DETERMINATION OF TRACE ELEMENTS IN HAIR FROM RESIDENTS OF NAKURU DISTRICT USING ENERGY DISPERSIVE X-RAY FLUORESCENCE ANALYSIS.

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A Thesis submitted in partial fulfilment of the requirement for the award of the degree of Masters of Science in the Department of Physics Egerton University-Njoro.

DECLARATION

This thesis is my original work and has not been submitted for the award of a degree in any other University .

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This Thesis has been submitted with our approval as the University supervisors.

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Abstract

A method of Energy dispersive X-ray Fluorescence analysis of hair samples for trace elements by acid digestion and heavy metal co-precipitation with with sodium diethyl-dithiocarbomate (NaDDTc) was applied for the analysis of trace elements in hair. Detection Limits of the method for most elements was in the range 0.4 - 3.5 µg/g. The RSDs (relative standard deviations) of the concentration values for most element of analytical interest was 15% or better.

Data for trace element concentrations in hair has been analysed and compared with those from different sampling locations and it appears that most of the essential trace elements i.e., Mn, Co, Ni and Se are constant in all the donor groups and their mean concentrations are 20.2, 2.7, 7.1 and 1.3 µg/g respectively. The essential elements Fe and Cu on the other hand show marked variability akin to that displayed by the non essential trace elements As, Hg and Pb.

Variation of the concentrations of the Elements Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Hg, Se and Pb with age and Sex was investigated. It was found that Cr, Mn, Co, Zn and Se did not vary appreciably with donor Age. The concentrations of elements Fe, Cu, Pb and Hg increased with donor age while Ni decreased with Age. No significant

difference was observed between the elemental concentrations for Male and Female donors at the 95% confidence level.

The correlation of each pair of elements in the hair samples for the whole population was investigated and revealed that Cu was positively correlated ($p < 0.001$) with As, Hg and Pb and was negatively correlated with Se. Mercury and Lead were found to be highly correlated.

Principal component Analysis of all the sample population considered revealed that Cu, Pb, Hg and As are highly loaded in component 1 which accounted for 43.63% of the Total variance, this component was attributed to anthropogenic sources. The elements Ni and Fe were found to be highly loaded in component 2 which accounted for 22.14% of the total variance.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

During the past few years, human hair has been recognised as an invaluable tissue for monitoring human environmental exposure. It provides good indications of exposure to many toxic metals over periods of time upto a year or more, and might as well be used in epidemiological studies related to environmental pollution (Corridan, 1978). The sequential accumulation of a number of elements, and in particular the fact that the concentrations of trace elements in hair are at least an order of magnitude higher than in body fluids and other accessible tissue, as well as the simple and easy preparation of the specimen reveal some of the reasons of undertaking this study. Scalp hair is a protein tissue with a very low metabolic activity and is readily available from different population groups (Eltayeb and Van Grieken, 1989). Moreover, hair can easily be sampled and conveniently stored for analysis. These advantages have made trace element analyses of hair attract interest in many disciplines including environmental, forensic and medical sciences (Valkovic, 1977).

Most studies on trace element analyses of hair are confined mainly to Caucasian or Asiatic populations and very few studies exist on African or Negroid populations. In this work preliminary data on hair samples from Kenyan populations, mainly Negroid in origin is presented.

Various analytical techniques have been used for the analysis of trace elements in human hair. Some of these include., Instrumental Neutron Activation Analysis (INAA), Atomic Absorption Spectroscopy (AAS), Differential Pulse Anodic Stripping Voltammetry (DPASV), Proton Induced X-ray Emission spectroscopy (PIXE), X-ray Fluorescence Analysis (XRF), etc. In this study XRF has been chosen as the technique for trace element analysis. The XRF techniques used for the analysis of hair, require mounting the specimens directly on the spectrometer. However, African or Negroid hair is kinky and therefore difficult to mount directly on the spectrometer (Eltayeb and Van Grieken, 1989). In this study, the hair samples were digested prior to the analysis to prepare a suitable target. Ideally, it is desirable to prepare the sample in a form suitable for the analysis so that inter-element and absorption effects are negligible and that the lowest possible detection Limits are achieved. Analytes which can be deposited as a thin layer on a suitable substrate can meet these requirements. Precipitation of the elements in solution by the addition of an organic chelating reagent followed

by filtration of the precipitate on a millipore membrane yields solid targets which are ideal for XRF. Among the wide spectrum of organic chelating reagents used for the precipitation of the metals in solution, the dithiocarbamates seem to be the most popular in view of the low solubility of their metal chelates (Luke, 1968).

This study has been accomplished by analysing head hair samples collected from residents of Nakuru District to establish concentration levels of the trace elements. From these concentrations values of the various elements measured, it has been possible to establish the level of toxic metals due to environmental pollution and those of essential trace elements. Variation of trace elements concentrations with Age and Sex have also been studied.

1.2 LITERATURE REVIEW

In recent years there has been much interest in the use of hair as a biopsy material and a number of reviews and detailed studies of the elements found in hair have been published (Campbel, 1981). Studies have shown that the level of trace elements found at a given location in the hair reflects the concentration at the time the hair was formed (Walter, 1982).

The absorption of air pollutants by hair has been demonstrated by Rendic et al, (1976), who measured the X-ray intensities of lead, bromine, arsenic and strontium relative to that of zinc and observed a monotonic increase in the ratios i.e., concentrations, with distance from the scalp. Stauber and Florence, (1989) studied Mn in scalp hair and looked at the problems of exogenous Mn and the implications for its monitoring in Groove Eylandt Aborigines. They found that high scalp hair Mn values were due to exogenous sources. They also found that the Aborigines from the non-Manganese areas had 2 µg/g Mn in hair. Caucasians living in the same Manganese rich area had 2.5 µg/g Mn in hair compared to 0.5 µg/g Mn in non manganese areas.

A study of the source of external metal contamination has been done by Rikuo et al, (1988). They examined the sources of external metal contamination of hair

experimentally exposed hair samples to soils, hot water from a water boiler for domestic use and household dust as well as fumes in the Kitchen. They found that concentration of Cu in hair increased markedly only when the hair was exposed to hot water from the boiler. Iron concentration in hair increased markedly after exposure to wet soil and increased slightly after exposure to hot water from the boiler. When the hair was exposed to household dust and fumes, Zn showed a slight increase but Cu and Fe revealed no change at all.

Not many investigations of the variation of the trace element contents of the human hair with sex have been done. However, the most extensive work reported was by Schroeder and Nason (1969), in which they investigated eight trace elements in hair samples of 126 male and 55 female subjects .

Schroeder and Nason, (1969) have also analyzed hair for subjects ranging from the age of 1 year to the age of 102 years using Atomic absorption spectrophotometry. When mean concentrations of the trace elements in the hair of the subjects in the first three decades of life were compared with those of subjects aged 40 to 70 years, differences occurred in female hair only with respect to Cu and Pb while Cd values were considerably lower in older persons.

Schuhmacher et al, (1991) evaluated the concentration of lead in children`s hair as related to exposure in taragona province -Spain. The influence of Age, Sex, Hair Colour and Family occupation was also evaluated. Girls had more Lead in their hair than boys (10.54 µg/g against 6.55 µg/g) and the Lead levels decreased with age. Michael and Friedrich (1990) reported that Females had more Zn in both scalp hair and Pubic hair than males. Further sex related differences were only observed in Pubic hair with higher Cd, Cu and Pb levels for Males compared with Females. This study was carried out in Germany in the Urban and rural areas surrounding Bonn.

Iron, Copper, Zinc and Lead in hair from Sudanese population of different age groups have been studied by Eltayeb and Van Grieken, (1990). The variation of Fe, Cu and Pb content of hair with Age were investigated. The averages of the elemental concentration in each group were compared with other age groups and with values from elsewhere. Correlation of various elements in hair were also investigated and they found that Fe in Sudanese population showed an extraordinarily high concentration which they concluded could have been due to dust contamination in the dry and bare environment of Sudan.

Othman and Spyrou, (1980) studied the abundance of some elements in hair and nail from a selected population of Kenya -Machakos District using Instrumental Neutron

Activation Analysis (INAA). The population chosen was involved in agricultural activities and it was supposedly free of industrial effluent. They studied the variation of elemental concentrations with age and sex and concluded that no significant difference in elemental concentration in hair was found between male and female populations for the elements studied except for Ca and Mn. Nonetheless, there were some differences within the two groups when divided by Age and in particular for Zn. They also found that despite a carefully selected "normal" population group, from a small and closed community, the dispersion of elemental concentration levels in hair and nail was large. A study was done by Michalis and Xenophan, (1990) to investigate the trace metals in scalp hair of Greek agricultural workers in order to determine "base line" values of hair metal concentration in unpolluted non-industrialized areas of Greece. They found statistically significant ($p < 0.05$) sex difference for Cd and Ni. They also found high positive correlations between Pb, Cr and Cu. High negative correlations were also reported between Cd and Zn.

An intercomparative analysis of the concentration of heavy metals i.e., Zn, Cd, Pb, Hg, Cu, Fe and Ca in head hair of a randomly selected sample of Kenyan people using Atomic Absorption Spectrometry (AAS) and Differential pulse Anodic Stripping Voltammetry (DPASV) was carried out

by Wandiga and Jumba (1982), from the study they found that the recalculated body burden ratios of Cd to Zn and Pb to Fe revealed no unusual health impairement symptoms which suggested a relatively clean environment in Kenya. Factors affecting the simultaneous determination of Cu, Pb, Cd and Zn concentrations in human head hair using DPASV method have also been considered by Wandiga And Jumba (1982).

Other work includes measurement of variation in Lead concentrations along a single strand of hair determined using Atomic spectrometry by Renshaw et al., (1972) and measurement of various trace elements in human hair as reported by Valkovic et al, (1973) who used proton induced X-ray emission (PIXE).

Toxic effects due to heavy metals have received much attention. Some of the heavy metals extensively studied include mercury and lead whereby it has been shown that lead concentrations in hair vary widely depending upon the diet and environment of the subject. Kopito et al, (1967;1969), have shown that the measurement of Lead in hair is a particularly useful diagnostic aid for even mild plumbism. In his study, Kopito explored the quantitative analysis of Lead in hair as an aid in the diagnosis of chronic and mild lead poisoning in children.

Head hair samples have been used as indicators of environmental pollution by Corridan, (1974). He gave a

brief account of problems which may arise from open cast metal mining to indicate environmental pollution. He did his study in an area in Rural Ireland, where he noted comparatively high levels of Arsenic, normal copper and Zinc levels and low levels of lead and mercury. All the metals with the exception of Arsenic were analyzed using AAS while Arsenic was determined by calorimetric method using Silver Diethyldithio-Carbamate as a reagent. Wet oxidation was used in preparation of all the samples. It was found that hair provides good indicators of exposure to many toxic metals over periods of up to a year or more and might well be used in epidemiological studies related to environmental pollution.

Health aspects of burning coal have been investigated by Valadimir and Karel, (1977). They determined Arsenic concentration in hair, urine and blood samples taken from 10 year old boys residing in a region polluted by Arsenic. They found hair to be a suitable material for estimation of non occupational exposure to this toxic agent, inspite of some problems associated with the decontamination procedure. They reported that analysis of urine and blood is complicated by several technical difficulties such as collection and transportation of the material. Apart from the above mentioned difficulties the data from urine were not as conclusive and demonstrative as were those from the hair analysis.

The possibility of exogenous exposure has led to substantial controversy concerning the reliability of hair analysis as a measure of the absorbed dose (Jenkins, 1979). Such exogenous contamination can occur when dust or vapours from the occupational environment adhere to the hair surface and are then incorporated into the hair matrix by diffusion. Even when meticulous and thorough washing procedures are employed, determining whether the heavy metal content of hair is associated with systematic distribution of the metals in hair during its formation or external contamination of already formed hair cannot be completely ascertained because there does not exist a precise physical border separating exogenous and endogenous contribution to measured values (Hopes, 1977; Chiltleborough, 1980). In the work reported by Al-shahristani and Shihab, (1974), hair samples were thoroughly washed with detergent solution and rinsed with distilled water and alcohol. The effect of washing was checked on identical samples and the mercury in hair was unaffected by washing. However, due to the forementioned controversy, there is a need to standardise the method of hair washing. In an attempt to do this, the IAEA (International Atomic Energy Agency) advisory group has recommended the use of Acetone, three portions of double distilled water and then rinsing with acetone (Yu, 1977). This is the procedure which was followed in this work.

Sample preparation depends on the type of analysis to be made. A quantitative analysis by x-ray fluorescence can be made on any solid or liquid as long as the elements are present in measurable quantities and the specimen can be properly positioned in the measuring position. African or Negroid hair is kinky and is difficult to position it properly in the sample holder for irradiation (Eltayeb and Van Grieken, 1989) and hence the hair samples have to be digested to prepare liquid samples. In the case of liquid samples it has been shown that it is possible to gather some elements, for precipitation, using various chelating agents (Luke, 1968). For the digested hair solution, APDC (ammonium pyrrolidine dithiocarbamate) and NaDDTC (Sodium diethyl dithiocarbamate) have been used widely as chelating agents for the co-precipitation of the trace elements. Eltayeb and Van Grieken, (1989), used APDC for the precipitation of trace elements in hair samples obtained from Sudanese populations. Since most precipitates, no matter how insoluble they are, have a certain solubility and sometimes also exhibit supersaturation, it is necessary to use enough suitable coprecipitant to ensure quantitative recovery of the trace elements. In most instances, the precipitation of trace elements will be complete shortly after the addition of the precipitate. However since this is not always true, the practice in this work has been to allow the solution

to stand for 15 minutes after the addition of coprecipitant prior to filtration (Luke, 1967).

In hair sample preparation, a variety of digestion mixtures have been used to break down organic matter under different conditions including the use of HNO_3 - HCl_4O (Whitehead, 1978 ; Eltayeb and Van Grieken, 1989); overnight UV radiation of the sample in H_2O_2 (Gardner and Stiff, 1975), HNO_3 -H_2O_2 (Bruno et al, 1978), H_2SO_4 - $KMnO_4$ (Pye Unicam Ltd., 1975) dry ashing with H_2SO_4 -HNO_3 dry ashing with Na_2CO_3 flux on platinum crucible (Lai and Fung, 1978) and HCl- H_2O_2-HNO_3 (Wandiga and Jumba, 1982). Dissolution of biological materials with various acid combinations followed by preconcentration of the metals in solution on cation exchanger Resin for XRF measurements has also been described by Kingston, (1981). In this work the digestion mixture used was that of Nitric with Hydrochloric acid in the ratio of 3:1.

Various analytical techniques have been used for the analysis of trace elements in human hair e.g., Instrumental Neutron Activation Analysis (Othman and Spyrou, 1980), Atomic Absorption Spectroscopy and Differential Pulse Anodic Stripping Voltammetry (Wandiga and Jumba 1982), Proton Induced X-ray Emission spectroscopy (Campbel et al, 1981), X-ray Fluorescence Analysis (Eltayeb and Van Grieken 1989) etc.,. In this study XRF has been chosen as the technique of analysis.

1.3 PURPOSE OF STUDY

Trace elements in human hair are gaining importance in nutritional studies(essential trace elements) and in toxicology (toxic metals) (Walter, 1982).Trace elements can be divided into two groups ie, dietary essentials and non essential trace elements. The non essential trace elements are those acquired by the body as environmental contaminations. Some of these elements are classified as toxic elements.

Some trace elements have important effects on the functioning of living organisms and are known to be essential to man. These elements include Fe ,Zn, Cu, Mn, I, Mo, Cr and Se (Laker, 1982). The majority of these essential trace elements, serve chiefly as key components in enzyme systems of proteins with vital functions. Enzymes in which metals are tightly inco-oporated are called metaloenzymes, since the metal is usually embedded deep inside the structure of the protein. If the metal atom is removed, the protein usually losses its capacity to function as an enzyme (Valkovic, 1977). A surfeit or deficiency of any essential trace element can be wholly or partially responsible for a number of disorders (Laker, 1982), thus it is important to determine the level of these essential trace elements in the human body.

The non essential trace elements are acquired by the

body as an environmental contamination. Some of these trace elements e.g., As, Cd, Hg and Pb are classified as toxic elements (Valkovic, 1977). It is the purpose of this study to investigate the presence of these elements and determine their concentrations in head hair. It is important to determine the trace element concentration for the residents of Nakuru District so that the data is available for comparison with samples taken from other populations as well as for studies of environmental nature or metabolism in the same population. This study also aims at finding or establishing any relationship between trace element concentrations and sex (or age) in order to understand their influence if any, on the results e.g., if there is a decrease or increase in the concentrations of the trace elements, one would like to know whether the change is due to age, sex or environmental factors.

CHAPTER TWO

2.0 THE BASIC PRINCIPLES OF XRF ANALYSIS

2.1 INTRODUCTION.

Various techniques have been used for the analysis of hair. Some of these techniques include: Instrumental Neutron activation analysis (INAA), Atomic absorption spectroscopy (AAS) and Photon induced x-ray fluorescence analysis (XRF).

The instrumental form of neutron activation analysis is based on the detection of induced gamma ray radiation using gamma ray detectors e.g., NaI(Ti) crystals or Ge(Li) semiconductors and multi-channel analyzer (usually computer based analyzer systems). All stable Isotopes are capable of capturing thermal neutrons but with characteristic probability that varies widely from one nucleus to another. The resulting compound nucleus is radioactive, its later decay product can be detected by activation analysis. In practical analytical applications a comparison method is used. When samples are to be analyzed for one or more elements, standard reference samples of these elements are activated at the same time as the unknown and then irradiated in an identical manner. A detailed description of the neutron -activation analysis

of hair samples for Cr, Fe, Co, Zn, Se, Ag, Sb, Au and Hg is presented by Obrusnik et al, (1972).

AAS makes use of the fact that free atoms of an element absorb light at wavelengths characteristic of that element and that the extent of absorption is a measure of the concentrations of these atoms in the light path . In atomic absorption one element is measured at a time (incase a multi-element lamp is not used) and the analysis of the elements requires a lot of time and a bundle of hair to be analyzed. According to (Valkovic, 1977) sample preparation is very critical since washing removes different elements in the hair at different rates. Superficial hair contaminants such as dirt and dust are easily removable by a variety of procedures.

The X-ray fluorescence technique is an analytical technique which is very suitable for trace element analysis of heavy metals in environmental samples. The technique complements other instrumental and classical analytical methods due to its indisputably advantageous characteristics e.g., simple sample preparation, Analysis of most elements beyond aluminium and low detection limits in the region of 10 µg/g or less. It is a relatively non destructive technique and is used for multi- elemental analysis of the sample with relatively good selectivity.

In this work, the analysis of the samples was done by X-ray fluorescence technique because of the above quoted

advantages as compared to the other techniques.

The X-ray fluorescence spectrometer and support accessories are available at the centre for Nuclear Science Techniques of the University of Nairobi

2.2 The basic theory of X-ray fluorescence analysis (XRFA)

The X-ray fluorescence analysis technique used is based on the measurement of the characteristic X-ray radiation emitted by a constituent element of the sample excited in a certain manner.

The principle of X-ray fluorescence spectroscopy is illustrated in the Figure 1 below (Jenkins, 1981). The figure shows an arbitrary division according to modes of exciting characteristic X-rays within the sample i.e., from accelerators, radioactive sources or from an x-ray tube.

2.2.1 Sources of Excitation of Characteristic X-rays

There are different modes of exciting the sample so as to obtain the characteristic X-ray radiations as can be seen in Figure 1. Almost any high energy photon will act as a source for the production of characteristic X-rays provided that it is sufficiently energetic to eject an electron from the appropriate atomic shell of the element to be excited. The minimum energy required is simply the binding energy of the electron in a given shell.

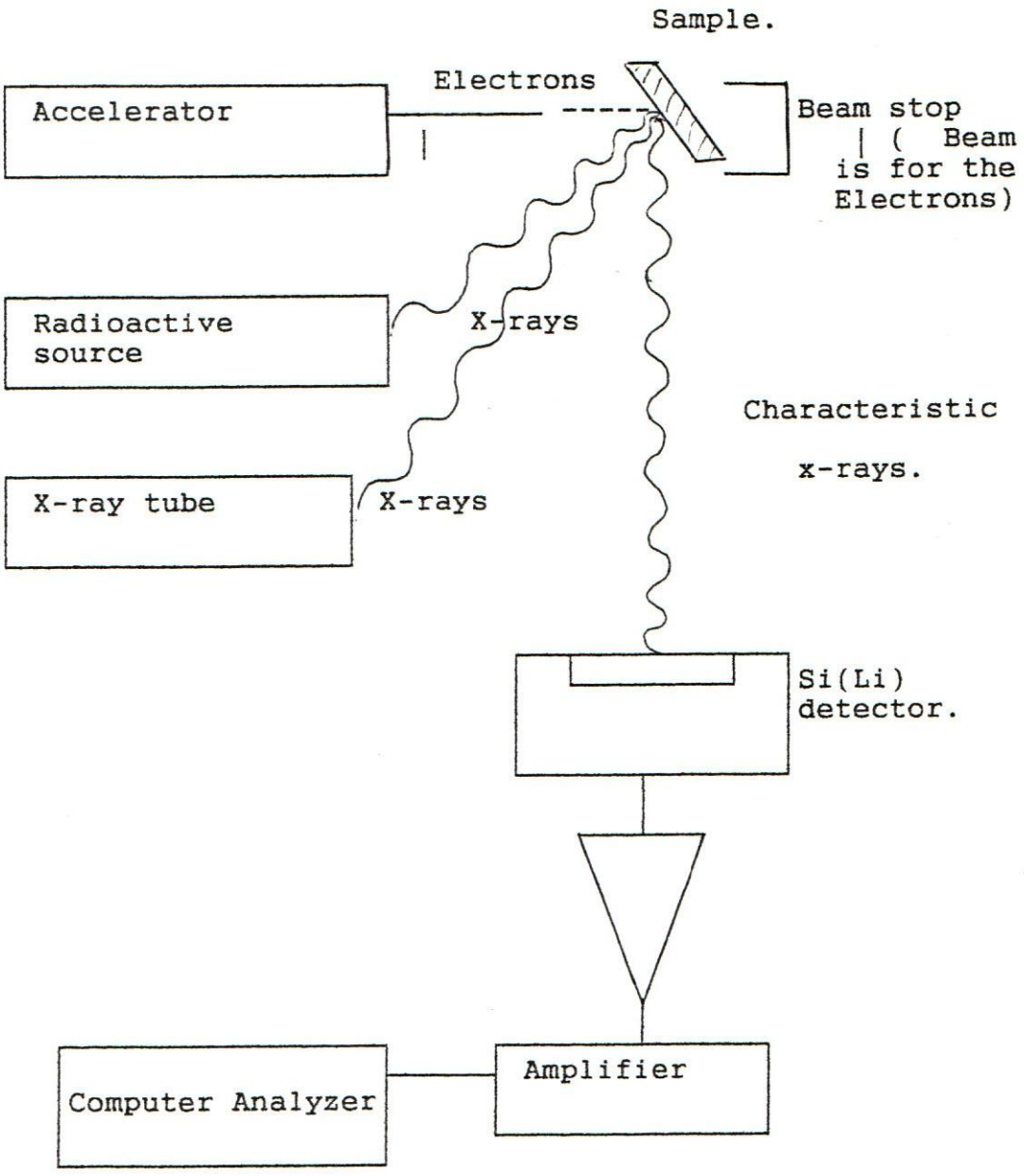


Fig 1 : Principle and sources of X-ray fluorescence spectroscopy.

Within an X-ray tube, electrons are emitted from a heated filament (Cathode) and accelerated towards the target (Anode) by a potential difference of upto 50Kv. A wide variety of X-ray tube Anodes materials are available. The more commonly used anodes include Chromium, Rhodium, tungsten and Molybdenum. A Molybdenum target was used as the anode material in the X-ray tube for sample excitation in this work.

When the electron reaches the anode it has already acquired a kinetic energy equivalent to :

$$E = eV$$

Most of the power transferred to the accelerated electron is dissipated as heat in the anode and only a small fraction of the power results in the emission of x-rays. Therefore the X-ray tube has to be continuously cooled. In our case, the X-ray tube was water cooled.
When the electrons strike the target they produce two types of radiations i.e., characteristic x-rays and Bremsstrahlung. Characteristic x-rays are emitted when the impinging photon energy is greater than the electron binding energy of a particular atomic shell of the anode material. In a small fraction of the cases, the photon is deflected by the nuclei of the anode where energy can vary from zero upto the full energy of incident photon. Thus a continuum of X-ray photon energies is generated as electrons bombard the X-ray tube anode. This continuum is

referred to as Bremsstrahlung (Robley, 1955).

Both the characteristic X-rays and Bremsstrahlung radiation can be used to excite the specimen in the Monochromatic and Broadband Excitation respectively. In this work, Monochromatic excitation was used to excite the analyte specimen.

For monochromatic excitation using X-ray tube, the characteristic X-ray lines are used to excite the trace elements in the specimen. The elimination of Bremsstrahlung contribution in the regions containing trace element peaks involves using some type of selective filtering or use of secondary fluorescers. The filtering technique together with secondary flourescers target material of Mo was used in this work.

The filter has a narrow band or window of high transmission in the vicinity of the Characteristic Mo K lines. When used with a Mo anode X-ray tube, the filter will pass Mo K lines from the anode and severely attenuate the Bremssrahlung continuum (Jenkins et al, 1981). These Mo K lines are then used to irradiate the sample.

The excitation radiation from a radioisotope is produced when an unstable nuclear isotope decays into a different isotope. This second isotope or daughter nucleus, may also be unstable and decay into yet another isotope. The radioactive decay scheme may involve only parent or daughter relationship or can encompass along

chain of decay sequences.

The radioisotopes commonly used in X-ray fluorescence spectrometry emit photons in the form of γ-rays from the nucleus or characteristic X-rays from the Atomic shells. Most γ-ray and X-ray emitting sources are monochromatic or nearly Monochromatic in that they emit photons having only one or a few discrete energies.

Some of the most widely used radioisotopes for X-ray fluorescence specrometry include ^{55} Fe, ^{109} Cd, ^{125}I and ^{241} Am. ^{55} Fe, is useful for exciting the light element K Lines from Sodium to Titanium. The Silver K lines from ^{109} Cd, are effective for exciting the medium Atomic number elements K lines while the 88.2 KeV γ-ray from ^{109} Cd is effective for exciting the K lines from Heavy metals.

When a sample is bombarded with charged particles such as protons, α -particles or electrons from the accelerators, electrons are ejected from the inner shells of the atom while the incoming particle loses some or all its energy. The characteristic X-rays produced are then used for elemental analysis of the specimen.

2.2.2 Modes of interaction of X-rays with matter

The excitation and consequent de-excitation of an atom will depend on the mode of interaction of the incident radiation with matter through.,

(i) Photo-electric interaction and

(ii) Scattering which is either coherent or incoherent.

2.2.2.1 Photo-electric interaction

This occurs when the incoming radiation interacts with the atom and ejects an electron from an inner shell which is subsequently replaced by an electron from a higher shell with emission of characteristic X-rays.

If a photon of energy E strikes a bound electron and the energy of the photon is greater than the binding energy (Φ) of the electron in its shell, then it is possible for the electron to absorb the total energy of the photon. The photon disappears in this process and its energy is transferred to the electron which is ejected from its shell. The ejected electron is called a Photo electron and the interaction is called the Photo Electric effect. The photo electron is emitted with energy ($E - \Phi$) where E is the original photon Energy and Φ is the binding energy of the electron in its shell.

The vacancy left in the shell after the electron has been ejected represents an unstable situation and consequently, an electron from a shell with lower binding energy will transfer to fill the vacancy. The energy released may be in form of a characteristic X-ray photon

and the probability that a characteristic X-ray will be emitted once a vacancy has been created is described by the fluorescence yield (Jenkins et al, 1981). Alternatively, the energy may be absorbed and an electron is released. This process is known as the Auger effect and the electron released is referred to as the Auger electron.

Therefore in the Auger effect two electrons are expelled simultaneously from the same atom in a single elementary action. One of the electron is the normal K photoelectron and the other is the auger electron from the L shell (Rajam, 1964). The Auger effect is more common in elements of low atomic number because the electrons are relatively loosely bound and the characteristic photons are more readily absorbed.

This Photo-electric interaction has a definite probability which is energy dependent and is denoted by σ_{ph} (E). The characteristic X-rays are generated as a result of photoelectric interaction.

2.2.2.2 Incoherent (Compton) scattering

This occurs when an X-ray Photon collides with a loosely bound or free electron and looses part of its energy to the electron such that the scattered photon moves at an angle θ from its initial direction (Fig. 2).

Compton scattering involves the light atoms and an increase in the wavelength of the photon i.e., a decrease in the photon energy. This is so because when a photon of primary energy ($h\nu$) collides with a weakly bound electron, assumed to be at rest, the incident photon energy is partly converted into the kinetic energy of the electron (as in the Figure 2 below).

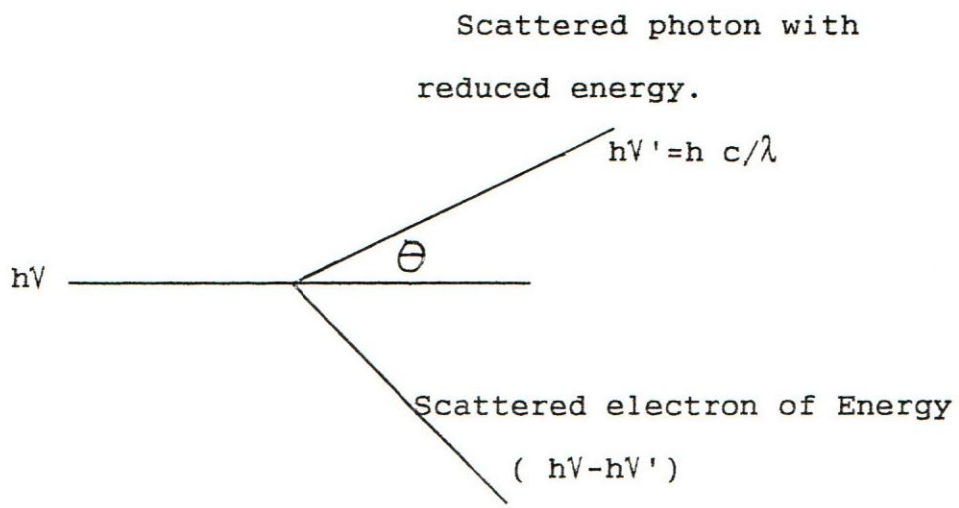


Fig 2: Momentum exchange during compton scattering.

This compton scattering has a probability which is energy dependent and increases as the energy of the X-ray photons increases i.e., as the wavelength of the X-rays decreases.

From the conservation laws of energy and momentum, it can be shown that the compton Shift, $\Delta\lambda$ is given by the

equation

$$\Delta\lambda = \frac{h}{mc}(1-\cos\theta)$$

where h is the Planks constant, c the velocity of light, m mass of the electron and θ the angle through which the radiation is scattered.

2.2.2.3 Coherent (or Rayleigh) scattering

Coherent scattering arises when an X-ray photon collides with an electron and is deviated without loss of energy, the corresponding wavelength remaining unchanged. As the energy of the photon increases, the interaction becomes inelastic since the photons are able to `knock off' the electrons from the atom.

Coherent scattering involves incident low energy photons being scattered by elements with higher atomic number, with its probability increasing as more tightly bound atomic electrons are involved.

The coherent and incoherent scattering of the excitation spectrum generally provides background contributions which tend to interfere with the analysis of characteristic X-rays .

2.2.3 Sample Matrix effects

The relationship between intensity and concentration of elements of interest may be seriously affected by the interference and enhancement effects caused by the matrix elements (Pinta, 1982).

These matrix effects in XRF method consists of the influence of the variations in the mineralogical composition of the sample on the fluorescence radiation intensity of element of interest. The matrix effects can be broadly classified in two categories i.e., those due to Elemental interactions and the Particle size effect. Those due to elemental interactions are further subdivided into Absorption and Enhancement.

2.2.3.1 Elemental interactions

(a) Absorption

In absorption, the matrix elements absorb both the exciting and the fluorescent radiation, thus reducing the final intensity of radiation reaching the detector. This effect is very noticeable when the mass absorption coefficient of the analyte for its own radiation is much greater than that of the matrix elements (Serman, 1955)

(b) Enhancement effects.

The enhancement effects consists of additional excitation of the atoms of the analyte element by the fluorescent radiation of some of the other constituent elements. During this process, there is excitation of the lighter atoms by the fluorescent radiation of the heavier elements. Therefore the intensity of the desired elements depends on the heavier elements present in the sample. This effect is more pronounced when the element to be enhanced is present in, small concentration and the element which can enhance is present in major concentrations.

2.3.2.2 Particle size effects

If the sample has different sized particles then the measured intensities would not be representative of the whole sample. This is because the specimen will not be completely homogeneous as required for the XRF analysis. A detailed description of the matrix effects is given by Shiraiwa and Fujino, (1966) and Sparks, (1975)

The above effects are considered negligible when dealing with thin, diluted samples of the order of a few hundreds μg/g for most elements (Pinta, 1982).

2.3 The Basic equation for X-ray fluorescence analysis

In X-ray fluorescence spetrometry, it is the intensity of the fluoresced characteristic X-rays from the spectrum which provides the analytical signal for qualitative and quantitative analysis.

In the derivation of the basic equation for the X-ray fluorescence analysis, the specimen is assumed to be homogeneous, has a flat surface and that the excitation radiation is practically monochromatic. Fig 3 shows the geometry of the excitation radiation, sample and detector as used in the derivation of the basic XRF equation.

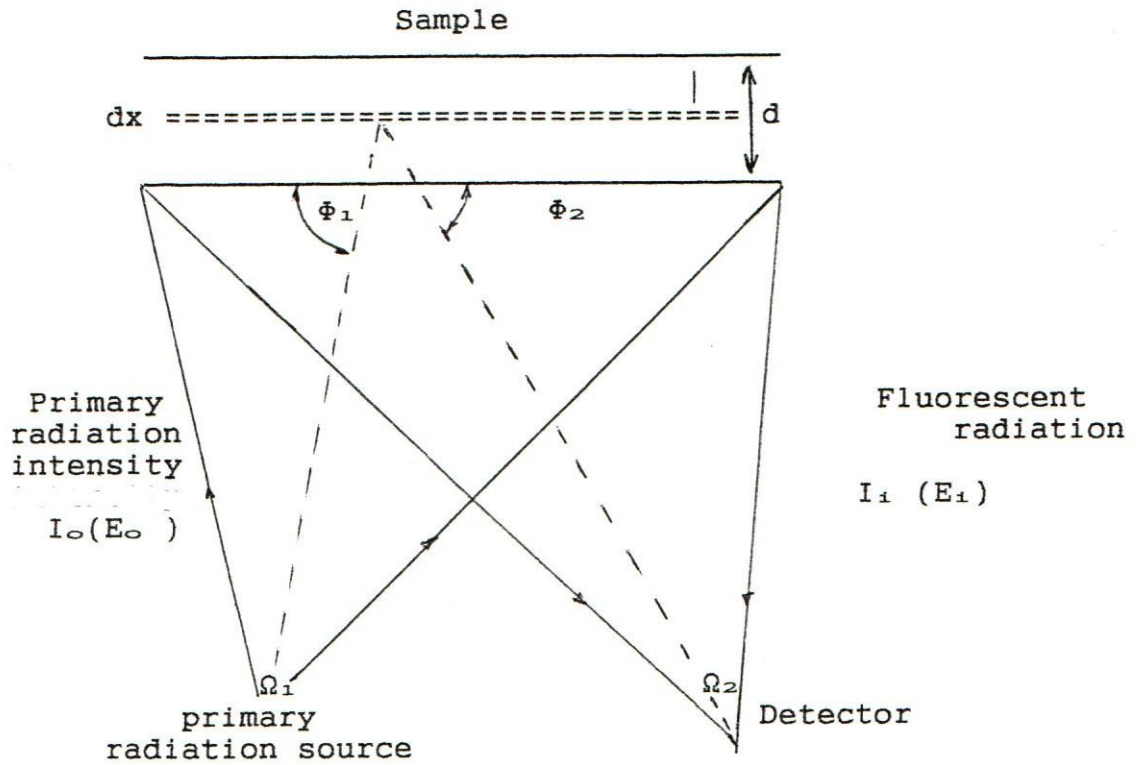
If we consider a sample of thickness `d' and density `ρ', and assume that element `i' is homogeneously distributed within the sample, then the penetration of the radiation obeys the Beer Lambert's law (Rajam, 1964) i.e.,

$$I = I_o e^{-\mu x \rho} \quad \ldots\ldots 1$$

where μ is the mass absorption coefficient.

The probability of exciting the K X-rays of energy E_i from element `i' in the layer at depth `x' within a thickness `dx' and detecting these fluoresced X-rays is given by a product of three probabilities i.e.,

Fig 3: The Geometry of the source, sample and the detector as used in the derivation of the Basic X-ray Fluorescence Equation.



(a) The probability that a part of primary radiation of initial intensity I_o will reach a depth `x'. is given as:

$$P_1 = \Omega_1 I_o \exp-(\mu_1 \, csc\Phi_1 \, \rho \, x) \quad \ldots\ldots\ldots\ldots\ldots\ldots$$

where Ω_1 is the solid angle of the incident primary radiation as seen by the sample.

(b) The probability that atoms of element `i' in the layer `dx' will be excited through photo-electric effect by primary radiation and that in the de-excitation process, which follows, fluorescence K X-rays of energy E_1 will be emitted i.e.,

$$P_2 = \sigma_i^{ph}(E_o) \, \rho_1 \, csc\Phi_1 \, (1-\frac{1}{J_x})^i \, \omega_k^i \, f_k^i \, dx \quad \ldots\ldots 3$$

(c) The probability that the fluorescence K X-rays of energy E_1 will pass through a part of the sample, reach the detector and be detected i.e.,

$$P_3 = \Omega_2 \, \eta(E_1) \, \exp-(\mu_2 \, csc\Phi_2 \, \rho \, x) \quad \ldots\ldots\ldots\ldots\ldots 4$$

in which Ω_2 is the solid angle through which the detector 'sees' the sample.

The contribution of the fluorescent X-rays in a layer of thickness `dx' at depth `x' is given by the product of the above three probabilities i.e.,

$$dI = P_1 \, P_2 \, P_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 5$$

Integrating equation 5 over the sample thickness `d ' and multiplying the numerator and denominator by d one

obtains :

$$I = G_o K_i \rho_i d\left(\frac{1-\exp(-a\rho d)}{a\rho d}\right) \quad \ldots\ldots 6$$

where

$$G_o = I_o \Omega_1 \Omega_2 \operatorname{csc} \Phi_1 \quad \ldots\ldots 7$$

G_o is the geometrical factor determined experimentally by the measurement of intensities of single element standards of known mass per unit area.

and

K_i is the relative excitation detection efficiency for element i and is given as:

$$K_i = \sigma_i^{ph}(E_o)\left(1-\frac{1}{J_k}\right)^i f_k^i \omega_k^i \eta(E_i) \quad \ldots\ldots 8$$

In which

$\sigma^{ph}{}_i$ (E_o) is the photo-electric cross-section of element `i' for the primary radiation of energy E. The values of $\sigma^{ph}{}_i$ (E_o) are tabulated in (McMaster et al, 1969).

$(1-1/J_k)^i$ is the relative probability for excitation of K-shell of element i.

$\omega^i{}_k$ is the fluorescence yield of element `i' for the K shell.

$\eta(E_i)$ is the relative efficiency of the detector at energy E_i.

f^i_K is the ratio of the intensity of a given K or L line to the intensity of the whole series and is give as:-

$$f_k^i = \frac{P(k_\alpha)}{[\,P(k_\alpha) + P(k_\beta)\,]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 9$$

In which

P(k_α) and P(k -) are the probabilities of producing k_α and k- x-rays respectively.

The quantities $\sigma^{ph}{}_i\ (E_o)$, $(1-1/J_K)^i$, $\omega^i{}_K$, $\eta(E_i)$ and $f^i{}_K$ are known as the Fundamental parameters and their values are obtainable from literature (Storm and Israel, 1967) except for values which can be calculated and checked by measurements e.g., $\eta(E_i)$.

a(E) is the total mass attenuation coefficients and is given by

$$a(E_i) = \mu_s(E_o)\csc\Phi_1 + \mu_s(E_i)\csc\Phi_2 \quad \ldots\ldots 10$$

Where $\mu_s\ (E_i\ )$ is the absorption coefficient for the fluorescent X-rays in the sample which is an additive quantity and can be expressed by the respective coefficients in pure elements as:

$$\mu_s\ (E_i\ ) = \sum_i^n \mu_i\ C_i$$

C_i is the concentration of element `i' in the sample.

μ_i is the attenuation coefficient of element `i'.

μ_s (E_o) is the absorption coefficient for the primary excitation

E_o is the primary exciting energy.

E_i is the characteristic energy of element `i'.

Φ_1 and Φ_2 are the angles formed by the primary incident and emergent characteristic radiations with the sample surface.

d is the thickness of the sample.

ρ_i is the partial density of element `i' in g/cm^3.

ρ is the mean density of the sample in g/cm^3.

The expression

$$\frac{(1-\exp(-a\rho d))}{a\rho d}$$

in the basic equation for XRF analysis is referred to as the **Absorption correction factor** and accounts for the attenuation of the primary and the secondary radiation in the sample.

The basic equation takes the following forms when the concept of Thick, Transparent and Thin samples is introduced.

2.3.1 Thick Samples

In thick samples the term $a\rho d$ is considered to be large so that the term

$$\frac{[1-e^{(-a\rho d)}]}{a\rho d} \approx \frac{1}{a\rho d}$$

Hence the approximate equation for a thick sample becomes

$$I_i(E) = G_o K_i C_i \frac{1}{a} \quad \ldots\ldots\ldots\ldots\ldots 11$$

where

$$C_i = \frac{\rho_i d}{\rho d}$$

A specimen is considered infinitely thick if increasing its thickness does not result in an increase of intensity of fluorescent radiation.

2.3.2 Transparent Samples

These include samples of intermediate thickness and are usually analyzed using the equation

$$I_i(E) = G_o K_i (\rho d)_i \frac{[1-e^{(-a\rho d)}]}{a\rho d} \quad \ldots\ldots 12$$

$0.1 < a\rho d < 2$

2.3.3 Thin Samples

In thin samples, the term $a\rho d$ is considered to be small ($a\rho d << 1$) and hence the term $[1 - \exp(-a\rho d)]$ is approximated as $a\rho d$. The basic equation then reduces to

$$I_i(E) = G_o K_i (\rho d)_i \quad \ldots\ldots 13$$

which is the equation for a thin sample.

The value $(\rho d)_i$ in equation (13) which is in mass per unit area of element 'i' is the concentration to be determined.

Equation (13) was used in the quantification of the concentration of trace elements in hair since the hair samples were prepared prior to irradiation to form a thin sample.

The units of the concentration used for the hair sample were µg/g and hence equation (13) was multiplied by the area of sample and divided by mass of the hair sample used to obtain equation 14.

$$C_i = \frac{\pi D^2 I_i(E)}{4 K_i G_o g} \quad \ldots\ldots 14$$

Where D is the diameter of the sample precipitate in cm and g is the mass of the hair sample used in grammes.

CHAPTER THREE

3.0 EXPERIMENTAL

3.1 XRF INSTRUMENTATION

The X-ray spectrometer used consisted of the following components i,e., The X-ray excitation source, Si(Li) Detector, EG & G preamplifier, Canberra amplifier model 2020, S-100 multichannel card interfaced to a PC computer for spectral data acquisition and storage.

The source used was an X-ray tube with a Molybdenum target.

The detector used in this work was a Canberra Si(Li) detector with a resolution (i.e., Full width of the peak at Half Maximum) of 200eV at the Mn K_{α} Energy (5.9 KeV).

The detector diode is fabricated from a cylindrical sector of a single crystal of P- I-N structure. The crystal is Silicon and adequately covers the energy range of analytical interest (Jenkins et al, 1981). When operated with normal reverse bias of approximately 1000 V the diode is depleted of the charge carriers and becomes in effect a solid state ionization chamber. X-ray photons enter the detector through the front contact and interact primarily by the photo electric process to produce a cloud of ionization in the form of electron- hole pairs. The

number of Electron hole pairs produced, 'n' is proportional to the photon Energy E i.e.,

$$n = \frac{E}{\epsilon}$$

where ϵ = 3.8 eV is the energy required to produce one electron hole pair in Si.

The front contact of the detector is negatively charged while the rear contact is grounded, thus the holes drift to the front while the electrons drift to the rear contact. The total charge Q collected at the rear contact is equivalent to

$$Q = \frac{E}{\epsilon} q_e$$

Where q_e is the charge of a single electron.

The rear contact of the detector is connected to the pre amplifier input so that the charge collected is stored in a capacitor C_f to produce a Voltage pulse of amplitude V_o, according to ,

$$V_o = \frac{E}{\epsilon} \frac{q_e}{C_f}$$

This charge is swept from the detector by the high voltage applied across it to the pre-amplifier.

The detector is operated at 77 K to lower the Lithium mobility in the crystal and to reduce the thermal noise

which would be caused by excessive diode reverse leakage current at high temperatures. In our case the Detector temperature was maintained at 77 K by the use of Liquid Nitrogen.

The pre-amplifier is responsible for collecting the charge swept from the detector on a feedback capacitor to produce an output pulse whose voltage amplitude is proportional to the original X-ray photon energy.

The signal from the pre-amplifier is small and has a low signal to noise ratio, consequently the slow pulse shaping amplifier serves two functions. First, it amplifies the pulse to the 0 to 10 V pulse height range so that the pulse height analysis can be performed. Secondly, it uses band filters which suppress extremely low and extremely high frequencies where the signal to noise ratio is poorest for improved energy resolution.

The Multi-channel analyzer measures the height of the amplifier output pulses and represents these amplitudes by an integer number. The number of times a pulse of the same height range has been detected is accumulated in the corresponding analysis memory address to represent the pulse height distribution. The pulse height distribution is equivalent to an X-ray spectrum because of the linear relationship between the energy of the absorbed X-ray photon in the detector and resulting pulse height. It is from this X-ray spectrum that qualitative and quantitative

analyses of the elements of interest in the sample is done after MCA calibration.

The detector and associated electronics were interfaced to a 486 PC computer based S-100 multi-channel analyzer for spectral data collection and storage.

A detailed description of the XRF instrumentation is available from Jenkins et al, (1981).

3.2 Spectral and Quantitative Analysis

Spectral analysis was accomplished by use of the AXIL computer program (Analysis of X-ray spectrum by Iterated Least Square fitting) which utilizes least square fitting procedure for spectrum analysis.

The principle of Least square fitting is used for spectrum analysis. The recorded X-ray spectrum is considered to be a Histogram with the X-axis representing Energy intervals which are referred to as channels. The Y -axis represents the Intensity (number of counts recorded in time t) in each energy interval.

The intensity peaks are therefore assumed to be presented by gausians and the background by a straight line or a polynomial.

The AXIL program assumes that the intensity peaks can be approximated by a Gaussian shape i.e., for a region of spectrum containing the background and n overlapping

peaks, the function could be described by the equation.,

$$Y(x) = f_o(x) + \sum y_i(x)$$

Where $f_o(x)$ describes the background while $y_i(x)$ describes one of the peaks i.e.,

$$y_k^i(x) = \frac{A_i}{\sigma_i\sqrt{2\pi}} \exp-\left(\frac{(E_i-E_p)^2}{2\sigma_i^2}\right)$$

where

A_i is the area of the peak.

σ_i is the standard deviation or full width at half maximum (FWHM) of peak 'i'.

E_p is the position of the peak.

E_i is the energy of the peak.

If the background function is a quadratic function then, $f_o(x)$ takes the form.,

$$f_o(x) = C_o + C_1E + C_2E^2$$

Thus the fitted spectrum is of the form :

$$Y_i(x) = \sum_1^n C_i\, y_i(x)$$

where the y_i are predetermined functions and the C_i are the parameters to be determined by optimizing the fit i.e

$$\chi^2 = \sum_i^n \frac{[Y_i - Y(x_i)]^2}{Y_i}$$

where

y_i is the measured data, $Y(x_i)$ is the calculated value and n is the number of data points i.e; the number of parameters or degrees of freedom used in the fit (Van Espen, 1977).

The intensities of the measured photopeaks, for elements of interest were converted to their corresponding concentrations using the Quantitative Analysis of Environmental sample software that uses fundamental parameter equation for thin samples as described earlier in chapter Two.

3.3 Sample collection.

The hair samples analyzed were collected from four groups of donors. Two of these groups A and B are located in Nakuru town while the third group (C) is located near Njoro Town. The fourth group (D) is located about 15 kilometres from Njoro Town. Additional samples were collected from Egerton University students who were used as controls. The hair samples were clipped from the nape of the neck using stainless steel scissors and then stored in polythene bags. The polythene bags were labelled according to Age, Sex and location of the donor.

3.4 Evaluation of the preconcentration and digestion method

3.4.1 Sample digestion

In the digestion procedure 0.3g of the Certified Reference hair Material (CRM) (received from Shanghai research institute of Nuclear Science Academia Sinica - China) was carefully weighed and transferred to a pyrex combustion flask which had been thoroughly washed and soaked over night in a 10% solution of HNO_3 in water. The flask was rinsed with double distilled water before transferring the sample into it. Analytical grade concentrated Nitric and Hydrochloric acids were added singly in the ratio of 3:1. After the addition of nitric acid, the sample was allowed to stand for 20 minutes to allow for the oxidation of the organic materials present before adding the Hydrochloric acid (Voinovitch, 1988). The resultant mixture was then heated until the specimen was completely digested and the resultant solution allowed to cool to ambient temperature. After cooling, a few millilitres of double distilled water were then added to the solution before heating it again to boil off any excess acid. Blanks for the specimens were prepared in parallel following the procedure described.

3.4.2 Sample pre-concentration and precipitation

The solution resulting from the digestion was made up to 100ml with double distilled water and the pH adjusted between 5-6 by placing the beaker containing the solution in a desiccator into which fresh ammonia solution had been poured.

The dissolved elements were precipitated by addition of a 10ml aliquot of freshly prepared 2% (w/v) NaDDTC solution following the procedure described by Holynska et al, (1987). No co-precipitant was added due to the presence of Iron in the hair which acted as a carrier. The NaDDTC chelates were then filtered, on a 47mm diameter millepore membrane filter of 0.45µm pore size. The filter membrane substrate was then placed in a petri dish and left to dry over night at ambient temperature and then placed between two aluminium rings on a mylar backing and irradiated for 2500 seconds at a voltage setting of 35 Kv and a current of 20 mA.

3.4.3 Elemental Recovery

In order to evaluate the efficiency of the preconcentration /precipitation procedure, recovery rates for the Elements Mn, Ti, Fe, Co, Ni, Cu, Zn and Pb were determined on samples prepared by diluting standard stock

solutions to prepare mixed solutions containing amounts ranging from 5µg to 45µg of the analyte element. The solutions were then prepared for irradiation as described previously. All targets were irradiated for 3000 seconds and the elemental quantities determined using equation (13). The ratio of experimental elemental concentration to the known concentration was then used as a measure of the recovery or scavenging action of the NaDDTc at pH 5-6.

3.4.4 Detection Limits

The detection limit for each element was determined from the Certified Reference Hair Material (CRM). Six samples of the hair CRM were digested and precipitated as described in the experimental procedure. Blank samples were also prepared in parallel. These were filtered and dried as described earlier, then irradiated for 3000 seconds and at a Voltage of 35 Kv and tube Current of 20mA.

Elemental intensities as a function of the concentrations were plotted and the slope of the calibration curves determined from a least square fit of the data. Detection limits were then calculated according to the equation:-

$$DL = \frac{3}{m}\left(\sqrt{\frac{C_b}{T_b}}\right)$$

where C_b is the background counts,

T_b is the background time and

m is the Sensitivity expressed in count rate per unit elemental concentration.

3.4.5 Precision and Accuracy of the method

The overall precision of the method was evaluated by analysing six targets prepared from 300mg aliquot of the hair certified reference material. The reference hair material was dried in an oven at 90 °C for 4 hours before digestion as recommended in the certificate. 300mg of the sample was accurately weighed and digested with nitric and Hydrochloric acid following the procedure described earlier.

The accuracy of the preconcentration method using NaDDTc was evaluated by analysing the elemental concentrations in the 300mg aliquot of hair CRM for the elements Cr, Mn, Fe, Co, Ni, Cu Zn ,As, Hg, Se and Pb and comparing with those from the reference certificate.

3.5 HAIR SAMPLE PREPARATION

3.5.1 Hair Sample Cleaning

After removing any visible contaminants,the hair samples were washed in acetone, followed by three portions of double distilled water and again in acetone and the washing decanted. Each wash lasted for about 10 minutes with continuous stirring. This is the washing procedure recommended by the IAEA advisory Group (Yu, 1977). This washing procedure was undertaken to remove hair oils, dust, dead skin etc., which adhere to the specimen sometimes covering a considerable part of its surface area. After the washing, the hair samples were dried in their washing flasks in an oven for 6 hours at a temperature of 80° C.

3.5.2 Hair Sample Digestion.

In the digestion method, 0.1g of hair was carefully weighed and transferred to a pyrex combustion flask which was thoroughly washed and soaked over night in acidic water. The flask was rinsed with double distilled water before transferring the sample into it. Analytical grade concentrated Nitric and Hydrochloric acids were added in the ratio of 3:1 respectively. Each acid was added singly

to the sample but in the order of Nitric then Hydrochloric acid. After the addition of nitric acid, the sample was allowed to stand for 10 minutes to allow for the oxidation of the organic materials present before adding the Hydrochloric acid. The resulting mixture was then heated until all the hair had been completely digested and the supernatant solution allowed to cool to ambient temperature. After cooling, a few millilitres of double distilled water were added to the solution before heating it again to boil off any excess acid in the solution. A blank sample, which consisted of the digestion mixture without the sample, was also prepared in parallel. The resultant solution was then precipitated and irradiated following the procedure described previously.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION.

4.1 Evaluation of the preconcentration and digestion method

4.1.1 Recovery and Detection limits

Most of the elements of were quantitatively analyzed with recoveries better than 87% (Table 4.1) with the exception of Ni and Zn where the recoveries were 83 and 82 % respectively. The element Co showed enhanced recovery above the 100% level indicating that some kind of contamination occurred. An analysis of the blank confirmed this suspicion though it was not possible to locate the source of contamination unambiguously .An additional contribution could possibly have been due to spectral overlap of Fe K~ and Co Kα making deconvolution for Co Kα difficult and uncertain.

The detection limits obtained were below 1 µg/g for most of the elements apart from Fe which was 3.5 µg/g.

4.1.2 Accuracy of the Method

From Table 4.2, it is evident that the preconcentration and digestion procedures are reliable as

reasonable agreement between the experimental values and the certified values is realized. For all elements, no significant difference is obtained at the 99% confidence level ($p < 0.01$) with the exception of the elements Cr, As and Pb. Nonetheless the discrepancies found between the experimental and certified values for As and Pb are probably due to the fact that the elements As and Pb have some contamination as well as the As $K\alpha$ and Pb $L\alpha$ peak overlap which makes a precise intensity extraction using the AXIL fitting procedure somewhat difficult.

Table 4.1 : Percentage mean recovery rates and Detection Limits obtained for the elements considered.

Element	Mean Recovery(%)	Detection Limit (µg/g)
Mn	99.2 ± 7.4	0.98
Ti	87.4 ± 6.6	ND
Fe	87.5 ± 2.5	3.56
Co	108.0 ± 10.9	0.53
Ni	83.1 ± 5.2	0.43
Cu	89.7 ± 3.1	0.60
Zn	81.5 ± 5.5	0.94
Pb	92.0 ± 2.9	0.90

ND - Not Determined

Table 4.2: Comparison of the experimental Elemental results obtained with the certified values for the Hair Certified reference material (CRM).

Element	analytical value (µg/g)	certified value (µg/g)
Cr	3.42 ± 0.29	4.77 ± 0.38
Mn	2.95 ± 1.95	2.94 ± 0.2
Fe	74.22 ± 8.5	71.2 ± 6.6
Co	0.34 ± 0.159	0.135 ± 0.008
Ni	3.89 ± 0.45	3.17 ± 0.46
Cu	20.57 ± 1.44	23.0 ± 1.4
Zn	165.0 ± 25.0	189.0 ± 8.0
As	1.72 ± 0.128	0.59 ± 0.07
Hg	3.13 ± 0.6	2.16 ± 0.21
Se	0.43 ± 0.173	0.58 ± 0.05
Pb	27.9 ± 0.9	7.2 ± 0.7

4.1.3 Precision

The mean Relative Standard Deviations (R.S.D) obtained were circa 11% for most of the elements between Cr and Pb indicating that the method can satisfactorily be used for determination of these elements in hair. Mn, Co and Se had RSDs above 30%. This is probably due to the fact that these elements were being determined close to their limits of determination hence an RSD of about 50% is expected.

4.2 Hair Samples

4.2.1 Trace Elements and Location

The trace element concentrations were grouped according to the location of the donor i.e., groups A, B, C and the controls. The summary statistics for the concentrations of all the groups are shown in Table 4.3 while the results for each individual group are shown in Tables A1.1 to A1.5. Table 4.4 shows the comparison of our results with those reported by Wandiga and Jumba, (1982).

From the summary Statistics for the concentrations of the elements in the analyzed samples (Table 4.3, A1.1 to A1.5), it is evident that the standard deviations of some elements eg., Fe, Zn and Cu are rather high. This is

possible as trace elements in hair are very individualistic and variation may occur from one strand to another and from one place to another on the head (Valcovic, 1977).

Large variations between the concentrations of one individual to another may be attributed to the possibility that most of the weights taken for the hair specimen from various donors may have consisted of hair segments that were different with respect to the distance from the scalp. This is because trace element content of hair is known to vary with the distance from the scalp (Campbell et al., 1981; Walta, 1982; Renshaw, 1972).

A statistical analysis of the data (t-test) reveals a significant difference at the 95% confidence level for elements Co, Ni Cu and Pb when the mean concentrations of Group A donors are compared with those of the control group. On the other hand Ni, Cu and Pb were significantly elevated in the control group while Co was higher in Group A donors. No significant difference was found between the two groups for the elements Cr, Mn, Fe, Zn, As, Se and Hg.

When the mean hair concentration levels for Group B donors were compared with those of the control, differences ($p < 0.05$) occur for the elements Cu, As, Hg, Se and Pb, which are significant.

In the case of Group C donors, significant differences were noted for the elements Fe, Cu, Zn and As.

When mean values for these elements were compared with those obtained for the control, Fe and Zn were significantly higher in Group C while Cu and As were lower.

From the brief statistical survey, it is clear that there is a significant variation of the non essential trace elements (Hg, As and Pb) from one location to another. The essential trace elements e.g., Mn, Co, Zn and Se do not differ very much from one donor group to the other. However, the mean concentrations of the essential trace elements Fe and Cu in all the Group donors differ appreciably from those obtained for the control group.

From Fig 4 which shows the variation of elemental concentration versus donor Location for the elements As, Pb and Hg, it is clear that these elements have their highest levels in the control group. This is then followed by group A and C while B has the lowest Level. This variation is not entirely unexpected since As, Pb and Hg are non essential trace elements and the elevated values probably reflect some environmental exposure which may be anthropogenic in origin particularly in the case of Pb and Hg.

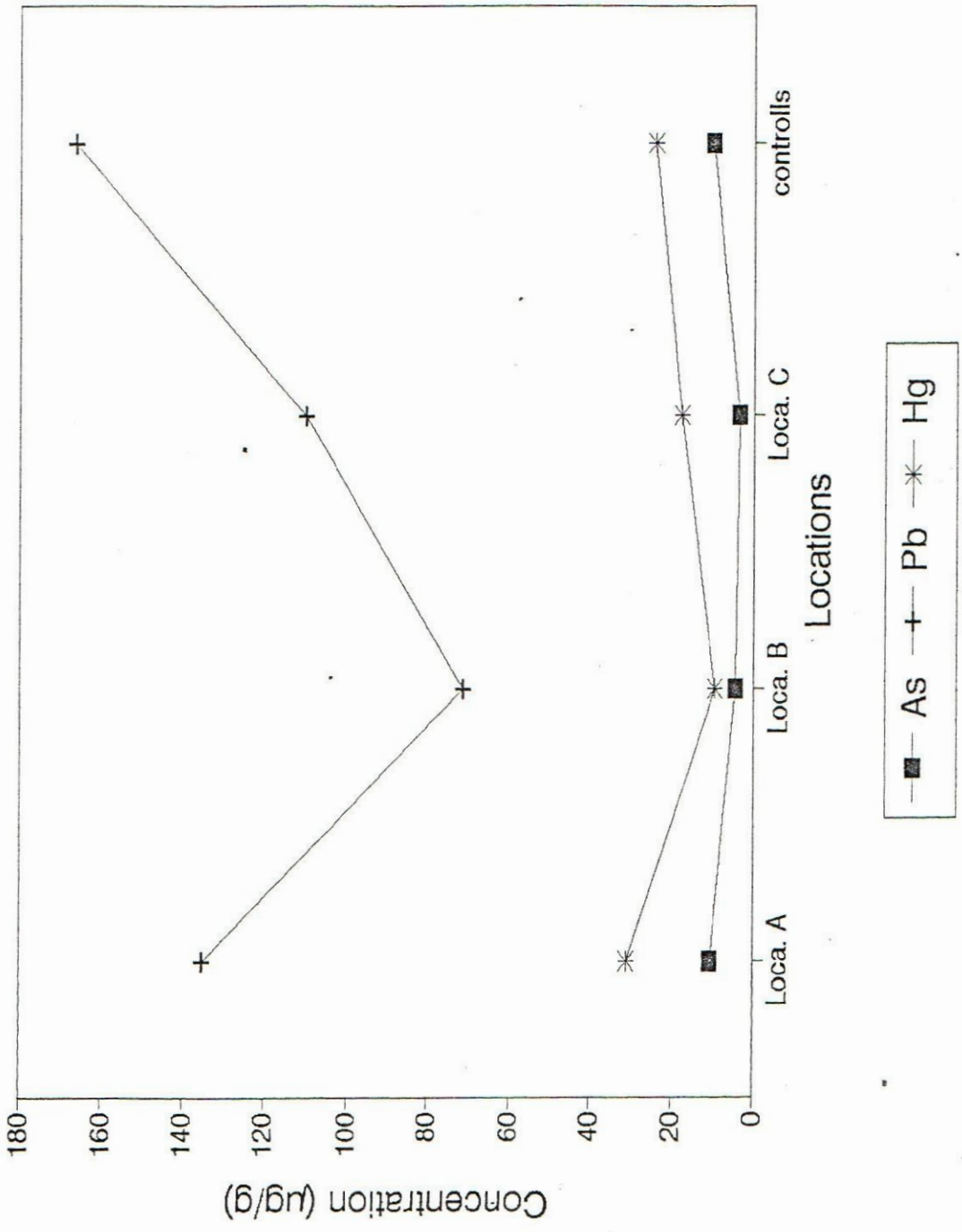


Fig 4: Variation of Concentration (µg/g) with donor Location for As, Hg and Pb.

Table 4.3: Comparison of Elemental Arithmetic mean concentrations (µg/g) in different groups (i.e., Donors of Locations A, B and C and Controls).

El.	A ± S.D n=26	B ± S.D n=18	C ± S.D n=15	control ± S.D n=8
Cr	21.9 ± 25.1	6.7 ± 6.6	11.0 ± 11.5	10.2 ± 2.5
Mn	22.1 ± 10.2	14.5 ± 10.4	25.3 ± 15.7	18.8 ± 16.7
Fe	289.0 ± 172.7	441.8 ± 216.4	596.7 ± 248.0	280.1 ± 160.1
Co	4.5 ± 0.7	2.9 ± 2.1	2.0 ± 1.4	1.3 ± 0.4
Ni	3.9 ± 2.6	7.1 ± 4.6	6.4 ± 5.2	11.1 ± 5.0
Cu	114.9 ± 13.6	42.3 ± 22.1	87.9 ± 37.7	171.6 ± 37.8
Zn	90.6 ± 50.3	169.3 ± 65.5	185.8 ± 65.2	106.3 ± 40.2
As	10.6 ± 7.0	4.1 ± 3.5	3.1 ± 2.6	10.0 ± 5.8
Hg	31.2 ± 8.8	9.1 ± 3.3	17.5 ± 9.7	24.2 ± 5.4
Se	0.8 ± 0.7	2.2 ± 1.9	1.6 ± 2.7	0.7 ± 0.2
Pb	135.1 ± 40.8	71.4 ± 46.5	110.1 ± 57.3	166.4 ± 42.5

El. refers to Element.

Table 4.4: Comparison of the results (concentrations µg/g) reported by Jumba and Wandiga (1982) using AAS with our results.

El.	Wandiga (µg/g)	Group A ±S.D (µg/g)	Group B ±S.D (µ/g/g)	Group C ±S.D (µg/g)	Control ±S.D (µg/g)
Zn	196.0 ± 32	90.6 ± 50.3	164.3 ± 65.5	185.8 ± 65.2	106.3 ± 40.2
Pb	52.0 ± 12.5	135.1 ± 40.8	71.4 ± 46.5	110.1 ± 57.3	166.4 ± 42.5
Cu	24.0 ± 6.2	114.9 ± 13.6	42.3 ± 22.1	87.9 ± 37.7	171.6 ± 37.8
Hg	12.2 ± 1.7	31.2 ± 8.8	9.2 ± 3.2	17.5 ± 9.7	24.2 ± 5.4
Fe	177.0	289.0 ±172.7	441.8 ±216.0	596.7 ±248.0	280.1 ±160.1

The values obtained for Mn were fairly constant in all the four groups with the highest value of (25.3 ± 15.7 µg/g) being in group C and the lowest value being 14.5 µg/g for group B. Othman and Spyrou, (1980) reported a mean value of 14 ± 11 µg/g for Mn in a study done in Machakos District of Kenya. This is consistent with our values which fall within their reported range particularly for group B and the control. The fact that the Mn concentration is fairly constant in the three donor groups and the control suggests that these concentration levels are probably dietary in origin.

Co and Ni levels were also fairly constant in all the groups. The highest value of Co (4.5 µg/g) was obtained from group A donors while the lowest value of (1.3 ± 0.4 µg/g) was obtained from the control group. In their study, Wandiga and Jumba (1982) did not consider these two elements neither did Othman and spyrou (1980) hence comparison of our data with any other existing data for Kenyan population was not possible. However, values in the range of 3.4 to 0.45 µg/g for Ni have been reported by Yurachek et al, (1969) for Caucasian populations.

Comparison of the data obtained in Group C with that obtained by Jumba and Wandiga, (1982) (see Table 4.4) shows that Zn is within the reported range while Cu and Fe are elevated in all the groups particularly in Group C. The Mean concentrations obtained for the Elements Fe and

Cu (Table 4.3) are higher than the values reported by Yuracheck et al, (1969) who reported values ranging from 12 - 2.72 µg/g for Fe while Cu was in the range 8.7 - 16.2 µg/g. These high values could indicate exposure of the donors to these two elements from the Environment. In the same study, Yuracheck reported values for Zn within a range of (181 - 246 µg/g), However our data for Zn is still somewhat lower and may probably reflect indigenous dietary factors.

Consideration of the mean concentrations obtained for Fe among the four locations reveal that Fe is significantly higher in Group C. These donors reside near a food Canning factory and hence traces of Fe could have been deposited on to their hair. The levels of Cu obtained in our study are high in all the Locations when compared to the value reported by Wandiga and Jumba (1982). The minimum value (42.3 µg/g) obtained in our results is higher by a factor of about two than that reported by Jumba and Wandiga. Copper is found in all human tissues (Harrison, 1969) and elevated levels of copper may occur from contamination of food stored or cooked in copper containers which are not kept clean or from ingestion of food to which copper has been added to keep the green colour of vegetables (Simpson, 1965). However these are not plausible reasons for our case since kenyan populations neither cook in Copper utensils nor add Copper

to their food.

Arsenic concentration is lowest in Group C (3.1 µg/g) and Highest in Group A (10.6 µg/g). Normal human hair has been reported to contain 0.3 to 0.7 µg/g of As (Underwood, 1971), while according to Smith (1964) and Bencko (1977), hair Arsenic concentrations greater than 3µg/g indicate extended exposure to Arsenic in cases of group examination. Thus it appears that the As levels obtained in our study (including the control Group) tend to point to some As exposure for Kenyan populations resident in Nakuru District. This view is supported by Hammer et al, (1971) who have shown that enhanced As values in hair reflect community exposure to Arsenic.

The amount of Pb reported by Jumba and Wandiga (1982) i.e., 52.0 ± 12.5µg/g falls below that obtained for Group A (135.1 ± 40.8 µg/g), the control group (166.4 ± 42.5µg/g) and Group C (110.1 ± 57.3 µg/g) but is within the range obtained for Group B (71.4 ± 46.5 µg/g). The high values of Pb obtained in these groups could be due to the fact that all these groups are located on a busy road and hence Pb from Automobiles could have been deposited on the hair of the donors. According to Valcovic (1975), Lead is normally present in the hair of healthy subjects but its concentration varies widely depending on the diet and environment.

Mercury is Lowest in Group B (9.2 µg/g) and highest

(31.2 µg/g) in Group A donors. The lowest value of Mercury (9.2 µg/g) falls within the range reported by Jumba and Wandiga (12.12 ±1.78 µg/g) while the highest value is above the range. High values obtained from our control group could possibly reflect the use of chemical agents applied to hair by the donors since the use of hair care products has been reported to have an influence on Mercury levels in hair (Yamaguchi et al, 1978).

4.2.2 Trace Elements and Age

The specimens were grouped according to age of the donor in intervals of five years i.e., Group1 :15 to 19 years, Group 2 :20 to 24 years, Group 3: 25 to 29 years, Group 4: 30 to 34 years, Group 5: 35 to 39 years, Group 6: 40 to 44 years. The variation of trace element concentration with donor Age group is shown in Table 4.5 while plots of Trace element concentrations versus donor age group are shown in Figures 5 to 10.

Table 4.5: Variation of Trace elements concentration (µg/g) with donor Age Group.

El.	Group1 n = 10	Group2 n =20	Group3 n = 8	Group4 n = 10	Group5 n =10	Group6 n = 9
Cr	14.6 ± 2.4	12.2 ± 2.4	11.7 ± 4.7	17.8 ± 6.2	5.2 ± 2.3	10.0 ± 3.0
Mn	19.4 ± 3.3	18.2 ± 6.3	17.6 ± 4.5	24.8 ± 5.5	18.8 ± 8.4	25.0 ± 8.2
Fe	269.9 ± 183.0	388.3 ± 83.4	461.4 ± 84.3	400.7 ± 61.9	599.0 ± 98.1	443.9 ± 103.6
Co	3.8 ± 1.5	2.6 ± 0.9	1.5 ± 0.6	1.5 ± 0.4	1.9 ± 0.8	2.1 ± 1.4
Ni	11.1 ± 2.6	7.4 ± 2.2	13.2 ± 6.1	3.0 ± 1.0	5.7 ± 1.9	5.0 ± 2.7
Cu	45.6 ± 26.0	89.1 ± 26.5	87.8 ± 38.8	102.9 ± 19.2	88.7 ± 17.0	116.9 ± 20.3
Zn	156.7 ± 41.8	153.7 ± 28.6	111.1 ± 33.2	105.8 ± 35.6	150.7 ± 31.8	119.2 ± 69.4
As	5.1 ± 3.6	6.7 ± 2.3	5.2 ± 0.9	5.4 ± 3.2	5.0 ± 1.7	11.5 ± 9.0
Hg	11.6 ± 6.2	17.8 ± 4.5	14.2 ± 8.9	24.7 ± 5.3	26.6 ± 9.3	29.9 ± 14.0
Se	1.8 ± 0.8	1.9 ± 1.0	2.6 ± 2.3	1.4 ± 0.2	0.8 ± 0.2	0.3 ± 0.1
Pb	49.6 ± 34.5	101.2 ± 27.1	103.1 ± 32.6	142.8 ± 11.7	135.9 ± 23.4	139.3 ± 20.1

El refers to Element

n refers to the number of samples.

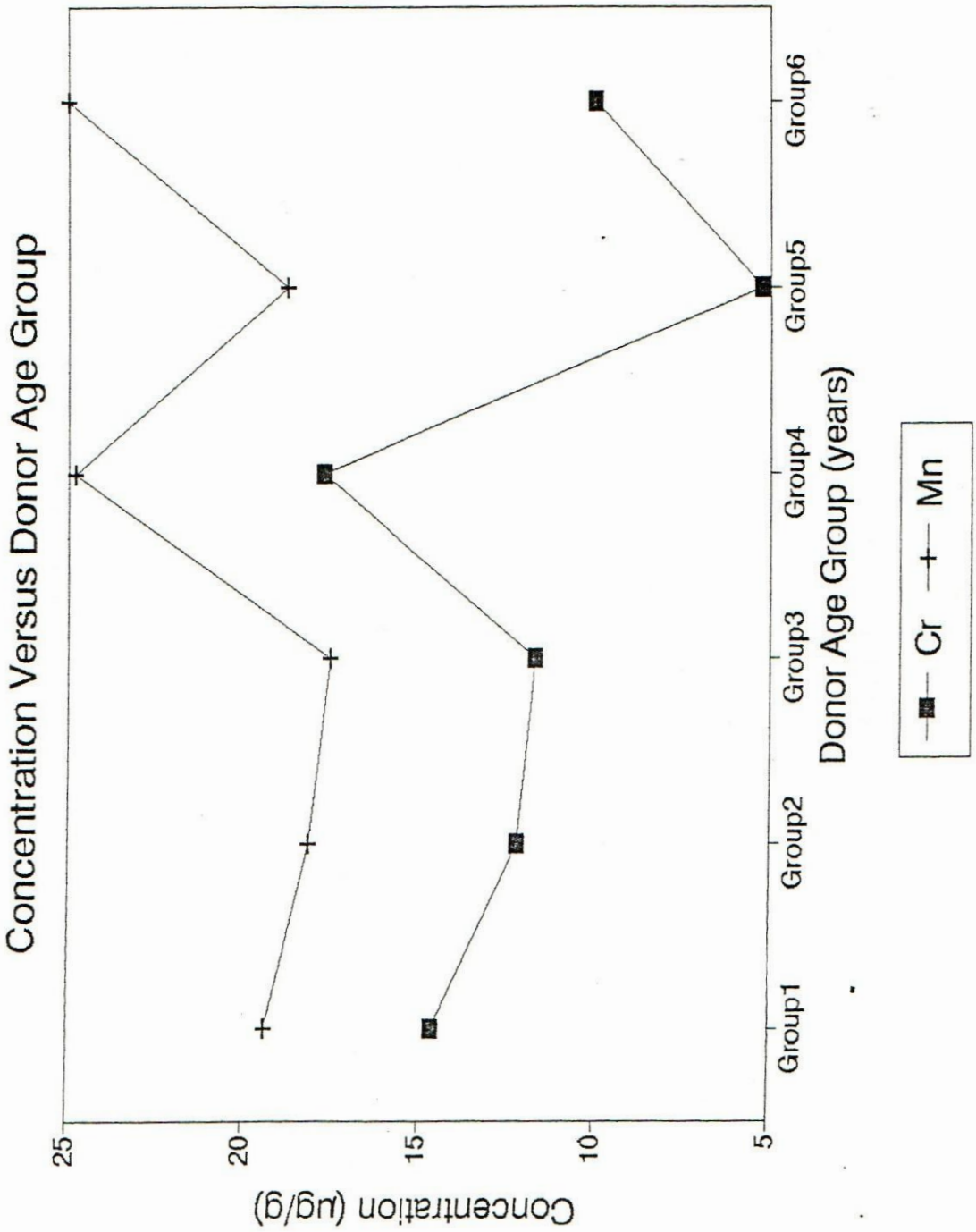


Fig 5: Variation of concentration (µg/g) with donor Age group for Cr and Mn.

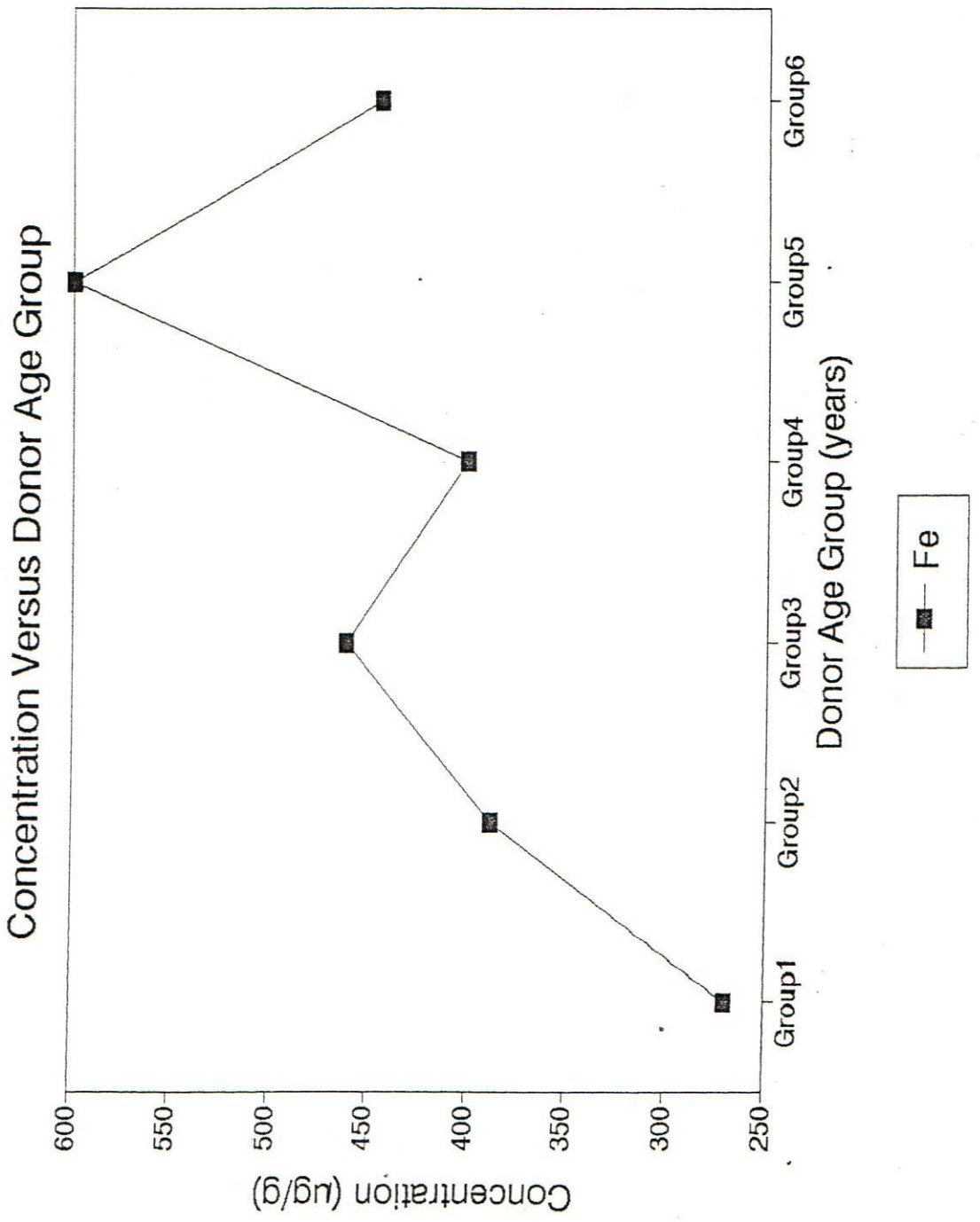


Fig 6: Variation of Concentation ($\mu g/g$) with donor age group for Fe.

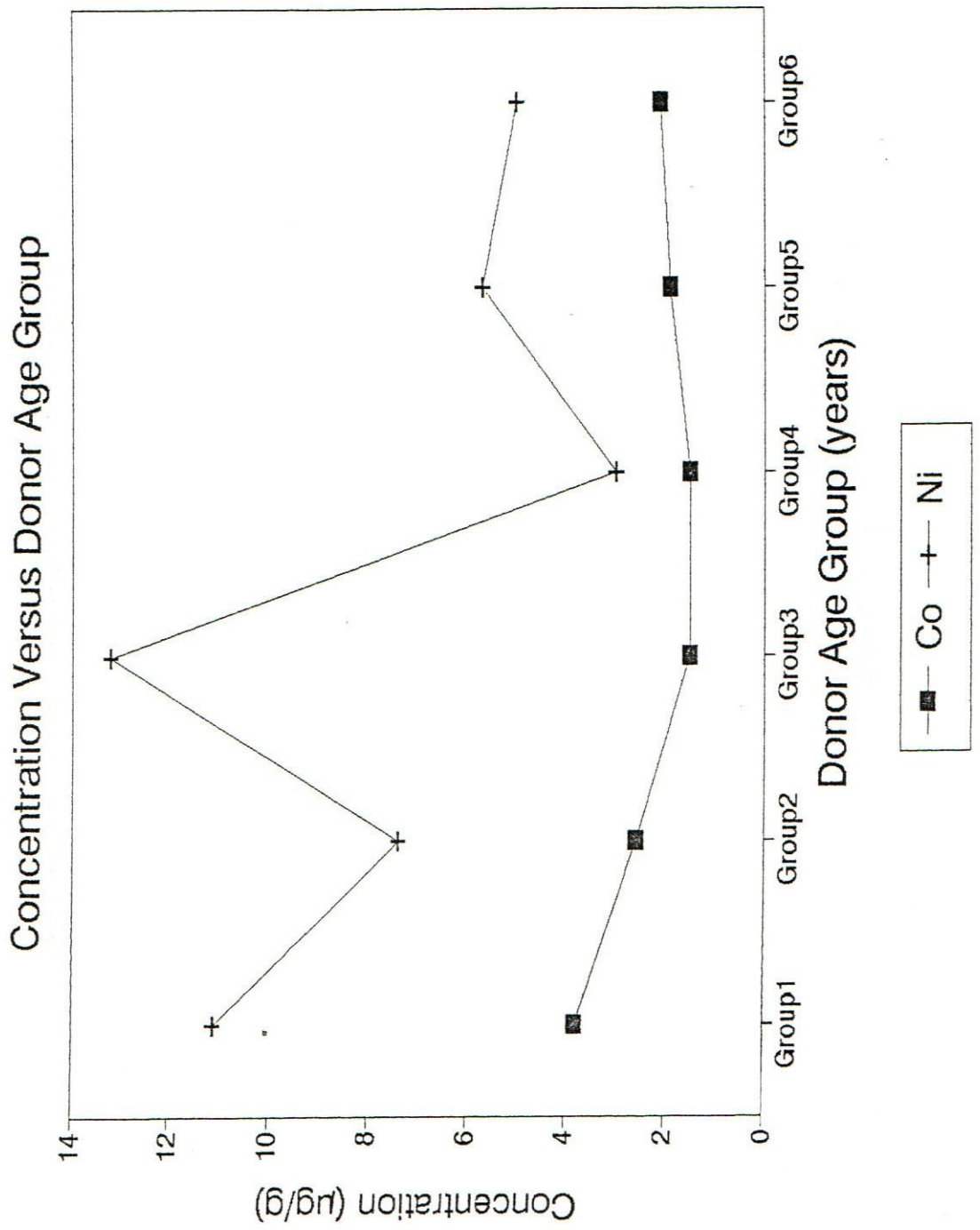


Fig 7: Variation of Concentration ($\mu g/g$) with donor age group for Ni and Co.

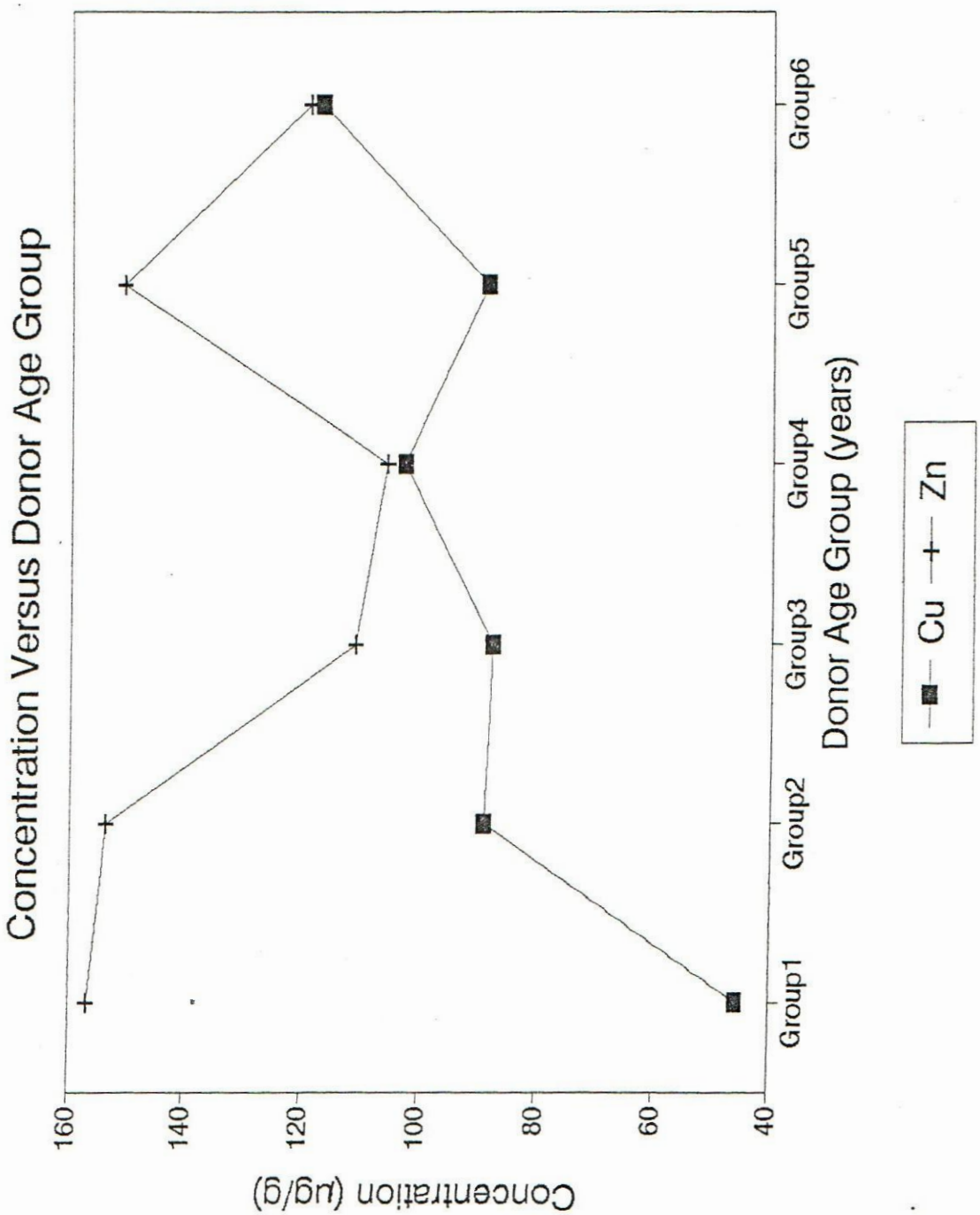


Fig 8: Variation of Concentration (µg/g) with donor age group for Zn and Cu.

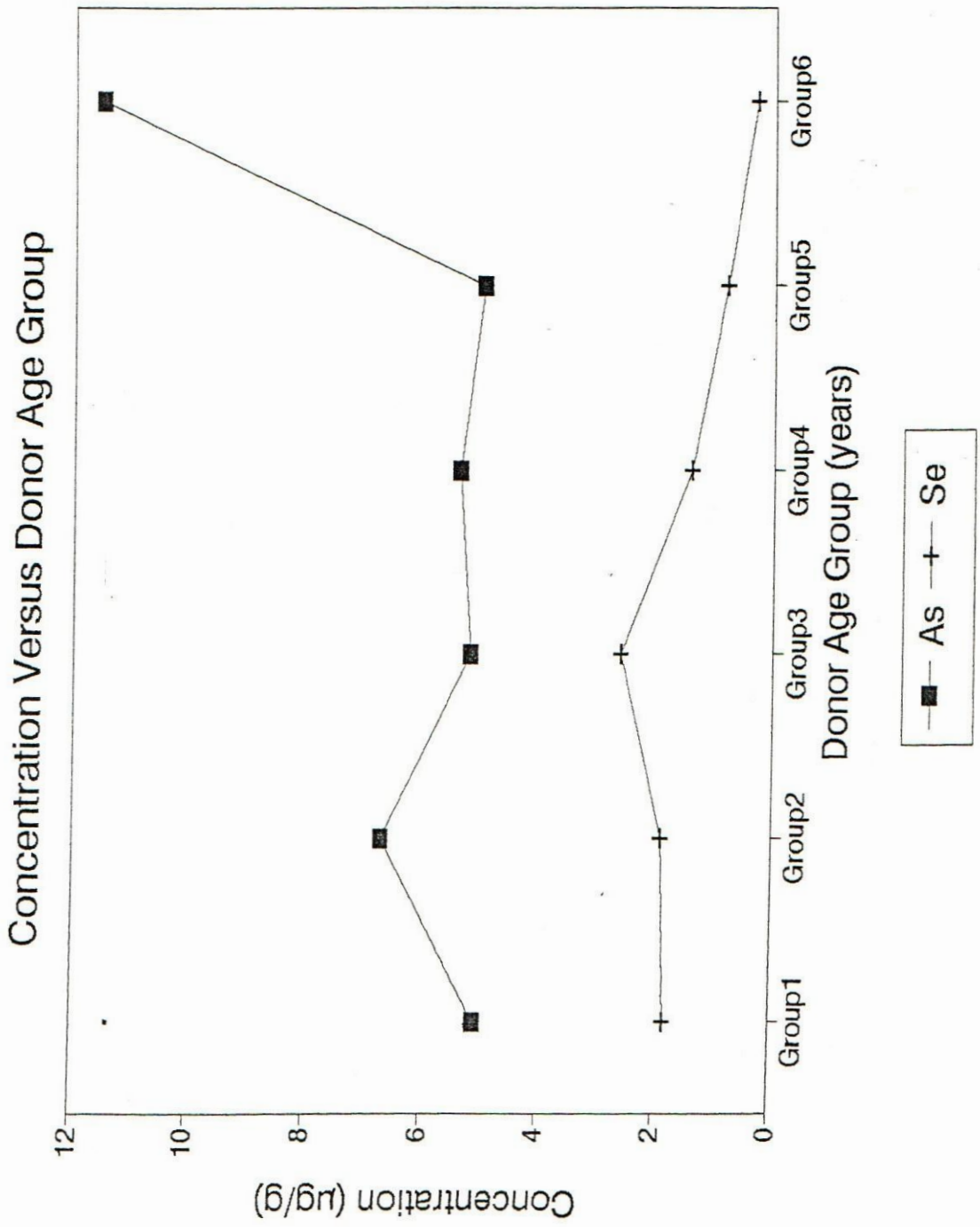


Fig 9: Variation of Concentration (µg/g) with donor age group for Se and As.

Fig 5 shows the variation of concentration with donor age group for Cr and Mn. From the graph it is evident that both Cr and Mn fluctuates about a mean of 12 µg/g and 21 µg/g respectively with the highest deviation for Cr occurring between ages 30 - 34 years of 18 µg/g and lowest at ages 35 - 39 years of 5 µg/g. In general, we can say that Cr is constant at 12 µg/g but varies in the range 5 to 18 µg/g over the age 20 to 45 years. It can also be observed that both elements tend to have high and low concentrations corresponding to the same age group eg., Mn has a high concentration in group4 and so does Cr. Both elements have low concentrations in group 5.

Variation of the concentration with age for Fe is shown in Fig 6. Its variation starts with a low concentration value of 259.9µg/g in group 1, and then gradually increases to 600µg/g in group 5 before decreasing to 443 µg/g in donor age group 6. In contrast to our observations, Eltayeb and Van Grieken, (1990) in a study done on Sudanese population reported a decrease in concentration of Fe from a mean of 505 µg/g for children and adolescents to a mean of 161 µg/g for old Farmers.

The general variation of the concentration of Ni with Age (Fig. 7) shows that there is a general decrease of concentration with donor age group, with the exception of an elevated concentration of 13.2 µg/g in donor age Group 3. From the study done by Shroeder and Nason, (1969) no

observable variation was noted for Ni when mean concentrations of the trace elements in the hair of subjects in the first three decade of life were compared with those of the subjects aged 40 and 70 years. However our observation differs from theirs probably due to the range of age considered. There appears to be no variation in the concentration of Co with age (Fig. 7).

The Zn concentration is fairly constant with the variation of age and appears to fluctuate about a possible mean of 135µg/g (Fig. 8). This is in agreement with the study done by Schroeder and Nason, (1969) who examined scatter diagrams and curves constructed from the mean of all values and age and failed to reveal significant changes with age in Zn.

The concentration of Cu start increasing from a value of 45.6 µg/g in Donor age group 1 and rises gradually to a value of 119 µg/g in donor age group 6 (Fig 8). Eltayeb and Van Grieken, (1990) observed a slight decrease in concentration with age for Cu from 18.5 to 12.4 µg/g, in contrast to our results which seem to indicate an increase of Cu levels in hair with donor age.

Se in hair appears to behave like Co and Zn which were fairly constant. The variation is fairly constant with an elevated concentration in donor age group 3 (Fig.9).

Arsenic displays no variation of concentration with

donor age as can be observed in Fig 9. However, there is an elevated concentration corresponding to donor age group 6. This could be attributed to the fact that As is a non Essential trace element which could have been deposited on the hair of the group 6 donors after prolonged exposure to this element.

Fig. 10 shows the variation of concentration with age for Pb and Hg. In case of Pb, the concentration gradually increases from a value of 49.6 µg/g in donor group1 to a high value of 142.8 µg/g in donor group4 before it stabilises at a value of about 135 µg/g in donor group 6. The data reported by Peterring et al, (1973) showed that Pb declined from a high value of 25µg/g at the age of 2 years to a low value of 10 µg/g at the age of 85 years. This was in the case of males. This trend was not observed in our results within the range considered.

There is a gradual increase of Mercury from 11.6 µg/g in donor age group 1 to 29.9 µg/g in donor age group 6 with an exception of donor group 3 which has a decreased value of 14.2µg/g.

4.2.3 Trace element and Sex

Male and female hair concentrations from all the locations were averaged and the results tabulated as shown in Table 4.6. A plot of the elemental log concentrations

against the elements for both Males and Females is shown in Figure 11.

The elements Cr and Fe were slightly higher in men than in female whereas the elements Cu, Zn, Hg and Pb were elevated slightly in female than in males (Table 6)

Using statistical methods, a comparison of the arithmetic mean and the standard deviation of trace element concentration between male and female for the population was carried out to test the significance of the difference between means of the concentrations. It was observed that no significant difference existed between concentrations of the two groups at the 95% confidence level. This is in contrast with the results reported by Schroeder and Nason, (1969) who observed a higher concentration in Cu, Co and Ni in Female than in Male. Our results reveal that these three elements are also slightly higher in female than in male though the difference is not significant. However, they also observed no significant difference in the concentration between Male and Female for the elements Zn, Cr and Pb. This observation agrees with our Data at the 95% confidence level.

Our observations tally with the observation made by Othman and Spyrou, (1980) who also found no significant difference, except for Ca and Mn, in elemental concentrations in hair between male and female populations of Machakos District Kenya.

Table 4.6: Comparison of the trace elements concentrations with sex.

El.	Male Mean Conc.± S.D (µg/g)	n	Female Mean Conc.±S.D (µg/g)	n
Cr	13.4 ± 7.7	50	11.2 ± 8.6	16
Mn	19.2 ± 12.7	51	19.1 ± 12.9	18
Fe	429.6 ± 225.1	54	388.7 ± 146.0	20
Co	2.4 ± 1.8	52	3.0 ± 3.2	19
Ni	8.2 ± 5.9	46	8.3 ± 5.3	16
Cu	84.6 ± 46.9	53	92.6 ± 38.4	19
Zn	139.9 ± 69.6	54	164.0 ± 58.7	20
As	5.6 ± 5.6	54	3.4 ± 4.5	19
Hg	19.4 ± 12.2	52	21.8 ± 10.1	20
Se	0.8 ±	52	1.3 ± 1.3	20
Pb	106.9 ± 58.0	54	124.8 ± 51.6	20

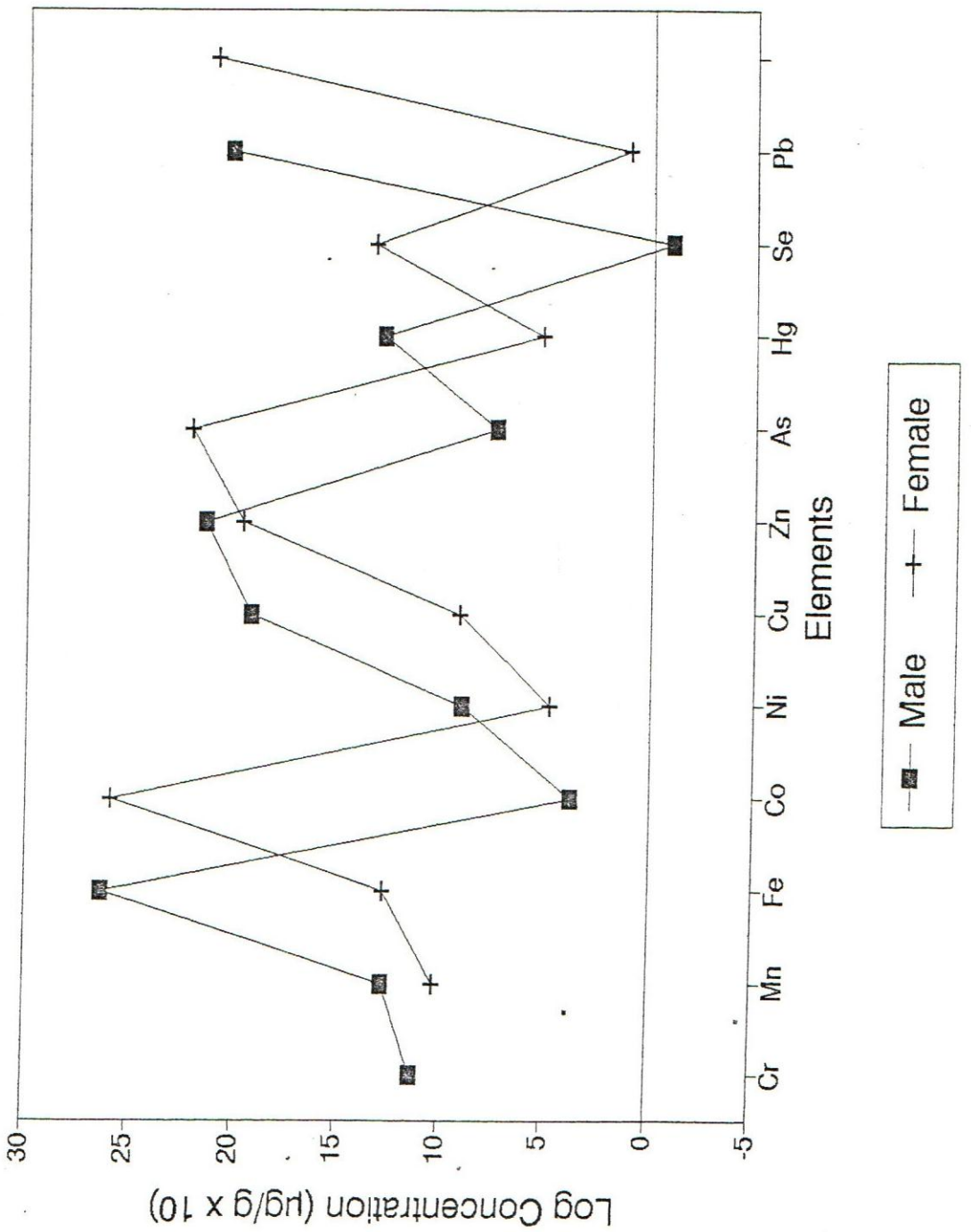


Fig 11: Elemental concentration (µg/g) for Males And Females between Ages 15 to 44.

Wilhelm and Ohnesorge, (1990) reported that Females had more Zn in both scalp hair and Pubic hair than males. Further sex related differences were only observed in Pubic hair with higher Cd, Cu and Pb levels for Males compared with Females. This study was carried out in Germany in the Urban and rural areas surrounding Bonn.

4.2.4 Correlation analysis of Trace elements in hair

In order to examine any relationship that may exist among trace element, correlation test of all possible pairs of elements were applied. This was done because there are reported results which suggest (Ryabukhin, 1978 and Moon et al, 1988) that metal accumulation, or patterns of metal accumulation, in human scalp hair may have value in tracing environmental exposure from industrial sources. We examined metal correlation matrices in greater detail in order to examine any relationship that may exist among the trace elements. The correlation analysis was done for every Location and for the whole population. Table 4.7 shows the correlation matrix for the whole population considered while a plot of the significantly correlated pairs of Trace elements at the 99% confidence level is shown in Figure 12.

Table 4.7: Pearson correlation matrix for the whole population.

	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Hg	Se	Pb
Cr	1.00										
Mn	0.14	1.00									
Fe	-0.28	0.17	1.00								
Co	-0.26	-0.63*	-0.29	1.00							
Ni	0.04	-0.58*	-0.78*	0.65*	1.00						
Cu	-0.11	0.33	-0.06	-0.36	0.01	1.00					
Zn	-0.25	-0.26	0.68*	-0.04	-0.32	-0.52	1.00				
As	0.17	0.39	-0.20	-0.55	-0.17	0.78*	-0.65*	1.00			
Hg	0.01	0.44	-0.15	-0.44	-0.04	0.97*	-0.65*	0.85*	1.00		
Se	-0.20	-0.75*	0.04	0.74*	0.33	-0.65*	0.44	-0.60*	-0.75*	1.00	
Pb	-0.14	0.34	0.14	-0.36	-0.17	0.96*	-0.48	0.77*	0.94*	-0.61*	1.00

(*: $r > 0.35$, $p < 0.001$)

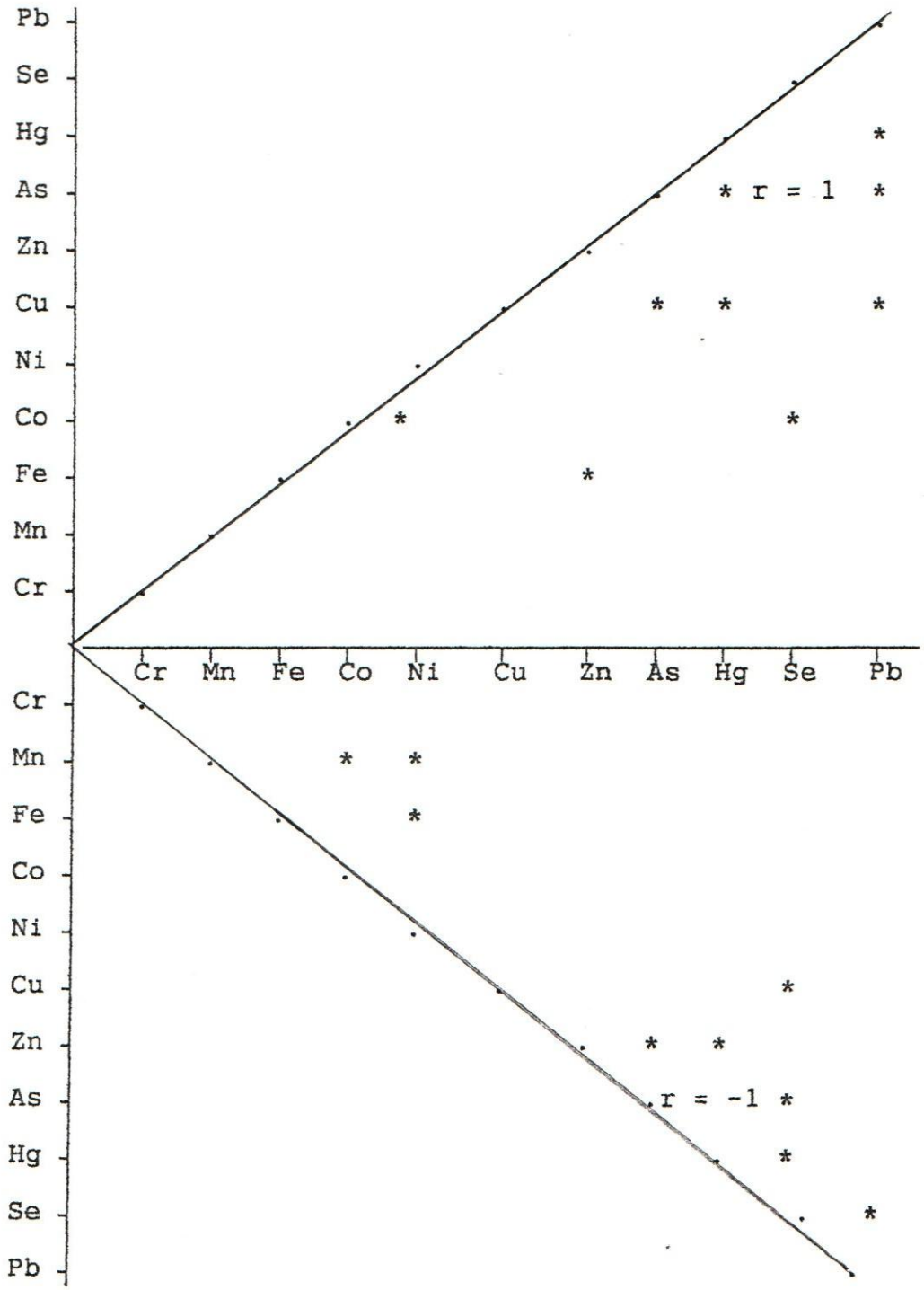


Fig 12: Significantly correlated pairs of Trace Elements at 99% confidence level.

The elements Cu and Pb were significantly correlated in all the four locations apart from Location A and also in the whole population. This is in line with the results reported by Baker et al, (1976) who found positive correlations between Cu and each of Pb and Zn. He also reported a negative correlation between Zn and Pb. Creason and Co- workers, (1975) have also reported that the elements that best correlate in children and adults are Cd, Pb and Cu. Michalis and Xenophan, (1990) also found high positive correlations between Pb, Cr and Cu.

Another set of significantly correlated elements revealed in this analysis is that of cu and Fe which was significantly correlated in three of the five locations considered i.e., locations A ,B and C. No significant correlation was observed between the two elements in the other locations and in the whole population.

When the whole population was considered, nineteen significant ($p< 0.001$) correlation pairs were observed (Table 4.7) where Cu was positively correlated with As, Hg and Pb and negatively correlated with Se. Arsenic was positively correlated with both Hg and Pb while negatively correlated with Se. Mercury and Lead were also found to be highly correlated.

The hair samples were thoroughly washed as described in the experimental procedure hence it is unlikely that

these significant correlations are due to dust or soil contamination. The results indicate that the correlation matrices may be of value in assessing simultaneous exposure to various trace elements in the environment.

4.2.5 Principal component extraction of trace elements in hair

In an effort to understand the above intercorrelations better, principal component analysis was performed for all the locations as well as for the whole population. The principal component analysis was performed on the correlation matrix with extraction of five components (accounting for 84.24% of the total variance) under Varimax rotation. The rotated component loadings for the whole population are shown in Table 4.8. A five component solution was arrived at by considering the number of components accounting for more than 80 % of the total variance.

Table 4.8 : Five component loadings accounting for 84.24% of the total variance for the total population.

Components	1	2	3	4	5
Variance(%)	43.63	20.14	12.05	4.67	3.75
Variables					
Cu	0.943	0.037	0.219	0.121	0.200
Pb	0.930	0.243	0.166	0.117	0.142
Hg	0.872	0.039	0.341	0.046	0.342
As	0.626	0.070	0.337	-0.020	0.492
Ni	0.094	-0.690	-0.532	-0.037	0.311
Fe	0.037	0.649	0.087	0.117	-0.726
Co	-0.220	-0.056	-0.858	0.123	0.178
Se	-0.489	-0.003	-0.829	0.091	-0.161
Mn	0.105	0.489	0.792	-0.083	0.225
Cr	-0.063	-0.133	0.162	-0.954	0.152
Zn	-0.385	0.078	-0.074	0.139	-0.885

Principal component Analysis of all the population considered revealed that Cu, Pb, Hg and As were highly loaded in component 1 (Table 4.8) which accounts for 43.63% of the total variance. Hg, As and Pb are non essential trace elements and their concentration in the environment could be high due to the activities of man. Hence component 1 may have been due to the anthropogenic sources. The elements Ni and Fe are highly loaded in component 2 which accounts for 22.14% of the total variance. Component 2 may probably be attributed to the drinking water which happen to contain a fairly high amount of Fe. The essential trace elements Se, Co and Mn are highly loaded in component 3 which accounts for 12.05% of the total variance. These elements are easily obtained from the diet and hence factor 3 could probably be associated with diet. The components 4 and 5 account for very low percentage of variance and it is difficult to explain these two components.

When principal component analysis of individual location was considered, it was observed that different elements are highly loaded in different components, this suggests that there are variations in sources of particular elements from location to location implying that there is an environmental Factor involved. It also reveals that there is a variation of elemental concentration from one location to the other.

CHAPTER FIVE

5.0 CONCLUSIONS AND RESEARCH RECOMMENDATIONS

5.1 CONCLUSIONS

Energy dispersive X-ray Fluorescence analysis combined with mineral acid digestion and co-precipitation with NaDDTC was successfully applied for the analysis of trace elements in hair.

Most of the Elements considered were quantitatively retained with recoveries better than 87% while the Detection limits are in the range 3.5 to 0.4 µg/g for most of the elements considered while Pb was somewhat higher. Comparison of the analytical values with the Certified values revealed no significant difference at the 99% confidence level ($p< 0.01$) for most of the Elements. The RSDs obtained were circa 11% for most of the elements indicating that the method could satisfactorily be used for the determination of these elements in Hair.

Comparison of the data obtained for donors from the four locations in Nakuru District suggests that there is possible Arsenic exposure in these four donor communities groups. The concentrations of the toxic Elements e.g., Pb and Hg were found to be elevated in group A donors and the controls. A part from Fe and Cu which were elevated in

group C donors only, the other essential trace elements did not differ appreciably in the donors. The marked variation of the concentrations of the elements As, Pb, Hg ,Fe and Cu in the donors shows that an environmental factors may be responsible for this variability.

It was established that the concentration of the elements Cr, Mn, Co, Zn and Se do not vary appreciably with donor Age. The concentrations of elements Fe, Cu, Pb and Hg increased with donor age while Ni decreased with Age. No significant difference was observed between the elemental concentrations of both Male and Female donors at the 95% confidence level. However, the elements Cr and Fe were slightly higher in men than in female whereas the elements Cu, Zn, Hg and Pb were elevated slightly in female than in males.

The correlation of each pair of elements in the hair samples for the whole population was investigated and revealed that Cu was positively correlated ($p < 0.001$) with As, Hg and Pb while it was negatively correlated with Se. Arsenic was found to be positively correlated with both Hg and Pb and is negatively correlated with Se. Mercury and Lead were also found to be highly correlated with each other.

Principal component Analysis of all the population considered revealed that Cu, Pb, Hg and As were highly loaded in component 1 which accounted for 43.63% of the

Total variance, This component was attributed to the anthropogenic sources. The elements Ni and Fe were found to be highly loaded in component 2 which accounted for 22.14% of the total variance. Component 2 was associated with the drinking water which happen to contain a reasonable amount of Fe.

From correlation and Principal component analysis it can be concluded that since the elements Cu, Pb and Hg are highly correlated and loaded on the same component, their increase with donor age could possibly be associated with prolonged exposure of the donor to these elements.

5.2 RESEARCH RECOMMENDATIONS

(1) It is necessary to establish the trace element concentrations in Air by analysing the Aerosols. A study of the correlation between the concentration of trace elements in Hair and Air should then be done so that the effect of trace elements concentrations in Air to the levels of trace elements in Hair can be assessed.

(2) A study has been done on the determination of trace elements in surface and ground waters in Nakuru district. It would be worthwhile to establish the contribution of the reported trace element

concentrations in water to the reported values of trace element concentrations in Hair.

Recommendations 1 and 2 should be able to help us establish which of the two, Air and Water, made the greatest contribution to the levels of metals found in the Hair.

(3) In order to have a comprehensive report on the variation of trace element concentration with donor age, a study should be done using samples donated by young children of below 15 years and adults of over 45 years who were not considered in this study.

(4) A study of the variation of trace element concentrations with distance from the scalp would be appropriate in order to establish any variation. A reported variation would help us establish whether there has been a deposition of these elements in hair.

(5) Concentrations of trace elements in the chemical agents applied to the Hair should be established.From the established levels, it would be possible to find out whether these levels have any effect on the trace elements concentrations reported for the samples from Nakuru District.

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APPENDIX

TYPICAL RESULTS OBTAINED FROM VARIOUS LOCATIONS.

Table A1.1: Typical results from location A for the elemental analysis of Hair with EDXRF (age) of donors was between 19 and 43).

El.	Range (µg/g)	A. Mean ± S.D (µg/g)	G.Mean	Median
Cr	102.3 - 4.1	21.9 ± 25.1	12.7	14.1
Mn	36.7 - 3.1	22.1 ± 10.2	18.6	24.0
Fe	761.0 - 201.6	289.0 ± 172.7	382.5	359.1
Co	5.5 - 3.6	4.5 ± 0.7	4.4	4.3
Ni	8.0 - 0.7	3.9 ± 2.6	4.1	5.1
Cu	129.7 - 86.6	114.9 ± 13.6	114.1	117.5
Zn	167.9 - 12.1	90.6 ± 50.3	70.2	96.8
As	25.7 - 2.6	10.6 ± 7.0	8.3	8.6
Hg	50.6 - 14.9	31.2 ± 8.8	29.9	31.8
Se	2.6 - 0.2	0.8 ± 0.7	0.7	0.6
Pb	188.9 - 121.5	135.1 ± 40.8	144.5	136.8

El. = Element.

Table A1.2 : Typical results from Location B for the elemental analysis of hair with EDXRF (Age was between 20 and 35 years).

El.	Range (µg/g)	A. Mean ±S.D (µg/g)	G.Mean (µg/g)	Median (µg/g)
Cr	21.0 - 0.6	6.7 ± 6.64	3.4	4.3
Mn	38.9 - 1.3	14.5 ± 10.39	10.6	11.0
Fe	780.0 - 201.6	441.8 ± 216.41	388.5	415.5
Co	7.9 - 0.1	2.9 ± 2.12	2.0	2.9
Ni	15.8 - 0.5	7.1 ± 4.56	5.0	7.5
Cu	70.0 - 19.2	42.3 ± 22.05	36.9	27.9
Zn	272.1 - 62.5	169.3 ± 65.50	150.2	131.1
As	12.5 - 1.4	4.1 ± 3.49	3.0	2.6
Hg	14.8 - 5.5	9.1 ± 3.29	8.6	7.8
Se	5.9 - 0.0	2.2 ± 1.88	1.2	1.8
Pb	135.7 - 21.1	71.4 ± 46.46	55.0	70.5

Table A1.3: Typical results from Location C for Elemental Analysis of Hair with EDXRF (age of the donors was between 18 and 30 years).

El.	Range (µg/g)	A.Mean ±S.D (µg/g)	G.Mean (µg/g)	Median (µg/g)
Cr	40.0 - 0.2	11.0 ± 11.5	4.2	7.3
Mn	48.1 - 2.8	25.3 ± 15.7	19.9	28.7
Fe	1197.1 -199.6	596.7 ± 248.0	508.2	561.2
Co	15.6 - 3.1	2.0 ± 1.4	5.0	4.3
Ni	19.6 - 0.7	6.4 ± 5.2	6.1	6.7
Cu	198.8 - 39.7	87.9 ± 37.7	80.8	83.6
Zn	257.4 - 45.6	185.8 ± 65.2	153.6	190.6
As	8.1 - 0.1	3.1 ± 2.6	2.8	2.4
Hg	31.6 - 5.0	17.5 ± 9.7	15.9	20.5
Se	6.2 - 0.3	1.4 ± 1.6	0.8	0.7
Pb	245.4 - 33.1	110.1 ± 57.3	95.8	118.3

Table A1.4: Summary of the results obtained from samples of the Location D donors (Age was between 16 and 20 years.

El.	Range µg/g	A.Mean ±S.D µg/g	G.Mean µg/g	Median (µg/g)
Cr	25.9 - 5.3	13.2 ± 5.6	12.1	11.6
Mn	30.0 - 2.2	17.3 ± 8.8	13.4	19.3
Fe	472.2 - 117.5	257.7 ± 126.4	228.8	227.7
Co	7.3 - 0.4	3.2 ± 2.3	2.2	2.7
Ni	17.5 - 5.1	11.6 ± 3.5	11.0	10.8
Cu	79.4 - 22.9	40.4 ± 20.2	33.3	29.5
Zn	210.8 - 31.8	134.6 ± 62.6	133.6	144.0
As	10.6 - 0.4	3.7 ± 3.1	2.7	2.6
Hg	25.5 - 5.3	11.0 ± 5.7	9.4	8.2
Se	5.4 - 0.3	1.9 ± 1.5	1.3	1.6
Pb	113.9 - 15.3	47.5 ± 36.9	35.5	23.4

Table A1.5: Typical results from the controls for the Elemental analysis of Hair with EDXRF (Age was between 21 and 28 years).

El.	Range (µg/g)	A. Mean ±S.D (µg/g)	G.Mean (µg/g)	Median (µg/g)
Cr	21.0 - 0.4	10.2 ± 2.5	9.9	10.0
Mn	43.3 - 1.6	18.8 ± 16.7	9.7	15.1
Fe	552.0 - 45.2	280.1 ± 160.1	208.1	288.9
Co	2.5 - 0.6	1.3 ± 0.4	1.2	1.2
Ni	17.2 - 0.7	11.1 ± 5.0	9.9	11.2
Cu	230.1 - 86.6	171.6 ± 37.8	167.7	163.9
Zn	193.6 - 20.2	106.3 ± 40.2	95.6	118.9
As	19.7 - 1.2	10.0 ± 5.8	8.6	10.8
Hg	91.8 - 19.5	34.2 ± 21.8	29.8	23.3
Se	2.5 - 0.3	0.7 ± 0.2	0.8	0.7
Pb	229.1 -112.9	166.4 ± 42.5	161.2	158.5