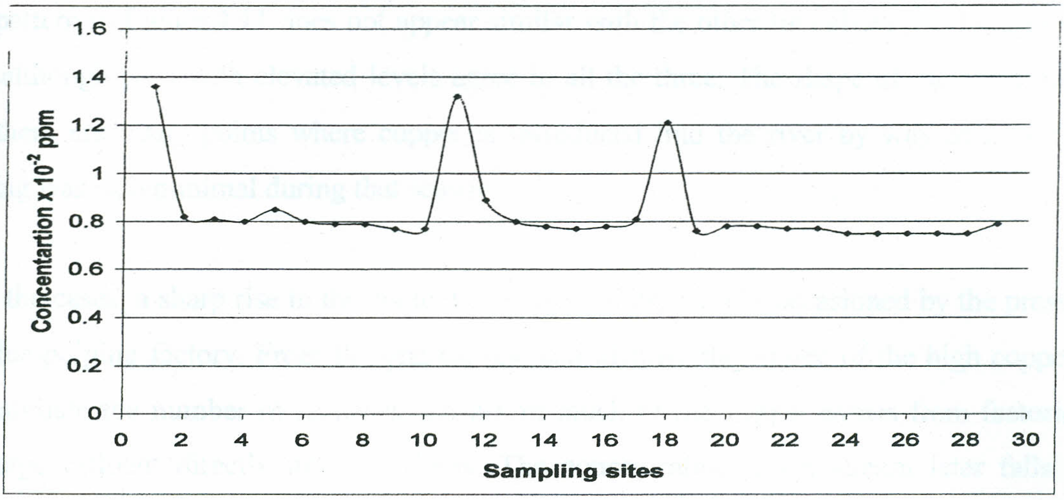
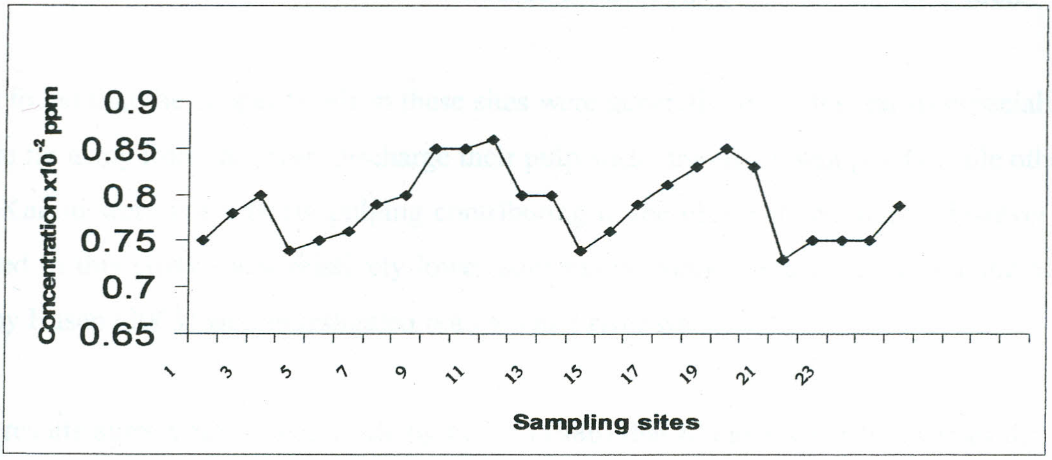


Figure 4.9 Concentration of copper at different sampling sites along KRB after the long rains.



igure 4.10 Concentration of copper in the water at different sampling sites along 2KRB in the ry season



gure 4.11 Concentration of copper in the water at different sampling sites along 3KRB during e short rains

gures 4.9-4.11 represent the distribution of copper in the upper region. This portion, between amiti/Riuru Road Bridge to Kiroma has several factories. They are Kamwaki, Sasini, Kiroeri, itamaiyu, Kiroma, Mbo-Kamiti, Surwa, Ngurunga, Gatunda Katito, Muhunu and Migaa. The ttern of graphs in Figures 4.9 and 4.10 are similar. These samples were taken when coffee rvesting was active. Sampling sites with elevated levels correspond to locations close to the ctories, as is the case in Figures 4.9 and 4.10.

The pattern in Figure 4.11 does not appear similar with the other two above i.e. Figures 4.9 and 4.10 although sites with elevated levels agree in all the three. The shape of the graph indicates that there are many points where copper is introduced into the river by way of runoff water. Pulping was also minimal during that season

In all the cases, a sharp rise in the content of copper in the river is occasioned by the presence of a coffee-pulping factory. From the graphs, one can identify the source of the high copper level and estimate the number of factories. Therefore much of the copper comes from factories that discharge effluent directly into the rivers. The concentration down stream later falls to the baseline level after the effluent is discharged in the river. This could be due to a number of factors such as the pH. The later was found to be generally above the neutral value leading to precipitation of any soluble copper salt introduced contributing to the low concentration.

It was found that the copper levels in these sites were generally low. Most farms especially those hat are far away from the river, discharge their pulp waste into treatment ponds while others like Mboi-Kamiti were not actively pulping contributing to the observations made. However results obtained in this study show relatively lower contents of copper as compared to a similar study done by Hasan (2003) who investigated both sea and river water.

These results agree with studies made by Payne (1986) that Mauree River that passes through an ndustrial complex and areas of high agriculture activity had similar values. The concentration in his river may be said to be normal (WHO, 1990). Result of dilution effects of some several maller streams joining the Kamiti river that do not have factories discharging into them had no oticeable effects.

Results for both water samples and sediments are given in Tables 4.9 and 4.10 respectively.

Table 4.9 Average concentration of copper in the water sampled in all the three seasons

SEASON	Copper levels x10^{-2} (µg/ml)	
	KRB	TRB
May (After long rains)	0.98 ± 0.034	1.01 ± 0.006
August (The dry season)	0.83 ± 0.046	0.3 ± 0.006
November (The short rains)	0.81 ± 0.006	0.76 ± 0.004

Table 4.10 Average concentration of copper in the sediments sampled in all the three seasons

SEASON	Copper levels x10^{-2} (µg/ ml)	
	KRB	TRB
May (After long rains)	7.01 ± 1.01	10.31 ±. 958
August (The dry season)	3.04 ± 0.413	2.96 ± 0.088
November (The short rains)	0.06 ± 0.475	0.12 ±0.532

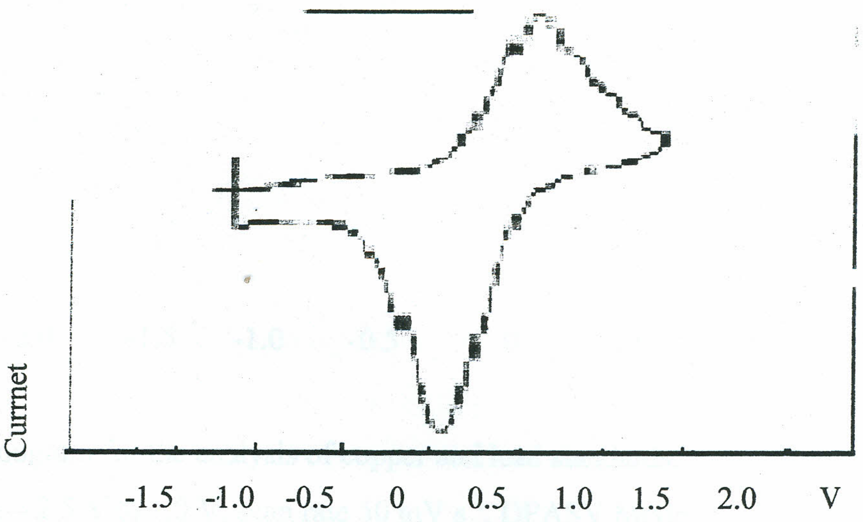
The average levels of copper were found to be low during the short rain season. This was attributed to the fact that runoff water was low and also the fact that there was no coffee harvesting in that period. As a result there was no migration of copper from the fields to the river and addition by effluent was low as well.

The results of total copper obtained in this study were subjected to ANOVA (one factor). Sampling was done in three different seasons. It was expected that the seasons would be a source of variation. ANOVA indicated there was indeed a significant difference in concentration between seasons based on F-test values obtained. The calculated value was 3.2390 as opposed to the critical value which was 3.0589. This confirms that there was significant difference as expected. During the rainy season there was a high concentration of copper compared to the dry season. High copper content during the rainy season may be attributed to surface runoff when fungicides from the field are swept to the river. It coincided with coffee picking season, which lead to high discharge of effluents to the river. During the dry season there was no surface runoff and the coffee processing was low hence less effluent discharges from factories. This can explain the low copper concentration reported in the water samples during the dry season.

Similar analysis was done to find out if there was a variation between the two regions where sampling was carried out. The calculated F value was 3.5332 as opposed to 4.0129 which is the critical value is low. This implies that sampling sites were not a source of variation. Similar results would not be expected for the case of sediments. This is because sediments at the bottom of the river are not as mobile as the water. Runoff water usually ferry soils from the farms, which lead to siltation but water flows down the river mixing homogeneously and carrying along the dissolved minerals. This was in agreement with what was obtained by the ANOVA tests. The F calculated value was greater than F critical as shown in Tables C1 to C4 in appendix C.

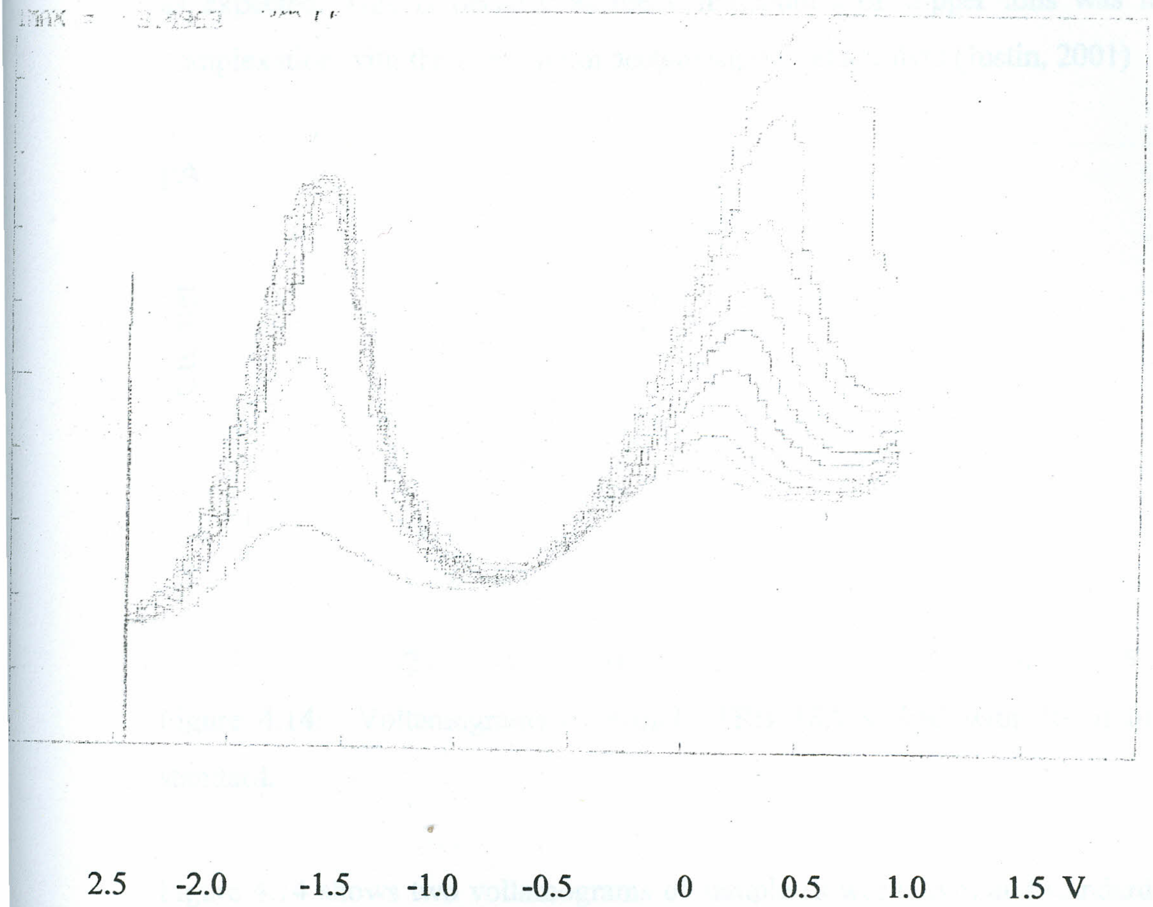
4.3 Labile fraction of copper

Before the labile fraction was determined, several voltamograms were obtained to optimize and calibrate the instrument. Test example runs were made and respective voltamograms acquired as shown Figure 4.12.



Figures 4.12 Cyclic Voltamogram (CV) for 50 μl of 0.05 M copper solution
Instrument settings –1.6 V to 2.0 V, scan rate 50 mV s^{-1} , DPASV (CV Mode)

yclic Voltammetry is a tool used for fundamental and diagnostic studies that provide qualitive formation about electrochemical processes and to validate the equipment. The difference tween cathodic and anodic peak potentials should be 0.591 to 0.684 for one electron transfer ard, 1987). In this study it was observed that reduction potential for the forward and reverse ins were 0.05 V and 0.72 V respectively. This gave a difference of 0.67 V, which is acceptable, l validate the instrument for analysis as with reference to manufacturers specifications inceton EG&G manual, 1988)



e 4.13 Voltamogram for the analysis of copper and lead standards.
ument settings—2.5 V to 1.0 V, scan rate 50 mV s^{-1}, DPASV Mode

ixture of lead with copper and a standard solution was introduced into the instrument and al voltamograms as shown in Figure 4.13 were obtained. The voltamograms indicate that the instrument was selective to various metals. The reduction potential of copper was 0.05 V while lead was reduced at –1.75 V versus silver –silver chloride electrode. The equipment was also found to be sensitive to differences in concentration. The concentration of lead was changed three times and the instrument responded to the change as shown in the Figure 4.13 at the reduction potential of –1.75 V. The concentration of copper was varied ten times by spiking 10 µ ml each time and the instrument responded to it as it appears at a reduction potential of 0.05 V. The instrument could therefore be used as an analytical tool to determine the concentration of copper. However it was noticed that there was shift in the reduction potentials to more positive as expected. This is because as the concentration of copper ions was increased, there was complexation with the ammonium acetate support electrolyte (Justin, 2001)

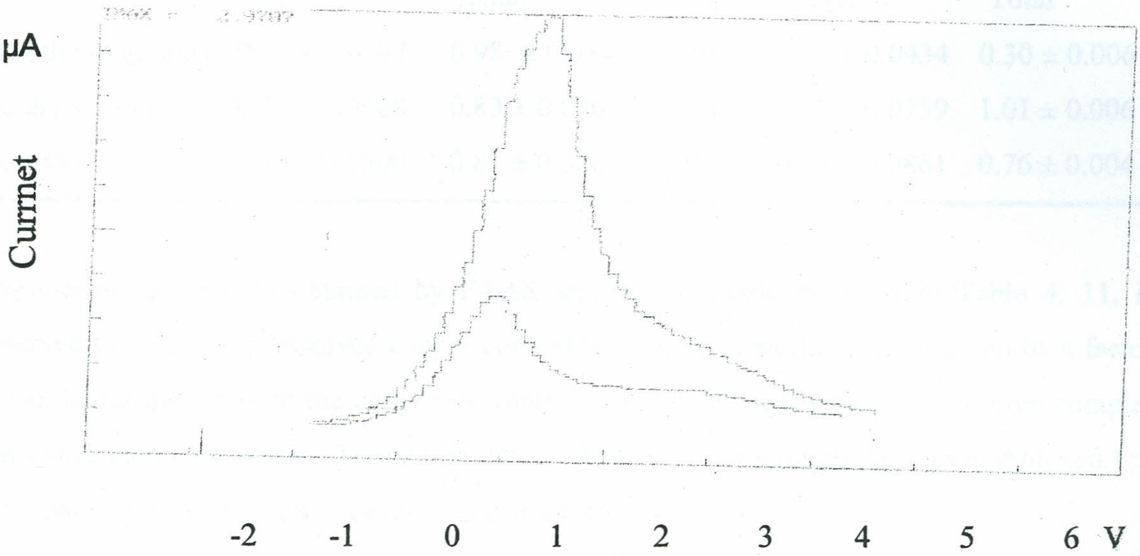


Figure 4.14: Voltamograms of sample TRB 18A spiked with 10 µl of 100µg/ ml copper standard.

Figure 4.14 shows two voltamograms of sample (lower curve) and standard plus sample (upper curve) demonstrating how the concentration of various samples was quantified (standard addition method) by getting the peak heights of sample and standard. The concentration of the unknown was determined by using equation 5 found in section 2.3.3.2. In this case, sample TRB 18 was found to contain 5.6 x10^{-3} µg/ ml of copper ions.

The determination of copper content was carried out using two different methods. FAAS was used to determine the total copper while voltammetry was used to determine the labile fraction. Average values of both the labile form and the total content of copper obtained for all the seasons were compared to establish the fraction of the labile form found in the water and are presented in Table 4.11 below.

Table 4.11 Composition of labile forms and total content of copper in water within the three seasons

SEASON	Content of copper (µg/ml x 10^{-2})					
	KRB			TRB		
	Labile	Total	%	Labile	Total	%
After the long rains	0.56 ± 0.0897	0.98 ± 0.034	57%	0.20 ± 0.0434	0.30 ± 0.006	33%
The dry season	0.30 ± 0.0288	0.83 ± 0.046	36%	0.47± 0.0759	1.01 ± 0.006	46%
Short rains	0.48 ± 0.0500	0.81 ± 0.006	59%	0.24 ± 0.0861	0.76 ± 0.004	31%

Considering the results obtained by FAAS and voltammetric methods in Table 4. 11, it is observed that the total dissolved copper content was higher than the labile fraction by a factor 2. It was found that 49% of the copper in solution was labile, that is free or in simple complexes that could easily dissociate. The other 51% could have been strongly held in complexed forms such that it was not available for reduction at the electrode.

The statistical analysis (one-way ANOVA) confirmed that a significant variation existed between the two methods. This is because the calculated F value of 15.2703 was very high as opposed to 3.0976 which is the critical value. The variation is due to the fact that voltammetric method measures the "free copper" and the copper complexed by simple ligands (the species which can be reduced at the electrode surface), while FAAS measures all forms of copper irrespective of their speciation.

4.4 Voltammetric determination of formation constants

Stability constant values were obtained by measurements of shifts in peak potential ΔE_p of the various voltamograms obtained. This was made possible with the help of the Ligane equation, equation 20 discussed in section 2.3.3.4.

Table 4.12 below gives the values of the stability constant of copper with the ligands studied by measurement of shift in peak potential and available literature values presented.

Table 4.12 Stability constant values obtained by voltammometric method.

Copper-Ligand	Ratio (Cu : L)	ΔE mV	Log β_1	Literature values
Proline	1:1	2.9	5.07	-
Glycine	1:1	5.10	8.27	8.22 (Martell, 1964)
Catechol	1:1	6.7	12.9	13.7 (Martell, 1964)
Catechol	1:2	13.2	24.5	23.65 (Martell, 1964)

The log β values obtained by this method compared very well with what are documented in literature (Martell, 1964).

Figure 4.15 below shows an example of a voltamogram acquired from Cu (II)-proline mixture among the many that were recorded. The mixture was made in 0.1M solution of potassium nitrate.

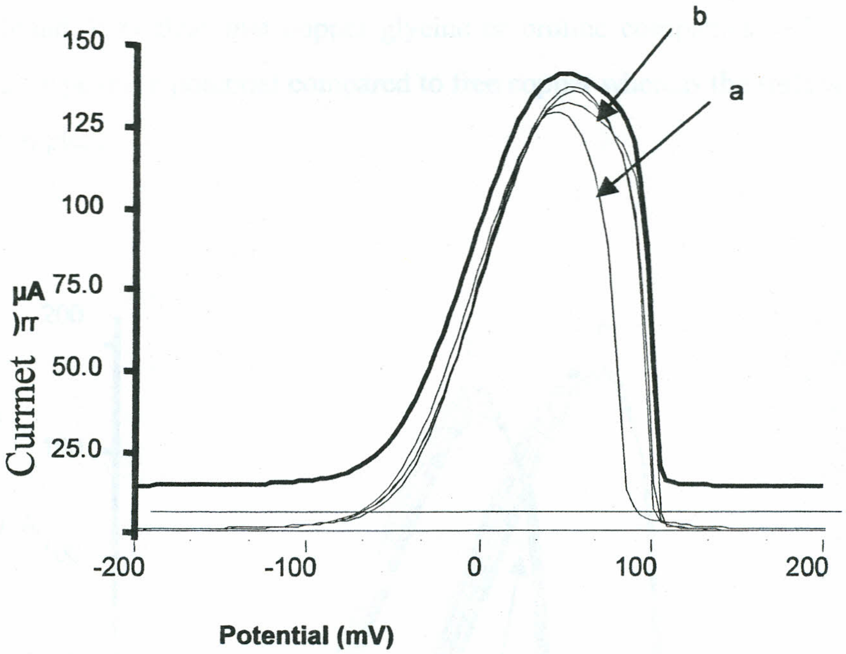


Figure 4.15: Voltammograms of copper-proline mixture at ratio Cu: proline 1:1

Where

a= Free copper
b= Copper :proline at ratio 1 : 1

Figure 4.16 show voltamograms of copper, and copper complex with ratio 1 : 2 and excess ligand. It is clear that copper glycine or proline complexes had their shift in peak potential to more positive potential compared to free copper whereas the shift was more negative for catechol complex.

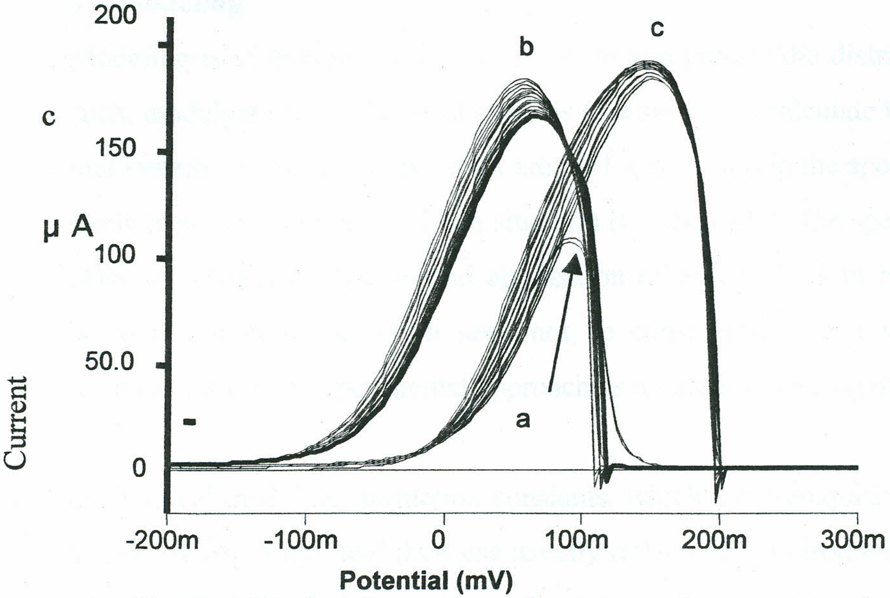


Figure 4.16 A voltamogram of copper, and copper complex with ratio 1 : 2 and excess ligand

Where

a= Free copper

b= Copper :catechol at ratio 1 : 2 followed by excess catechol

c= Copper: glycine at ratio 1:2 followed by excess glycine

A study by Justin (2001) revealed that copper glycine and proline complexes had their peak potential shift to a more positive than the free copper. Similar observations have been reported that the complexation of metal ions by even simple amino acids such as glycine or cysteine results in a more anodic peak. This was attributed to the transformation of a cupric glycate or cystinate complex. Other studies also observed a positive shift in peak potential, which they attributed to the formation of copper (I)-cystine complex (Bai *et al.,* 1998).

On the same voltamogram, Figure 4.16, it was observed that the potential did not shift further upon increasing the copper: ligand ratio beyond 1:2. This is because copper can only bind with twice its number of moles of the ligand (Nakamura *et al.,* 1990). The shift remained constant even when the Cu : L ratio was raised to 1:30.

4.5 Modeling

Modeling is to simulate the natural system and predict the distribution of chemical species. As such, modeling uses a chemical analysis of a system to calculate the distribution of the species in that system. It has found important areas of application in the speciation of chemical components likely to be present under a given situation (Cross, 1986). The species are identified and chemical behavior predicted. The second application of modeling is in the prediction of environmental behavior for systems, which need not be considered over a very long timescale or are too complex for direct experimental approach as it enables building of a realistic picture.

In chemical modeling, formation constants, which are prerequisite in modeling, are generated in laboratory conditions and their use usually reflects the environment with a great deal of accuracy (Cross, 1986). Stability constant values are a good measure of the stability of the complex species; it describes the competition of metal ions with protons for binding. The higher the stability constant value, the higher the possibility of complex formation.

Many formation constants reported in literature are at a temperature of 298.15 K. However, most reactions in the environment occur at a different temperature, and it is important to be able to estimate that stability constant value at the temperature of interest. For correction of equilibrium constants, this can be done using the Van Hoff equation 54 (Ure, 1997).

$$\ln K = \frac{-\Delta G}{RT} = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} \qquad (55)$$

where

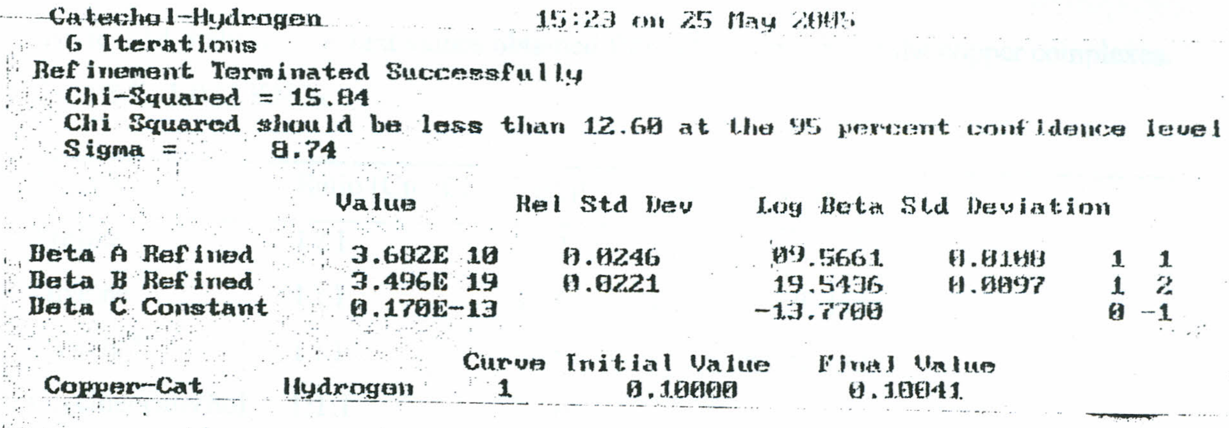
ΔH= standard enthalpy change

ΔS=standard entropy change

If it is assumed that ΔH and ΔS are independent of temperature then K can be calculated for the required temperature using equation 55 below. This assumption is valid for temperatures up to 398 K (Ure, 1997).

$$\ln K_1 - \ln K_2 = \frac{-\Delta H}{RT}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{56}$$

For the three ligands system studied, glycine, (catechol and proline), formation constants were calculated from potentiometric titration curves of HL_n in both the presence and absence of the metal ions at 25 ^{0} C (298 K) and ionic strength maintained with 0.1M KNO_3. Changes in pH were monitored using a glass microelectrode on a molspin pH meter. Protonation constant values for the ligands were calculated from the pH titration curves using a superquard program for each of the titration. An example of a refined calculation as it was performed is given Figure 4.17 below.



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 Catechol-Hydrogen                   15:23 on 25 May 2005
   6 Iterations
  Refinement Terminated Successfully
   Chi-Squared = 15.84
   Chi Squared should be less than 12.60 at the 95 percent confidence level
   Sigma =        8.74

                   Value        Rel Std Dev     Log Beta Std Deviation

 Beta A Refined     3.602E 10    0.0246          09.5661   0.0100    1  1
 Beta B Refined     3.496E 19    0.0221          19.5436   0.0097    1  2
 Beta C Constant    0.170E-13                   -13.7700             0 -1

                              Curve Initial Value   Final Value
  Copper-Cat      Hydrogen     1        0.10000      0.10041
```

Figure 4.17 Refined stability constant values for deprotonation of catechol.

A similar operation was done to the other titration curves and their respective formation (stability) values obtained. Stability constant values for the ligands alone and copper with the ligands studied were determined in a similar procedure and are shown in table 4.13 and 4.14

respectively. The values obtained in this study by potentiometric analysis for both ligands and complexes compared very well with literature values.

Table 4.13 Protonation constant values obtained from pH-titrimetry for the ligands studied. T=298 K, 1.0 M KNO_3

Ligand	Log β_1	Literature values
Proline	11.02	10. 68 (Martell, 1964)
Glycine	9.39	9.64 (Martell, 1964)
Catechol	9.58	-

Results in Table 4.13 indicate that for ligands such as glycine and proline the calculated protonation constants, compares well with literature values (Hogfeldt *et al*, 1971).

Table 4.14 Stability constant values obtained from pH-titrimetry for the copper complexes. T=298 K, 1.0 M KNO_3

Ligand	Ratio (Cu : L)	Log β_1	Literature values
Proline	1 : 1	5.02	-
Glycine	1 : 1	8.24	8.22 (Martell, 1964)
Catechol	1 : 1	12.52	13.7 (Martell, 1964)
Glycine/ catechol	1:1:1	26.79	-

Similarly, results in Table 4.14 indicate that Stability constants for the complexes studied such as copper glycine and catechol complexes provide stability constants, which are as close to values found in literature (Martell, 1964)

Figures 4.18-4.20 represents the species distribution as calculated using the stability constant values obtained in this work for the various single ligand or mixed ligand complexes of copper at T= 298 K and I= 1.0 M KNO_3.

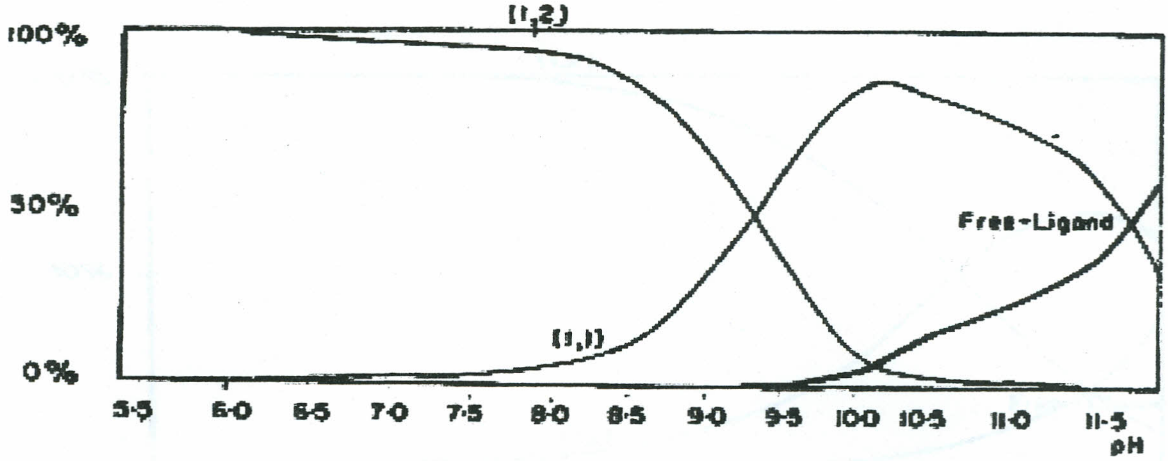

Figure 4.18 Speciation diagram for deprotonation of glycine.

The diagram in figure in Figure 4.18 represents the deprotonation of glycine. It was observed that glycine exists as diprotic ligand, H_2L, up to a pH of 8.0 after which it looses one proton due to the high concentration of OH^- ions and this will result it's complexation with any available Lewis acid like a metal ion. In the diagram (1,2) represent glycine (H_2NCH_2COOH), (1,1) glycate ion ($H_2NCH_2COO^-$).

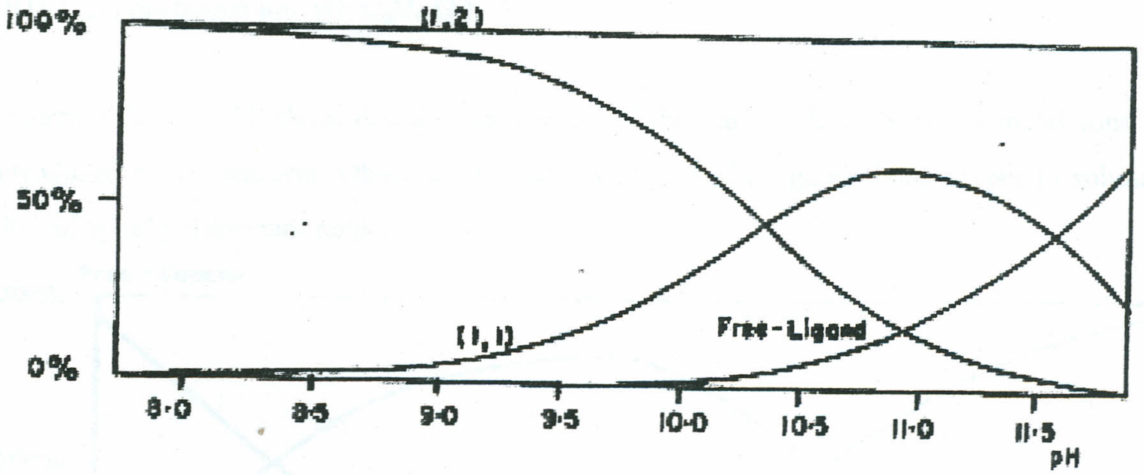

Figure 4.19: Speciation diagram for deprotonation of proline.

Proline, as shown in Figure 4.19, starts to deprotonate at pH 9, which is higher than the other two because the leaving groups are not favored unlike others since it is a secondary amine. In the diagram (1,2) represent proline (HNC_4H_7COOH), (1,1) prolate ion (($HNC_4H_7COO^-$).

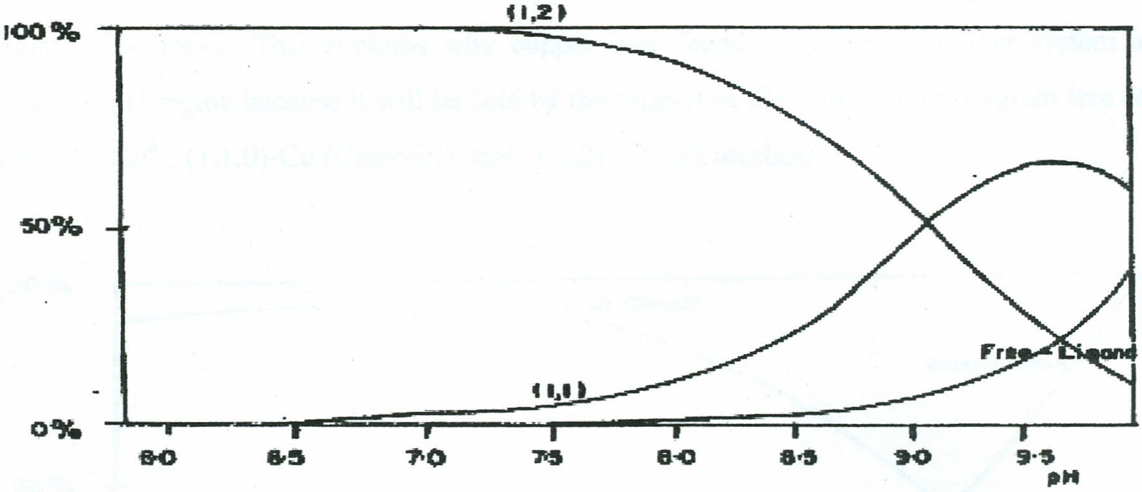

Figure 4.20: Speciation diagram for deprotonation of catechol.

For the case of catechol represented in Figure 4.20, the ligand exists as H_2L up to a pH of 7.5, which is lower than glycine. Catechol has an aromatic ring hence it has better leaving groups than glycine and therefore a better deprotonation. In the diagram (1,2) represent catechol (HO C_6H_4 OH), (1,1) phenolate ion (O^- C_6H_4 O^-).

Figures 4.21 to 4.23 show distribution curves of the various ligands when metal ions were introduced in the reaction. Observations made were that the ligands hold copper in solution at physiologically important ranges.

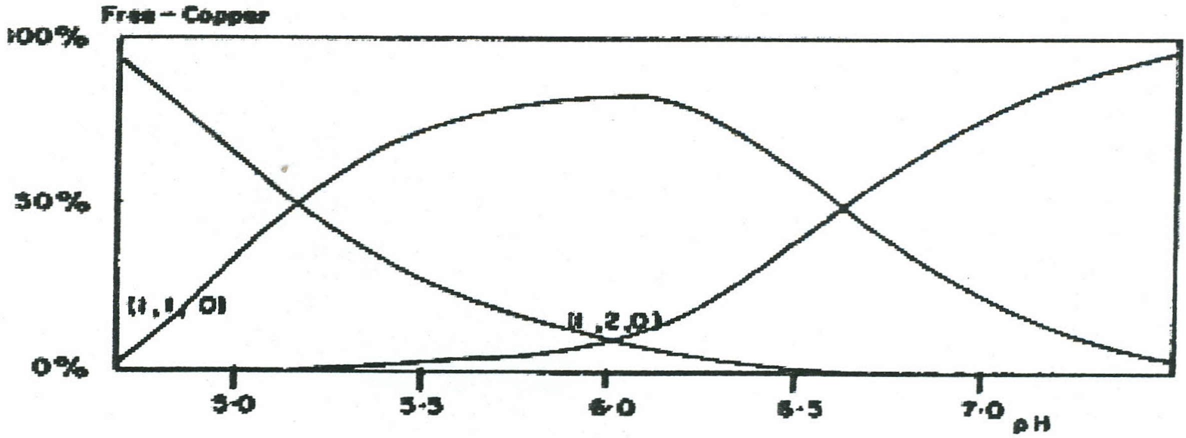

Figure 4.21: Speciation diagram for copper catechol mixture.

A mixture of copper: catechol formed complexes at a pH range of between 4.7-7.5 as shown in Figure 4.21 above. This explains why copper was found in solution in river system at the observed pH region because it will be held by the ligands in the water. In the diagram free copper represent Cu^{2+}, (1,1,0)-Cu (Catechol), and (1,2,0)- Cu 2(Catechol).

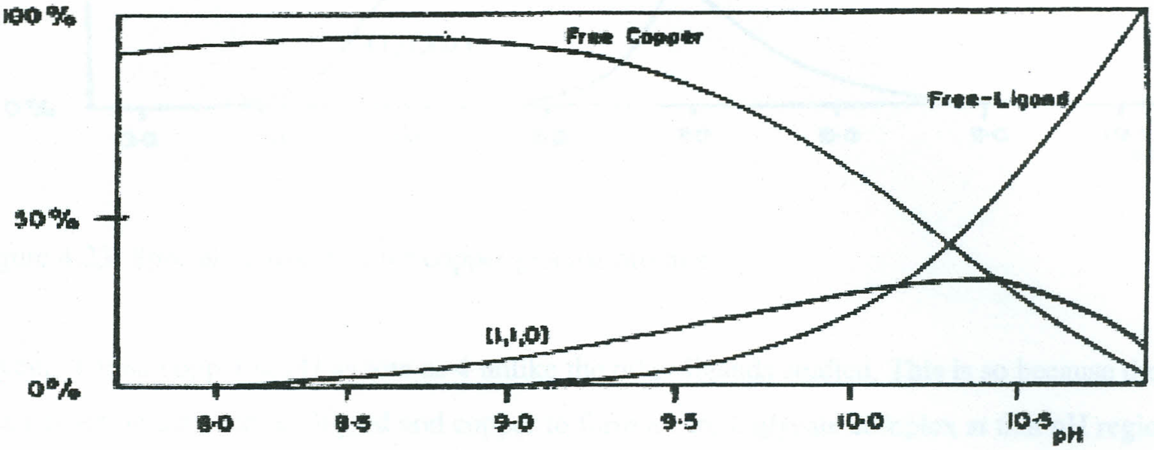

Figure 4.22 Speciation diagram for copper proline mixture.

Proline has a poor complexing ability with copper as shown in the speciation diagram 4.22. This is because proline does ionise fully as shown in Figure 4.19. The copper prolate complex will also be very lowbecause very litte proline will be available to bind it. In the diagram free copper represent Cu^{2+}, (1,1,0)-Cu (proline) complex, and (1,2,0)- Cu 2(proline) complex.

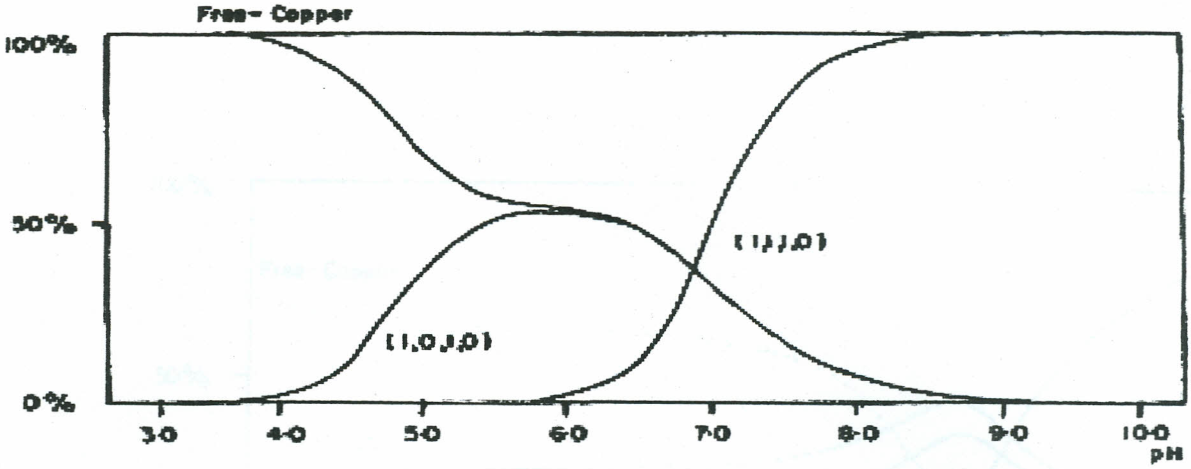

Figure 4.23: Speciation diagram for copper glycine mixture.

Glycine bound copper at pH as low as 4 unlike the other ligands studied. This is so because there was a reaction between the ligand and copper to form a cupric glycate complex at that pH region, which later is transformed to copper (I)- glycate complex (Bai *et al.,* 1998). In the diagram free copper represent Cu^{2+}, (1,1,0)-Cu (glycate) complex, and (1,2,0)-Cu 2(glycate) complex.

Figures 4.24 to 4.26 shows the distribution curves of copper and a mixture of at least two ligands.

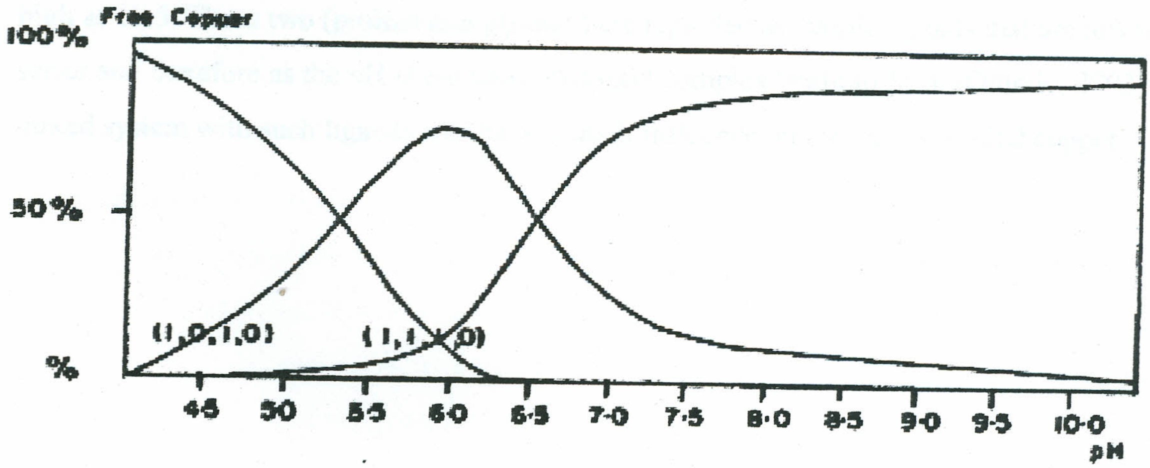

Figure 4.24: Speciation diagram for copper- proline-catechol mixture.- Cu^{2+} -Free copper, (1,0,1,0)-Cu:catechol, and (1,1,1,0)- Cu catechol:proloine.

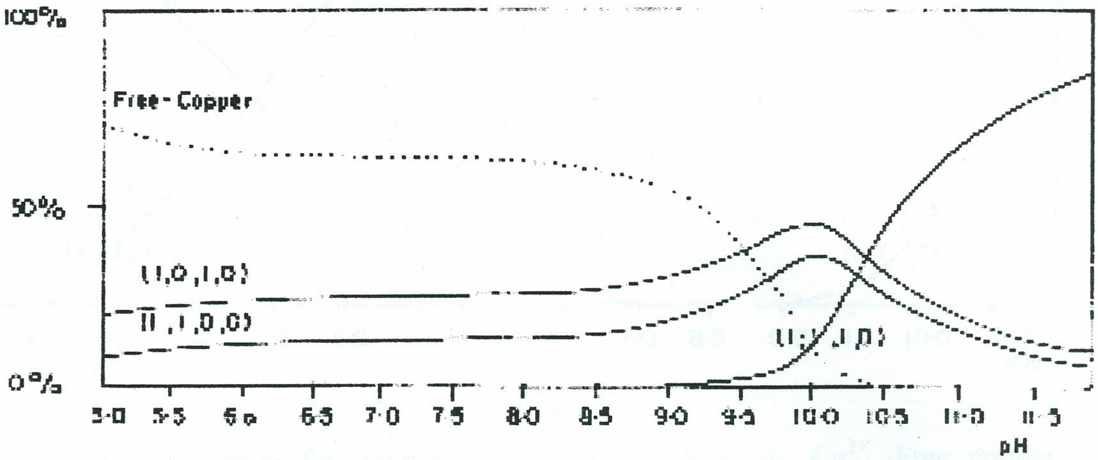

Figure 4.25 Speciation diagram for copper- proline-glycine mixture. Cu^{2+} -Free copper, (1,0,1,0)-Cu:proline, (1,1,0,0)- Cu:glycine., (1,1.1,0) Cu: glycine: proline

Figure 4.25 is a copper: proline: glycine system. The results show that these ligands had a low ability to bind copper at a wide pH range as copper free ions were found in solution at a pH as high as 10.5. These two (proline and glycine) are regarded as simple ligands that are low in the series and therefore as the pH is elevated, hydroxyl complex begin to form (Onindo, 2003). A mixed system with such ligands will have a small influence on the ability to bind copper.

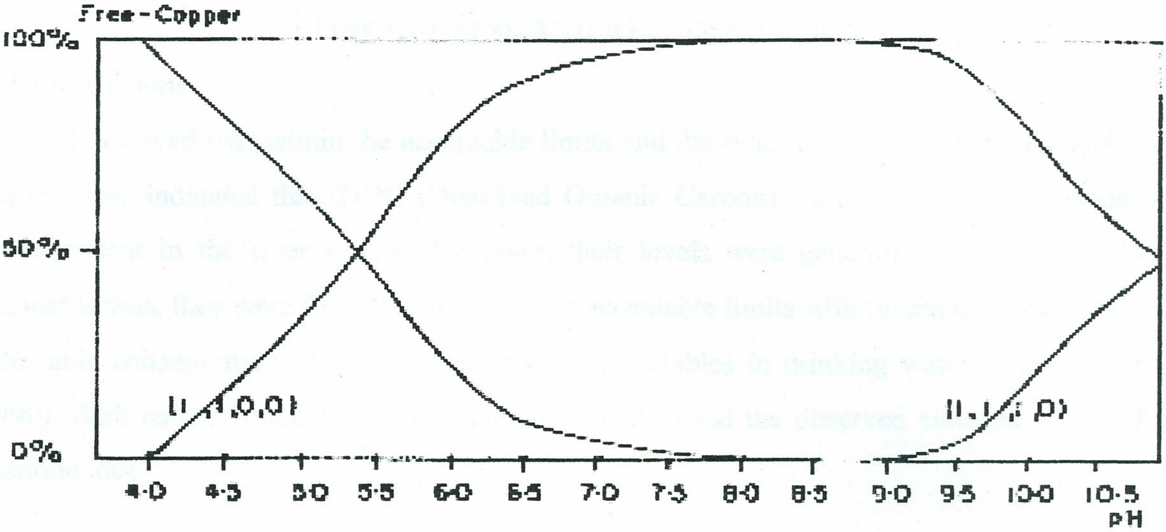

Figure 4.26: Speciation diagram for copper catechol-glycine Mixture. Cu^{2+} -Free copper, (1,1,1,0)-Cu: catechol, (1,1.1,0) Cu: catechol:glycine

A mixture of copper: catechol:glycine appeared to bind copper at a wider pH range (4.5-11.5) than a copper: catechol system in figure 4.21. This demonstrates that such a mixed system will have a high influence on the ability to bind copper due to the high stability constant value. On all situations where there is catechol as a ligand, irrespective of it being singly or mixed copper is bound at pH range that is safe for human consumption as shown in Figures 4.21, 4.24 and 4.26.

The distribution diagrams shown in Figures 4.21 to 4.26 give the proportion of given specie as a function of the total copper or ligand and pH. The results show the tendency of the ligands to form complexes with copper in the specified pH ranges. The presence of various complexes at different pH environment is as displayed in the speciation diagrams. From these results, it shows that catechol/copper exists, as the highest fraction at most pH ranges irrespective of wheather it is the sole ligand or mixed with another ligand. This could be explained by the high stability constant of copper-catechol complex. Proline-glycine does not bind the copper because they are relatively lower complexing agents as compared to catechol as they are only bi-dented whereas catechol is polydentate.

CHAPTER FIVE

CONCLUSIONS AND RECOMEDATIONS

5.1 Conclusions

The pH observed was within the acceptable limits and the other physico-chemical parameters of water tested indicated that, DOC (Dissolved Organic Carbon), chloride ions and fluoride ions were present in the river system. However, their levels were generally low based on WHO standards thus, they were found to be within the acceptable limits with reference to the maximum allowable concentration of selected water quality variables in drinking water (Maybeck *et al.*, 1990). Both natural sources and the fungicides contributed the observed elevated levels of the chloride ions.

Copper was found to be dissolved in Kamiti river water, however the average content of copper in the water was lower than the maximum contaminant level and so is not a threat to life except for a situation of possible accumulation in the body on prolonged use. Noticeably high levels were observed at points where the effluent was discharging directly to the river. The levels at those points were as high as 1.9×10^{-3} µg/ml which is enough to threaten the survival of fish and other lower life forms in the river.

The study shows that the copper in the water result mainly from effluents from the coffee pulping factories. There is nothing else to suggest another source of copper such as mining activity along this river. Therefore it can be concluded that coffee farming is the source of introduction of the vice into the river system by either factory effluents or run off water from the fields.

The content of the labile fraction of copper was found to be 49% of the total copper content. The analysis was carried at a pH of 5.5. Results from modeling speciation, refer to speciation diagrams found in Figures 4.21-4.26, show that at around the same pH value, the content of free copper and copper complexed with simple ligands closely agree.

Despite the fact that modeling was carried out in the laboratory where the system was at equilibrium and relatively higher ionic strength as opposed to the river system which was dynamic with multiple ligand system the results from the laboratory, Figure 4.21 give a likely scenario of the distribution of copper and the complexes in the natural waters.

Catechol formed stable copper complexes at a wide pH range when mixed with other ligands or alone as shown in the speciation diagrams 4.24 and 4.26. This reveals that its presence in natural waters may be able to bind ions such as copper to a safe level.

5.2 Recommendations and further work

Coffee pulp is usually a waste product from the factories. Studies (Kiptoo, 2001) have shown that the product cannot be used as an animal fodder because of the presence of catechins. This product has however been used as manure or mulching in farms. Considering the high value of the formation constant of copper and catechol, 26.79, a lot of copper is held by the pulp therefore there is a likelihood of re-introduction of copper in the fields. A study should be carried out to find out the quantity of copper that find it's way back into the fields as a result of this application.

Lower life forms such as fish are very vulnerable to the contents of copper observed in this study. A survey should be carried out to find out if their survival is threatened by introduction of the vice into their habitat.

Treatment ponds should be encouraged and a legistration be put in place such that no factory discharges effluent in the rivers directly but through the ponds to give ample time to have dissolved copper in the wastewaters equilibrate with catechins and be bound to safe complexes.

Results obtained in this research should be communicated to the general public by way of seminars and to relevant government organs so as to advice farmers for the necessary action to be taken.

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APPENDICES

Appendix A

Table A 1: Raw data table for the total parameters analysed.

Key

TRB-Thika Road bridge

KRB-Kamiti road bridge

AAS -Conc of total copper

pol Conc of labile copper

Chloride- Conc of Cl^-

Flouride- Conc of F^-

Sediments- Conc of total copper in sediments

SAMPLE	NUMBER	pol $x10^{-2}$	AAS $x10^{-2}$	DOC$x10^{-2}$	pH	Chloride	Flouride	sediments
KRB	1A	1.78	1.978799	9	7.87	15.46321	1.666802	0
KRB	1B	2.186857	2.54417	8.375	7.87	17.39465	1.309273	0
KRB	2A tr	1.78	26.60777	9.625	7.8	18.09066	2.357218	0
KRB	2B	1.43824	1.55477	10.25	7.8	9.285321	1.940173	0
KRB	3A	1.424	1.282	8.375	7.66	9.285321	2.223474	0
KRB	3B	1.674048	1.282	8.375	7.64	10.44511	1.888	0
KRB	4A	0.826839	0.8	9.625	7.64	10.86305	1.699573	0
KRB	4B	0.936148	0.75	7.75	7.66	12.2199	1.910186	0
KRB	5A	0.108348	0.282686	6.5	8.66	9.656852	2.138553	13.19159
KRB	5B	0.127143	0.2424	10.25	7.71	9.285321	1.477261	15.52804
KRB	6A	0.029667	0.141343	10.875	7.71	10.44511	2.258378	11.13551
KRB	6B	0.037474	0.141343	12.125	7.71	13.74623	2.232149	13.9865
KRB	7A	3.139273	0.282686	12.75	7.71	14.29626	2.001566	0
KRB	7B	0.219882	0.261	10.25	7.7	14.86829	2.375648	0
KRB	8A	0.127143	0.141343	8.375	7.71	12.70885	2.214832	0

KRB	8B	0.057419	0.706714	12.125	7.72	8.928083	2.197649	0
KRB	9A	0.130244	0.021	12.75	7.72	8.58459	2.403564	0
KRB	9B	0.158757	0.2582	15.25	7.58	10.86305	1.426384	0
KRB	10A	0.291273	0.565	15.25	7.59	12.70885	1.55397	0
KRB	10B	0.081344	0.565	11.5	7.44	11.74976	2.232149	12.21028
KRB	11A	0.108537	0.565	10.875	7.44	12.2199	1.460103	0.38785
KRB	11B	0.477561	0.424028	12.125	7.44	14.86829	1.910186	3.051402
KRB	12A	0.116548	0.989399	11.5	7.44	16.08194	1.086058	13.00467
KRB	12B	0.146456	0.424028	9.625	7.44	11.29771	2.064902	4.675
KRB	13A	0.044318	0.424028	10.875	7.44	10.86305	1.851595	3.004673
KRB	13B	0.039556	0.424028	6.5	7.44	11.29771	1.426384	3.892523
KRB	14A	0.15575	0.141343	8.375	7.73	12.2199	1.426384	5.808411
KRB	14B	0.167529	0.19894	14	7.52	18.09066	2.121962	6.667
KRB	15A	0.726053	0.848057	10.875	7.51	18.09066	1.83009	6.074766
KRB	15B**	0.716341	0.848057	10.875	7.49	14.86829	2.1055	17.53738
KRB	16A	0.170479	0.156537	7.75	7.83	13.21737	2.072958	0
KRB	16B	0.129612	0.156537	7.125	7.83	11.74976	1.415318	0
KRB	17A	0.0712	1.55477	15.25	7.67	8.58459	1.371907	0
KRB	17B	0.068323	1.696113	6.5	7.72	12.70885	1.962971	33.34217
KRB	18A	0.118667	0.848057	7.125	7.6	18.09066	1.962971	31.05607
KRB	18B	0.138444	0.989399	7.75	7.6	16.08194	1.83009	0
KRB	19A	0.141213	1.272085	24	7.76	11.29771	1.794802	0
RB	19B	0.1068	1.130742	10.25	7.76	14.29626	2.172125	0
RB	20A	0.162522	0.141343	7.75	7.88	13.21737	1.615677	0
RB	20B	0.139377	0.141343	7.125	7.88	10.44511	1.437536	0
RB	21A	0.89	0.141343	6.5	7.88	11.29771	2.412942	31.05607
RB	21B	0.837647	0.141343	6.5	7.88	14.29626	1.910186	20.48131
RB	22A	0.371974	0.424028	4	7.88	10.86305	2.172125	13.75234
RB	22B	0.2848	0.424028	6.5	7.88	11.74976	2.172125	0
RB	23A	0.130244	0.12	5.25	8	9.285321	1.902762	43.48598

KRB	23B	0.130244	0.12	5.875	8	8.928083	1.837231	21.74299
KRB	24A	0.118904	0.141343	5.875	7.37	10.44511	1.560033	24.3271
KRB	24B	0.090978	0.14	9	7.37	10.86305	1.483024	0
KRB	25A	2.797143	2.826855	7.75	7.37	10.04325	1.660324	0
KRB	25B	2.808444	2.826855	7.75	7.6	10.04325	1.666802	23.95327
KRB	26A	0.115822	0.565371	12.005	7.88	16.72542	2.320786	0.060748
KRB	26B	0.1068	0.565371	12.125	7.8	16.72542	1.85882	0
KRB	27A	1.909451	2.120141		7.78	10.04325	1.866072	0
KRB	27B	1.844493	2.120141		7.75	18.81452	2.172125	1.602804
KRB	28A	0.079111	0.565371		7.3	11.29771	1.49462	0
KRB	28B	0.04657	0.989399		7.37	16.72542	2.172125	0
KRB	29A	0.038883	0.16		7.37	12.2199	2.206224	13.00467
KRB	29B	0.10041	0.16		7.37	12.70885	2.26719	0
KRB	30A	0.125081	1.978799		7.6	11.74976	2.258378	0
		0.331864				10.04325	2.460384	0
Kiihu f.d.		0.230353	2.720848		7.7	12.70885	2.121962	0
Cave		0.012508	0.144		7.2	11.29771	2.403564	0
TRB	1A	0.091145	0.14	5.25	7.59	11.29771	1.955342	0
TRB	1B	0.013699	0.141343	6.5	7.61	12.2199	2.232149	0
TRB	2A	0.009828	0.141343	6.5	7.59	12.70885	2.403564	0
TRB	2B	0.013308	0.156	5.875	7.61	12.2199	1.506306	0
TRB	3A	0.01312	0.56	6.5	7.61	12.2199	1.512183	0
TRB	3B	0.012449	0.141343	2.125	7.65	7.337782	2.357218	0
TRB	4A	0.01513	0.1421	13.375	7.61	7.78881	0.87555	0.03271
TRB	4B	0.01513	0.141343	2.75	7.66	16.72542	0.327316	0.03271
TRB	5A	0.014571	0.156	4	7.66	15.46321	1.174024	0
TRB	5B	0.014035	0.141343	5.875	7.68	16.08194	1.220644	2.023364
TRB	6A	0.016144	0.141343	2.125	7.6	14.29626	0.683285	0
TRB	6B	0.013938	0.142	5.875	7.6	12.2199	1.032849	0

TRB	7A	0.015193	0.142	6.5	7.6	10.44511	1.491132	0
TRB	7B	0.015575	0.142	5.875	7.67	13.21737	0.569005	0
TRB	8A	0.016811	0.141343	5.875	7.68	12.70885	0.562396	0
TRB	8B	0.012373	0.144	7.125	7.68	10.44511	0.569005	15.66822
TRB	9A	0.022886	0.282686	9.625	7.85	10.44511	0.955089	0
TRB	9B	0.022375	0.241	14	7.62	10.04325	1.426384	0
TRB	10A	0.013098	0.141	5.875	8.16	10.44511	1.426384	0
TRB	10B	0.010986	0.141	3.375	8.05	11.29771	1.040519	10.6215
TRB	11A	0.012945	0.141	5.25	8.12	9.656852	1.028434	11.92991
TRB	11B	0.012838	0.141	3.375	8.08	9.656852	0.702166	12.91121
TRB	12A	0.046842	0.421	4	8.14	11.74976	1.443145	13.79907
TRB	12B	0.048887	0.424028	5.875	8.02	10.86305	0.925794	14.5
TRB	13A	1.157	1.130742	4.625	7.63	10.86305	0.624747	15.43458
TRB	13B	1.082432	1.130742	5.25	7.75	10.44511	0.943997	17.11682
TRB	14A	0.014669	0.141	5.875	7.57	10.04325	0.603231	50.52336
TRB	14B	0.013138	0.141	7.75	7.6	10.44511	0.925794	73.14019
TRB	15A	0.146524	1.696113	6.5	7.6	9.285321	0.429883	37.03738
TRB	15Btr	0.18597	1.696113	4.625	7.6	7.337782	1.178605	36.57009
TRB	16A	1.302439	1.696113	12.125	7.57	7.631388	1.060977	39.37383
TRB	16B	1.529999	1.71	9	7.24	8.928083	0.466515	72.20561
TRB	17A	0.111563	1.1307	6.5	7.25	9.656852	0.662327	59.86916
TRB	17B	0.113507	1.130742	5.875	7.56	12.70885	1.220644	10.29439
TRB	18A	0.561087	0.424028	6.5	7.56	11.74976	0.500388	34.04673
TRB	18B	0.436759	0.424028	6.5	7.16	8.928083	0.911485	3.658879
TRB	19A	0.254449	0.282686	8.375	7.16	10.44511	0.977667	3.658879
TRB	19B	0.050377	0.2827	5.875	7.27	9.285321	0.993015	2.756669
TRB	20A	0.025044	0.282714	7.75	7.27	8.58459	1.415318	2.556
TRB	20B	0.026612	0.282729	7.75	7.36	10.04325	1.851595	2.7896
TRB	21A	0.073151	0.565371	10.875	7.27	8.928083	1.851595	2.3337
TRB	21B	0.0712	0.565371	4	7.27	7.936741	1.615677	1.996

TRB	22A	0.097534	0.8	9	7.27	7.337782	1.160389	4.96729
TRB	22B	0.098889	0.767	9.625	8.16	8.928083	1.107412	9.172897
TRB	23A	0.052013	0.848057	6.5	8.45	8.928083	1.254374	0
TRB	23B	0.06411	0.84	6.5	8.12	8.58459	1.355973	0
TRB	24A	0.017327	0.424028	7.125	8.28	8.58459	0.272572	0
TRB	24B	0.13411	0.424028	4	8.14	12.2199	1.560033	0
TRB	25A	0.162743	0.989399	7.125	8.02	10.44511	1.49462	0
TRB	25B	0.154783	0.989399	6.5	7.90	16.08194	1.560033	0
TRB	26A	0.089	0.989399	2.75	8.32	8.58459	1.773957	32.92523
TRB	26B	0.065441	0.989399	9.625	7.45	12.70885	1.699573	4.96729
TRB	27A	0.237333	0.848057	9.625	7.37	10.44511	1.679834	0
TRB	27B	0.175303	0.848057	5.875	7.99	8.928083	0.943997	0
TRB	28A	0.167925	1.696113	4.4	7.6	9.285321	1.044579	0
TRB	28B	0.345259	1.696113	4.7	7.64	9.285321	1.142454	0
TRB	29A	1.71325	0.848057	4.05	7.4	8.254312	1.201778	0
TRB	29B	0.89	1.696113	0	7.48	9.285321	0.528425	7.397196
TRB	30A	0.116662	1.978799	3.0769	7.51	7.337782	1.274066	4.5
TRB	30B	0.11259	1.696113	6.1538	7.5	8.254312	1.443145	16.32243
		0.117952				10.04325	0.312372	1.976636
Munene f.d		2.120141		1.413428	7.6	7.631388	0.394588	0
		0.017332		15.3846	7.6	9.285321	0.783057	0
2TRB	1A	0.127758	0.14	5.25	7.8	8.254312	457875.6	0
2TRB	1B	0.731071	0.141343	5.25	7.8	17.39465	457875.6	0
2TRB	2A	0.785294	0.141343	5.25	7.8	17.39465	1.107412	0
2TRB	2B	0.121364	0.156	4	7.88	15.46321	0.883528	0
2TRB	3A	0.127143	0.56	4.625	7.8	15.46321	1.01649	0
2TRB	4A	0.051149	0.141343	4.625	7.8	14.86829	0.904414	0
2TRB	4B	0.052976	0.1421	5.25	7.8	14.29626	3.493091	0

2TRB	5A	0.038072	0.141343	5.25	8	13.74623	2.786897	0
2TRB	5B	0.055625	0.156	3.375	7.8	14.29626	0.230547	0
2TRB	6A	0.07513	0.141343	3.375	7.8	13.74623	0.238771	0
2TRB	6B	0.058976	0.141343	4.625	7.8	13.21737	0.306349	0
2TRB	7A	0.536871	0.142	4.625	7.8	14.86829	0.367877	0
2TRB	7B	0.535816	0.142	4.625	7.8	15.46321	0.429883	0
2TRB	8A	0.269697	0.142	4.625	7.8	26.78189	1.815893	0
2TRB	8B	0.395556	0.141343	4.625	7.8	26.78189	2.249601	0
2TRB	9A	0.404545	0.144	4.625	7.9	16.72542	0.353827	0
2TRB	9B	1.615185	0.282686	7.125	7.8	21.16455	0.135228	0
2TRB	10A	1.992826	0.241	7.125	7.8	10.04325	0.143362	0.014019
2TRB	10B	1.854167	0.141	7.125	7.8	7.337782	2.214832	0.481308
2TRB	11A	2.879412	0.141	7.125	7.8	10.04325	2.394223	0.294393
2TRB	11B	3.087188	0.141	4	7.8	10.04325	2.690916	0.014019
2TRB	12A	0.378582	0.141	4	7.8	9.656852	2.776066	0.014019
2TRB	12B	0.378582	0.421	5.25	7.8	8.928083	2.743823	0
2TRB	13A	0.520566	0.424028	5.875	7.8	13.74623	3.071855	0
2TRB	13B	0.43511	1.130742	4.625	7.9	15.46321	2.977633	0
2TRB	14A	0.509727	1.130742	4	7.9	17.39465	3.012622	0
2TRB	14B	0.485455	0.141	5.25	7.95	22.89215	0.313591	0
2TRB	15A	0.278125	0.141	5.25	7.95	14.86829	0.283396	0
2TRB	15B	0.068462	1.696113	3.375	7.9	16.72542	0.886975	0
2TRB	16A	1.970714	1.696113	3.375	7.9	13.21737	3.294899	0
2TRB	16B	1.854167	1.696113	4.625	7.9	13.21737	3.269337	0
2TRB	17A	0.139063	1.71	4	7.9	11.29771	3.359681	0
2TRB	17B	0.178	1.1307	5.25	7.9	10.86305	1.169461	0
2TRB	18A	0.09634	1.130742	5.875	7.9	16.72542	2.284916	1.556075
2TRB	18B	0.087165	0.424028	5.875	7.9	13.74623	2.293831	0.901869
2TRB	19A	0.092525	0.424028	4.625	7.9	14.86829	1.962971	0.481308
2TRB	19B	0.114958	0.282686	8.375	7.9	10.44511	1.31951	0.38785

2TRB	20A	0.089	0.2827	9	7.9	13.21737	0.817328	0
2TRB	20B	0.085404	0.282714	7.75	7.9	11.29771	0.605585	0
2TRB	21A	0.212386	0.282729	6.5	7.9	17.39465	0.571225	0
2TRB	21B	0.176859	0.565371	5.875	7.9	16.72542	3.243973	0
2TRB	22A tr	0.124817	0.565371	5.25	7.9	18.09066	3.25663	0
2TRB	22B	0.131218	0.8	7.75	7.9	16.72542	0.367877	0
2TRB	23A	0.15026	0.767	8.375	7.9	19.56734	0.294649	0
2TRB	23B	0.148333	0.848057	5.875	7.74	20.35028	0.532556	0
2TRB	24A	0.258387	0.84	6.5	7.74	25.7515	0.471997	0
2TRB	24B	0.25121	0.424028	2.75	7.74	20.35028	0.56021	0
2TRB	25A	0.392647	0.424028	4	7.76	11.29771	0.627185	0
2TRB	25B	0.483696	0.989399	4.625	7.76	11.29771	1.706205	0
2TRB	26A	0.490408	0.989399	4	7.77	16.72542	2.329841	0
2TRB	26B	0.320088	0.989399	5.25	7.72	18.09066	3.132251	0
2TRB	27A	0.272742	0.989399	5.25	7.70	23.80812		0
2TRB	27B	0.292031	0.848057	6.5	7.66	11.97865	1.506306	0.005674
2TRB	28A	0.462451	0.848057	5.25	7.8	10.44511	1.340224	0.38785
2TRB	28B	0.309911	1.696113	5.25	7.8	10.44511	1.304185	0.098786
2TRB	29A	0.322843	1.696113	5.25	7.9	10.04325	1.590705	0.098786
2TRB	29B	0.39663	0	4.625	7.76	10.86305	1.529952	0.098712
2TRB	30A	0.478375	0.848057	3.375	7.72	10.04325	1.443145	0
2TRB	30B	0.33375	1.696113	3.761	7.34	10.44511	1.488811	0
Munene		0.422179	1.978799	6.662	7.71	10.04325	1.64104	0.481308
2KRB	1A	0.124186	0.424	3.21	7.24	11.29771	1.388028	0.07674
2KRB	1B	0.12642	1.848057	3.32	7.21	10.04325	1.382633	0.06754
2KRB	2A	0.121364	1.848057	0	7.15	10.04325	1.304185	4.96729
2KRB	2B	0.056636	0.684806	3.0769	7.71	10.86305	1.471519	4.96729
2KRB	3A	0.056636	0.713074	6.1538	7.72	10.04325	1.426384	9.172897
2KRB	3B	0.454468	0.582	9.2307	7.45	10.44511	1.55397	9.172897

2KRB	4A	0.454468	0.6282	1.23076	7.44	13.21737	1.371907	8.7862
2KRB	4B	0.323636	0.411307	1.53846	7.45	10.04325	1.382633	8.7862
2KRB	5A	0.323636	0.384205	9.04	7.43	11.29771	1.4658	9.6985
2KRB	5B	0.197778	0.141343	10.25	7.8	10.04325	1.335015	8.342
2KRB	6A	0.139063	0.141343	9	7.8	9.656852	1.294067	6.57942
2KRB	6B	0.408525	0.40141	7.125	7.8	7.337782	1.518083	0
2KRB	7A	0.479231	0.40142	7.75	7.8	9.285321	1.596912	0
2KRB	7B	0.224945	0.265371	5.25	7.8	10.86305	1.590705	0
2KRB	8A	0.227557	0.240283	6.5	7.58	10.86305	1.355973	0
2KRB	8B	0.144759	0.170671	10.875	7.58	9.656852	1.512183	0
2KRB	9A	0.143971	0.170671	10.25	7.6	10.04325	1.366575	0
2KRB	9B	0.087255	0.424028	4.625	7.47	7.936741	1.4658	0
2KRB	10A	0.089	0.424028	5.25	7.47	10.04325	1.304185	0.07564
2KRB	10B	1.204118	1.413428	5.875	7.47	8.928083	1.590705	0.0564
2KRB	11A	1.227586	1.413428	6.5	7.47	9.285321	1.49462	1.0076
2KRB	11B	0.924231	1.424028	5.25	7.47	8.928083	1.603142	0.9876
2KRB	12A	0.900854	1.424028	5.25	7.47	8.928083	1.653871	2.356
2KRB	12B	0.169524	2.402827	12.75	7.47	10.04325	2.499008	2.78563
2KRB	13A	0.347317	1.978799	12.125	7.9	10.04325	1.932632	3.67543
2KRB	13B	0.178	2.424028	12.125	7.48	4.765839	2.450822	9.8765
2KRB	14A	0.148333	2.240283	10.875	7.48	7.936741	3.333616	11.3452
2KRB	14B	0.11926	0.141343	10.875	7.48	8.58459	0.089495	8.9
2KRB	15A	0.131788	0.141343	10.875	7.48	10.04325	3.32066	7.785
2KRB	15B	0.364754	0.282686	12.75	7.48	9.285321	3.617684	8.97654
2KRB	16A	0.364754	0.282686	12.75	7.48	12.2199	3.688812	7.77543
2KRB	16B	0.042788	0.141343	11.5	8.02	10.86305	3.835292	0
2KRB	17A	0.235588	0.141343	12.125	8.02	11.29771	2.412942	0
2KRB	17B	0.560934	0.706714	12.75	8.09	11.74976	2.863909	0
2KRB	18A	0.564103	0.424028	12.75	8.09	11.29771	3.674475	0
2KRB	18B	0.549706	0.565371	6.5	7.49	11.29771	2.412942	0

2KRB	19A	0.445	0.565371	5.875	7.5	14.86829	0.56021	0
2KRB	19B	0.084494	0.565371	5.25	7.58	14.86829	0.598551	0
2KRB	20A	0.109877	0.565371	4	7.62	13.74623	0.434934	0
2KRB	20B	0.079307	0.565371	4	7.62	13.74623	0.622319	0
2KRB	21A	0.091753	0.282686	5.25	7.38	7.337782	3.282093	0
2KRB	21B	0.162294	0.141343	9.625	7.67	7.936741	0.186825	0
2KRB	22A	0.157059	0.141343	10.875	7.67	8.58459	0.210796	0
2KRB	22B	0.231771	0.282686	8.375	7.35	7.936741	0.555864	0
2KRB	23A	0.233625	0.565371	7.75	7.37	9.656852	1.024437	0
2KRB	23B	0.21863	0.282686	9	7.35	8.254312	3.452521	0
2KRB	24A	0.228304	0.282686	9	7.35	9.656852	2.743823	0
2KRB	24B	0.411241	0.565371	8.375	7.35	10.04325	0.438335	0
2KRB	25A	0.422373	0.565371	8.375	7.35	9.285321	0.373652	0
2KRB	25B	0.044011	0.565371	6.5	7.41	8.58459	0.231447	0
2KRB	26A	0.044011	0.506714	6.5	7.41	8.254312	0.174178	0
2KRB	26B	0.472469	0.424028	6.5	7.18	8.58459	0.394588	0
2KRB	27A	0.374365	0.424028	6.5	7.18	11.74976	0.492654	0
2KRB	27B	0.280411	0.282686	12.75	7.41	12.70885	457875.6	0
2KRB	28A	0.163678	0.282686	12.75	7.41	14.860	457875.6	0
2KRB	28B	0.305522	0.282686	6.5	7.41	15.77	457875.6	0
2KRB	29A	0.216795	0.282686	5.25	7.41	15.4721	1.371907	0
2KRB	29B/	0.206105	2.826855	3.375	7.41	16.72542	457875.6	0
	Cave	0.201421		3.375	7.46	16.72542	0.759039	0

3TRB	1	1.489038	0.424028	4	7.87	15.46321	0.759039	0
3TRB	2	3.745417	0.424028	4	7.89	18.09066	457875.6	0
3TRB	3	1.820455	0.424028	5.875	7.15	18.09066	457875.6	0
3TRB	4	1.335	0.424028	6.5	7.1	18.09066	457875.6	0
3TRB	5	1.367963	0.424028	5.25	7.45	18.09066	457875.6	0

3TRB	6	1.287321	0.424028	4.625	7.45	7.337782	457875.6	0
3TRB	7	1.403462	0.1413	12.75	7.23	10.44511	457875.6	0
3TRB	8	1.288966	0.141343	12.75	7.23	14.86829	457875.6	0
3TRB	9	2.67	0.565371	7.75	7.45	11.29771	457875.6	0
3TRB	10	1.613125	0.565371	6.5	7.45	11.29771	457875.6	0
3TRB	11	1.631667	0.565371	5.875	7.45	11.74976	457875.6	11.05433
3TRB	12	1.275667	0.565371	5.25	7.45	11.29771	457875.6	10.00764
3TRB	13	1.820455	0.565371	7.75	7.45	10.44511	457875.6	13.7864
3TRB	14	1.335	0.565371	8.375	7.45	7.337782	457875.6	12.89675
3TRB	15	1.335	0.565371	5.875	7.44	9.656852	457875.6	16.98762
3TRB	16	1.367963	0.424028	6.5	7.41	11.29771	457875.6	17.6743
3TRB	17	1.367963	0.282686	2.75	7.5	13.74623	457875.6	17.6743
3TRB	18	1.287321	0.565371	4	7.45	26.78189	457875.6	17.6743
3TRB	19	0.974189	0.565371	4.625	7.43	22.89215	457875.6	15.9876
3TRB	20	1.275667	0.565371	4	7.4	16.08194	457875.6	0
3TRB	21	0.038696	0.424028	5.25	7.38	18.09066	457875.6	0
3TRB	22	0.41474	0.424028	5.25	7.34	14.86829	457875.6	0
3TRB	23	0.029116	0.424028	6.5	7.33	13.74623	457875.6	0
3TRB	24	0.035847	0.424028	10.25	7.33	17.39465	457875.6	0
3TRB	25	0.0356	0.424028	9	7.33	16.08194	457875.6	0
3TRB	26	0.038696	0.424028	7.125	7.33	13.21737	457875.6	0.056479
3TRB	27	0.038696	0.424028	7.75	7.33	14.29626	457875.6	0.9546
3TRB	28	0.824878	0.282686	5.25	7.38	17.39465	457875.6	0.0177
3TRB	29	1.186667	0.282686	6.5	7.76	16.72542	457875.6	16587

3KRB	1	0.147251	1.413428	6.5	7.19	14.29626	457875.6	1.566822
3KRB	2	0.091405	1.413428	5.25	7.3	11.74976	457875.6	0.045327
3KRB	3	0.17686	1.413428	5.25	7.4	11.29771	457875.6	0.05
3KRB	4	1.350893	0.141343	12.75	7.4	11.29771	457875.6	0.059346
3KRB	5	0.658229	0.141343	12.125	7.33	10.44511	457875.6	0.068692
3KRB	6	0.161818	0.141343	12.125	7.16	10.86305	457875.6	0.064019
3KRB	7	0.549706	0.141343	10.875	7.21	10.04325	457875.6	0.059346
3KRB	8	0.979	0.282686	10.875	7.34	8.928083	457875.6	0.91729
3KRB	9	0.091405	0.141343	10.875	7.81	10.44511	457875.6	0.931308
3KRB	10	0.476786	0.565371	12.75	7.88	10.04325	457875.6	1.557477
3KRB	11	0.193012	0.565371	9	7.6	9.285321	457875.6	0.059346
3KRB	12	0.41431	0.848057	9	7.65	11.29771	457875.6	0.064019
3KRB	13	0.393083	0.848057	8.375	7.7	11.74976	457875.6	0.059346
3KRB	14	0.40123	0.706714	8.375	7.67	10.44511	457875.6	0.068692
3KRB	15	0.347656	0.565371	6.5	7.9	11.29771	457875.6	0.068692
3KRB	16	0.361563	0.424028	6.5	8	15.46321	457875.6	0.059346
3KRB	17	0.33375	0.989399	6.5	8.02	16.72542	457875.6	0.059346
3KRB	18	0.181818	0.848057	6.5	8.01	8.58459	457875.6	0.068692
3KRB	19	0.617647	0.282686	12.75	8.41	7.837782	1.584523	0.059346
3KRB	20	1.1	0.282686	12.75	8.41	7.337782	1.584523	0.068692
3KRB	21	0.102703	0.5322	6.5	8.41	12.70885	457875.6	0.931308
3KRB	22	0.535714	0.5673	5.25	8.41	12.70885	1.780878	0.833178
3KRB	23	0.216867	0.316	3.375	8.41	12.70885	457875.6	0.973364
3KRB	24	0.465517	0.5103	3.375	8.41	13.21737	1.584523	0.078037
3KRB	25	0.441667	0.51683	4	7.45	12.2199	457875.6	0.059346
3KRB	26	0.45082	0.5168	4	7.23	12.70885	2.302781	0.073364
3KRB	27	0.390625	0.424	5.875	7.11	12.2199	457875.6	0.068692
3KRB	28	0.40625	0.424	6.5	7.5	11.74976	0.759039	0.05
3KRB	29	0.375	0.4238	5.25	7.43	11.29771	457875.6	0.078037

Appendix B

Table B1 Maximum allowable concentration of selected water quality variables in drinking water (Maybeck *et al* 1990)

Variable	WHO a	EC b
Turbidity(NTU)	5	4
pH	6.5-8.5	6.5-8.5
Nitrate as N μg/ml	10	
Nitrate μg/ml		50
Nitrite μg/ml		0.1
Phosphorus μg/ml		5.0
Sodium μg/ml	200	150-175
Chloride μg/ml	250	25
Sulphate μg/ml	400	25
Fluoride μg/ml	1.5	1.5-1.7
Aluminium μg/ml	0.2	0.2
Cadmium μg/ml	0.005	0.005
Copper μg/ml	1.0	0.1
Lead μg/ml	0.05	0.05
Manganese μg/ml	0.1	0.05
Dissolved Organic Carbon(DOC) μg/ml	400	

a World Health Organisation (WHO)

b European Community (EC)

NTU Nephelometric turbidity units

Appendix C

Statistical formulae

Mean

The mean level of each substance for each sample was calculated in duplicate. This was done using equation 66 given below

$$\overline{X = \sum_{i=1}^{i=n} \frac{(x_i - \mu)}{n}} \tag{57}$$

Where

x = mean

n = data size

i = i^{th} term

Standard deviation, s

This was used to measure dispersion of values about the mean (equation 51).

$$\overline{s = \sum_{i=1}^{i=n} \frac{(x-\mu)^2}{n-1}} \tag{58}$$

Correlation coefficient, r

The correlation coefficient was used to test linearlity within the correlation ranges used for the standard in the preparation of calibration curves equation 52.

$$r = \frac{\sum_{i=1}^{i=n}(X_i - \bar{X}\,X)^2(Y_i - \bar{Y})^2}{\sqrt{\sum_{i=1}^{i=n}(X - \bar{X})^2(Y - \bar{Y})}} \tag{59}$$

Where X and Y represent two parameters or two sets of data being correlated and n is the number of the values. The degree of correlation is treated as fair for 0.90 < r > 0.95, good for 0.95 < r > 0.99 and it is excellent for all values of r > 0.99 (Miller 1988).

Table C 1 Analysis of variation Water samples

ANOVA: Single Factor

(Water)

SUMMARY

Groups	Count	Sum	Average	Variance
S1	59	0.703124	0.011917	0.001186
S2	59	0.377471	0.006398	3.51E-05
S3	29	0.15689	0.00541	1.44E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001226	2	0.000613	1.239023	0.292736	3.058928
Within Groups	0.071224	144	0.000495			
Total	0.072449	146				

Where S1= after the long rain

S2= during the dry season

S3= During short rains

Table C 2 Analysis of variation between instruments on water

Anova: Single

Factor (Sediment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S1	30	88.81308411	2.960436	27.77707846
S2	31	534.3831776	17.23817	488.0981854
S3	32	4.144859813	0.129527	0.10964084

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F*
Between Groups	5243.454	2	2621.727	15.27033933	1.94939E-06	
Within Groups	15451.88	90	171.6876			
Total	20695.33	92				

Where

S1= after the long rain

S2= during the dry season

S3= During short rains

Table C 3 Analysis of variation between Regions

ANOVA: Single Factor

(Regions)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
K1	29	224.5234	7.742184982	160.7256577
K2	29	82.22897	2.835481792	47.48926977

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F*
Between Groups	349.0981749	1	349.0981749	3.353248292	0.072394	4.0
Within Groups	5830.017969	56	104.1074637			

Where

K1=lower region

K2= upper region

Table C 4 Analysis of variation between seasons on sediments

ANOVA: Single
Factor (Sediment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S1	30	88.81308411	2.960436	27.77707846
S2	31	534.3831776	17.23817	488.0981854
S3	32	4.144859813	0.129527	0.10964084

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F*
Between Groups	5243.454	2	2621.727	15.27033933	1.94939E-06	
Within Groups	15451.88	90	171.6876			
Total	20695.33	92				

Where

S1= after the long rain

S2= During the dry season

S3= During short rains