HEAVY METAL ANALYSIS OF CONSUMABLE CLAYS AND TISSUE SAMPLES OF CLAY FED RATS BY X-RAY FLUORESCENCE.

BY

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A thesis submitted in partial fulfilment for the Degree of Master of Science in the University of Nairobi.

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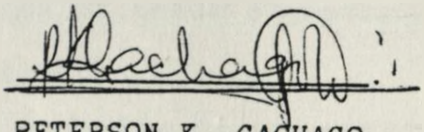
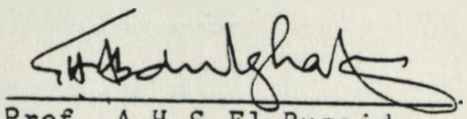
This thesis is my original work and has not been presented for a degree in any other university.



PETERSON K. GACHAGO

This thesis has been submitted for examination with my approval as a university supervisor.



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DEDICATION.

This thesis is dedicated to my daughter, Sylvia who came into my life in the course of this work.

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SUMMARY.

The consumption of clay by pregnant women is not abnormal and has been in existence for a long time. The habit is believed to be due to lack of enough iron in the body. However, while serving the good purpose of replacing body nutrients, excessive consumption of this clay may increase to toxic levels the content of some harmful trace elements in the body tissues.

In this study, consumable clay samples obtained from Gikomba Market, Nairobi (GIK I and GIK II) and a shop along Biashara street (Mahti sample, imported from India) were analysed for trace metal content using x-ray fluorescence technique. Similarly, tissues from rats fed with Mahti clay and control group were analysed.

The results of the clay analysis showed the following elements to be present in all the clay samples: Iron (2.65 - 3.83) x 10^4 µg/g, Zinc (33.0 -133.7 µg/g), Lead (5.5 -15.3 µg/g), Zirconium (54.8 - 1500.0 µg/g), Manganese (45.6 - 1310.0 µg/g), Strontium (29.1 - 46.8µg/g), Gallium (8.5 - 30.0 µg/g), Yttrium (8.5 - 79.9 µg/g), Titanium (0.287 - 4.86) x 10^4 µg/g and thorium (3.5 - 26.7 µg/g). In addition, the Mahti sample had substantial amounts of vanadium, nickel, chromium and bromine while the Gikomba samples had calcium and rubidium.

The rats were fed with food supplements of the Mahti sample for 37 days (phase 1), 74 days (phase 2) and 110 days (phase 3). After every phase, the following tissues were dissected: liver, spleen, kidney, heart, whole blood and brain. In the liver, levels of: iron (226.5 - 544.1 µg/g), nickel (0.74 - 1.83 µg/g), lead (0 - 2.8 µg/g), selenium (0.8 - 1.6 µg/g) and manganese (1.3 - 7.8 µg/g) increased progressively from phases 1 to 3. Similarly, levels of: iron (655.4 - 2272.8 µg/g), lead (1.9 - 2.5 µg/g) and manganese (3.4 -11.8 µg/g) increased in the spleen.

Lead and manganese were also found to increase in the kidneys (phase 1 to 3) of the clay fed rats. In these tissues, there were significant differences between the clay fed and the controls which had lower values. A significant difference ($p < 0.01$) between the controls and clay fed rats was found in the liver (phase 1 and 3) and the spleen (phase 3). In the other organs including the heart, the brain and the blood, there was no major increase during the entire feeding period.

The effect of mahti sample on growth rate was also assessed. The rats were weighed twice a week. During the period of the study, the clay fed rats increased weight by 77.5% (phase 1), 160.5% (phase 2) and 204.0% (phase 3) while the control group increased by 105.0%

(phase 1), 190.1% (phase 2) and 231.5% (phase 3). The consumption of this clay may have led to the reduced growth in the clay fed rats.

CHAPTER ONE

1.0 INTRODUCTION

Fifty percent of the 103 chemical elements found in nature are present in human body tissues and fluids [1]. It is well recognized that small quantities of trace metals contribute remarkably to healthy plant and animal life. These essential biological metals, as they have come to be referred, are infact the inorganic counterparts of essential biological organic nutrients, namely the vitamins. Unlike the vitamins, they cannot be synthesized by living organisms. Their only source thus remains the environment [2]. For humans and most animals, this is through consumption of natural foods and other food products containing these metals.

It is a well known fact that craving for certain types of things or foods in pregnant women is not abnormal. In Kenya, many such instances have been observed in both rural areas and urban centres like Nairobi. Expectant mothers have been seen eating soils which are got from many sources such as new construction sites, quarries, mud houses and ant hills. In urban areas such as Nairobi, the soil is sold in shops and City markets i.e Gikomba market. The Mahti soil obtained in one of the shops neatly packed in polythene bags is being imported from India. Since elemental contamination can occur to both mother

and the growing foetus, the interest in this work is to check the changes observed in the levels of trace metals in the body organs of rats fed with a supplement diet with this consumable clay.

Geophagia (clay eating) is associated with an interesting syndrome of Iron deficiency, hypogonadism, iron deficiency anaemia and dwarfism [3,4]. The female in her reproductive years represents the second major target for iron deficiency. Not only is her daily requirement greater than that of the male, but her daily iron intake is lower. The problem is more acute in poorer families since the mother may deprive herself of food in favour of the children.

The most strenuous nutritional challenge takes place during pregnancy. It requires an initial store of some 500 mg iron in addition to an enhanced absorption. Pregnancy is the equivalent of a 1200 to 1500 ml blood loss for those women not taking supplemental iron. In such cases, iron deficiency frequently ensues [5].

Iron supplementation is most often used on pregnant women, and has been found to improve not only the haemoglobin levels but also other indices of iron nutrition [6]. A number of iron preparations such as ferrous sulphate, ferrous gluconate and ferrous succinate have been developed and are now available at government hospitals, private hospitals and health

centres. It is interesting to note that even after the introduction of these iron preparations women continue craving for soils. Recently, multi-element iron preparations have been made and are available at the hospitals.

It is only recently that geophagia (clay eating) has been appreciated in the pathogenesis of iron deficiency. The habit has been implicated in Pica [7] which is predominantly a disorder of children and pregnant women. Pica has been recognised in many parts of the world including United States [8], Turkey [8] and Iran [10].

It has been observed that clay eating may affect the metabolism of other cations. Minnich et al. [9] showed that ingestion of iron with certain clays resulted in markedly decreased absorption of iron. Potassium may also be adsorbed to clay. Weakness and myalgia due to hypokalemia were observed in a woman who consumed at least three handfuls of clay daily [4]. Clay eating is more prevalent in those of low socio-economic status. In a certain study in rural Mississippi [7], approximately 40% of poor pregnant women admitted to being habitual eaters of laundry starch, and 27% admitted habitual clay ingestion. In Southern United States, it was reported that at least 25% of poor school children had recently eaten dirt or clay [8].

1.1 Distribution of trace elements in soils and body organs.

1.1.1 Iron

Soils.

The heavy metal content in market-garden soils in Canada has been reviewed by Chattopadhyay and Jervis [11] and a range of 1.8 - 2.8% iron was obtained. Research work by Koons and Helmke [12] on the analysis of standard soils established iron concentration to range from 2.35 - 6.00%. In another study based on topsoils and herbage of north-west Pembrokeshire, Wilkins [13] reported mean iron concentration to be 5.7% as Fe_2O_3. Kline and Brar [14] working on soils from different countries have obtained the following mean iron concentrations: Malaysia (2.7%), Norway (2.0%), Australia (5.9%), Tokyo (6.4%), Brazil (0.8%) and Lebanon (1.4%). The same authors have reported high concentrations of iron in Southern Zimbabwe (9.6%) and Hawaii (18.2%). In Kenya, Maina [15] has obtained a mean of 40,088 mg/kg for the sewage sludges in Kariobangi and values of 41,775 and 15,620 mg/kg were obtained in manure and fertilizer respectively.

Organs.

Iron is very essential for all forms of life. The estimated daily human iron requirement ranges from 7-20mg(menstruating women), 20-48mg(pregnant women) and

10-20mg(adolescents) [7]. Most of the body iron exists in complex forms bound to protein, either as porphyrin or heme compounds or non-heme protein bound compounds such as ferritin or transferrin. The demands for iron are greatest during the period of rapid growth and throughout the child-bearing age [16]. Among the body organs and tissues, the liver and the spleen usually carry the highest iron concentrations followed by the kidney, heart, skeletal muscles and the brain. In man, the liver has a high storage capacity of iron and the total iron content may rise to 10g in certain disease states. In pregnant animals near term (last stage of pregnancy), a relatively large proportion of plasma iron may go to the placenta [16].

Deficiency of iron leads to anemias [7]. Iron deficiency is much more common in women than in men since additional iron losses occur due to menstruation, pregnancy, parturition and lactation.

Excess of iron causes hemosiderosis and haemochromatosis [3]. Increased intestinal absorption of a normal dietary iron may be responsible for the excessive iron accumulation found in idiopathic haemochromatosis, occasionally in chronic alcoholism with Laennec´s cirrhosis and in certain types of porphyria. It has also been shown that excessive accumulation of iron might in itself inhibit heme synthesis. Excessive Fe(III) may inhibit

aminolevunilic acid (ALA) synthesis in vitro, but has no effect on ferrocheratase [7]. Extensive deposition of iron pigment in numerous organs has been found in persons with Kaschin-Beck disease [17]. Acute toxic poisoning leads to vomiting, pallor, shock, haematemesis, circulating collapse and coma.

1.1.2 Nickel.

Soils.

Nickel levels in the agricultural soils of Ontario have been found to vary quite widely and values ranging from 1.3-6560 ppm have been reported [18]. In the agricultural soils of Manitoba, Mills et al. [19] obtained a range of 20-145 ppm and a mean of 40.5 ppm. Nalovic and Pinta [20] working on various soils of Madagascar obtained the following ranges: ferrallitic soils(14-530 ppm), ferruginous (3-82 ppm) and alluvial soils(20-220 ppm). In the loamy and clay soils of New Zealand a range of 9-110 ppm has been reported [21]. Elsewhere values ranging from 3.6-8.1 ppm have been obtained [11]. Values on Kenyan soils were unnavailable.

Organs.

The specific biological function or functions of nickel in the body are not well known. The element was found to be distributed throughout the body without particular concentration in any tissue or organ. The

reported range of values for human tissues are as follows: lung,0.03-1.46; liver,0.02-0.05; and heart,0.02-0.03 ppm dry weight [16]. Higher values have been reported for these tissues and for kidney and spleen in rats [22]. Dietary nickel is poorly absorbed in the body. Most of it is excreted in the faeces, with smaller amounts appearing in the urine and sweat.

However, deficiency leads to impaired growth and reproductive performance in rats. In a certain study, nickel deficient rats were less active, had a rough hair coat and weighed less at weaning [7]. Nickel is a relatively non-toxic element. Rats exposed to 5 ppm nickel as a soluble salt in drinking water did not show any signs of toxicity [22].

1.1.3 Copper.

Soils.

The quantities of total copper in Israel soils have been estimated to lie between the limits of 14.0-61.5 ppm [23]. For Canadian soils, Mckeague and Wolynetz [24] have reported a mean of 22 ppm. In Nigerian soils, copper levels have been found to vary quite widely, and values ranging from 7-72 ppm are not uncommon [25]. For surface soils, Osiname et al. [26] working with some Western-Nigerian soils reported a range between 3-19 ppm and a mean value of 11.1 ppm. From the compilation of the analysis of Danish arable

soils, Tjell et al. [27] found a mean of 13.2 ppm for normal soils and a mean 12.5 ppm for total soils. In a study done in Nakuru, Kenya, Maroko [28] has reported a range of 35.0-597.0 µg/g in topsoils of well managed coffee farms. A range of 8.0-20.0 µg/g in neglected farms was also reported. The high copper levels in well managed coffee farms was attributed to the continued use of copper biocides.

Organs

Copper is an essential element for life. A lot of information is available on uptake and transport in the body [16]. Body copper content is normal between 100-500 mg with the highest concentration in the locus of the brain stem [7]. The distribution of total body copper among the tissues varies with species, age and copper status of the animal. In a certain study, the following mean concentrations in adult humans was reported: Liver, 14.7±3.8; Brain, 5.6±0.2; Kidney, 2.1±0.4 µgCu/g wet weight [16]. The normal range of concentration of copper in the blood of healthy animals can be given as 0.5-1.5 µgCu/ml with a high proportion of values lying between 0.8-1.2µg/ml [7]. In the liver, copper retention is influenced by the levels of zinc and iron and of calcium carbonate in the diet. High intakes of zinc depresses both copper and iron absorption [29].

Deficiency of copper leads to defects in the stabilisation of the fibrous protein of the connective tissue in a number of animals. It is characterized by anaemia, hypocupremia, and low serum and copper levels.

Excessive accumulation of copper leads to hepatic cirrhosis, lack of coordination, severe tremors and progressive mental deterioration. In humans, Wilson's disease (hepatolenticular degeneration) is characterized by an accumulation of potentially toxic levels of copper in both the liver and brain [17].

1.1.4 Zinc.

Soils.

The zinc content of soils varies widely with the average concentration ranging from 50-100 ppm [30]. Ravikovitch et al. [23] working on soils of Israel (ferralsols) reported higher values ranging from 200-214 ppm. In a study of surface soils of various countries, the following mean values were reported: Gleysols(Chad), 25-300 ppm [21]; Histosols and other organic soils(Denmark), 48-130 ppm [27]; Kostanozems and brown soils(Chad), 25-100 ppm [21]. In the analysis of standard soils using neutron activation analysis, Koons et al. [12] reported a range of 55-168 ppm. In Kenya, Onyari [31] has reported a mean of 3.40-256.3 ppm while working on sediments from Winam Gulf of L. Vicoria.

Organs.

Zinc is widely distributed and occurs in relatively high concentrations throughout the body [32]. The whole body of adult man is estimated to contain 1.4-2.3 g of zinc. In rats, the following zinc concentrations of normal tissues have been reported: Kidney(23); Heart(21); Liver(30); Spleen(24) and Brain(18) ppm fresh weight [16,32,33]. Similarly, levels of zinc in the body tissues of humans [16] and monkeys [16,32] have been reported. Levels of zinc in the whole blood of various animals have also been studied. In adult rabbit, average zinc levels are 2.5 µg/ml while a mean of 8.8 ± 0.2 µg/ml has been obtained for human beings [16].

Zinc deficiency leads to growth retardation and skeletal maturation. In pregnant rats it was shown to cause fetal abnormalities, behavioral impairment in the offspring and difficulty in parturition in the mother [34].

Excess of zinc results in reduced growth, anaemia, poor production and decreased activity of the liver catalase and cytochrome oxidase. Acute poisoning leads to vomiting, dehydration, electrolyte imbalance, abdominal pain, nausea, lethargy, dizziness and muscular incoordination.

1.1.5 Lead.

Soils.

Lead occurs mainly as Pb^{2+}. It's other oxidation state, +4 is also known and it forms other compounds which are quite insoluble in natural waters. The approximate mean values in soils have been reported as 15-25 ppm [35]. In a study of world soils, Aubert and Pinta [36] estimated a range of 15-25 ppm. In the agricultural soils of Ontario, the mean natural background levels of lead was reported as 14.1 ppm [18] while a range of 4.3-9.6 ppm [37] has been estimated for the cultivated soils in Alberta. Elsewhere, ranges of 5-50 ppm and 11.3-17.3 ppm have been reported [24,27].

Organs.

The total body burden of lead in normal adult man ranges from 50-400 mg with a mean of less than 200 mg [38]. Some years ago, lead in human soft tissues was reported to range from 0.13-0.50 ppm in the brain and from 1.3-1.7 ppm (wet weight) in the liver [16]. The pattern of distribution of lead with the highest levels in the bones and lowest in the muscles is similar in normal laboratory animals to that in human beings [39]. In rats, the average whole blood levels of lead have been given as 0.26 ppm when consuming a normal diet [16].

Lead is known to accumulate in the body more rapidly than it is excreted [40]. It produces a continuum of effects primarily on the haematopoietic system, the nervous system and the kidneys. In a certain study, it was shown that rats fed on diets low in calcium exhibited markedly increased lead retention in various tissues including blood, soft tissues and bone [17]. Heme synthesis is affected by the inhibition of δ-aminolevulinic acid(δ-ALA) dehydratase and ferrocheratase, the enzyme controlling the incorporation of iron into the heme molecule. Chronic lead poisoning is characterized by neurological defects, renal tubular dysfunction and anaemia.

1.1.6 Selenium.

Organs.

Selenium is widely distributed in body organs in concentrations that vary with the organ and the chemical form of selenium in the diet. In mice fed alfalfa containing nutritional levels of selenium, the distribution was as follows starting with the highest [41]:

Kidney >liver >heart >spleen >brain

The distribution of selenium in human tissues was examined by Schroeder et al. [42] who obtained the following mean values: kidney(1.09), liver(0.54), spleen(0.34), heart(0.28) and brain(0.13) ppm wet

weight. In whole human blood selenium levels ranged from 0.10-0.34 µg/g while a range of 0.06-0.20(mean 0.10) µg/g was reported for sheep grazing pastures of normal selenium status [16].

The absorption, retention and distribution of Se in the body varies with the chemical forms and amounts ingested and with the dietary levels of other elements such as arsenic and mercury. Deficiency leads to liver necrosis in rats, pancreatic fibrosis in poultry and muscular dystrophy (white muscle disease) in lambs and calves. The consumption of seleniferous diets interferes with the normal development of the embryo in rats, pigs, sheep and cattle [16]. In a certain study significant accumulation of radio-selenium by the liver and the kidneys after intravenous injections of selenite or selenomethionine was demonstrated [41].

Chronic selenium poisoning is characterized by dullness, emaciation, roughness of coat, atrophy of the liver and anaemia. In acute selenium poisoning, animals suffer from blindness, abdominal pain, grating of teeth and some degree of paralysis.

1.1.7 Rubidium.

Soils.

Data on the amounts of Rubidium in soils is rather scarce and meagre. A range of 33-270 ppm has been reported in a number of countries [43]. In

U.S.S.R, Rubidium levels in Chernozems have been reported to range from 57-90 ppm [43].

Organs.

Rubidium resembles Potassium in its pattern of distribution and excretion in the animal body. It does not accumulate in any particular organ or tissue and is normally relatively low in bones. In a recent study of human tissues, the following mean rubidium concentrations were reported: brain, 4.0±1.1; muscle, 5.0±0.3; kidney cortex, 5.2±0.5; liver, 7.0±1.0; whole blood, 2.7±0.04 ppm wet weight [16]. The Rubidium retained in the tissues as a result of high dietary intakes, is slowly lost from the body, mainly in the urine. The essential role of rubidium in animals has not been demonstrated although there are indications that it may substitute partially for potassium and is more toxic on low than on high potassium-diets.

1.1.8 Zirconium.

Soils.

Like Rubidium, there is very little data on the levels of zirconium in the soils. A range of 330-850 ppm have been obtained in New Zealand's top soils [21]. The mean zirconium levels in U.S.A soils have been reported as 224 ppm [43].

Organs.

The reported values for the zirconium levels in plants and animal tissues are highly discordant. In a certain study based on humans, the element was found to be present in all the organs examined including the brains and was reported to be especially high in the liver (6.3 ppm). In other tissues the levels were reported to lie between 1 and 3 ppm wet weight [16]. However in another study [16], much lower zirconium levels for human tissues were obtained. The brain, muscle, testis, ovary, liver, kidney and lungs all averaged between 0.01-0.06 ppm wet weight.

Zirconium compounds have a low order of toxicity for rats and mice when orally ingested. Schroeder et al. [44] obtained no growth effect on mice after adding 5 ppm zirconium as Zirconium sulphate to the drinking water.

1.1.9 Manganese.

Soils.

The approximate mean values of manganese in soils is reported as 500-1000 ppm [35]. Chattopadhyay and Jervis [11] found values ranging from 625-1430 ppm in Canadian soils(market-garden soils). In Kenya, manganese levels in sediments from several lakes are as follows [45]: L. Victoria, 275-285 ppm; L. Nakuru, 30 ppm; L. Naivasha, 29-35; L. Bogoria, 105-130 ppm.

Organs.

Manganese is widely distributed throughout the tissues and fluids, without notable concentration in any particular location. The variation among organs, species and with age have been found to be comparatively little. The mean manganese concentration in human tissues has been reported [16] as: Brain, 0.34 ppm; Heart, 0.23 ppm; Kidney, 0.93 ppm; liver, 1.68 ppm; Spleen, 0.22 ppm (all on fresh weight basis). The average levels of Manganese in unexposed rabbit tissues were reported to be 2.1 ppm(liver), 1.2 ppm(kidney) and 0.4 ppm(brain) wet weight [46]. Whole blood mean manganese levels have been reported as 9.8±0.4 µg/litre [16].

The deficiency of Manganese has been demonstrated in mice, rats, rabbits, pigs, goats and cattle. The main manifestations are impaired growth, skeletal abnormalities, ataxia of the newborn and defects in lipid and carbohydrate metabolism. For toxic levels to be realised, high dietary manganese levels are required as it is among the least toxic of the trace elements. The growth of rats was unaffected by dietary intakes as high as 1000-2000 ppm [16]. The adverse effects of excess manganese on growth were shown to be mainly a reflection of depressed appetite. Chronic manganese levels occur among miners following prolonged working with manganese ores.

1.2 Other elements.

The distribution of chromium, vanadium, strontium, calcium, yttrium, titanium, gallium, bromine, and thorium in various soils is shown in Table 1.1.

Data on the chromium and vanadium content of animal tissues are sparse and sporadic presumably as a consequence of analytical difficulties. There seems no doubt however that both elements are widely distributed throughout the human body in low concentrations without special concentration in any known tissue. In a certain study, the following mean vanadium levels were reported for human tissues [16]: brain, 0.030±0.008; liver, 0.04±0.01; muscle, 0.010±0.003 and lungs, 0.10±0.02 µg/g wet weight .

Chromium deficiency is characterised by impaired growth, disturbances in glucose, lipid, and protein metabolism. In the same way, vanadium deficiency is manifested in impaired growth and reproduction and disturbed lipid metabolism. A mutual antagonism between chromium and vanadium, or more particularly between chromate and vanadate has been demonstrated [16]. Studies have revealed that high intakes of chromium can prevent the growth depression and mortality of chicks associated with feeding 20ppm vanadium as vanadate.

The toxicity of chromium and vanadium have been

TABLE 1.1. The distribution of some trace elements in various soils.

Element	Soil	Country	Range	Mean	Ref.
Cr	Ferrallitic soil	Madagascar	20 - 930	------	20
	Alluvial soil	"	60 - 540	------	20
	Various soils	Bulgaria	55 - 1085	-----	47
V	Podzols&sand soils	N.Zealand	160 - 220	-----	21
	Loess & silty soils	"	-------	185	21
	Loamy & clay soils	Chad	15 - 50	-----	36
Sr	Loamy & clay soils	N.Zealand	18 - 86	----	21
	Organic soil	Canada	55.8 - 170.4	114	48
	Various soils	Great Britain	----	261	30
Ca	Various soils	Great Britain	0.13 - 2.4%	---	30
	Topsoils	Denmark	----	2.60%	27
	Market garden soils	-----	1.14 - 2.00%	21	11
Y	Uncultivated soils	U.S	------	23	43
	Cultivated soils	U.S(Missouri)	------	15	43

Table 1.1(cont...)

Ti	Various soils	New Caledonia	0.2 - 0.5%	---	36
	"	Madagascar	0.065-1.00%	---	20
	Podzols & sandy soils	N.Zealand	0.37 - 1.70%	---	21
	loamy & clay soils	"	0.54 - 2.40%	---	21
Ga	Brown soils	Ghana	20 - 30	---	49
	Forest soils	"	<3 - 200	---	49
	Standard soil sample	Britain	----	21	50
Th	Various soils	Bulgaria	5.0 - 18.0	---	47
	Surface soils	Great Britain	---	10.5	30
	"	Canada	4.2 - 14.1	8.0	12
Br	Forest soils	Norway(eastern)	5 - 14	7	51
	"	Norway(Northern)	16 - 100	45	51
	Gleysoils	Great Britain	---	34	52
	Tuff	"	---	48	52

NB. --- Values not given.
(All values are in ppm unless otherwise stated).

reviewed by several workers [7,16]. Oral adminstration of 50 ppm of chromate has been associated with growth depression and liver and kidney damage in experimental animals [7]. As for Vanadium, dietary concentration of 5 ppm vanadium were reported toxic to chicks and at intakes of 50 ppm, the animals exhibited diarrhoea and mortality [53]. The hair of rats fed with 100 ppm vanadium exhibits a reduction in cystine content, suggesting that this element affects the reaction of Sulphur-containing compounds [16].

The elements strontium and calcium interact with each other primarily because of their similarity in metabolic behaviour. High dietary levels of strontium result in increased retention and absorption of strontium at the expense of calcium [54,55]. Raising dietary calcium intake reduces the degree of strontium retention. In a certain study, 0.1% strontium fed to rats in the presence of adequate calcium did not alter the daily weight gain or feed efficiency [54]. It therefore follows that the toxicity of strontium is dependent on the levels of calcium present in the diet. At present, there is no conclusive evidence that strontium is essential for living organisms.

The elements yttrium, titanium, gallium and bromine have not been shown to perform any vital function in animals or that they are a dietary essential for any living organism. Titanium and

bromine have been found to be widely distributed in animal tissues [16]. The toxicity of these elements has not been established as yet.

1.3 Objectives.

Consumable clay soils like other soils consist of trace elements some of which are useful to the body while others are not. It is also possible that some of these useful trace elements might be present in relatively high amounts as to cause accumulation in the body tissues. The aim of this project was therefore to:

1) carry out a detailed trace metal analysis of consumable clays sold in various parts of Nairobi. Hence establish the difference if any between consumable clay sold in Gikomba and that sold in City shops neatly packed in polyethylene bags (imported).
2) Feed consumable clay (Mahti) to young rats and note any difference in weight or behaviour between the controls and consumable clay fed.
3) Analyse the trace metal content of the body organs of controls and clay fed rats.

Results from 2 and 3 will help in assessing the possible consequences women would face as a result of consuming this clay.

CHAPTER TWO

2.0 THEORY OF THE ANALYTICAL TECHNIQUE.

2.1 Introduction

X-Ray fluorescence spectrometry is now widely accepted as a highly versatile and potentially accurate method of elemental analysis [56]. Previously, the most widely used spectrometers were of wavelength-dispersive type in which a crystal was used to diffract the X-rays according to Bragg's law.

The analytical capabilities of semiconductor detectors were first demonstrated in the mid 1960's [56]. This marked the development of an alternative method, energy-dispersive x-ray fluorescence analysis(EDXRFA), which enabled simultaneous recording of the entire fluorescent energy spectrum. The method had the advantage in that it allowed the use of weak excitation sources such as radioisotopes. Earlier, the use of polychromatic sources made the applicability of the fundamental parameter technique(FPT) a dream due to mathematical complexities. With the use of monochromatic radioisotope excitation sources, the technique could now be applied. The advantages of EDXRFA over other analytical techniques are:

1) the sample specimen may be in various forms such as solid, powder, liquid, slurry, etc.
2) multielemental analysis can be done,
3) the use of prepared set of standards in evaluation of elemental concentrations is minimal,
4) It is non-destructive,
5) It has a high sensitivity and requires a short time for analysis.

2.2 X-RAY FLUORESCENCE RADIATION.

2.2.1 Basic principles.

The characteristic radiation arises when electrons are ejected from the inner shells of atoms and electrons from outer levels fall into the vacancies. The ejected electrons are called photoelectrons and the interaction is called the photoelectric effect. Fig 2.1 illustrates this principle and indicates the more important of the transitions which are involved.

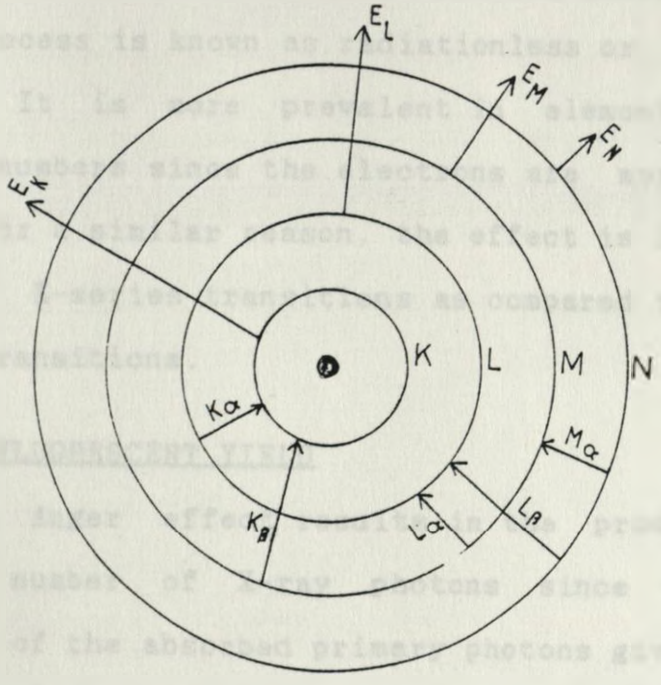


Fig 2.1. Transitions giving X-radiation.

For example, if an electron from the K-shell is ejected, the atom becomes unstable due to the presence of a positive hole in the K-shell. To regain stability, a succession of transitions (single or multiple) from outer orbitals follows. The selection

rules that determine the allowable transitions are as discussed previously [56].

The energy which an atom posses after an electron jump, for example L to K, is emitted as characteristic radiation. Alternatively, the energy may be used to reorganize the electron distribution within the atom itself leading to the ejection of one or more electrons from the outer shells. The probability of this type of ionisation will increase with a decrease in the difference of the corresponding energy states. This process is known as radiationless or the Auger effect. It is more prevalent in elements of low atomic numbers since the electrons are more loosely bound. For a similar reason, the effect is less marked for the K-series transitions as compared to the L-series transitions.

2.2.2 FLUORESCENT YIELD

The Auger effect results in the production of reduced number of X-ray photons since a certain fraction of the absorbed primary photons gives rise to Auger electrons. The probability that a characteristic X-ray will be emitted once a vacancy has been created is described by the fluorescent yield,w. The K-shell fluorescent yield, w_k is given by the equation:

$$w_k = \frac{E(n_k)_i}{N_k} = \frac{n_{k\alpha 1} + n_{k\alpha 2} + n_{k\beta 1} + \ldots\ldots}{N_k} \quad \ldots\ldots 2.1$$

where;

N_k is the rate at which K-shell vacancies are produced.

$(n_k)_i$ is the rate at which photons of spectral line i are emitted.

Similarly, the L and M fluorescent yields w_l and w_m are defined.

The fluorescent yield w, lies between 0 and 1. It decreases with atomic number since the probability of producing an Auger electron increases. For similar reasons, the L fluorescent yield values (w_l) are always less than corresponding fluorescent yield values (w_k).

Fig 2.2 shows approximate curves for w_k, w_l and w_m.

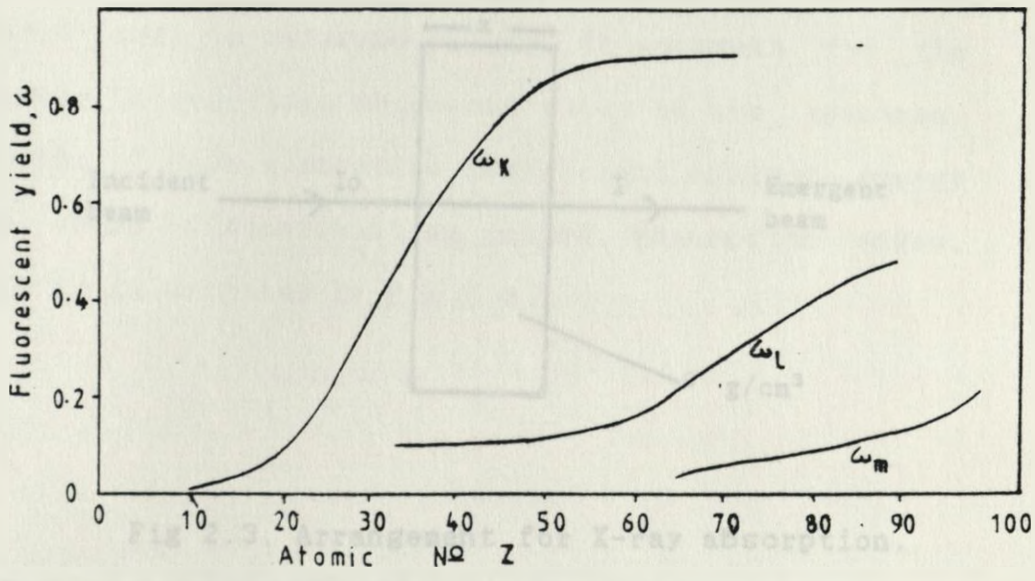


Fig 2.2. K, L and M fluorescent yields.

2.3 INTERACTION OF X-RAYS WITH MATTER.

2.3.1 Absorption.

When a beam of X-ray photons transverses matter, they are attenuated by an amount dependent upon the thickness and density of absorbing medium. The fraction of the photons passing through the material without interacting is described using the concept of a mass attenuation coefficient.

Supposing a monochromatic beam of X-rays of intensity I_o is incident upon a homogeneous absorber of thickness x (Fig 2.3). A certain fraction I will pass through the absorber whilst the remainder will be lost by photoelectric absorption or scatter.

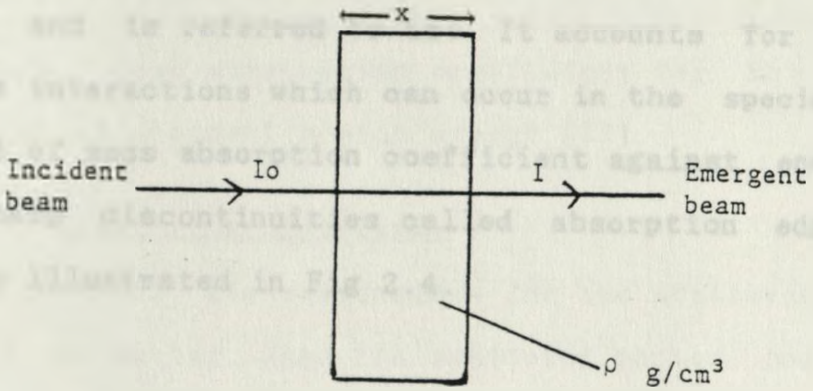


Fig 2.3. Arrangement for X-ray absorption.

The emergent intensity I, is given by

$$dI = -I\mu_x d_x \quad \ldots\ldots\ldots\ldots 2.2$$

where μ_x = linear absorption coefficient,

but the fraction of both absorbed and scattered photon is also dependent on mass, hence

$$dI = -I\mu_m d_m \quad \ldots\ldots\ldots\ldots 2.3$$

where μ_m is called the mass absorption coefficient.

The fraction ,(I) of photons transversing the absorber without being scattered or absorbed can be calculated by integrating dI between the limits 0 and x. Equation 2.2 becomes:

$$\ln I_x - \ln I_o = -\mu_x X \quad \ldots\ldots\ldots\ldots 2.4$$

but μ_x and μ_m are related by the following expression.

$\mu_x = \mu_m$, hence

$$I = I_o e^{(-\mu x)} \quad \ldots\ldots\ldots\ldots 2.5$$

This is an expression of Beer-Lambert law [57].

The mass attenuation coefficient is the most useful and is referred to as μ. It accounts for the various interactions which can occur in the specimen. A plot of mass absorption coefficient against energy has sharp discontinuities called absorption edges. This is illustrated in Fig 2.4.

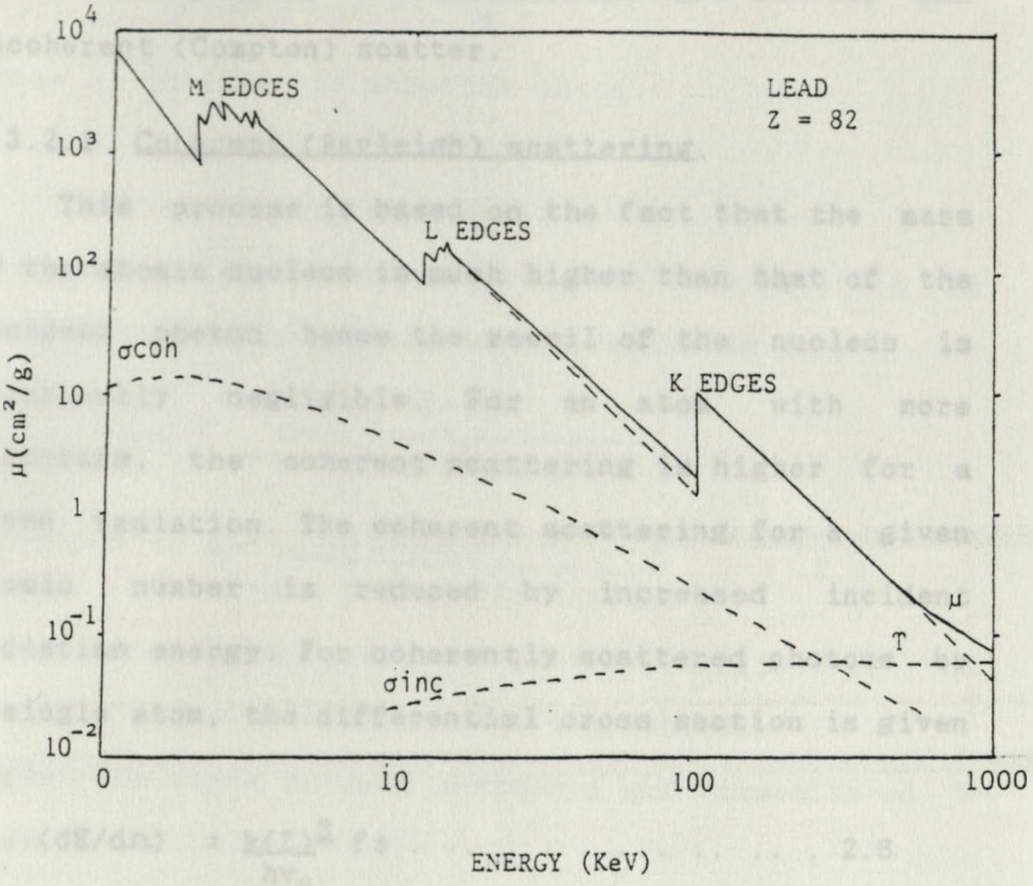


Fig 2.4. Mass attenuation coefficient for Pb as a function of incident photon energy [57].

2.3.2 Scattering of X-rays.

Electrons are responsible for the scattering of X-rays by matter. When the scattered photon has the same energy or wavelength as the incident photon, the scattering is called coherent. When the energy or wavelength of the scattered photon has been modified, the scattered X-ray is said to be incoherently or Compton scattered.

The scattering coefficient is made up of two

terms corresponding to coherent(Rayleigh) scatter and incoherent (Compton) scatter.

2.3.2.1 Coherent (Rayleigh) scattering.

This process is based on the fact that the mass of the atomic nucleus is much higher than that of the incident photon hence the recoil of the nucleus is practically negligible. For an atom with more electrons, the coherent scattering is higher for a given radiation. The coherent scattering for a given atomic number is reduced by increased incident radiation energy. For coherently scattered photons by a single atom, the differential cross section is given by;

$$(dE/d\Omega) = \frac{k(Z)^3}{hv_o} f\psi \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 2.6$$

Where k is a constant.

$f(\psi)$ is a function of scattering angle.

2.3.2.2 Incoherent (compton) scattering

In this process, the incident X-ray photon collides with a loosely bound electron in an outer orbit of the atom. Under the impact, the electron recoils leaving the atom and taking away some of the photon energy. The deflected electron results in loss of energy and hence increase in wavelength. This is illustrated by the following equation:

$$\Delta\lambda = \lambda_{inc} - \lambda_o = \frac{h(1-\cos\psi)}{M_o C} \quad \ldots\ldots 2.7$$

where, $\Delta\lambda$ is given in angstrom units.

h is Planck's constant

c is velocity of light.

For energy-dispersive analysis, the Compton shift is more conveniently applied in the following way:

$$E_{inc} = \frac{Eo}{1 + (Eo/Mo^2)(1-\cos\psi)}$$

$$= \frac{Eo}{1 + 0.001957Eo(1-\cos\psi)} \quad \ldots\ldots 2.8$$

where Eo and E_{inc} are the photon energies of the incident and scattered photons respectively.

ψ is the angle between scattered and unscattered X-rays.

2.4 XRFA Instrumentation.

Fig 2.5(b) illustrates a typical XRFA unit which was used in this project. A more detailed description of this unit has been previously discussed [58].

The system is composed of an X-ray source and an X-ray spectrometer. The spectrometer comprises of the following main parts:

1) solid state detector, silicon-lithium (Si(Li)) detector,
2) pre-amplifier,
3) amplifier and
4) a multichannel analyser(MCA).

A microcomputer with its input and output devices is connected to the spectrometer to assist in handling and analysis of the spectral data.

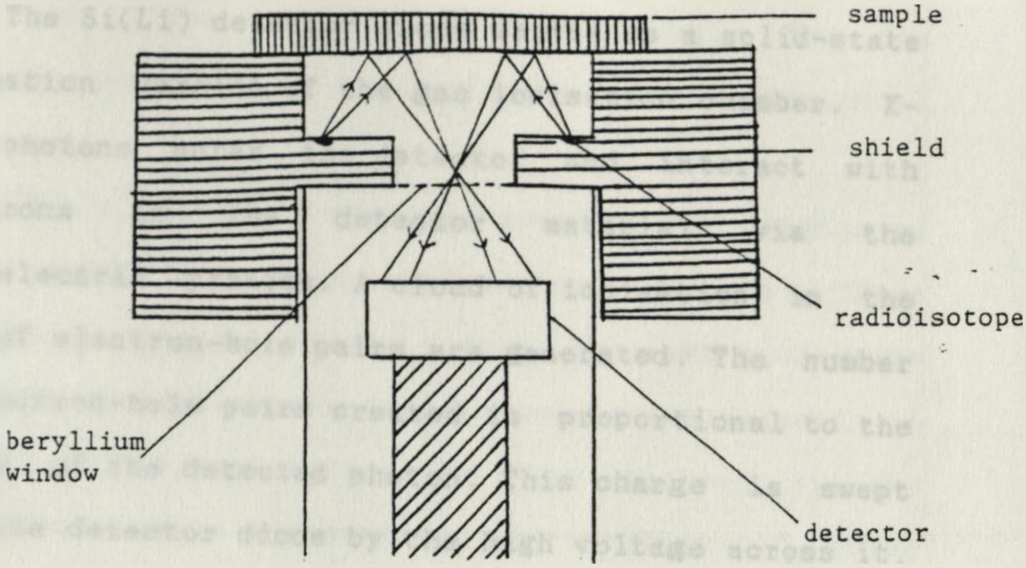


Fig. 2.5(a). Schematic representation of radioisotope excitation system with annular source configuration.

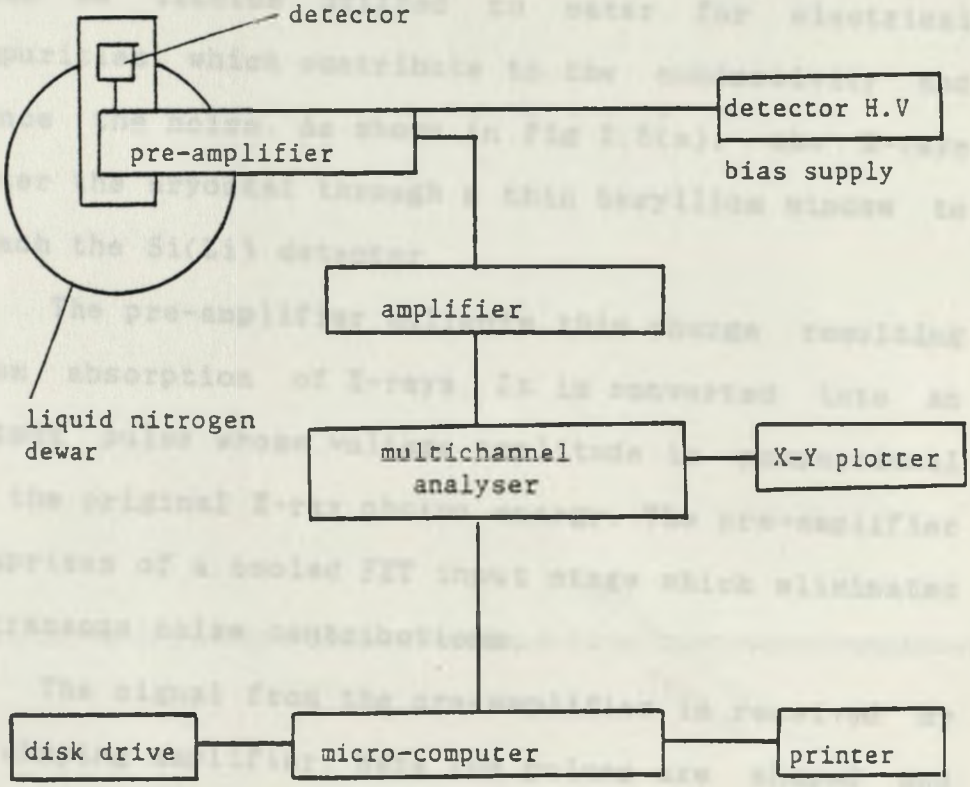


Fig. 2.5(b). Schematic representation of XRFA unit.

The Si(Li) detector diode serves as a solid-state ionisation version of the gas ionisation chamber. X-ray photons enter the detector and interact with electrons in the detector material via the photoelectric process. A cloud of ionisation in the form of electron-hole pairs are generated. The number of electron-hole pairs created is proportional to the energy of the detected photon. This charge is swept from the detector diode by the high voltage across it.

In normal cases, the Si(Li) detector is mounted in a light, vacuum cryostat and operated at the liquid nitrogen temperature(77K) [56]. This is found to be quite necessary to minimise the electronic noise added to the signal during the mentioned process. It must also be lithium drifted to cater for electrical impurities which contribute to the conductivity and hence the noise. As shown in Fig 2.5(a), the X-rays enter the cryostat through a thin beryllium window to reach the Si(Li) detector.

The pre-amplifier collects this charge resulting from absorption of X-rays. It is converted into an output pulse whose voltage amplitude is proportional to the original X-ray photon energy. The pre-amplifier comprises of a cooled FET input stage which eliminates extraneous noise contributions.

The signal from the pre-amplifier is received by a shaping amplifier. Here the pulses are shaped and

amplified linearly to make them suitable for the precise pulse-height analysis by the multichannel analyser.

The multichannel analyser measures the heights of each amplifier output pulse, and converts these amplitudes into an integer number. The number of times a pulse of each height is detected is accumulated in the memory address to form the X-ray spectrum. By the appropriate MCA calibration, the spectrum of pulse heights are converted to the X-ray energy spectrum.

2.5 QUANTITATIVE ANALYSIS

2.5.1 Fluorescence intensity for the characteristic X-rays.

In X-ray fluorescence spectrometry, it is the intensity of the fluoresced characteristic X-ray from the specimen which provides the analytical signal for both quantitative and qualitative analysis. The characteristic X-rays are generated as a result of a photoelectric interaction. Fig 2.6 defines the geometry of the excitation source, the specimen and the detector.

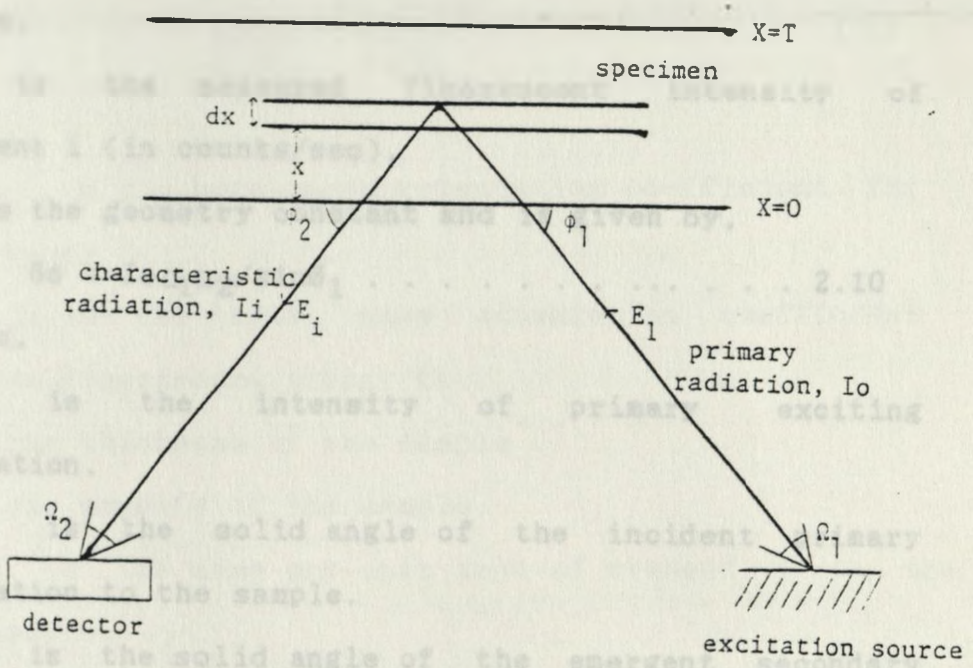


Fig 2.6. Arrangement of excitation source, specimen and detector.

The basic equation in the fundamental parameter approach has previously been discussed by various workers [58,59] for the case of monochromatic excitation. The derivations are based on the following assumptions:

1) a homogeneous sample where the density is well defined and constant throughout the sample.
2) a monochromatic source of radiation is used to excite characteristic X-rays from the sample.
3) the geometry of the sample, source and the orientation of the detector must be maintained.

The equation is given by;

$$Ii = Go.Ki\ E(Ei)\rho_i di.\ 1-\exp(-a\rho d)/a\rho d. \quad . \ . \ . \ .2.9$$

where,

Ii is the measured fluorescent intensity of element i (in counts/sec).

Go is the geometry constant and is given by,

$$Go = Io\,\Omega_1\Omega_2/\sin\emptyset_1 \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ 2.10$$

where,

Io is the intensity of primary exciting radiation.

Ω_1 is the solid angle of the incident primary radiation to the sample.

Ω_2 is the solid angle of the emergent secondary radiation from the sample.

Ki is the relative excitation detection efficiency given by:

$$Ki = \sigma_i^{ph}(Eo).P_i^s.W_i^s.f_s^i\,\varepsilon(E_i)\ (cm^2/g) \quad . \quad . \quad . \quad .2.11$$

where;

$\sigma_i^{ph}(Eo)$ is the photoelectric mass absorption coefficient of element i at energy Eo.

W_i^s is the fluorescent yield for element i in shell "S".

P_i^s is the fraction of photoelectric events which occur in shell "S".

f_s^i is the ratio of the intensity of a given K or L line to the intensity of the whole series.

E(Ei) is the relative efficiency of the detector for photons of energy Ei,

but,

$$a = \frac{\mu(Eo)}{\sin\phi_1} + \frac{\mu(Ei)}{\sin\phi_2} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad 2.12$$

where,

$\mu(Eo)$ is the total mass attenuation coefficient for the energy Eo

$\mu(Ei)$ is the total mass attenuation coefficient for the fluorescent energy Ei

d is the thickness of the sample .

ρ is the density of the sample.

$\rho_i d_i$ is the mass per unit area of element i in the sample.

ϕ_1 is the angle between mean direction of primary X-ray and sample surface

ϕ_2 is the emergent angle of secondary radiation with the sample

In equation 2.9 the factor 1-exp(-aρd)/aρd is called the absorption correction factor. The attenuation of the measured fluorescence intensity is accounted for by this factor.

This factor is dependent on the value of the product (aρd). For thin samples the value 1-exp(-a ρd) basically equals the value aρd when aρ d is small. In this case the absorption effects are negligible.

Equation 2.9 hence transforms into:

$$Ii = GoKi(\rho_i d) \quad \ldots\ldots\ldots\ldots 2.13$$

where $a\rho d < < 1$

The above equation is advantageous in that no intermediate standards or empirical coefficients are needed for any matrix [56].

For thick samples, the values of aρd are normally quite high. This reduces equation 2.9 to:

$$Ii = GoKi\ \alpha_i.1/a \quad \ldots\ldots\ldots\ldots\ldots\ldots 2.14$$

where $a\rho d > > 1$ and α_i is the concentration of the element (i)

2.6 Matrix effects.

Matrix effects constitute the major sources of errors in XRFA [57]. Their effects on the measured intensity may be categorized in the following way;

1) Elemental interactions

2) Particle size and surface effects.

Elemental interactions may further be divided into absorption and enhancements effects.

2.6.1 Elemental interactions.

a) Absorption.

Absorption effects take place when the characteristic radiation produced from an element in a matrix results in reduced number of characteristic photons leaving the sample. This is less than the number initially produced. As a result, there is a change in the mean absorption coefficients for both primary radiation $\mu_i(Eo)$ and the fluorescent (secondary) radiation $\mu_i(Ei)$ for the element in question. The relative influence of the radiation absorption (primary and secondary) can be seen in the following equation for monochromatic primary radiation.

$$Ii(Ei) = f(\rho idi, \mu i(Eo), \mu i(Ei)) \quad \ldots\ldots\ldots 2.15$$

where,

ρidi is the weight fraction of element i in the matrix.

This equation is a simplified form of equation 2.9.

In general, the secondary-absorption effect is usually more severe than the primary-absorption effect but is also more easily predicted, evaluated and corrected.

b) Enhancement effects

Enhancement effects occur when the matrix elements emit their own characteristic radiation which may lie on the short-wavelength side of the element absorption edge and hence excite the element to emit radiation in addition to that caused by primary X-rays. This implies that the number of characteristic photons actually measured is greater than that predicted by the following equation:

$$Ii(Ei) = (\rho i di, Io(Eo)) \quad \ldots\ldots\ldots\ldots .2.1$$

2.6.2 Particle size and surface effects.

In the derivation of the basic equation (2.9) a homogeneous, smooth sample was assumed, i.e the surface layer contains all elements present in the sample homogeneously distributed in their true concentration ratios.

In badly prepared samples, compositional variations in depth are present and the measured count data is not a representative of the whole sample. In a sample containing large and small grains, the effect would be to measure too much of the small grain and too little of the large grains. This occurs since the effective depth contributing to both wavelengths is less than the average particle size of the large grains.

Correction methods for matrix effects are discussed in chapter 3.

CHAPTER THREE

3.0 EXPERIMENTAL TECHNIQUES

Samples.

In this study, the following samples were analysed: consumable clays, rat food (rabbit pellets) and body organs of rats which included the liver, the spleen, the heart, the kidney and the brain. The whole blood of these rats was also analysed.

The trace metal content in these samples was determined. In addition, the growth rate and the behaviour of rats fed and not fed with consumable clay (Mahti) were assessed.

Apparatus.

1) A canberra S40 Multi-Channel Analyser (MCA). This was fitted with a Si(Li) ortec detector, a Dec Professional 350 series minicomputer and an XY-plotter.

The Si(Li) detector used was operated at a voltage of 1500V negative bias.

2) A Cd-109 radioisotope with a half-life of 453 days was used as the excitation source.

3) Energy calibration of the MCA was performed with the use of Fe-55 and Cd-109 point sources.

4) A Microprocessor-pocket-pH-mV-meter model pH 96.

5) A mechanical grinder (pulverisettee type 02.102).

Reagents and chemicals.

The following chemicals were used:

1) Perchrolic acid (70%)
2) Sulphuric acid
3) Hydrochrolic acid
4) Ammonia
5) Sodium molybdate
6) Sodium diethyldithiocarbamate (NaDDTC).

All these chemicals were of analytical grade.

3.1 SAMPLING.

3.1.1 Consumable clay and rabbit pellets.

Consumable Clay.

Consumable clay samples were obtained from three different vendors in Nairobi. The first two were from Gikomba Market while the third one was from a shop along Biashara Street. A total of 20 samples were bought from Gikomba Market. Each sample was approximately 125g. From Biashara Street, a total of 70 packets were bought which weighed 102g each. All the consumable clay samples were stored in polyethene bags to avoid trace metal contamination.

Rabbit Pellets.

These were obtained from Kenya Grain Growers Co-operative Union (KGGCU). About 500.0g of them were taken and put in polyethene bags for storage prior to analysis.

3.1.2 Animal Selection.

28 Female Fisher strain baby rats were bought from the National Public Health Laboratories (NPHL) in Nairobi. They were about 4 weeks old with an average weight of 74±10g. There were two experimental groups, 11 controls and 17 experimental rats. The two groups were housed in different wooden cages under constant light-dark conditions (12 hours on, 12 hours off)

3.1.3 Feeding and weighing.

The experimental group of rats received rabbit pellets which had been ground and mixed with consumable clay at a ratio of 3.7:1 and pressed into pellets (The mentioned ratio was arrived at after a series of experiments to determine the best ratio for the formation of pellets). The control group of rats received rat food with no consumable clay. Both groups received tapwater which was available to them ad libitum. The weights were taken twice a week. After 37 days of feeding (phase 1), 10 rats (4 controls and 6 consumable clay fed) were sacrificed. After 74 days

(phase 2), 10 other rats (4 controls and 6 consumable clay fed) were sacrificed. The remaining 8 rats (3 controls and 5 clay fed) were sacrificed after 110 days of feeding (phase 3). The choice of the rats during the time of sacrifice was done at random.

3.2 SAMPLE PREPARATION.

3.2.1 Consumable clay and rabbit pellets.

Consumable Clay.

The Consumable clay samples (in form of rocks) were first crushed using a mechanical grinder (pulveressette type 02.102). Further grinding by hand using a small agate mortar was done which reduced the particle size to approximately less than 50 microns. The sample material was then diluted with starch and thoroughly mixed. Thin pellets were then made by pressing a small amount of the diluted material between two stainless steel dies at a pressure of 3-5 tons. Thin pellets weighing between 0.13g - 0.18g were made.

Rabbit pellets.

About 3.0g of the pellets were crushed using a mortar and pestle then dried overnight at 75^{o}C to eliminate moisture. Further grinding was done and thin pellets of approximately 0.19g were made.

3.2.2 Organs.

3.2.2.1 Dissection and Storage.

Each rat was initially put in a desiccator containing diethyl-ether. After attaining a semi-conscious state, it was removed and the jugular vein was cut and the blood carefully drained into a clean glass bottle. The blood samples were kept at approximately zero degrees centigrade in an ice-box. The following organs were then dissected: the liver, the heart, the spleen, the kidney, and the brain. New ultra-clean stainless steel knives were used for each dissection. All organs were put separately in polythene bags to minimise trace metal contamination and stored at zero degrees centigrade in an ice-box [60]. The samples (blood and organs) were then transferred to a deep freezer at -18 degrees centigrade within 6 hours after sacrifice before digestion [60].

3.2.2.2 Treatment prior to analysis.

The organ samples were removed from deep-freezer and allowed to thaw for at least 1 1/2 hours. Since the concentration of trace elements within the organs are different [61], the following tissues were weighed wholly to eliminate sampling errors which could be introduced by these variations: the heart, the kidney, the spleen and the Brain. Polythene bags and plastic

tweezers were used throughout to avoid trace metal contamination.

The liver samples in sealed plastic bags were removed from deep-freezer and were broken while frozen into four pieces which were weighed and then put in 4 round bottomed flasks. This was found necessary to avoid explosions during digestion.

After thawing, the blood samples were shaken vigorously to homogenize the coagulated blood. Clean conical flasks were weighed and about 1 ml of the coagulated blood was put into them. The weight of the blood was then noted.

3.3 Wet digestion procedure (organs and blood).

Digestion Mixture.

10.0g of sodium molybdate were dissolved in 150.0ml of double-distilled water and 150.0ml of H_2SO_4 (analar grade) was then added. After cooling, 200.0ml of 70% $HCLO_4$ (A.R) was added (exothermic reaction). This mixture was used for digestion [62]

Procedure.

About 1-2 g of coagulated blood or tissue was put in a round bottomed flask and 3.0ml of the digestion mixture was added. The flask was heated in an oil bath to a temperature of 170 degrees centigrade and kept at this temperature until the solution became clear (the blood samples were clear but slightly yellow due most

probably to iron). The solution was cooled and diluted with distilled de-ionised water about four times and boiled to remove chlorine.

The round bottomed flasks were then put at the internal step of a desiccator containing 250 - 500ml of concentrated NH_4OH. It was then closed and the samples were left for approximately 14 hours. The samples were then removed and the volumes adjusted accordingly.

The pH of the solution was adjusted to a value of 5.0 and 10ml of freshly prepared 2% sodium diethyldithio-carbamate (NaDDTC) solution was added as the precipitating agent [63]. The resulting precipitate was allowed to stand for about 15 minutes to age. It was then filtered through a millipore(0.45 micron pore diameter) filter paper. The filter paper with the precipitate was air-dried and analysed by X-ray fluorescence analysis(XRFA).

3.4 Analysis.

The samples were irradiated with a ^{109}cd (10mci) excitation source. The X-rays were detected in an ORTEC Si(Li) detector located inside a cryostat with 25 micron beryllium window entrance. The XRFA spectra was collected by Canberra S40 multichannel analyser (MCA). A preset time of 1000 - 2500 seconds was found adequate for consumable clay samples and rabbit

pellets. For body organs, a preset time of 4000 seconds and above was found appropriate. The spectra so obtained were stored in the Dec PRO 350 computer for off-line analysis. The program used was a linear Least Square Program, QXAS [64]. With the help of a multi-element standard sample (target), whose elemental composition was known, the absorption correction parameters (a_{pd}) were obtained. A graph of log a_{pd} Vs log E (E is energy of element) was plotted and the a_{pd}'s for the elements in the samples were obtained. Calculations were done using equation 2.9. A plot of log E Vs log a_{pd} is shown in appendix 1A for the Gikomba I sample. The respective spectrum as well as that of the Mahti clay are given in Appendices 2A and 3A.

Liquid samples were treated as thin samples, $a_{pd} << 1$. Hence using equation 2.13, the concentrations of each element present were calculated. The spectra representing a few of the tissue samples are as shown in Appendices 4A, 5A, 6A, 7A and 8A.

3.5 Correction methods for matrix absorption effect.

Correction methods for matrix effects are well established. A comprehensive discussion on these methods is well documented in references 56, 65. In this study, matrix effects due to enhancement

effects were reduced by diluting the sample material with pure starch as previously reviewed [58]. It has therefore been assumed that matrix correction refers only to absorption correction which is represented in basic equation 2.9 by the factor

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

In this study, the technique used involved the measurement at five x-ray energies, with and without the sample from a target placed at the back of the sample of known mass per unit area as previously discussed [58,66,67]. The multi-element target contain elements whose characteristic x-rays are within the range of interest. Values of $(a\rho d)_i$ for elements in target were obtained from the following equation:

$$(a\rho d)_i = \log_e\left(\frac{I_o i}{I_2 i - I_1 i}\right)$$

and plotted on a log-log scale from which $a\rho d$ values of interest in the sample were then interpolated.

Using equation 2.9 the absorption correction factor,

$A_{corr.} = \frac{1 - \exp(-a\rho d)}{a\rho d}$

approaches 1 for a thin sample. The uncorrected concentration,

$\alpha_{uncorr.} = \frac{(\rho d)_i}{(\rho d)_s}$ was hence obtained

where $(\rho d)_i$ is mass per unit area of element i and $(\rho d)_s$ is the mass per unit area of sample. The $(a\rho\, d)_i$

values were calculated by an iteration process based on the following equation:

$$(a\rho d)_i' = (a\rho d)_i(o)\{1 - (1-1/jk)\,\alpha_{uncorr.}\}$$

The new $(a\rho d)_i'$ was then used to generate the $A_{corr.}$ factor which was used to calculate the corrected concentration.

Corr. concentration, $\alpha_{corr.}$

$$= \alpha_{uncorr.} \times \frac{1}{A_{corr.}} \times \text{dilution factor.}$$

where dilution factor,

$$= \frac{\text{weight of sample + weight of dilutant}}{\text{weight of sample}}$$

In case of liquid samples, $A_{corr.}$ was assumed to be equal to 1 since the samples are very thin and matrix effects were minimal.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION.

4.1 Accuracy of the method.

The accuracy of this method has been reviewed by several workers [15,66]. In this study, it was assessed by analysing the following International Atomic Energy Agency(IAEA) Certified Reference Materials(CRM's): Soil-7, Freeze dried animal blood, A-13 and Animal muscle, H-4. Soil-7 sample was treated as for the consumable clay while freeze dried animal blood and Animal muscle were treated as for the tissues samples. The results obtained were compared with the certified values from the respective certificates as shown in Table 4.1a and 4.1b. The results are shown together with the confidence limits stated in the CRM certificates for the determined elements.

From the results, it is found that the concentration values obtained by XRF are in good agreement with CRM values. In the analysis of Soil-7, accuracies of order less than 14% or better were obtained for most of the elements. Overall, a total error of 7.8 % was obtained.

For the other reference materials, acceptable accuracies of order less than 19% or better were

TABLE 4.1a. Results of Certified Reference Material(CRM); Soil-7 by use of XRFA.
(all values in µg/g unless otherwise stated).

	XRFA results	Certified results		
Element	mean,s.d * (n=3)	Recommended conc. *	Confidence limit	Relative error
Ca	(15.2 ± 1.4)%	16.3%	(15.7 - 17.4)%	6.7 %
Fe	(2.53 ± 0.05)%	2.58%	(2.54 - 2.67)%	2.7 %
Mn	582.1 ± 18.7	631.0	604.0 - 650.0	7.7 %
Rb	57.9 ± 7.1	51.0	47.0 - 56.0	13.5 %
Sr	104.0 ± 2.6	108.0	103.0 - 114.0	3.7 %
Zr	178.5 ± 1.5	185.0	188.0 - 201.0	3.5 %
Zn	115.0 ± 7.4	104.0	101.0 - 113.0	10.6 % b 7.8 %

s.d - standard deviation of replicate analysis.
* - expressed on dry weight basis.
b - total error(%)

TABLE 4.1b. Results of Certified reference material(CRM); Freeze dried animal blood and Animal muscle by use of XRFA. (All values in µg/g unless otherwise stated).

Element	XRFA results mean,s.d * (n=3)	Certified results Recommended conc. *	Confidence limit	Relative error
	FREEZE DRIED ANIMAL BLOOD, A-13.			
Cu	3.7 ± 0.3	4.3	3.7 - 4.8	14.0 %
Fe	(2.25 ± 0.02)%	2.40%	(2.2 - 2.5)%	6.3 %
Ni	0.83 ± 0.14	1.00	0.6 - 1.4	17.0 %
Zn	15.4 ± 3.1	13.0	12 - 14	18.5 % 14.7 % [b]
	ANIMAL MUSCLE, H-4			
Cu	4.2 ± 0.1	4.0	1.7 - 11.5	5.0 %
Fe	45.0 ± 0.6	49.0	36.5 - 83.0	8.2 %
Zn	87.3 ± 1.1	86.0	43.0- 228.0	1.5 %
Se	0.23 ± 0.01	0.28	0.14 - 0.43	17.9%
Mn	0.38 ± 0.10	0.52	0.1 -3.88	26.9% 10.2% [c]

s.d - standard deviation of replicate analysis.
* - expressed on dry weight basis.
b - total error(%)
c - Manganese value not included in the total error.

obtained. A total error of 14.7 % was calculated for the Freeze dried animal blood, A-13 and 10.2% for the Animal muscle. Discrepancies were however noted in Animal muscle in the determination of manganese. A negative deviation was obtained which could have resulted from the high pH (5.0) used during precipitation.

4.2 Levels of trace elements in consumable clay and rabbit pellets.

The results of the three samples analysed are presented in Tables 4.2, 4.3 and 4.4. There was no significant differences between GIK I and GIK II samples. This is as expected since the samples had a similar origin. Levels of trace elements in the Mahti sample varied greatly with those of GIK I and GIK II. The elements nickel, vanadium, chromium and bromine were not detected in the Gikomba samples. In addition, calcium in Gikomba samples was relatively in high concentrations but was not detected in the Mahti sample. The differences could be attributed to geological differences.

Table 4.5 shows the levels of trace elements in rabbit pellets. A comparison between rabbit pellets and Mahti sample indicates that the elements Vanadium, chromium, gallium, yttrium, titanium, thorium and bromine were not present in rabbit pellets. Apart

TABLE 4.2. Levels of trace elements in Gikomba I clay. (Values in ug/g unless otherwise stated).

Element	Mean, ± s.d
Fe	3.74 ± 0.04(%)
Ni	nd
Cu	15.0 ± 4.5
Zn	92.5 ± 8.3
Pb	10.0 ± 4.0
Zr	1500.0 ± 20.0
Mn	722.1 ± 55.5
V	nd
Cr	nd
Sr	29.1 ± 5.5
Ga	30.0 ± 6.0
Y	89.6 ± 9.6
Ti	0.287 ± 0.022(%)
Th	26.7 ± 3.5
Br	nd
Ca	3330.0 ± 320.0
Rb	196.0 ± 8.0

(Values reported average of three replicates).
s.d= standard deviation.
n.d= non-detected.

TABLE 4.3. Levels of trace elements in Gikomba II clay. (Values in µg/g unless otherwise stated).

Element	Mean, ± s.d
Fe	3.83 ± 0.04(%)
Ni	nd
Cu	nd
Zn	133.7 ± 10.0
Pb	15.3 ± 4.5
Zr	1380.0 ± 20.0
Mn	1310.0 ± 50.0
V	nd
Cr	nd
Sr	38.4 ± 2.3
Ga	27.9 ± 3.4
Y	78.7 ± 5.9
Ti	0.294 ± 0.016(%)
Th	22.2 ± 4.2
Br	nd
Ca	3740.0 ± 480.0
Rb	189.1 ± 6.8

(Values reported average of three replicates).
s.d= standard deviation.
nd= non-detected.

TABLE 4.4. Levels of trace elements in Mahti clay. (Values in µg/g unless otherwise stated).

Element	Mean, ± s.d
Fe	2.65 ± 0.03%
Ni	79.5 ± 10.0
Cu	21.7 ± 0.5
Zn	33.0 ± 1.6
Pb	5.5 ± 0.6
Zr	548.4 ± 2.2
Mn	456.6 ± 9.7
V	< or = 394 ± 42.4
Cr	< or = 356.7 ± 52.0
Sr	46.8 ± 2.1
Ga	45.4 ± 6.1
Y	8.5 ± 0.6
Ti	4.86 ± 0.07(%)
Th	3.5 ± 0.6
Br	7.7 ± 0.8

Values reported average of three replicates.
s.d -standard deviation.

TABLE 4.5. Levels of trace elements in rabbit pellets. (Values in µg/g unless otherwise stated).

Element	Mean, ± s.d
Fe	3610.0 ± 40.0
Ni	12.5 ± 1.1
Cu	81.6 ± 0.3
Zn	535.0 ± 23.0
Pb	1.1 ± 0.6
Zr	7.7 ± 0.4
Mn	1337.0 ± 15.0
V	nd
Cr	nd
Sr	592.0 ± 22.0
Ga	nd
Y	nd
Ti	nd
Br	nd
Ca	5.40 ± 0.03(%)
K	4.90 ± 0.01(%)
Rb	86.6 ± 4.0(%)
Se	1.1 ± 0.4

(Values reported average of three replicates).
s.d= standard deviation.
nd= not detected

from the elements analysed in the Mahti sample, the elements calcium, potasium, rubidium and selenium were detected in the rabbit pellets.

4.2.1 Iron.

High values of iron were obtained in all the three consumable clay samples as shown in Tables 4.2, 4.3 and 4.4. The Gikomba I & II samples had the highest values (3.74 ± 0.04% and 3.83 ± 0.04% respectively) while the Mahti sample had the lowest (2.65 ± 0.03)%. These values are quite high and comparable to those of other soils [11,12]. They are also comparable to those values obtained in the sewage sludges (40,088 mg/kg) in Kariobangi and values of 41,775 and 15,620 mg/kg obtained in manure and fertilizer respectively [15]. A daily dietary allowance of 18 mg have been recommended for both pregnant and lactating women [16].

The elemental variation of iron in both controls and clay fed rats is shown in Table 4.6 and Figure 4.1. The spleen had the highest levels of iron. The values (on fresh weight basis) were: 655.4 µg/g(phase 1), 1257.7 µg/g(phase 2) and 2272.8 µg/g(phase 3) for the clay fed rats. Iron levels in the spleen and the liver of both group of animals continued to increase with continued feeding. This is well illustrated in Figure 4.1. In the liver, a significant difference (p

TABLE 4.6. Levels of iron in the liver, spleen, kidney, heart, brain and blood of controls and clay fed rats(ug/g wet weight).

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
LIVER	n=2	n=3	*	*	n=3	n=5
	137.1 ± 23.5	226.5[a] ± 2.7			315.5 ± 25.2	544.1[a] ± 41.0
SPLEEN	n=4	n=5	n=4	n=3	n=2	n=5
	456.8 ±35.4	655.4 ±32.7	1169.0 ±223.6	1257.7 ±191.3	1927.0 ±26.3	2272.8[a] ±200.6
KIDNEY	n=3	n=4	n=4	n=5	n=3	n=5
	78.7 ±17.5	104.1 ±20.7	195.0 ±20.8	214.3 ±26.5	165.1 ±10.0	196.6[a] ±9.0

TABLE 4.6(cont...)

	PHASE 1(37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
HEART	n=4	n=5	n=3	n=5	n=3	n=5
	164.8 ±11.5	144.7 ±21.5	170.7 ±11.1	157.7 ±8.6	179.5 ±12.6	162.2 ±15.6
BRAIN	n=3	n=3	n=4	n=6	n=3	n=5
	70.9 ±9.6	75.6 ±10.3	49.5 ±4.5	48.1 ±3.9	88.6 ±14.2	68.8 ±11.2
BLOOD	n=3	n=4	n=4	n=6	n=3	n=5
	533.5 ±83.5	553.0 ±30.9	458.4 ±58.9	514.8 ±20.9	512.8 ±30.8	432.8 ±76.0

s.d = standard deviation
* = samples lost after explosion during digestion.
a = $p < 0.01$ (for two-tailed areas).

< 0.01) between the controls and clay fed rats was noted even after feeding for 37 days. In the spleen, the difference was only significant during the last phase of the study. The high levels of iron in the consumable clay (Mahti sample) could have led to accumulation of iron in these body organs.

The Iron in the liver is stored as hemosiderin and ferritin [16]. It has been shown that iron deposited in the liver and spleen of rats is utilized for the demands of erythropoiesis and placental iron transfer to the fetus [16]. This has the implications that there are chances of iron toxicity to a growing fetus if the mother eats a lot of clay. Excessive dietary iron intake is reported to be the cause of abnormal iron accumulation in a condition described as "Bantu Siderosis" occurring in South Africa [7].

In the kidneys, slightly elevated levels were obtained for the clay fed rats. The values (fresh weight basis) were: 104.1 µg/g(phase 1), 214.3 µg/g (phase 2) and 196.6 µg/g(phase 3) as compared to 78.7 µg/g(phase 1), 195.0 µg/g(phase 2) and 165.1 µg/g(phase 3). Levels of iron decreased during the last phase of the study showing that iron didn't accumulate on continued feeding (Fig. 4.1). However a significant difference ($p < 0.01$) between the two groups was evident during this last phase.

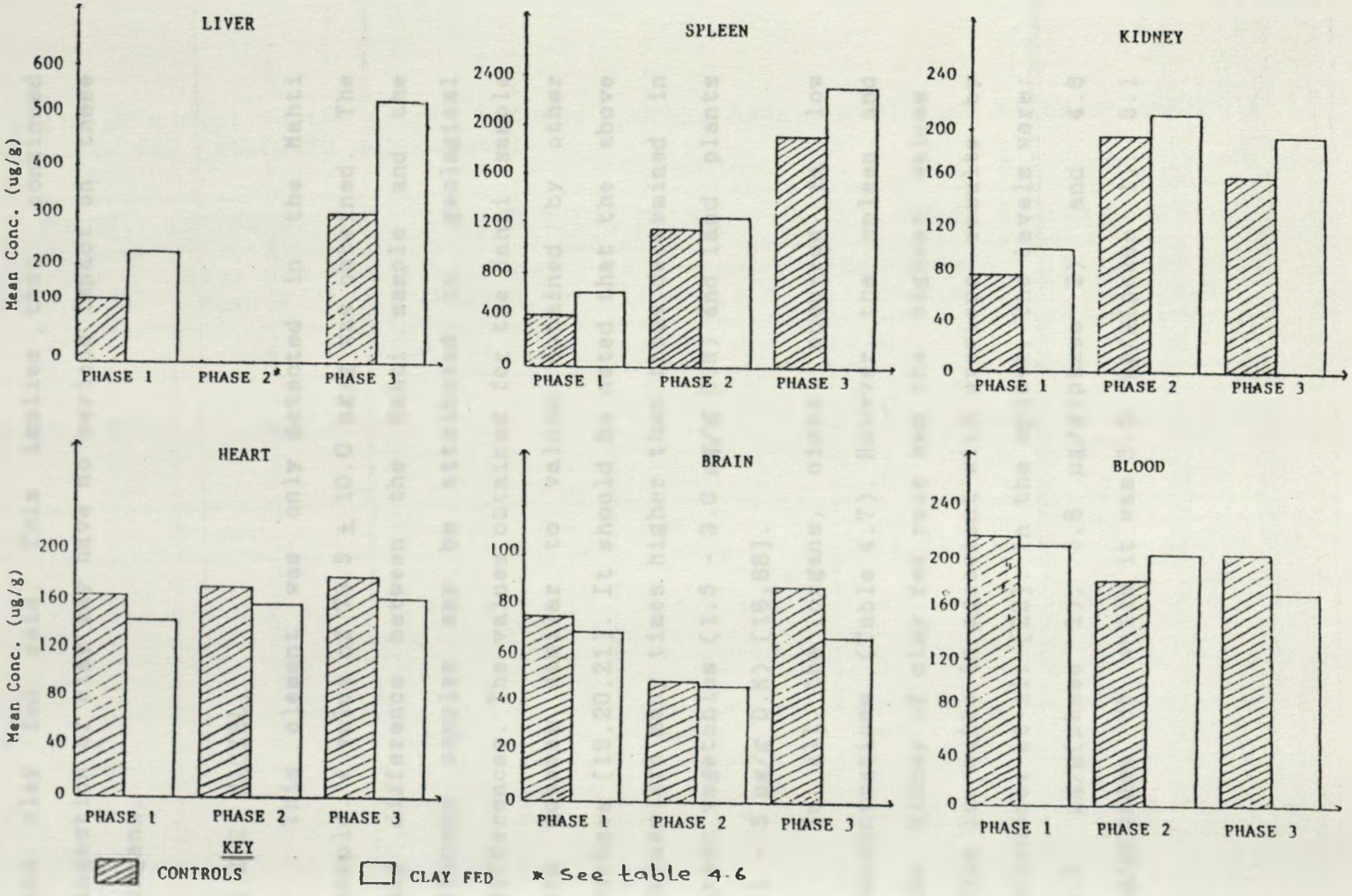


Fig: 4.1. The variation of iron in rat organs

In the heart, brain and the blood, there was no major accumulation of iron. In the heart, the level were: 164.8 µg/g(phase 1) and 179.5 µg/g(phase 3) for the clay fed rats. In these tissues, there was no significant difference ($p > 0.05$) between the controls and clay fed rats. This implies that continued ingestion of clay may have no serious impact on these organs.

4.2.2 NICKEL.

This element was only detected in the Mahti sample. A value of 79.5 ± 10.0 µg/g was obtained. The big difference between the Mahti sample and the Gikomba samples may be attributed to geological differences. The values obtained for the Mahti sample are however similar to values obtained by other workers [19,20,21]. It should be noted that the above values are many times higher than those determined in green vegetables (1.5 - 3.0 µg/g D.M) and land plants (1 - 5 µg/g D.M) [16,68].

In all the organs, nickel occurred in low concentrations (Table 4.7). However, the spleen and the kidney of clay fed rats had the highest values. This is quite in agreement with previous results by Schroeder et al. [22]. In the spleen, the levels were: 4.2 µg/g(phase 1), 5.6 µg/g(phase 2) and 4.6 µg/g(phase 3) while it was 3.5 µg/g(phase 1), 5.1

TABLE 4.7. Levels of Nickel in the Liver, spleen, kidney, heart, brain and blood of controls and clay fed rats(ug/g wet weight).

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
LIVER	n=2	n=4	*	*	n=2	n=3
	0.81 ± 0.18	0.74 ± 0.14			0.70 ± 0.25	1.83 ± 0.21 [c]
SPLEEN	n=2	n=3	n=4	n=5	n=2	n=4
	2.0 ± 0.3	4.2 ± 0.3 [b]	4.3 ± 1.0	5.6 ± 1.0 [e]	3.2 ± 0.3	4.6 ± 0.9 [d]
KIDNEY	n=2	n=4	n=3	n=4	n=2	n=3
	3.1 ± 1.5	3.5 ± 0.9	3.0 ± 0.9	5.1 ± 0.7 [d]	0.7 ± 0.1	2.7 ± 0.6 [c]

TABLE 4.7(Nickel...)

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
HEART	n=3	n=3	n=3	n=3	n=2	n=4
	1.3 ± 0.2	1.6 ± 0.2	2.8 ± 0.5	2.6 ± 0.5	1.4 ± 0.1	1.4 ± 0.2
BRAIN	n=3	n=3	n=2	n=3	n=3	n=5
	0.63 ± 0.19	0.66 ± 0.16	0.14 ± 0.04	0.15 ± 0.03	0.1 -0.5[a]	0 -0.31[a]
BLOOD	n=2	n=3	n=3	n=4	n=3	n=3
	1.7 ± 0.5	2.0 ± 0.5	1.6 ± 0.2	1.7 ± 0.5	2.1 ± 0.3	1.4 ± 0.2[d]

*- samples lost after explosion during digestion.
a- range reported.
b- $p < 0.01$ (two-tailed areas).
c- $p < 0.02$ (two-tailed areas).
d- $p < 0.05$ "
e- $p < 0.10$ "

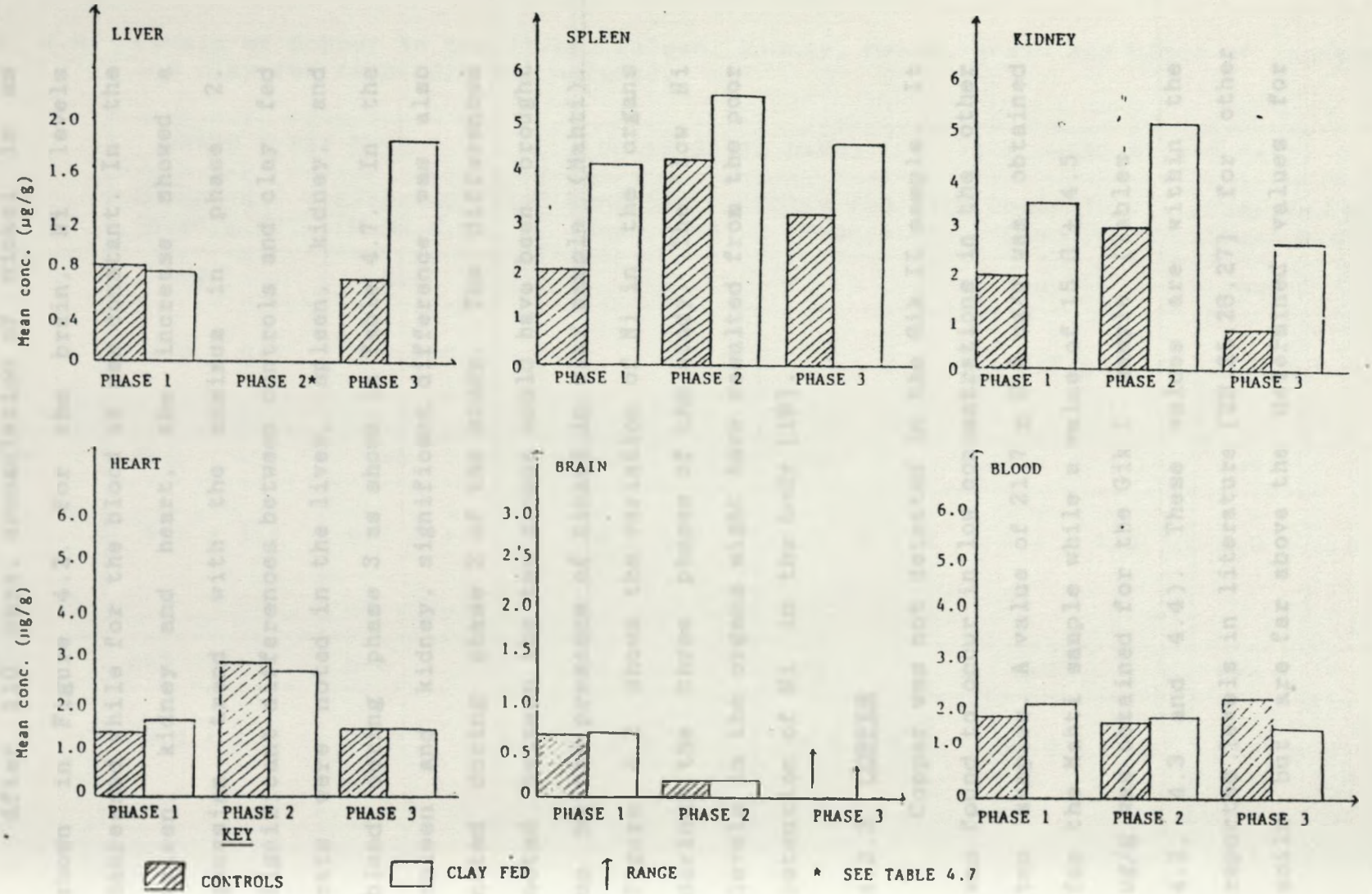


Fig. 4.2. The variation of nickel in rat organs

ug/g(phase 2) and 2.7 ug/g(phase 3) in the kidneys of consumable clay fed rats.

After 110 days, accumulation of nickel is as shown in Figure 4.2. For the brain, Ni levels decreased while for the blood it was constant. In the spleen, kidney and heart, the increase showed a gaussian trend with the maximum in phase 2. Significant differences between controls and clay fed rats were noted in the liver, spleen, kidney, and blood during phase 3 as shown in Table 4.7. In the spleen and kidney, significant difference was also noted during phase 2 of the study. The differences noted between the two groups could have been brought up by the presence of nickel in clay sample (Mahti). Figure 4.2 shows the variation of Ni in the organs during the three phases of the study. The low Ni levels in the organs might have resulted from the poor retention of Ni in the body [16].

4.2.3 COPPER

Copper was not detected in the Gik II sample. It was found to occur in low concentrations in the other two samples. A value of 21.7 $\pm$ 0.5 ug/g was obtained for the Mahti sample while a value of 15.0 $\pm$ 4.5 ug/g was obtained for the Gik I sample (Tables 4.2, 4.3 and 4.4). These values are within the reported levels in literature [23,25,26,27] for other soils but are far above the determined values for

TABLE 4.8. Levels of Copper in the liver, spleen, kidney, heart, brain and blood of controls and clay fed rats(ug/g wet weight).

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
LIVER	n=2	n=4	*	*	n=3	n=5
	6.6 ± 0.7	5.6 ± 0.6			6.2 ± 1.7	7.0 ± 0.9
SPLEEN	n=4	n=5	n=3	n=4	n=3	n=5
	6.0 ± 1.5	6.7 ± 1.3	9.6 ± 0.8	8.1 ± 1.2	8.4 ± 1.0	7.0 ± 1.3
KIDNEY	n=3	n=4	n=4	n=5	n=3	n=4
	22.9 ± 2.0	24.2 ± 3.2	83.8 ± 13.3	40.6 ± 8.8 b	30.9 ± 1.5	41.7 ± 4.9 b

TABLE 4.8(cont...)

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
HEART	n=4	n=4	n=4	n=6	n=3	n=5
	8.5 ± 0.5	11.5 ± 0.7 b	8.8 ± 0.8	8.1 ± 1.3	8.3 ± 0.6	7.7 ± 0.7
BRAIN	n=4	n=6	n=4	n=6	n=3	n=5
	3.8 ± 0.5	3.6 ± 0.4	3.4 ± 0.6	3.1 ± 0.3	4.6 ± 0.4	4.5 ± 0.7
BLOOD	n=2	n=3	n=4	n=6	n=3	n=5
	3.4 ± 0.1	3.8 ± 0.6	3.3 ± 0.6	3.7 ± 0.5	4.5 ± 0.4	3.3 ± 0.4 b

s.d- standard deviation
*- samples lost after explosion during digestion.
b- $p < 0.01$

foodstuffs: cabbage leaves (0.33), potato tubers (0.38), tomato fruits (0.65) and bean pods (1.7) ug/g fresh weight [43].

Levels of Copper in the rat tissues are shown in Table 4.8. Higher levels of liver Copper (6.8 µg/g) were evident in the control group during phase 1 of study as compared to the lower levels (6.2 µg/g) obtained in phase 3 of the study. This is as expected since newborn rats have been shown to have higher liver Copper levels than the mature rats [16]. In the clay fed rats, higher Copper levels (7.0 ± 0.8 µg/g) was registered during the last phase of the study. This may have implications that Cu present in the Mahti sample may have contributed to the high Copper levels. Similarly, the significant difference ($p < 0.01$) reported for the heart during the first phase of the study may be explained. In all the three stages of feeding, there was no significant difference noticed for the liver, spleen and the brain between the two groups. In the kidney of the controls, the high Cu levels noted in phase 2 may have resulted from the low blood Cu levels. During phase 3 of the study, kidney Cu levels decreased considerably (30.9 ± 1.5 µg/g) while blood Copper levels increased (4.5 ± 0.4 µg/g). It is probable that during phase 2, a lot of Copper was being deposited while the vice versa was true during the phase 3 of the study.

Copper didn't accumulate in any of the organs analysed. In the spleen and the kidney, the increase was gaussian with a maxima in phase 2 (Fig. 4.3).

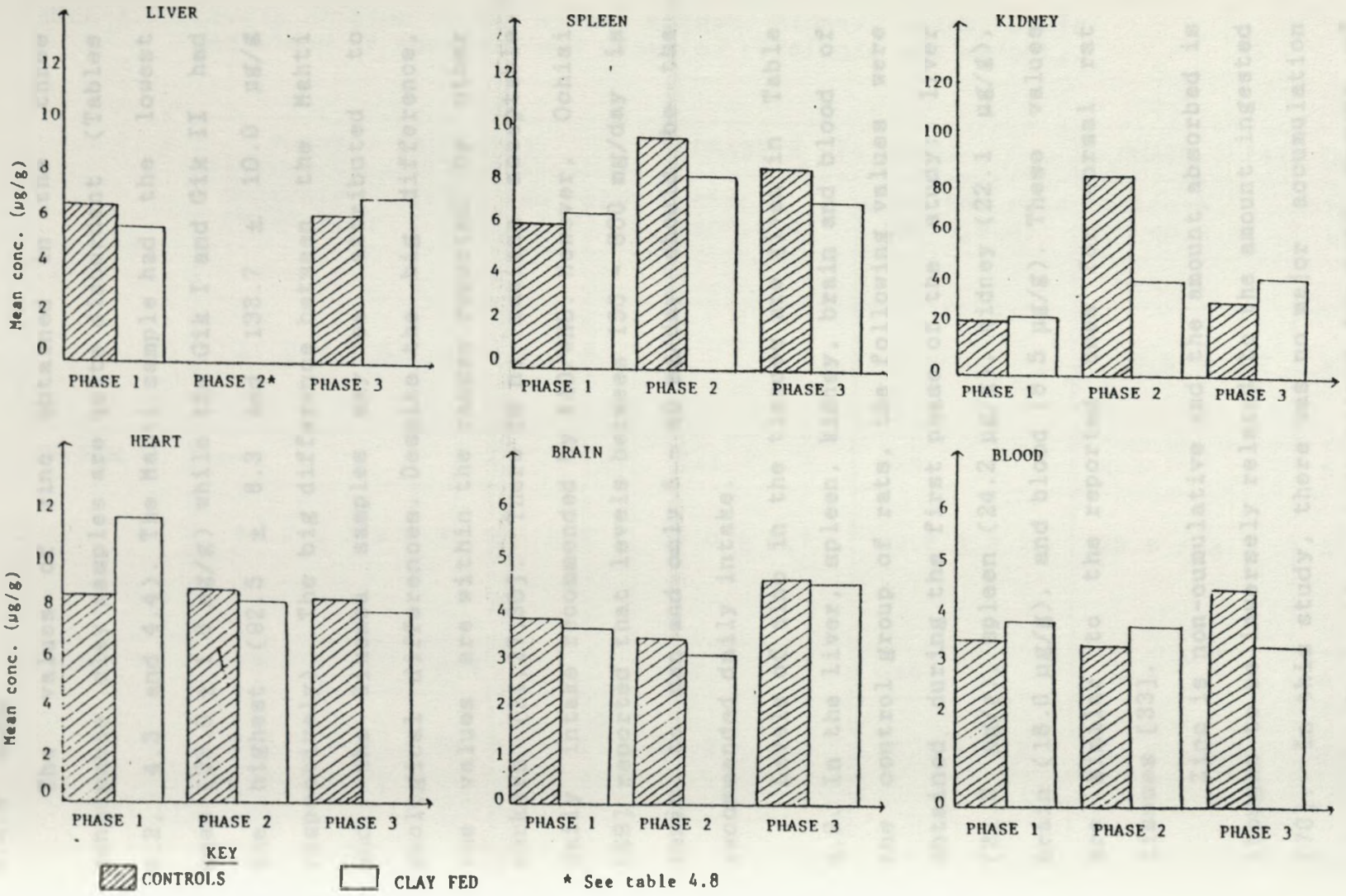


Fig. 4.3. The variation of copper in rat organs

4.2.4 ZINC

The values of zinc obtained in the three consumable clay samples are quite discordant (Tables 4.2, 4.3 and 4.4). The Mahti sample had the lowest mean (33.0 ± 1.6 µg/g) while the Gik I and Gik II had the highest (92.5 ± 8.3 and 133.7 ± 10.0 µg/g respectively). The big difference between the Mahti and the Gikomba samples may be attributed to geological differences. Despite the big difference, the values are within the ranges reported by other workers [21,27,30]. There is no maximum acceptable daily intake recommended by FAO/WHO. However, Ochiai [69] reported that levels between 150 - 600 mg/day is toxic to man and only 5 - 40 mg/day should be the recommended daily intake.

Levels of zinc in the tissues are given in Table 4.9. In the liver, spleen, kidney, brain and blood of the control group of rats, the following values were obtained during the first phase of the study: liver (31.0 µg/g), spleen (24.2 µg/g), kidney (22.1 µg/g), brain (18.0 µg/g), and blood (8.5 µg/g). These values are similar to the reported ones for normal rat tissues [33].

Zinc is non-cumulative and the amount absorbed is thought to be inversely related to the amount ingested [70]. In this study, there was no major accumulation in any of the tissues dissected for both groups of

TABLE 4.9. Levels of Zinc in the liver, spleen, kidney, heart, brain and blood of controls and clay fed rats(ug/g wet weight).

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
LIVER	n=2	n=5	*	*	n=3	n=5
	31.0 ± 1.8	27.0 ± 4.8			29.8 ± 6.2	35.1 ± 2.5
SPLEEN	n=4	n=4	n=3	n=5	n=3	n=4
	24.2 ± 3.9	28.3 ± 3.2	31.0 ± 0.7	29.6 ± 5.2	29.8 ± 3.5	31.6 ± 4.1
KIDNEY	n=3	n=4	n=4	n=6	n=3	n=5
	22.1 ± 2.8	34.3 ± 8.1 [c]	42.3 ± 7.2	39.1 ± 4.5	34.2 ± 2.8	31.5 ± 2.8

TABLE 4.9(cont...)

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
HEART	n=4	n=5	n=4	n=5	n=3	n=5
	24.7 ± 1.3	16.8 ± 3.9 [a]	25.6 ± 3.8	20.5 ± 2.6 [d]	23.8 ± 1.3	20.5 ± 3.0 [d]
BRAIN	n=4	n=6	n=4	n=6	n=3	n=5
	18.0 ± 1.5	15.5 ± 2.1 [d]	15.0 ± 1.1	12.2 ± 0.9 [a]	18.4 ± 3.0	18.4 ± 3.0
BLOOD	n=3	n=4	n=4	n=6	n=3	n=3
	8.5 ± 1.4	8.4 ± 1.2	7.5 ± 0.9	9.2 ± 2.0	7.9 ± 0.4	7.7 ± 1.5

*- samples lost after explosion during digestion.
s.d- standard deviation.
a- $p < 0.01$ (two-tailed areas).
c- $p < 0.05$ (two-tailed areas).
d- $P < 0.10$ "

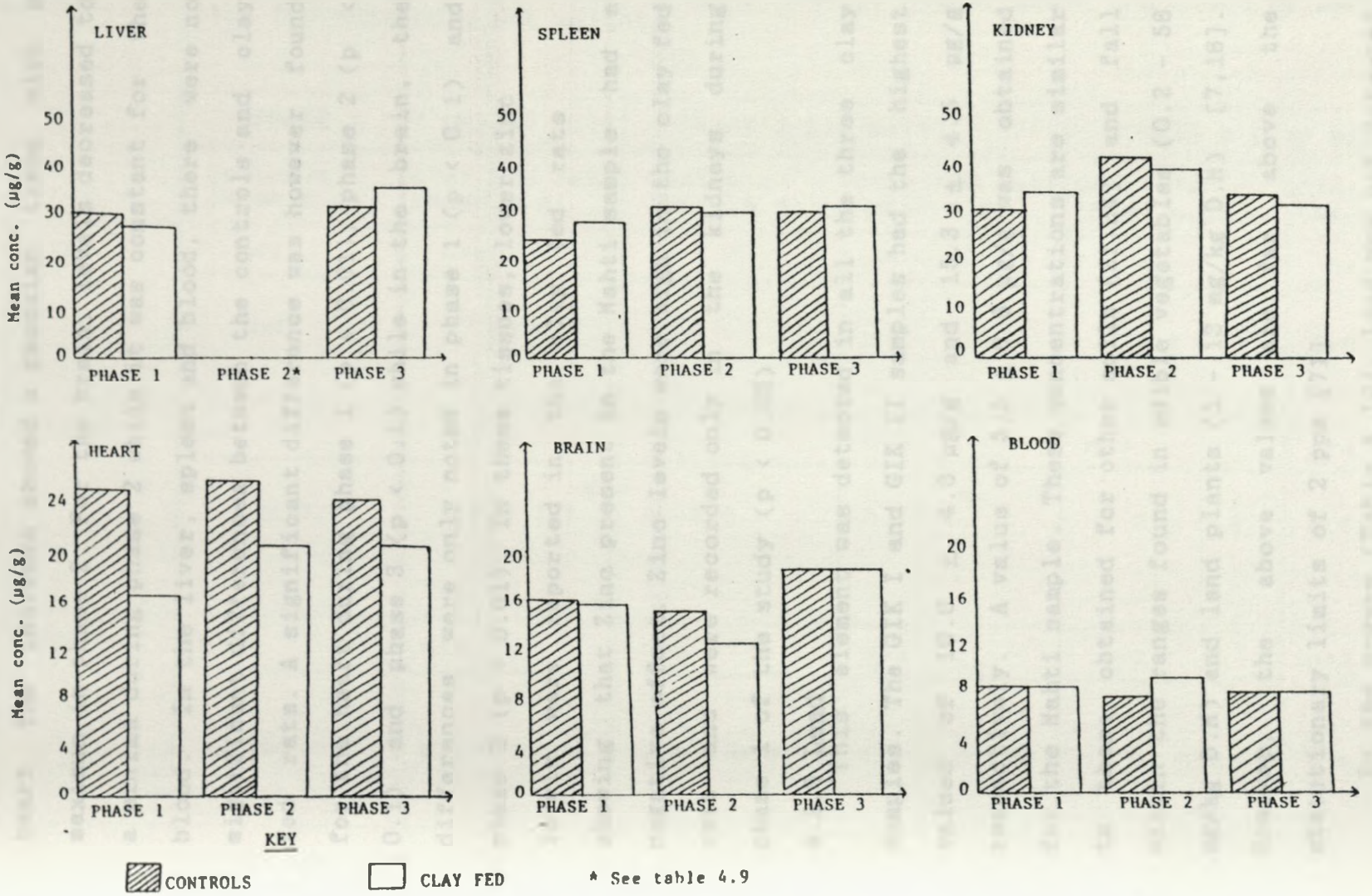


Fig. 4.4. The variation of zinc in rat organs

rats (Fig. 4.4). In the spleen, the kidney and the heart the increase showed a gaussian trend with a maximum in phase 2. For the brain, levels decreased to a minima during phase 2 while it was constant for the blood. In the liver, spleen and blood, there were no significant differences between the controls and clay fed rats. A significant difference was however found for the heart during phase 1 ($p < 0.01$), phase 2 ($p < 0.1$) and phase 3 ($p < 0.1$) while in the brain, the differences were only noted in phase 1 ($p < 0.1$) and phase 2 ($p < 0.01$). In these tissues, lower zinc levels were reported in the clay fed rats showing that Zinc present in the Mahti sample had a negative effect. Zinc levels were high in the clay fed rats and were recorded only in the kidneys during phase 1 of the study ($p < 0.05$).

4.2.5 Lead

This element was detected in all the three clay samples. The GIK I and GIK II samples had the highest values of 10.0 $\pm$ 4.0 µg/g and 15.3 $\pm$ 4.5 µg/g respectively. A value of 5.5 $\pm$ 0.6 µg/g was obtained for the Mahti sample. These concentrations are similar to those obtained for other soils [21,24] and fall within the ranges found in edible vegetables (0.2 - 56 mg/kg D.M) and land plants (1 - 13 mg/kg D.M) [7,16]. However, the above values are far above the statutionary limits of 2 ppm [71].

In the organs (Table 4.10), lead was not detected

TABLE 4.10. Levels of Lead in the liver, spleen, kidney, heart, brain and blood of controls and clay fed rats(ug/g wet weight).

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
LIVER	n=2	n=5	*	*	n=2	n=3
	nd	nd			1.4 ± 0.3	2.8 ± 0.7 [e]
SPLEEN	n=3	n=6	n=4	n=6	n=3	n=3
	1.4 ± 0.5	1.9 ± 1.1 [a]	0 -3.6	0 -3.1 [a]	2.2 ± 0.4	2.5 ± 1.0
KIDNEY	n=4	n=6	n=3	n=3	n=2	n=3
	nd	0 - 1.2	1.4 ± 0.3	1.7 ± 0.2	1.1 ± 0.3	1.8 ± 0.3 [e]

TABLE 4.10(cont...)

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
HEART	n=4	n=3	n=3	n=3	n=3	n=3
	0 - 1.9[a]	2.1 ± 0.6	0.9 ± 0.3	0.5 ± 0.1	0.38 ± 0.03	0.56 ± 0.22
BRAIN	n=4	n=6	n=4	n=4	n=3	n=5
	3.3 ± 0.3	3.8 ± 0.5	1.6 ± 0.2	1.7 ± 0.3	2.8 ± 0.3	2.8 ± 0.4
BLOOD	n=3	n=3	n=3	n=4	n=4	n=4
	2.2 ± 0.4	2.9 ± 0.6	0.5 ± 0.1	3.8 ± 0.3[b]	1.7 ± 0.3	2.0 ± 0.6

*- samples lost after explosion during digestion.
s.d- standard deviation.
a- range reported
b -p < 0.01
e -p < 0.10
nd -not detected.

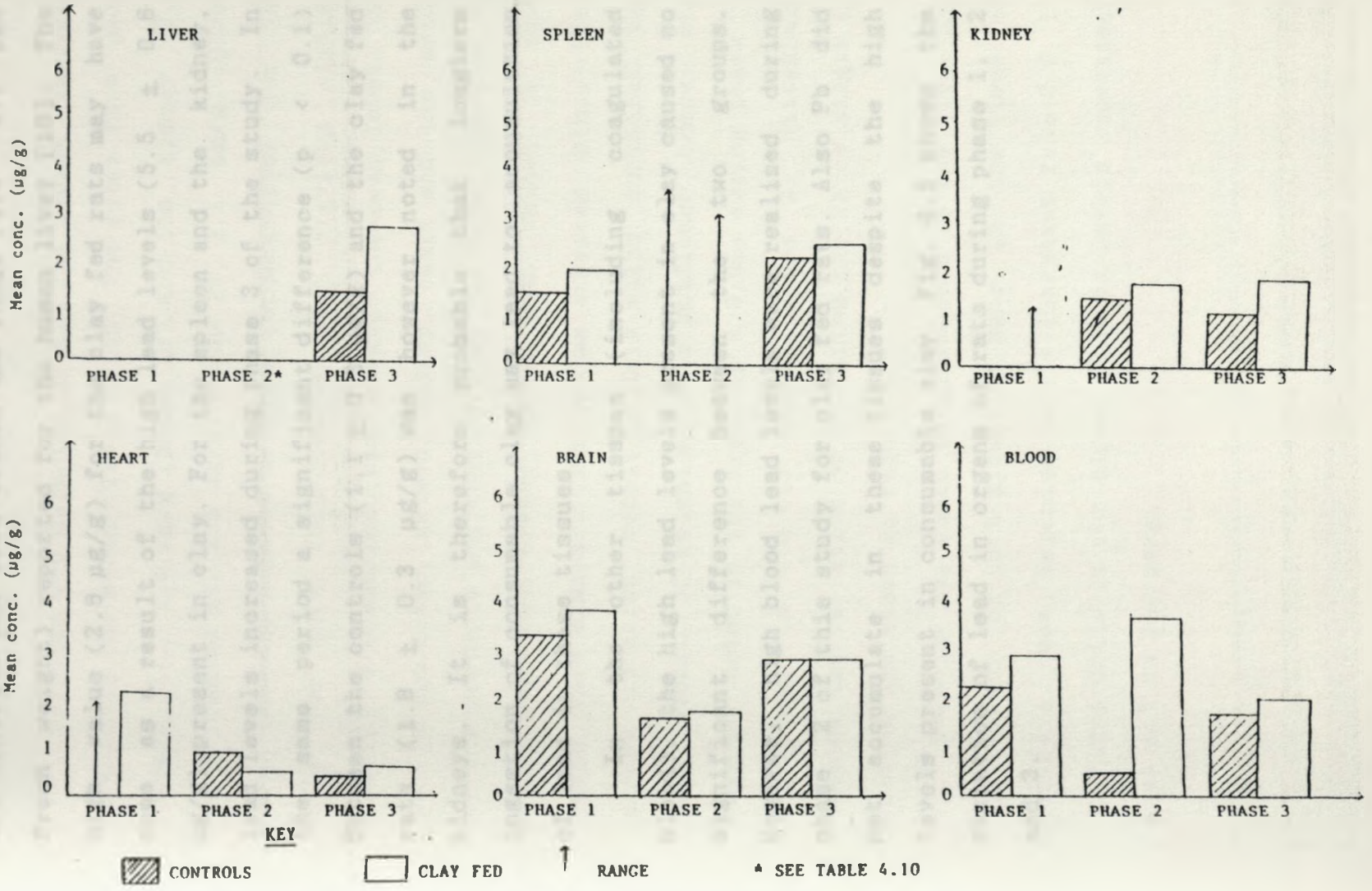


Fig. 4.5. The variation of lead in rat organs

in the liver during the first phase of the study. A significant difference ($p < 0.1$) was however noted during phase 3. A value of 1.4 µg/g was obtained for the controls and lies within the range (1.3 - 1.7 ppm fresh weight) reported for the human liver [16]. The high value (2.8 µg/g) for the clay fed rats may have come as a result of the high lead levels (5.5 $\pm$ 0.6 µg/g) present in clay. For the spleen and the kidney, lead levels increased during phase 3 of the study. In the same period a significant difference ($p < 0.1$) between the controls (1.1 $\pm$ 0.3 µg/g) and the clay fed rats (1.8 $\pm$ 0.3 µg/g) was however noted in the kidneys. It is therefore probable that longterm ingestion of consumable clay may lead to accumulation of lead in these tissues.

In the other tissues (including coagulated blood), the high lead levels present in clay caused no significant difference between the two groups. However, high blood lead levels were realised during phase 2 of this study for clay fed rats. Also Pb did not accumulate in these tissues despite the high levels present in consumable clay. Fig. 4.5 shows the variation of lead in organs of rats during phase 1, 2 and 3.

4.2.6 Selenium

Selenium was not detected in the consumable clay samples. A value of 1.1 ± 0.4 µg/g was obtained in the rabbit pellets.

Selenium was detected in all the organs analysed including the blood (Table 4.11). The liver (0.8 µg/g (phase 1), 1.6 µg/g (phase 3)) and the kidney (2.1 µg/g (phase 1), 1.6 µg/g (phase 2), 2.1 µg/g (phase 3)), had the highest Selenium concentration for the clay fed rats. This compares with previous studies [41,42]. A comparison made between the mean levels of Se in the liver, spleen, kidney, heart and the brain of the controls and that of clay fed rats indicated no significant difference as the mean values in most cases were similar. This was as expected since both groups received rabbit pellets which contained the same amount of selenium. A significant change was however noted in the brain ($p < 0.01$) during phase 1 and blood ($p < 0.01$) during phase 3. Histograms showing the variation of Se during the three stages of feeding are shown in Figure 4.6.

Selenium was found to accumulate in the liver and the kidney of both groups of rats. In the liver of controls, mean Selenium levels increased from 0.9 to 1.7 µg/g while in the kidney mean Se levels increased from 1.7 to 2.2 µg/g.

TABLE 4.11. Levels of Selenium in the liver, spleen, kidney, heart, brain and blood of controls and clay fed rats(ug/g wet weight).

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
LIVER	n=2	n=5	*	*	n=2	n=4
	0.9 ± 0.1	0.8 ± 0.2			1.7 ± 0.4	1.6 ± 0.4
SPLEEN	n=4	n=6	n=4	n=6	n=3	n=4
	0 -1.0	0 -0.8[a]	0 - 2.6	0 - 1.6[a]	0.4 ± 0.2	0.6 ± 0.1
KIDNEY	n=2	n=3	n=4	n=6	n=3	n=3
	1.7 ± 0.2	2.1 ± 0.7	1.6 ± 0.2	1.6 ± 0.5	2.2 ± 0.2	2.1 ± 0.3

TABLE 4.11(cont...)

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
HEART	n=3	n=3	n=2	n=4	n=2	n=3
	0.66 ± 0.14	0.88 ± 0.23	0.37 ± 0.01	0.43 ± 0.12	0.55 ± 0.36	0.48 ± 0.11
BRAIN	n=3	n=3	n=2	n=4	n=3	n=4
	0.83 ± 0.07	0.37 ± 0.10 b	0.66 ± 0.17	0.43± 0.08	0.52 ± 0.17	0.64 ± 0.11
BLOOD	n=3	n=3	n=3	n=3	n=3	n=4
	0.33 ± 0.03	0.59 ± 0.18	0.36 ± 0.08	0.42 ± 0.13	0.77 ± 0.16	0.35 ± 0.08 b

s.d- standard deviation
*- samples lost after explosion during digestion.

a -range reported
b -$p < 0.01$

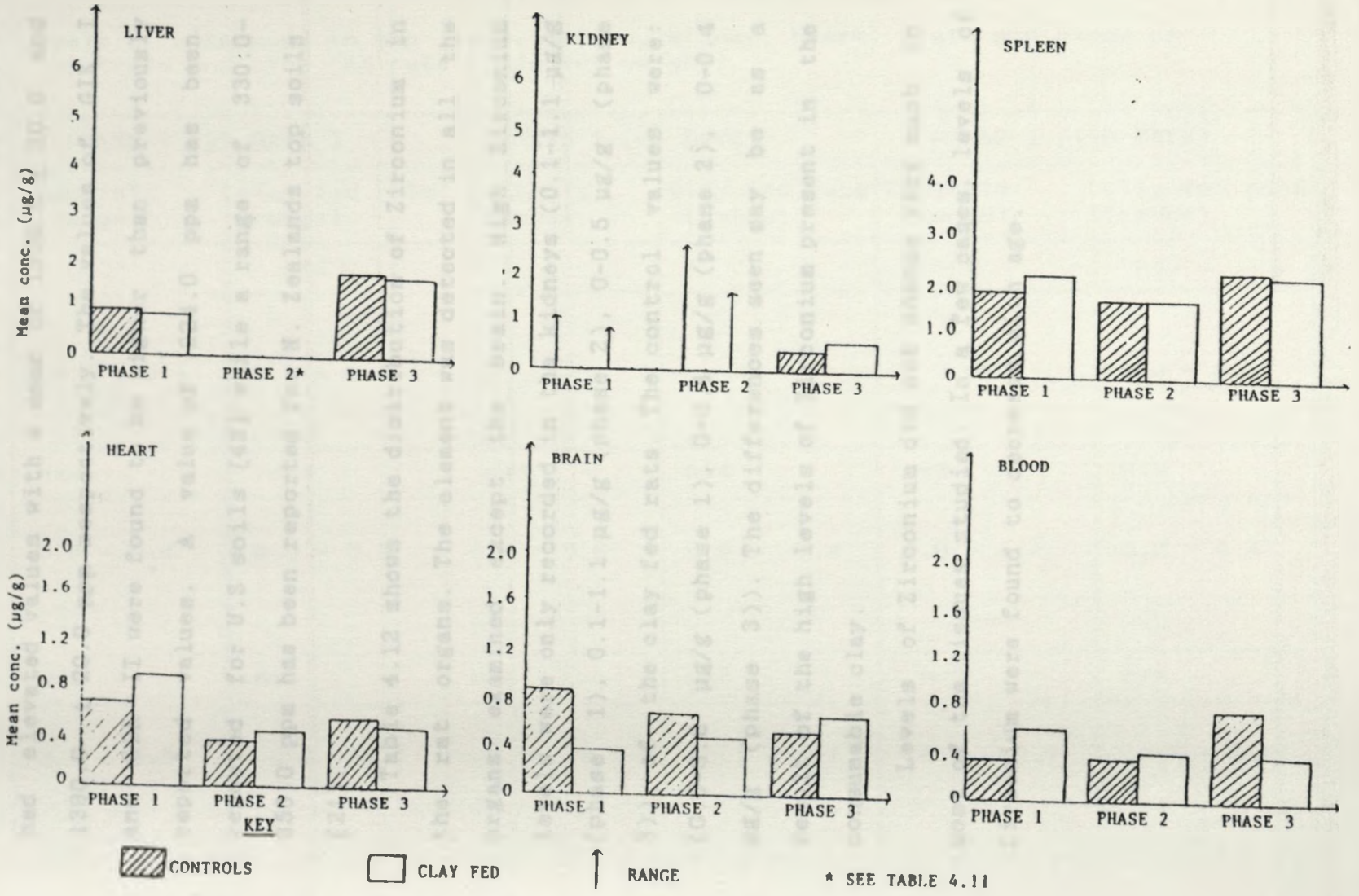


Fig. 4.6. The variation of selenium in rat organs

4.2.7 Zirconium

Zirconium concentration was lowest in the Mahti sample, 548.4 ± 2.2 µg/g. The GIK I and GIK II samples had elevated values with a mean of 1500.0 ± 20.0 and 1390.0 ± 20.0 ppm respectively. The values of GIK I and GIK II were found to be higher than previously reported values. A value of 224.0 ppm has been reported for U.S soils [43] while a range of 330.0-850.0 ppm has been reported for N. Zealands top soils [21].

Table 4.12 shows the distribution of Zirconium in the rat organs. The element was detected in all the organs examined except the brain. High Zirconium levels were only recorded in the kidneys (0.1-1.1 µg/g (phase 1), 0.1-1.1 µg/g (phase 2), 0-0.5 µg/g (phase 3)) of the clay fed rats. The control values were: (0.0-0.8 µg/g (phase 1), 0-0.4 µg/g (phase 2), 0-0.4 µg/g (phase 3)). The differences seen may be as a result of the high levels of Zirconium present in the consumable clay.

Levels of Zirconium did not change very much in most of the tissues studied. In a few cases, levels of Zirconium were found to decrease with age.

TABLE 4.12. Levels of zirconium in the liver, spleen, kidney, heart, brain and blood of controls and clay fed rats (ug/g wet weight).

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (range)	clay fed rats (range)	Controls (range)	Clay fed rats (range)	Controls (range)	Clay fed rats (range)
LIVER	n=2	n=5	*	*	n=3	n=5
	0.5 ± 0.1	0.3 ± 0.1 [a]			nd	0.0 - 0.1
SPLEEN	n=4	n=4	n=3	n=4	n=3	n=5
	0.0 - 0.4	0.0 - 0.8	0.0 - 0.5	0.0 - 0.3	0.0 - 1.1	0.0 - 0.4
KIDNEY	n=3	n=5	n=4	n=5	n=3	n=4
	0.1 - 0.6	0.0 - 1.1	0.0 - 0.4	0.1 - 1.1	0.0 - 0.4	0.0 - 0.5

TABLE 4.12(cont...)

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (range)	clay fed rats (range)	Controls (range)	Clay fed rats (range)	Controls (range)	Clay fed rats (range)
HEART	n=4	n=5	n=4	n=5	n=3	n=4
	0.1 - 0.4	0.0 - 0.2	0.0 - 0.7	0.0 - 0.4	0.0 - 0.2	nd
BRAIN	n=4	n=6	n=4	n=6	n=3	n=5
	nd	nd	nd	nd	nd	nd
BLOOD	n=3	n=4	n=3	n=6	n=3	n=5
	0.1 - 0.2	0.0 - 0.3	0.0 - 0.1	0.0 - 0.2	nd	0.0 - 0.3

*- samples lost after explosion during digestion.
nd - not detected
a - mean,s.d reported

4.2.8 Rubidium.

Results of Tables 4.2, 4.3 and 4.4 show that rubidium was not detected in the Mahti sample. In GIK I and Gik II, values of 186.0 ± 8.0 and 185.1 ± 6.8 µg/g were obtained respectively. These values are within the mean for soils of various countries which range from 33 to 270 ppm [43].

The distribution of rubidium in the rat organs is shown in Table 4.13. There were no major differences between the controls and the clay fed rats. This is because the rats received the same amount of rabbit pellets which was the source of rubidium (86.6 ± 4.0 µg/g). In all the tissues studied, there was no noticeable accumulation of rubidium. In the liver, the kidney and the heart, the ranges reported decreased with continued feeding.

TABLE 4.13. Levels of rubidium in the liver, spleen, kidney, heart, brain and blood of controls and clay fed rats (ug/g wet weight).

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (range)	clay fed rats (range)	Controls (range)	Clay fed rats (range)	Controls (range)	Clay fed rats (range)
LIVER	n=2	n=5	*	*	n=3	n=5
	0.1 - 0.5	0.0 -0.4			0.0 - 0.2	0.0 - 0.4
SPLEEN	n=4	n=6	n=4	n=5	n=3	n=5
	nd	nd	0.0 - 0.6	0.0 - 0.8	0.0 - 0.4	0.0 - 0.8
KIDNEY	n=4	n=6	n=3	n=5	n=3	n=4
	0.0 - 1.2	0.0 - 0.9	nd	0.0 - 0.20	0.0 - 0.2	0.0 - 0.1

TABLE 4.13(cont...)

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (range)	clay fed rats (range)	Controls (range)	Clay fed rats (range)	Controls (range)	Clay fed rats (range)
HEART	n=4	n=6	n=4	n=5	n=3	n=4
	0.0 - 0.5	0.0 - 0.8	0.0 - 0.3	nd	nd	nd
BRAIN	n=4	n=6	n=4	n=6	n=3	n=5
	0.0 -0.2	0.0 - 0.2	0.1 - 0.4	0.0 - 0.2	0.0 - 0.1	0.0 - 0.1
BLOOD	n=3	n=4	n=4	n=6	n=3	n=5
	nd	nd	nd	nd	nd	nd

*- samples lost after explosion during digestion.
nd - not detected

4.2.9 Manganese.

In this study, levels of manganese in the Mahti sample were quite low compared to the Gikomba samples (Tables 4.2, 4.3 and 4.4). A value of 456.6 ± 9.7 µg/g was recorded for the Mahti sample while a value of 722.1 ± 55.5 µg/g and 1310.0 ± 50.0 µg/g were recorded for the GIK I and GIK II samples respectively. The big difference between the Mahti sample and the gikomba samples may be attributed to geological differences. Despite this difference, the above values lie within the reported ranges for other soils [11,35].

In the organs (Table 4.14), manganese was found to be present in all the tissues analysed. In the liver, spleen and kidney, levels of manganese increased during the feeding period for both groups. This increase is illustrated in Figure 4.7. It is worth noting that high values (1.3 µg/g (phase 1), 7.8 µg/g (phase 3)) were recorded in the livers of clay fed rats as compared to the controls (1.2 µg/g(phase 1), 4.8 µg/g (phase 3)). This difference might have come about as a result of the manganese present in the clay (Mahti sample).

In all the tissues analysed, high levels of manganese were obtained. In a study on levels of manganese in unexposed tissues, the following average levels (wet weight) were reported: 2.1 ppm (liver), 1.2 ppm (kidney) and 0.4 ppm (brain) [46]. These

TABLE 4.14. Levels of manganese in the liver, spleen, kidney, heart, brain and blood of controls and clay fed rats(ug/g wet weight).

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls (Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
LIVER	n=2	n=4	*	*	n=3	n=4
	1.2 ± 0.1	1.3 ± 0.4			4.8 ± 1.3	7.8 ± 1.0
SPLEEN	n=2	n=3	n=3	n=5	n=3	n=5
	5.5 ± 1.4	3.4 ± 0.7	9.7 ± 3.0	7.1 ± 1.2	10.4 ± 1.6	11.8 ± 3.0
KIDNEY	n=2	n=3	n=4	n=3	n=3	n=4
	1.9 ± 0.4	2.2 ± 0.4	4.1 ± 0.6	6.2 ± 2.0	0.0 - 6.9 [a]	6.4 ± 1.8

TABLE 4.14(cont...)

	PHASE 1 (37 DAYS)		PHASE 2 (74 DAYS)		PHASE 3 (110 DAYS)	
	Controls ((Mean,s.d)	clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)	Controls (Mean,s.d)	Clay fed rats (Mean,s.d)
HEART	n=3	n=4	n=4	n=6	n=3	n=4
	2.2 ± 0.9	2.4 ± 0.6	0 - 5.2[a]	0.1 - 7.1[a]	3.0 ± 0.3	2.7 ± 0.6
BRAIN	n=3	n=4	n=3	n=4	n=3	n=5
	2.7 ± 0.9	3.1 ± 0.6	1.7 ± 0.4	1.4 ± 0.2	1.6 ± 0.5	1.4 ± 0.4
BLOOD	n=3	n=4	n=3	n=5	n=3	n=3
	3.6 ± 1.2	3.8 ± 0.9	3.2 ± 1.4	3.5 ± 1.0	3.5 ± 0.4	3.3 ± 0.8

* - samples lost after explosion during digestion.
s.d -standard deviation.
a - range reported.

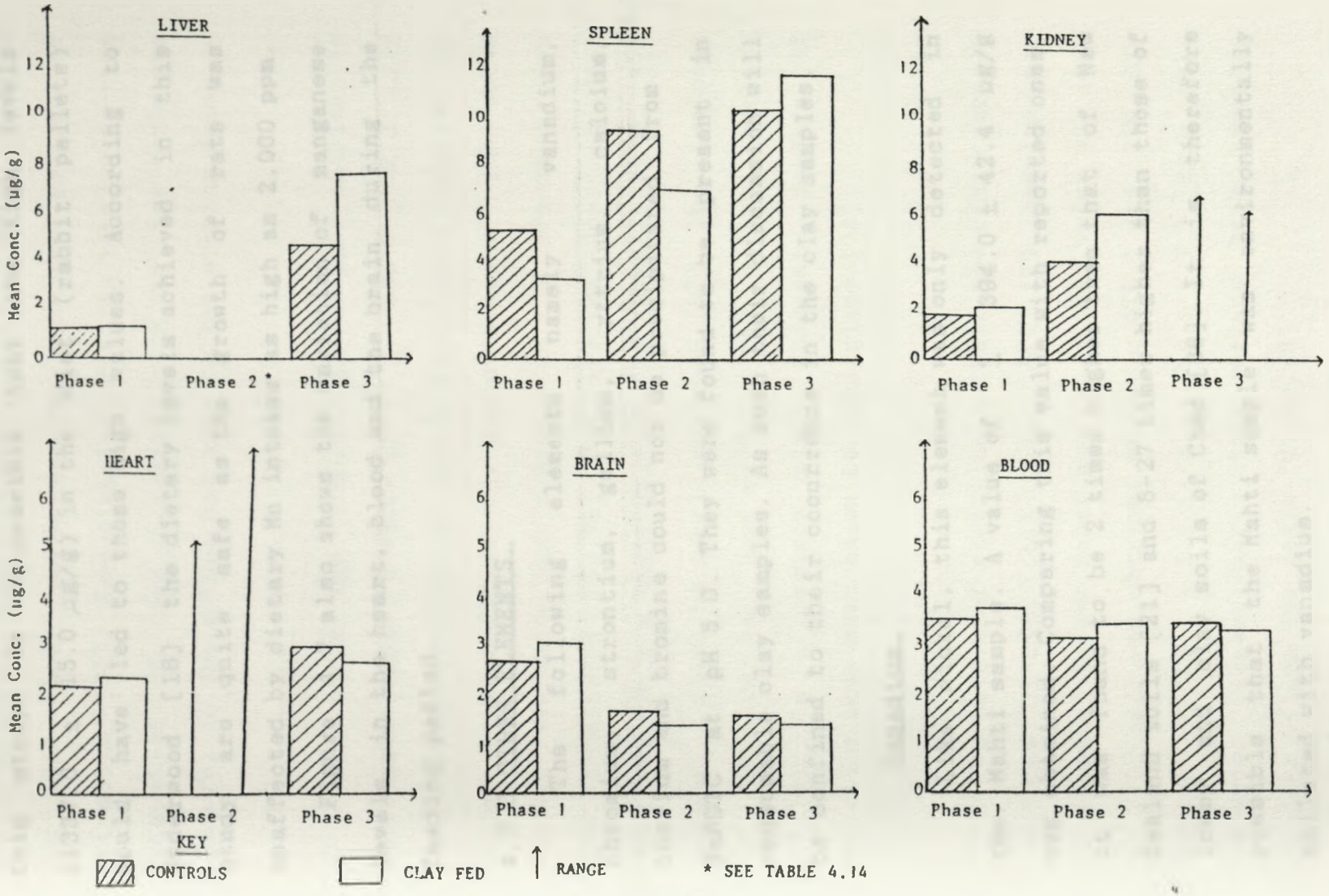


Fig. 4.7. The variation of manganese in rat organs

values are quite low compared to those obtained in this study. It is possible that the high levels (1337.0 ± 15.0 µg/g) in the diet (rabbit pellets) could have led to those high values. According to Underwood [16] the dietary levels achieved in this study are quite safe as the growth of rats was unaffected by dietary Mn intakes as high as 2,000 ppm.

Figure 4.7 also shows the variation of manganese levels in the heart, blood and the brain during the feeding period.

4.3.0 OTHER ELEMENTS.

The following elements namely vanadium, chromium, strontium, gallium, yttrium, calcium, thorium and bromine could not be precipitated from NaDDTC at pH 5.0. They were found to be present in consumable clay samples. As such their discussion will be confined to their occurrence in the clay samples.

Vanadium.

Like nickel, this element was only detected in the Mahti sample. A value of ≤ 394.0 ± 42.4 µg/g was obtained. Comparing this value with reported ones, it was found to be 2 times higher than that of New Zealand soils [21] and 8-27 times higher than those of loamy and clay soils of Chad [36]. It is therefore possible that the Mahti sample was environmentally polluted with vanadium.

Vanadium is a relatively toxic element. Studies on mice and rats have shown placenta passage of vanadium [72]. This clearly indicates that long-term ingestion of this consumable clay may not only be harmful to the mother but also to the growing fetus.

Chromium.

Like nickel and vanadium, it was only detected in the Mahti sample. A value of $\leq$ 356.7 $\pm$ 52.0 µg/g was obtained. Similar levels have been reported by other workers [20,47] for soils. However, the above values are quite high compared to the reported ones for foodstuffs [73,74]. The average American diet contains only a small quantity of chromium (5 - 115 µg/day), which is poorly absorbed [26]. The toxicity of chromium has been reviewed by several workers [7,16]. In a certain study, placenta passage of chromium have been shown to take place [74].

Strontium.

The levels of strontium in both the Mahti sample and the Gikomba (GIK I and GIK II) samples are comparable. In the Mahti sample, a mean value of 46.8 $\pm$ 2.1 µg/g was obtained while in GIK I and GIK II, values of 29.1 $\pm$ 5.5 µg/g and 38.4 $\pm$ 2.3 µg/g were recorded respectively. Other workers have reported similar values for soils [21,30,48] .

It has been reported that the uptake of lead and

strontium is increased by calcium defficiency [54,70]. This has the implications that consumers of the Mahti sample face the danger of lead and strontium toxicity if their normal diets contain little or no calcium.

Gallium.

The gallium status in soils has not been intensively studied. In this study, it was registered in all the samples with the Mahti sample having the highest concentration (45.4 ± 6.1 µg/g). The GIK I and GIK II had almost the same amount (30.0 ± 6.0 µg/g and 27.9 ± 3.4 µg/g respectively). These values are within the reported ranges for other soils [49,50].

Yttrium.

The element was detected in all the samples. The GIK I and GIK II had the highest concentration (89.6 ± 9.6 µg/g and 79.7 ± 5.9 µg/g respectively). A concentration of 8.5 ± 0.6 µg/g was recorded for the Mahti sample. Comparing this with the reported values [43,50], the GIK I and GIK II had mean values four times higher while the Mahti sample was only slightly below the reported values.

Titanium.

Titanium is a common constituent of rocks and ranges from 0.03 to 1.4%. In this study, it was found to be present in all the samples analysed. The Mahti sample had 4.86 ± 0.07 % while the GIK I and GIK II

had lesser values; 0.287 ± 0.022 and 0.294 ± 0.016 %. Values obtained for GIK I and GIK II are within the reported ranges [20,21,36,]. The Mahti sample had a value (4.86 ± 0.07)% well above the reported ranges. This may probably be due to geological differences.

Thorium.

Thorium was present in all the samples analysed. The GIK I and GIK II samples had the highest concentrations (26.7 ± 3.5 and 22.2 ± 4.2 µg/g respectively) while the Mahti sample had a mean of 3.5 ± 0.6 µg/g. The GIK I and GIK II samples had mean values well above the reported ranges for soils [12,30,47]. These figures are rather scaring in that thorium is known to be a radioactive element[75].

Bromine.

Bromine was only present in the Mahti sample. A value of 7.7 ± 0.7 µg/g was obtained. This is quite comparable to values reported by Lag [51] for forest soils. However other workers have obtained higher values [13].

4.4.0 WEIGHTS AND APPEARANCE.

To monitor the effect of Mahti on growth rate, the weight of the rats was recorded twice a week. The results of the weights are shown in Tables 4.15, 4.16 and 4.17. In phase 1 (Table 4.15), the control group of rats increased weight by 105.0% while the consumable clay fed rats increased by 77.5%. The average gain in weight was 77.5 g and 56.2 g respectively.

In phase 2 (Table 4.16), the control group of rats increased weight by 190.1% while the clay fed rats increased by 160.5%. The average gain in weight was 128.3 g and 111.1 g respectively.

A similar trend was observed in phase 3 (Table 4.17). The control group of rats increased weight by 231.2% while the clay fed rats increased by 204.0%. The average gain in weight was 166.0 g and 142.8 g respectively.

These results indicated that rats eating the Mahti soil had reduced growth. This is well illustrated in Figures 4.8, 4.9 and 4.10 which show the mean weight of rats versus days of feeding for the three phases of the study. The growth depression may be attributed to the high concentration of Vanadium in the Mahti sample as discussed in section 4.3.1 and also as discussed in previous studies [53,76].

It has previously been reported that deficiency

TABLE 4.15 WEIGHT(g) OF RATS TAKEN FOR 37 DAYS.

	CONTROLS					CLAY FED RATS						
Days	R4	R8	R11	R12	MEAN(g)	R2	R6	R8	R13	R15	R18	MEAN(g)
1	75	60	90	70	73.8	70	65	70	90	70	70	72.5
8	85	75	95	90	86.3	90	70	70	85	70	80	77.5
12	100	90	115	115	105.0	90	85	85	105	80	95	90.0
15	105	95	125	120	111.3	90	90	90	95	80	100	90.8
18	110	100	120	120	112.5	90	95	90	100	90	105	95.0
22	117	100	125	120	115.5	100	100	100	115	95	110	103.3
26	135	115	145	140	133.8	108	120	118	115	102	120	113.8
29	145	130	155	145	143.8	118	130	125	112	110	130	120.8
33	140	130	150	150	142.5	120	125	130	115	120	130	123.3
37	150	140	160	155	151.3	125	130	135	120	122	140	128.7

TABLE 4.16. WEIGHT(g) OF RATS TAKEN FOR 74 DAYS.

	CONTROLS					CLAY FED RATS.						
Days	R3	R5	R9	R10	MEAN(g)	R4	R7	R9	R11	R14	R17	MEAN(g)
1	70	60	70	70	67.5	70	55	65	80	70	75	69.2
8	90	75	85	85	83.8	70	60	70	100	65	85	75.0
12	115	85	105	105	102.5	90	70	85	115	75	100	89.2
15	110	85	110	105	102.5	100	80	90	125	80	110	97.5
18	120	90	115	110	108.8	100	78	95	130	80	115	99.7
22	120	105	120	115	115.0	105	80	100	135	90	115	104.2
26	140	113	125	130	127.0	120	110	120	160	95	135	123.3
29	145	120	142	140	136.8	130	120	125	160	115	140	131.7
33	140	125	150	135	137.5	132	115	125	155	118	140	130.8
37	145	140	160	150	148.8	135	125	130	165	128	145	138.0
40	155	150	165	155	156.3	150	140	140	170	130	155	147.5
43	157	160	170	160	161.8	155	145	150	170	140	155	152.5
47	157	165	175	160	164.3	157	150	152	180	147	165	158.5
50	160	175	190	165	172.5	170	155	152	185	152	162	162.7
55	160	178	190	165	173.3	165	160	152	185	147	170	163.2
57	162	185	190	170	176.8	168	160	160	190	155	165	166.3
61	170	200	192	175	184.3	180	165	160	195	170	180	175.0
66	170	200	190	175	183.8	170	170	165	210	170	178	177.2
68	175	195	205	180	188.8	175	175	170	200	170	188	179.7
71	175	202	205	185	191.8	180	173	170	200	172	185	180.0
74	178	205	215	185	195.8	182	175	170	200	175	180	180.3

TABLE 4.17. WEIGHT(g) OF RATS TAKEN FOR 110 DAYS.

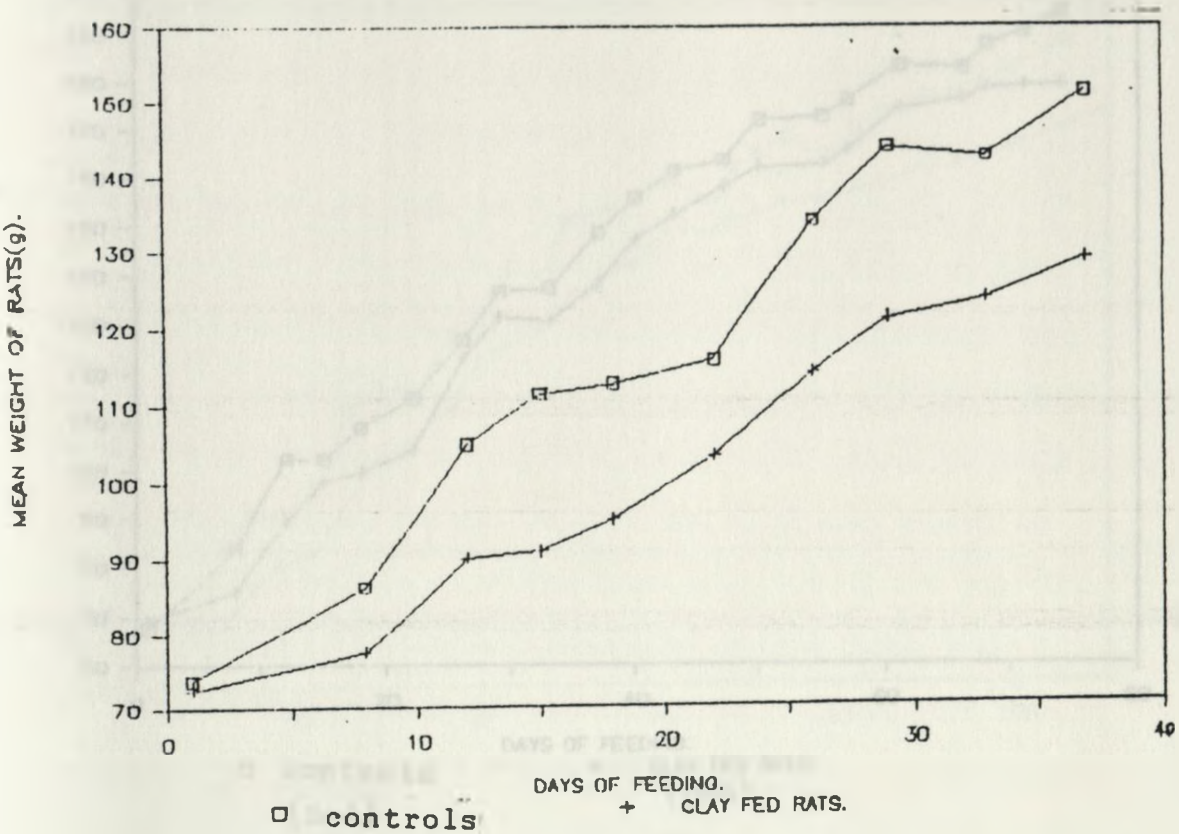
	CONTROLS				CLAY FED RATS					
Days	R1	R2	R7	MEAN	R3	R5	R10	R12	R16	MEAN
1	75	75	65	71.7	70	60	70	70	80	70.0
8	90	80	80	83.3	75	65	70	60	90	72.0
12	115	100	95	103.3	90	85	85	70	100	86.0
15	125	115	100	113.3	100	90	95	70	115	94.0
18	125	110	100	111.7	110	93	100	75	120	99.6
22	130	115	110	118.3	110	100	105	80	120	103.0
26	145	145	125	138.3	122	120	115	100	125	116.4
29	153	150	130	144.3	145	130	115	105	130	125.0
33	150	145	133	142.7	150	140	120	110	140	132.0
37	165	160	145	156.7	152	145	125	120	152	138.8
40	175	160	152	162.3	170	160	135	130	160	151.0
43	185	170	160	171.7	170	162	135	140	160	153.4
47	188	165	160	171.0	180	162	140	140	170	158.4
50	195	185	175	185.0	195	170	145	150	170	166.0
55	198	188	178	188.0	195	180	155	152	180	172.4
57	195	185	185	188.3	200	182	150	155	180	173.4
61	205	195	185	195.0	210	195	157	168	180	182.0
66	210	180	180	190.0	210	190	155	170	185	182.0
68	215	190	188	197.7	212	200	160	170	185	185.4
71	217	200	188	201.7	212	200	155	170	195	186.4
74	220	202	195	205.7	220	205	155	175	195	190.0
79	218	195	190	201.0	220	205	158	175	195	190.6
82	228	200	195	207.7	222	210	160	175	195	192.4
85	230	205	200	211.7	225	212	162	178	195	194.4
89	232	210	205	215.7	228	220	162	180	200	198.0
93	232	212	205	216.3	228	220	162	182	202	198.8
96	240	212	207	219.7	230	222	165	185	202	200.8
99	245	215	210	223.3	232	225	167	190	205	203.8
105	250	225	215	230.0	238	230	170	192	212	208.4
110	265	230	218	237.7	245	235	175	195	214	212.8

of iron, manganese, zinc and chromium leads to impaired growth [16]. Higher mean levels of iron (2.65 ± 0.03%), manganese (456.6 ± 9.7 µg/g), chromium (411.6±43.0 µg/g) and zinc (33.0±1.6 µg/g) were found in the Mahti sample while means of 3610.0±40.0 (iron), 1337.0±15.0 (manganese) and 535.0±23.0 (zinc) µg/g were found in rabbit pellets. These levels are high implying that the rats could not have been deficient of these elements.

Other changes noted were that the control group of rats had well-groomed fur, were very active while the clay fed rats appeared lethargic and with rough fur coats. It has been reported that the hair of rats fed with 100 ppm Vanadium exhibits a reduction in cystine content, suggesting that this element affects the reaction of sulfur-containing compounds [16]. This may probably be the reason why the clay fed rats had a rough hair. Similar changes have been reported in nickel deficient rats [6]. However in the analysis of nickel in the tissues, normal levels were found indicating that the rats were not nickel deficient.

Changes in the growth and appearance of hair has been noted in copper deficient rats [16]. In this study, there was plenty of copper in both the Mahti sample (21.7±0.5 µg/g) and rabbit pellets (91.6±0.3 µg/g). Data on the effect of other elements on growth rate was unavailable.

Fig. 4.8 Mean weight of rats versus days of feeding over a period of 37 days.



(n=4) (n=6)

Fig. 4.9 Mean weight of rats versus days of feeding over a period of 74 days.

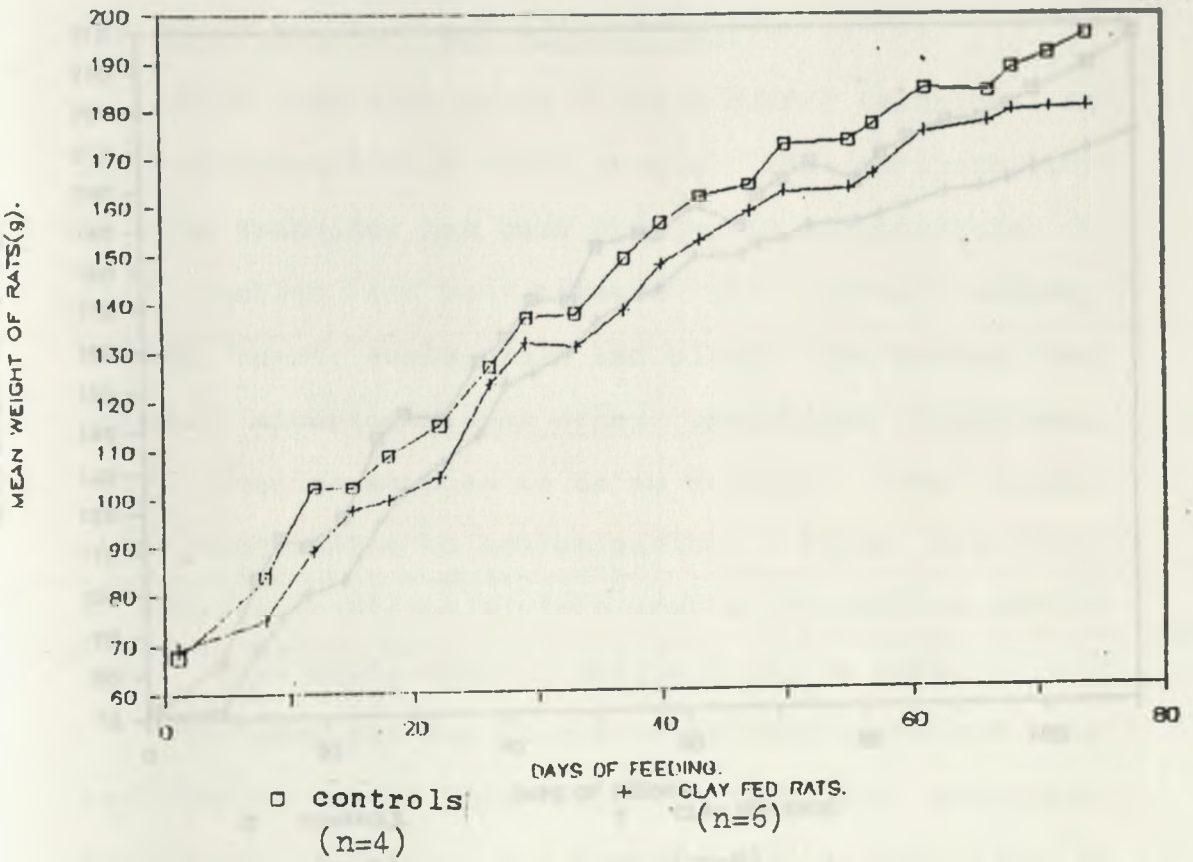
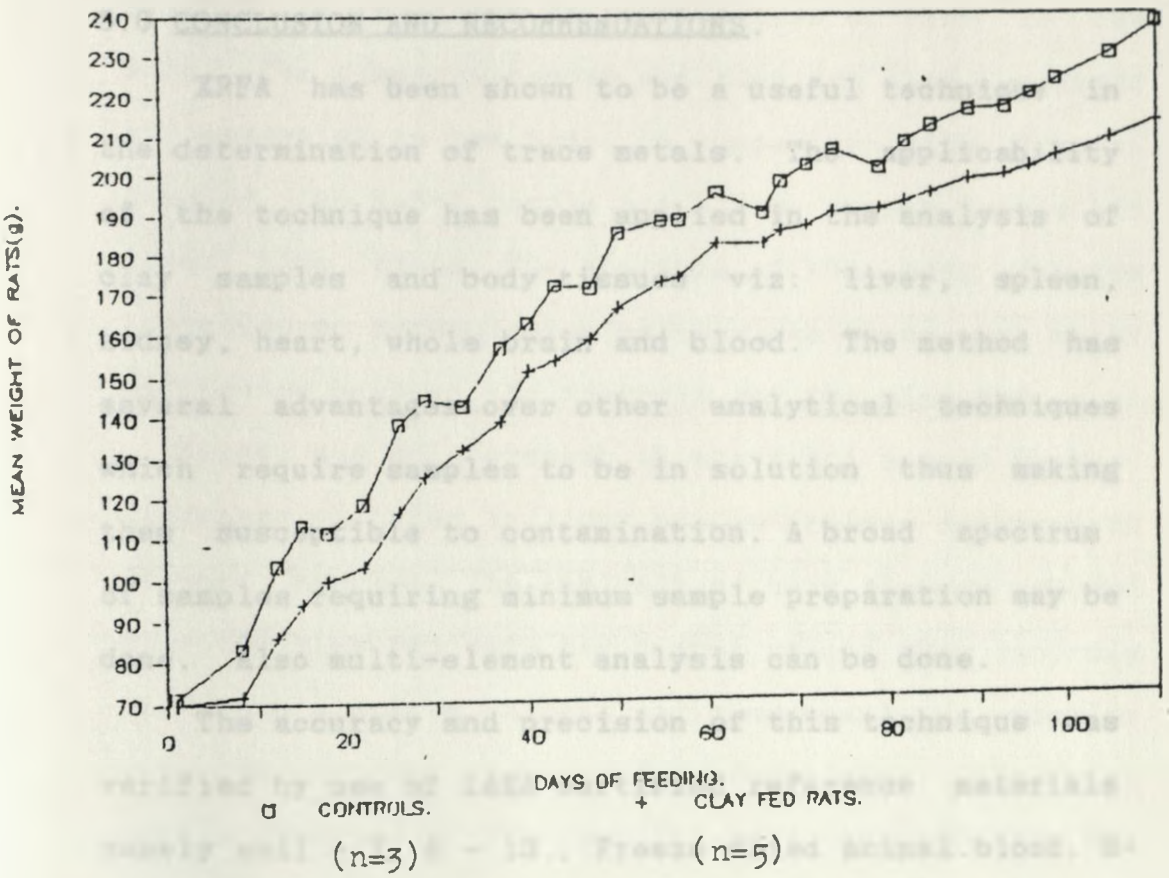


Fig. 4.10 Mean weight of rats versus days of feeding over a period of 110 days.



CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS.

XRFA has been shown to be a useful technique in the determination of trace metals. The applicability of the technique has been applied in the analysis of clay samples and body tissues viz: liver, spleen, kidney, heart, whole brain and blood. The method has several advantages over other analytical techniques which require samples to be in solution thus making them susceptible to contamination. A broad spectrum of samples requiring minimum sample preparation may be done. Also multi-element analysis can be done.

The accuracy and precision of this technique was verified by use of IAEA certified reference materials namely soil - 7, A - 13., Freeze dried animal blood, H-8 and Animal Muscle, H-4. The results of the clay analysis indicated the presence of more than 12 elements in each sample.

Iron is known to be essential to the body. However, when taken in large amounts, it can be toxic. In this study, iron was found to occur in relatively high concentrations in all the three clay samples. In the analysis of body tissues, it was found to accumulate mainly in the liver and the spleen. Since the consumers of this soil are mainly pregnant women

whose demand for Iron is high at this period, the problem of accumulation may not even exist. It is therefore possible that the consumption of clay may be helpful in replacing depleted stores of iron in the body. The elements copper and zinc are also regarded as essential to the body. In comparison with Gikomba samples, the Mahti sample had higher copper levels and lower zinc levels. The analysis of the body tissues indicated no accumulation neither was there any major difference between the controls and the clay fed.

Nickel was only present in the Mahti sample while manganese occurred in lower concentrations compared to Gikomba samples. The analysis of body tissues showed that the clay fed rats had higher values than controls for both elements (nickel and manganese). A similar case was evident in the spleen for nickel. The high levels obtained in the clay fed rats indicate that the consumption of clay increases levels of trace elements in these tissues.

Not all elements analysed in this study are useful to the body. Lead, chromium, thorium and vanadium are known to be toxic. The four elements were detected in the Mahti sample while chromium and vanadium were not detected in Gikomba samples. Levels of lead and thorium were found to be higher in Gikomba samples than in the Mahti sample. In the analysis of body tissues, lead was found to accumulate mainly in

the liver and the kidneys of clay fed rats. This may have the implications that continued ingestion of clay may lead to accumulation, after all, lead is not required during pregnancy. Lead poisoning has been diagonised in children with pica as a result of eating dirt or clay. The high levels of calcium present in Gikomba samples could be useful in reducing the absorption of lead in the body tissues. The fate of the elements namely chromium, thorium, and vanadium needs further investigation.

The elements rubidium, zirconium, strontium, gallium, yttrium, titanium and bromine were found to be present in consumable clay. These elements have not been shown to perform any vital function in animals nor are they a dietary essential for any living organism. Rubidium and zirconium were found to occur in low concentrations in the body tissues analysed in this study. The elements did not accumulate in the tissues neither was there any major difference between the controls and the clay fed rats. For the other elements (strontium, gallium, yttrium, titanium, and bromine), further research is necessary to determine their fate in the body tissues.

The consumption of clay may serve the good purpose of replacing depleted stores of useful body nutrients such as iron, copper and zinc. However, as a necessary precaution, it should be checked before

being allowed to the public to avoid any harmful deleterious effects that might result from the undesirable trace elements such as vanadium, lead, chromium and thorium present in it.

REFERENCES

1. Mclaren, D.S. *Medicine Digest*, 12(6), 4-9 (1986).

2. Davies, I.J.T. The Clinical Significance of Essential Biological Metals. William Heinemann Medical Books Ltd, London (1972).

3. Fairbanks, V,F., Fahey, J.L and Beutler, E. Clinical disorders of iron metabolism. 2nd Ed. Grune and Stratton, N.Y (1971).

4. Prasad, A.S. Zinc deficiency in human subjects. In: Zinc metabolism. A.S. Prasad, ed. Charles C. Thomas, Springfield, Illinois (1966).

5. Van Eijk, H.G., Kroos, M.J., Hoogendoorn, G.A and Wallenburg, H.C.S. *Clin. Chim. Acta* 83, 81-91 (1978).

6. Bothwell,T.H., Charlton, R.W., Cook, J.D and Finch, C.A. Iron metabolism in man. Blackwell Scientific Publications, Oxford, London (I979).

7. Prasad, A.S. Trace elements and Iron in human metabolism. Plenum Medical Company. N.Y. (1978).

8. Gutelius, M.F., Millican, F.K., Layman, E.M., Cohen, G.J. and Dublin, C.C. *Pediatrics*, **29**, 1018 - 1023 (1962).

9. Minnich, V., Okevogla, A., Tarcon, Y., Arcasoy, A., Cin, S., Yorukoglu, O., Renda, F and Demirag, B. *Amer. J. Clin. Nutrition.* **21**, 71-86 (1968).

10. Halstead, J.A., Smith, J.C.,Jr., and Irwin, M.I. *J. Nutr.* **104**, 345-378 (1974).

11. Chattopadhyay, A and Jervis, R.E. *Anal. Chem.* **46**, 1830 (1974).

12. Koons, R.D and Helmke, P.A., *Soil. Sci. Soc. Am. J.* **42**, 237 (1978).

13. Wilkins, C. *J. Agric. Sci. Camb.* **92**, 61 (1979).

14. Kline, J.R and Brar, S.S. *Soil. Sci. Soc. Amer. Proc.* **33**, 234 (1969).

15. Muchori, D.M. Heavy metal analysis of sewage sludge by X-ray Fluorescence Technique and the environment. University of Nairobi. M.Sc. Thesis (1984).

16. Underwood, E.J., Trace elements in human and animal nutrition. Academic Press, N.Y, London (1977).

17. Calabrese, E.J. Nutrition and environmental health: The influence of nutritional status on pollutant toxicity and carcinogenecity. Vol.2. John Willey and Sons, N.Y (1981).

18. Frank, R., Ishida, K and Suda, P. *Can. J. Soil. Sci.* **56**, 181 (1976).

19. Mills, J.G and Zwarich, M.A. *Can. J. Soil. Sci.* **124**, 152 (1977).

20. Nalovic, L and Pinta, M. *Geoderma,* **3**, 117 (1970).

21. Wells, N. *J. Soil. Sci.* **11**, 409 (1960).

22. Schroeder, H.A., Mitchener, M and Nason, A.P. *J. Nutr.* **104**, 239 (1974).

23. Ravikovitch, S., Margolin, M. and Navroth, J. *Soil. Sci.* **92**, 85 (1961).

24. Mckeague, J.A and Wolynetz, M.S. *Geoderma,* **24**, 299 (1980).

25. Udo, E.J., Ogumwale, J.A and Fagbami, A.A. *Commun. Soil. Sci. Plant. Anal.* **10**, 1385 (1979).

26. Osiname, O.A., Schutte, E.E and Corey, R.B. *J. Sci. Food Agric.* **24**, 1341 (1973).

27. Tjell, J.ch. and Hovmand, M.F. *Acta Agric. Scad.* 28, 81 (1978).

28. Maroko, J.B. Copper levels in soils and coffee plant from Bahati-Solai, Nakuru, Kenya. Kenya Coffee, Bulletin, pp.215 (1987).

29. Van Campen, D.R. *J. Nutr.* 88, 125-130 (1976).

30. Ure, A.M., Bacon, J.R., Berrow, M.L and Watt, J.J. *Geoderma,* 22, 1 (1979).

31. Onyari, J.M. The Conc. of Mn, Fe, Cu, Zn, Cd, and Pb in sediments from the Winam Gulf of L. Victoria and fish bought in Mombasa Town Market. University of Nairobi, M.Sc. Thesis (1985).

32. Macapinlac, M.P., Pearson, W,N., Barney, G.H and Darby, W.J. *J. Nutr.* 93, 511; 95, 569 (1967).

33. Mawson, C.A and Fischer, M.I. *Biochem. J.* 55, 696 (1953).

34. Prasad, A.S. Trace elements in human and disease. Vol(II). Ed. Academic Press, N.Y (1976).

35. Ferguson, J.E. Inorganic Chemistry and the Earth. Pergamon Series on Environmental Science Vol. 6 (1982).

36. Aubert, H., and Pinta, M. Trace elements in soils. Transl. L. Zuckerman and P. Segalen. Elsevier, Amsterdam (1977).

37. Dudas, M.J and Pawluk, S. *Can. J. Soil. Sci.* **57**, 329 (1977).

38. Hay, R.W. Bioinorganic chemistry. John Wiley and sons, N.Y (1984).

39. Hsu, F.S., Pond, W.G and Duncan, J.R. *J. Nutr.* **105**, 112 (1975).

40. Mohamad, A.B. Heavy metal polution in the urban environment: Environmental monitoring and assesment: Tropical Urban Applications. National workshorp. Univ. of Kebangsaan, Malysia (1986).

41. W.H.O. Environ. Health Criteria **58** (Selenium: Report of the 1987 joint UNEP/ILO/WHO Expert Committee). Geneva, WHO (1987).

42. Schroeder, H.A., Frost, D.V. and Balassa, J.J. *J. Chron. Dis.* **23**, 227-243 (1970).

43. Kabata-Pendias and Pendias, H. Trace Elements in Soils and Plants. CRC Press Inc. Boca Raton, Florida (1984).

44. Schroeder, H.A., Mitchener, M., Balassa, J.J., Kanisawa, M and Nason, A.P. *J. Nutr.* **95**, 95 (1968).

45. Wandiga, S.O., Molepo, J.M and Alala, L.N. *Kenya J. of Science and Technology*. Series A(1983) **4** (2): 89-94 (1983).

46. W.H.O. Environmental Health Criteria **17** (Manganese: Report of the 1981 joint UNEP/ILO/ WHO Expert Committee) Geneva, WHO (1981).

47. Nadenov, M. and Travesi, A. *Soil. Sci.* **124**, 152 (1977).

48. Mathur, S.P., Hamilton, H.A. and Preston, C.M. *Commun. Soil. Sci. Plant. Anal.* **10**, 1399 (1979).

49. Burridge, J.C. and Ahn, P.M. *J. Soil. Sci.* **16**, 296 (1965).

50. Ure, A.M. and Bacon, J.R. *Analyst,* **103**, 807 (1978).

51. Lag, J. and Steinnes, E. *Geoderma,* **16**, 317 (1976).

52. Wilkins, C. *J. Agric. Sci. Camb.* **90**, 109 (1978b).

53. Romoser, G.L., Dudley, W.A., Machlin, L.J and Loveless, L. *Poultry Sci.* **40**, 1171 (1961).

54. Colvin, L.B., Creger, C.R., Ferguson, T.M. and Crookshank, H.R. *Poultry Sci.* **51**, 576 (1972).

55. Weber, C.W., Doberenz, A.R., Wyckoff, R.W.G and Reid, B.L. *Poultry Sci.* **47**, 1318 (1968).

56. Jenkins, R. and De Vries, J.L. Practical X-ray Spectrometry. 2nd Ed. Springer-Verlag, N.Y. Inc. (1970).

57. Jenkins, R., Gould, R.W and Gedcke, D. Quantitative X-ray spectrometry. Marcel Dekker, Inc. N.Y (1981).

58. Kinyua, A.M. Multi-element analysis of solid and liquid samples by X-ray fluorescence (XRFA). Univ. of Nairobi, M.Sc. Thesis (1983).

59. Alala, L.N. Heavy metal concentration in Kenyan Lakes. Univ. of Nairobi, M.Sc. Thesis (1981).

60. IAEA Technical report series No. 197. Elemental analysis of biological materials. IAEA, Vienna (1980).

61. Hock, A., Demmel, U.,Schicha, H., Kasperek, K and Feinedegen, L.E. *Brain,* 98, 49 (1975).

62. Holynska, B. Biological material analysis. (Wet digestion procedure). Private Communication (1986).

63. Holynsika, B., Muia, L.M and Maina, D.M. *Appl. Radiat. Isot.* **38**, 45-47 (1978).

64. International Atomic Energy Agency (IAEA). Quantitative XRFA software. IAEA, Vienna (1985).

65. Bertin, E.P. Principles and practice of X-ray spectrometric analysis. 2nd Ed. Plenum Press, N.Y (1975).

66. Mangala, M.M. Multi-element energy dispersive x-ray fluorescence analysis of Kerio Valley fluorite ores and Mrima hill soil sediments. Univ. of Nairobi, M.Sc. Thesis (1987).

67. Giaugue, R.D., Garret, R.B and Goda, L.Y. *Anal. Chem.* **51**, 511-516 (1979).

68. Thomas, B., Roughan, J.A and Watters, E.D. *J. Sci. Food Agric.* **25**, 771 (1974).

69. Ochiai, E.I. Bioinorganic Chemistry. Allyn and Bacon, London, Rockleigh, N.J (1977).

70. Goyer, R.A and Myron, A.M. Toxicology of trace elements; Advances in Modern Toxicology, Vol. 2, Hemisphere Pub. Corp. Washington. Ed. (1977).

71. Thomas, B., Edmud, J.W., Curry, S.J. *J. Sci. Food. Agric.* **26**, 1 (1975).

72. W.H.O. Environmental Health Criteria 81 (Vanadium: Report of the 1988 joint UNEP/ILO/WHO Expert Committee). Geneva, WHO (1988).

73. Welch, R.M and Carry, E.E. *J. Agric. Food. Chem.* 23, 479 (1975).

74. W.H.O. Environmental Health Criteria 61 (Chromium: Report of the 1988 joint UNEP/ILO/ WHO Expert Committee). Geneva, WHO (1988).

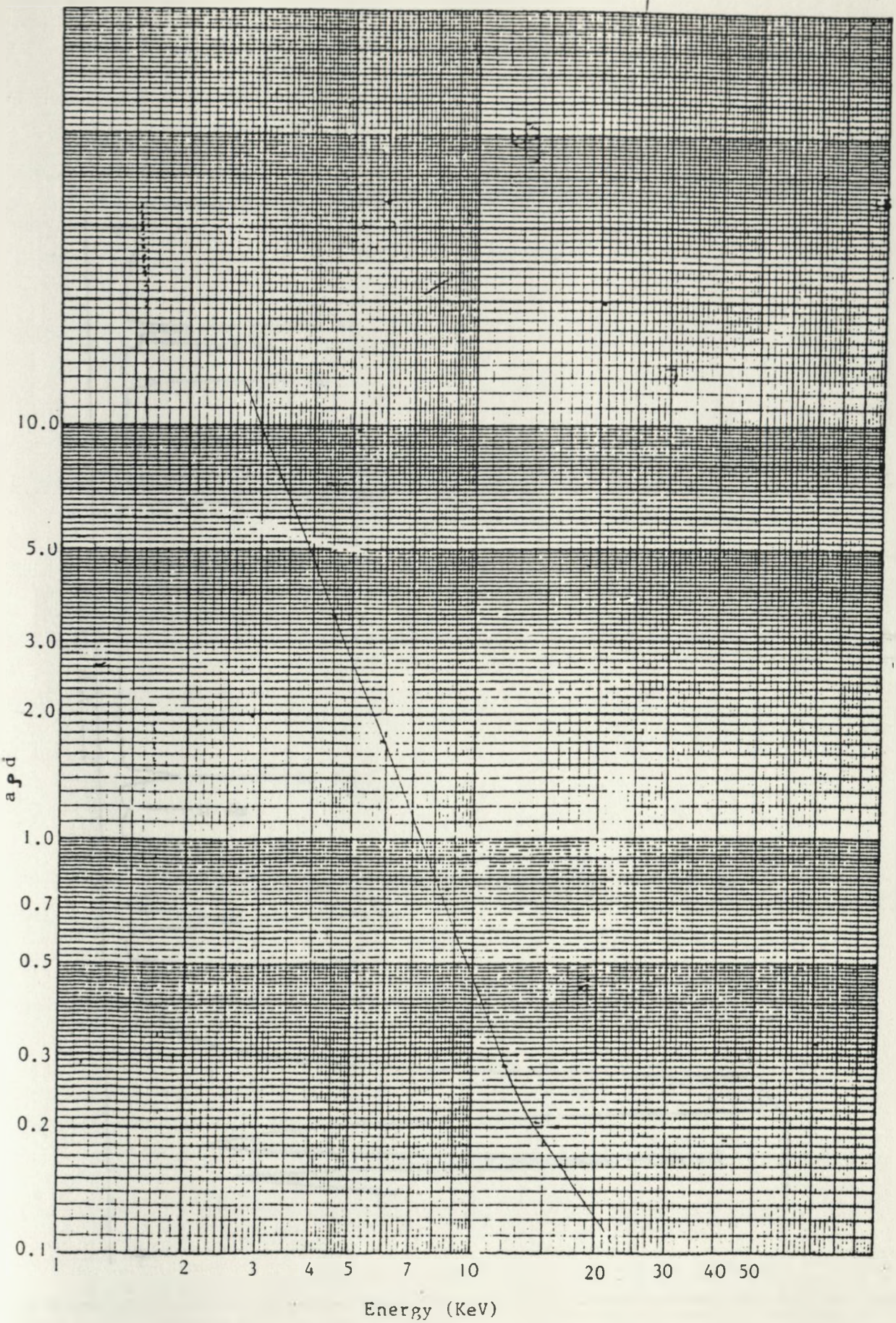
75. Mellor, J.W. A comprehensive treatise on inorganic and theoritical chemistry, Vol. VII. Longmans, Green and Co. London, N.Y, and Toronto (1957).

76. Nelson, T.S., Gillis, M.B and Peeler, H.T. *Poultry Sci.* 41, 519 (1962).

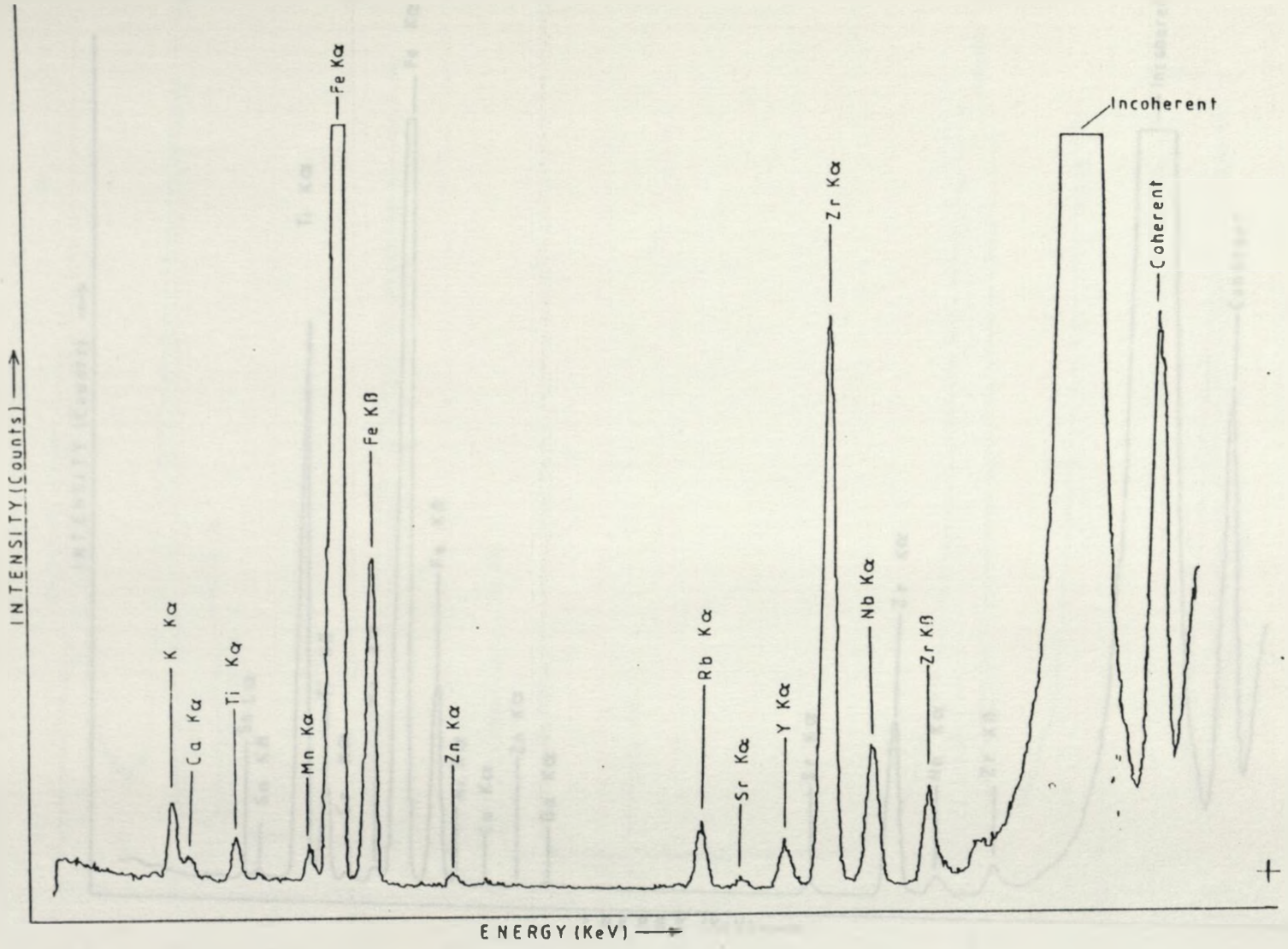
APPENDICES.

Appendix 1A indicates a plot of log $a_0 d$ versus log E. The rest of the appendices shows typical XRFA spectra of consumable clay and tissue samples obtained in this work. The element peaks are indicated by lines. Also indentified are the coherent and incoherent peaks.

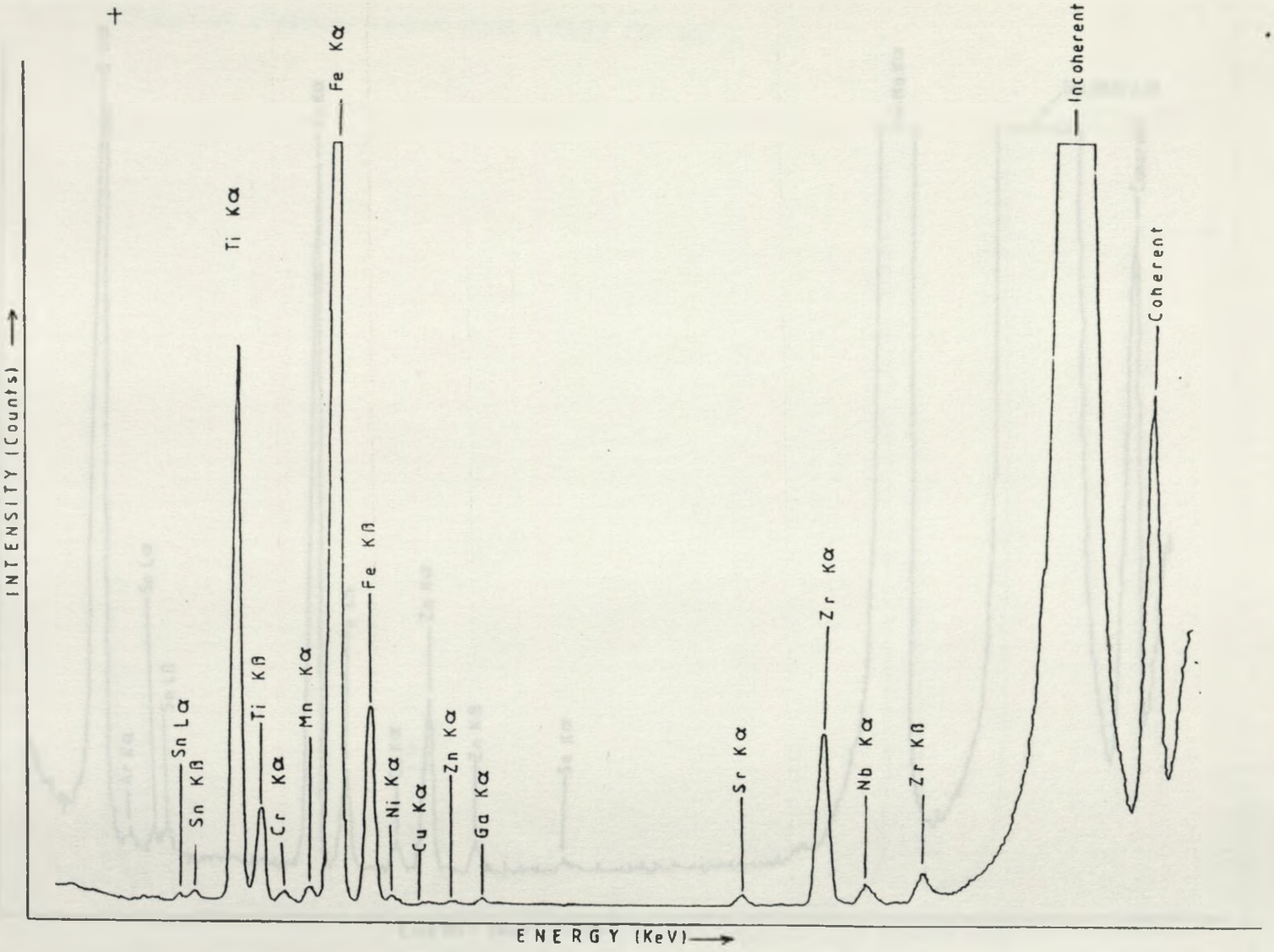
APPENDIX IA A GRAPH OF LOG $a\rho d$ VERSUS LOG E



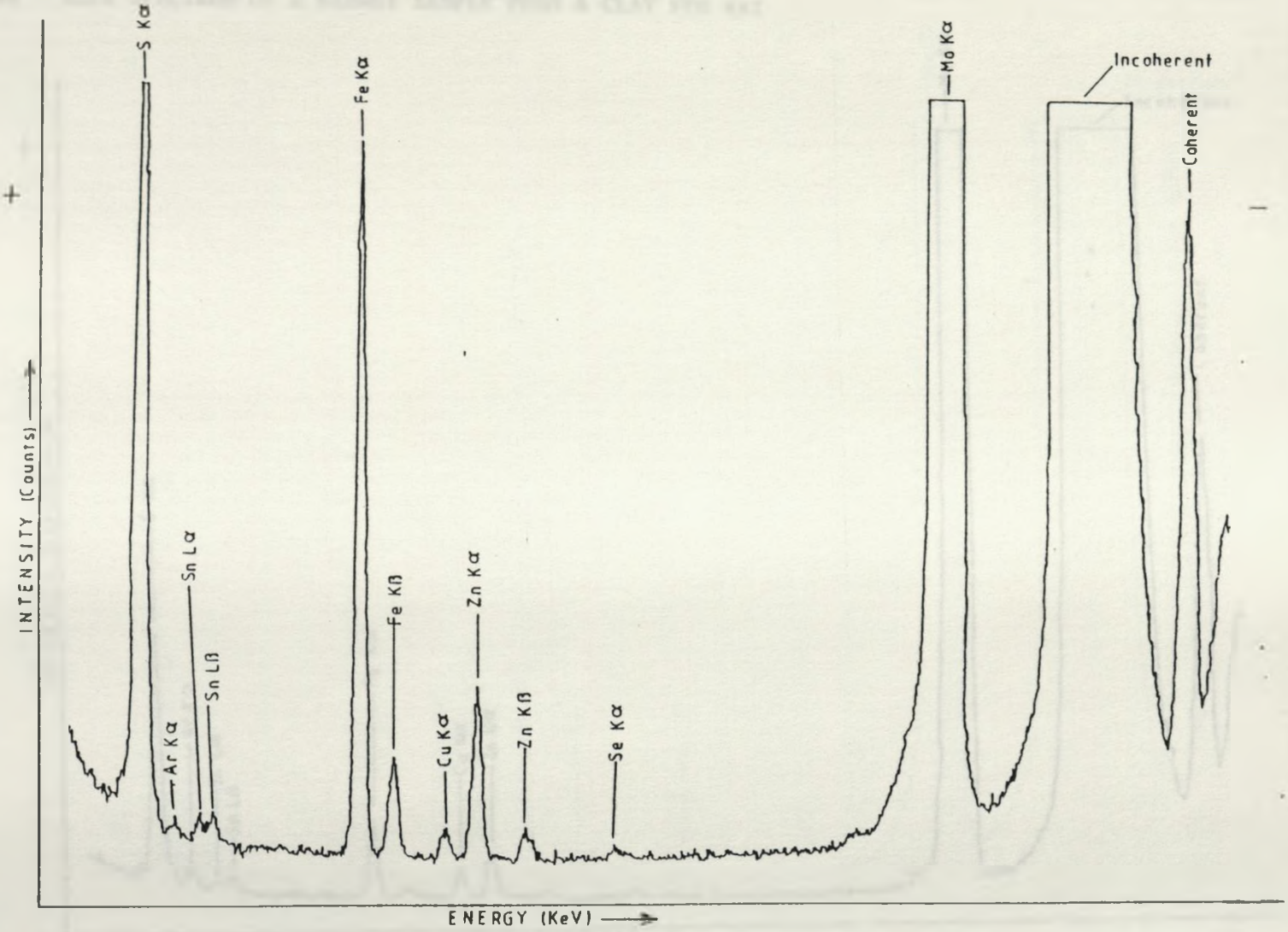
APPENDIX 2A XRFA SPECTRUM OF A CONSUMABLE CLAY FROM GIKOMBA MARKET



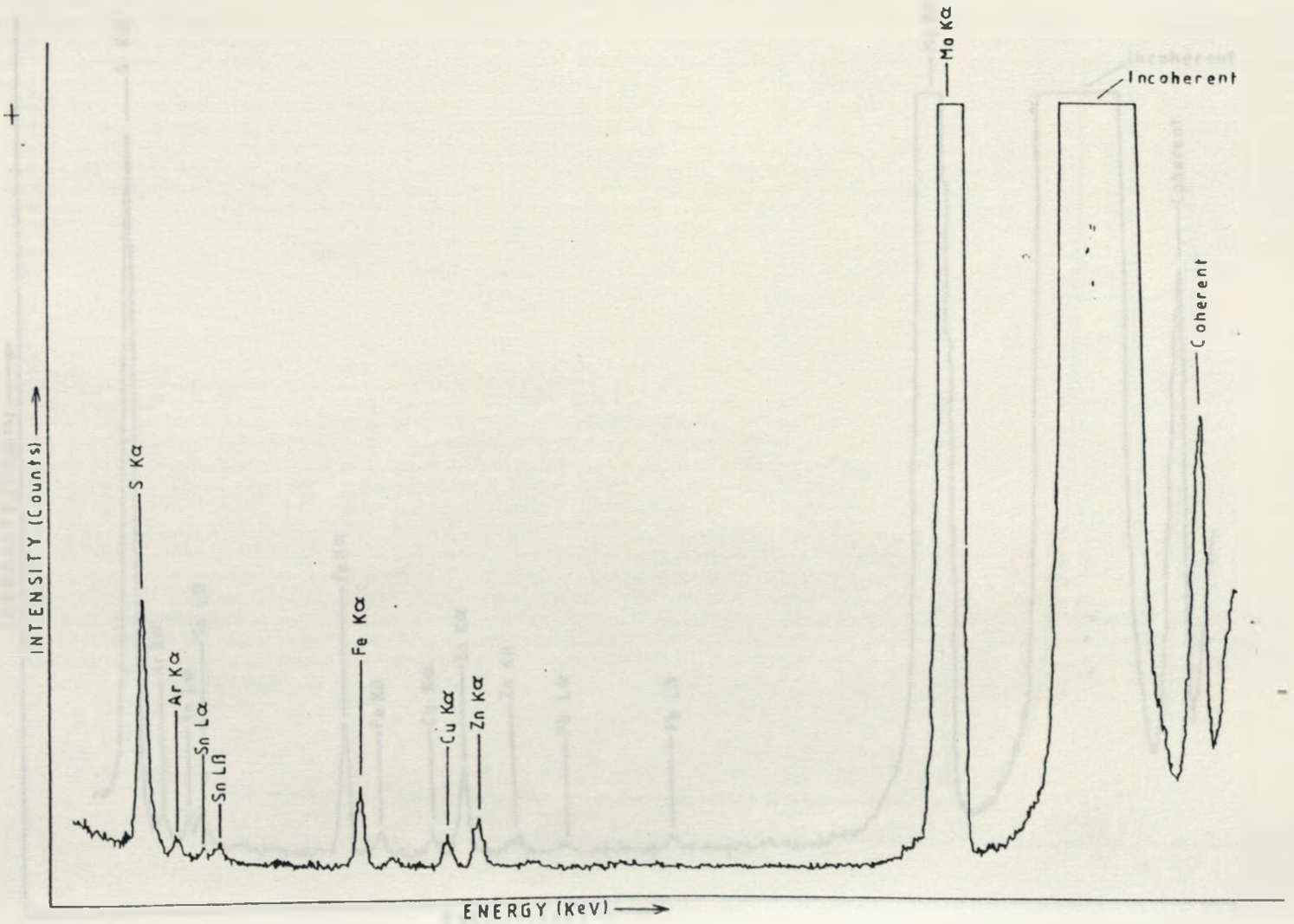
APPENDIX 3A XRFA SPECTRUM OF A CONSUMABLE CLAY FROM A SHOP ALONG BIASHARA STREET



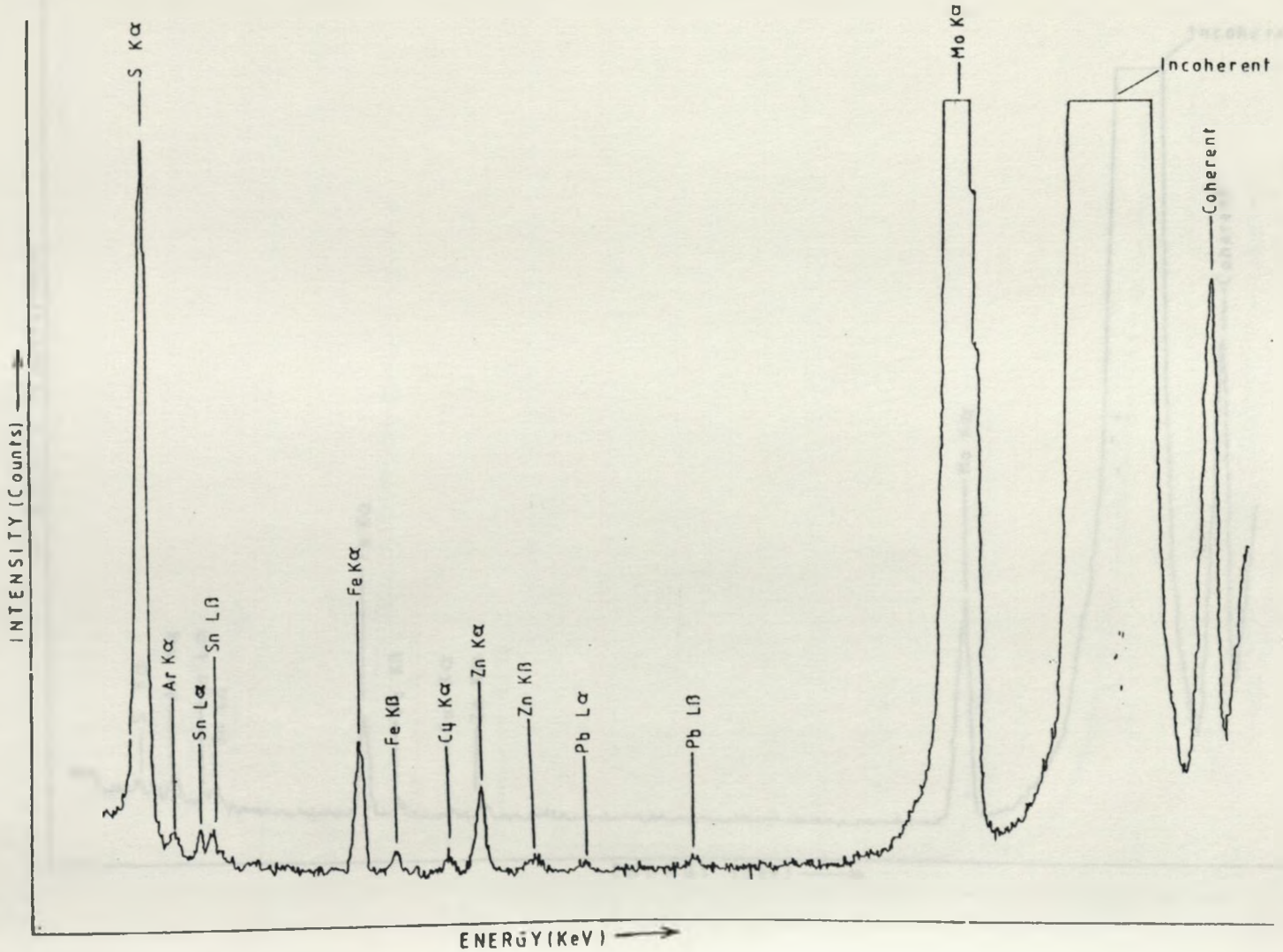
APPENDIX 4A XRFA SPECTRUM OF A LIVER SAMPLE FROM A CLAY FED RAT



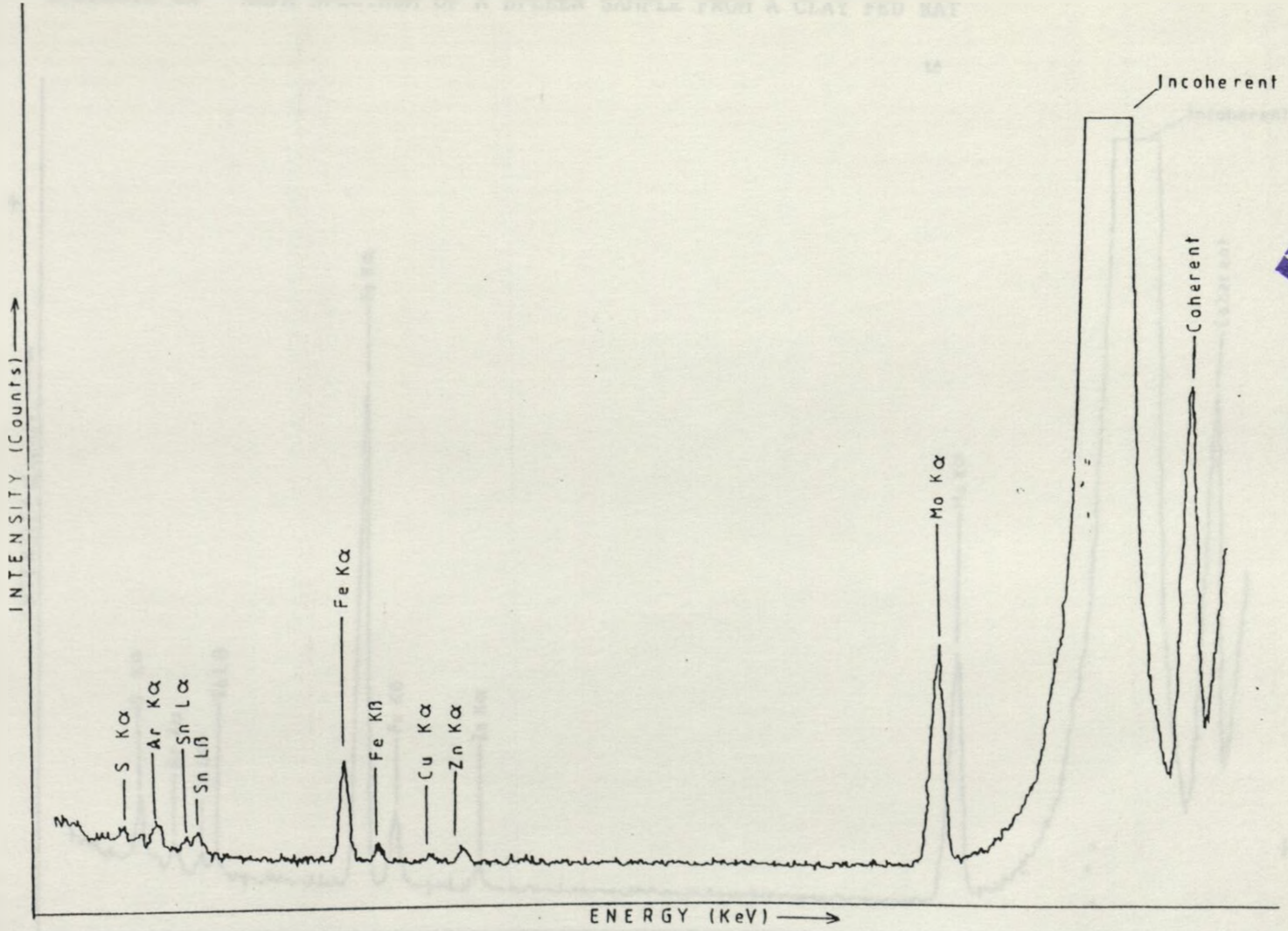
APPENDIX 5A XRFA SPECTRUM OF A KIDNEY SAMPLE FROM A CLAY FED RAT



APPENDIX 6A XRFA SPECTRUM OF A BRAIN SAMPLE FROM A CONTROL RAT



APPENDIX 7A XRFA SPECTRUM OF A HEART SAMPLE FROM A CLAY FED RAT



APPENDIX 8A XRFA SPECTRUM OF A SPLEEN SAMPLE FROM A CLAY FED RAT