UPTAKE OF HEAVY METALS BY VEGETABLES GROWN ON SEWAGE SLUDGE AMENDED SOILS

BY

BARUA, EJIDIUS NJOGU

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DECLARATION

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

Enbarua

Barua, Ejidius Njogu

This thesis has been submitted for examination with our approval as University Supervisors.

Professor David N. Kariuki
Chemistry Department
University of Nairobi

David Maina Muchori
Centre for Nuclear Science Techniques
Faculty of engineering
University of Nairobi

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Dedication

To my parents, Mr. Faustus Barua Njogu and Mrs. Susan Njoki Barua in memory of my brother who is not with us, the late master William Munene Barua.

ABSTRACT

The concentrations of some heavy metals in soil and plant tissue, resulting from land application of sewage-sludge, were examined by determining the levels of manganese (Mn), iron (Fe), Copper (Cu), zinc (Zn) and lead (Pb) in spinach (spinacea oleraceae) and kale (brassica oleraceae leaves and the total heavy metal content in the soils and sludges used.

The technique used in this analysis was Energy-Dispersive X-ray Fluorescence Spectrometry (EDXRFS). Excitation source used was a radio-isotope 109-cadmium (25 mCi) with a semi-conductor Silicon-lithium Si(Li) drifted detector. The vegetable samples were dried, ground and digested using a mixture of nitric and perchloric acids at a ratio of 3 :1. Ion precipitation was done by adding a non-selective precipitating agent, sodiumdiethyldithiocarbamate at a pH between 5 and 6 and later filtered through millipore filter membranes.

The samples used were from Nairobi, Nakuru and Kiambu. Those from Nairobi and Nakuru were sampled from farms where sludge from their respective municipalities had been used for many years. Kiambu samples were from farms where farmyard manure had been used to improve the soil quality.

An open air pot experiment was also carried out to investigate whether the uptake of these heavy metals increases with increase of sludge application. Sludge and soil were mixed at various percentages (dry weight basis) ranging from pure soil to pure sludge. Vegetables grown in these pots were analysed after maturity.

The general observation was that Nairobi vegetables had high levels of the trace elements compared to the vegetables from other regions.

The metal content in spinach from Nairobi ranged between the following values. Mn ($145 - 1230\mu gg^{-1}$), Fe ($490 - 990\mu gg^{-1}$), Cu ($16 - 35\mu gg^{-1}$), Zinc ($302 - 1486\mu gg^{-1}$) and Pb ($7 - 16\mu gg^{-1}$). In kale, the range was Mn ($110 - 371\mu gg^{-1}$ Fe($399 - 758\mu gg^{-1}$), Cu ($16 - 33\mu gg^{-1}$), Zn ($123 - 282\mu gg^{-1}$) and Pb ($6 - 11\mu gg^{-1}$).

All samples from Nakuru and Kiambu had lower values than those from Nairobi. In most cases kale had lower trace metal content than spinach from similar environments.

Increase in sludge material (w/w) in both fields had a corresponding increase in manganese copper and zinc content for both vegetables. In field one, increase in levels of Cu(6.8-25.9μgg-1) Zn ($152-459\mu gg^{-1}$)

were obtained in spinach while a similar increase in Cu (5.7-10.5 μgg^{-1}), Zn (53 - 142 μgg^{-1}) and Mn (6.4 - 35 μgg^{-1}) was obtained for kale.

Lead (Pb) levels in spinach and kale were less than 7 μgg^{-1} and 5.4 μgg^{-1} respectively even at the highest level of sludge application in both fields.

In field two, increase of Mn (254 - 1119 μgg^{-1}) Cu(15.3 - 31.2 μgg^{-1}) and Zn (137 - 1422 μgg^{-1}) was obtained in spinach.

In kale increase in Mn (8.5 - 276 μgg^{-1}), Cu (3.3 - 14.4 μgg^{-1}) and Zn (47 - 536 μgg^{-1}) was obtained.

Pure sludege from Kariobangi had trace average concentrations of elements at the following levels. Mn: 0.21%, Fe: 4.0%, Cu: 208 μgg^{-1}, Zn: 0.18% and Pb: 333 μgg^{-1}.

Nakuru sludge had Mn: 993μgg-1.Fe:0.38% Cu: 98 μgg^{-1}, Zn: 0.14% and Pb:85.5 μgg^{-1}

TABLE OF CONTENTS

CHAPTER 1

1. INTRODUCTION AND LITERATURE REVIEW

Among the Kenyan urban population, the most widely consumed green vegetables are kale and spinach, respectively. Kale is normally called "sukuma wiki" in the local swahili language which is spoken throughout the country. The vegetables - especially Kale - are enjoyed by people of all tirbes and races in the country. It therefore follows that most of the vegetables gardeners in the urban areas and their environs concentrate more on the growing of the above popular types of green vegetables.

The high growth rate of the towns in Kenya has resulted to the problem of waste disposal. These wastes include domestic and industrial effluents. The growth of the towns has also triggered the growth of the industrial sector. The increase in the number of processing and manufacturing plants has also increased the amount of industrial effluents.

All these effluents finally find their way into sewage treatment plants and hence the resultant sewage sludge produced carries a heavy load of heavy metals from these industrial effluents. The total content of these heavy and trace elements in the sludge generally depends on the activities of various industries within the sewage treatment plant's catchment area.

The treatment process generally is comprised of three major stages. The first stage in the purification process is settlement in large tanks where the bulk of the suspended solids sediments out. The resultant liquid is then passed directly to contact beds where it is aerated by percolation through layers of rock or slag. A layer of bacteria soon develops on the surfaces in the beds, which is capable of rapid oxidation of the organic matter in the effluent. Raw sludge is a liquid in which some pathogens may survive and some of the larger sewage disposal plants have facilities for anaerobic digestion of this material by microbial action. This is achieved at atmospheric temperatures in open tanks over periods of several months, or in enclosed tanks at a higher temperature ($35^{o}C$) over 3 - 5 weeks. Large quantities of methane and carbon dioxide are given off at this stage and the product becomes relatively inoffensive [6].

While the pathogens and much of the water in sewage sludge can be effectively dealt with during the treatment process, there is no practical way, at present, of removing metals absorbed by the sludge. The composition of sludge is extremely variable depending on the contribution made to the sewage from industrial areas. Sludge normally contains a wide range of trace elements of which many of them are non-essential and potentially toxic to plants and animals. The presence of factories producing metal rich effluents within the catchment area of a particular sewage disposal plant obviously make a great difference to the final metal content of the sewage sludge [6].

Any sewage treatment plant would have problems disposing these materials. This is due to the effects of the toxic heavy metals and pathogens contained in the sludge to the plants and animals within the disposal sites.

The advent of increased ecological concern has led the public to demand measures for decreasing environmental pollution. Legislation has been enacted that will stop incineration, ocean dumping, land filling and other wasteful and polluting practices [25]. There is also pressure from marine biologists and those concerned with prevention of river pollution who would like to see waste products disposal on agricultural land because they want to keep them away from water sources and estuaries [6]. This leaves few alternatives, with land application being one of the most ecologically and economically viable [23].

However, the use of sewage sludge especially as a fertilizer in growing the vegetable crops would also have some negative environmental implications. Uptake of heavy metals from the soil by many varieties of garden vegetables have been reported [8,14,20,24]. Consumption of products with elevated amounts of heavy elements can lead to various physiological impairments depending on the element. For example brain damage can be caused by lead poisoning. Mice treated with lead have increased susceptibility to infection. Cadmium has been associated with arterial hypertension and increased mortality [12].

Consumption of food crops with elevated levels of trace elements within a given area can also lead to the emergence of epidemics. Health and welfare workers in Japan, as a result of associating cadmium with the "Itai-Itai" disease epidemic in the Jinstu basin, consider a daily intake of 300 μg Cd/person the maximum acceptable rate. The locally produced rice in this epidemic area was identified as a major dietary source of cadmium for the diseased residents [3].

Agricultural use of sewage sludge is cost effective and offers the potential of recycling nutrients but sludges that contain large quantities of metals may inhibit crop growth adversely affecting long term productivity and lead to food chain accumulation of some metals [16]. Due to the recent rise in interest in vegetable gardening particularly in the large cities, articles have appeared in the popular press questioning the safety of produce from urban areas on the basis of possible heavy metal contamination [14].

1.1 Project Objectives

The tendency among vegetable gardeners within the sewage treatment plants areas in Kenya municipalities is to use the produced sludge as a fertilizer. Therefore, the objectives of this research were to investigate whether

a) there is any direct or indirect relationship of heavy metals in the plants and the soil with particular emphasis on sludge borne heavy metals.

b) the uptake of the heavy metals increases (or decreases) with the level of sewage-sludge addition to the soil.

c) the heavy metals in plants accumulate with plant age.

1.2. Past Usage of Sewage Sludge and its Effect on Heavy Metal Accumulation in Soils and Plants

The application of sewage sludge to agricultural land is presently emphasized as a means to relief the general waste accumulation problem. While the values of nitrogen and phosphorus are not objectionable, heavy metals contained in sludge have generally been viewed as a disadvantage considering the abundance in relation to their toxicity even at low concentration [19].

According to Neufeld, R.D. et al [21], the organic biomass which forms the body of the sludge effectively concentrate the heavy metals from the surrounding sewage liquid by a factor measured to be as high as 10^4. This indicates that in waste disposal, sludge functions as a natural sink and therefore applying sewage sludge to agricultural land gradually turns the soil into a new sink.

Other authors have different opinions as concerning the usage of sludge on agricultural land. Darmody, R.G. *et al* [15] considers its usage as one method of disposal of waste products while recycling the nutrients it contains. They also note that one constraint of this approach is the possible contamination of the human food chains with toxic substances such as heavy metals if food crops are grown on sludge treated soil. Excessive accumulation of heavy metals like zinc, copper, nickel and cadmium in the soils and the resultant phytotoxicity and metal uptake by plants is a limiting factor in application of sewage sludge to agricultural land. The heavy metal phytotoxicity has been demonstrated with plants grown in solution culture, in greenhouse pot experiments and field experiments. The different metals were found to vary significantly in their phytotoxic effects. In addition, the relative metal uptake and phytotoxicity were shown to be dependent on plant species as well as growth media [21].

Sludges from industrial areas contain heavy metals such as copper, zinc, cadmium and nickel in greater concentrations than those in agricultural soils. However, application of sewage sludge is beneficial to crops as a source of nutrients such as nitrogen and phosphorus both of which have fertilizer value . Schauer, P. S. et al [26] give an example of the United States of America where the use of municipal sewage sludge to increase yields of agronomic crops is widespread: It has become an attractive alternative in areas near sewage treatment plants due to its low cost and availability although there is insufficient municipal sewage to make a significant impact on total fertilizer needs of the United States agricultural system.

Other people use sewage due to shortage of water supply. Sewage is therefore used for the dual purpose of irrigation and providing major and micro-nutrients required for plant growth. In India for example, thirty two municipal committees are utilizing about 82,000 m^3 of partly treated sewage water per day for irrigating around 17,400 ha of agricultural land and it has been calculated that sewage available from big cities in India could annually contribute 33,000 tonnes of phosphorus and 20,000 tonnes of potassium. However, anthropogenic activities are known to mobilize certain trace and heavy metals at the same rates comparable with natural geochemical processes. As a result of this, waste water discharged from sewage system have become a recognised source of trace and heavy metals. Municipal water irrigation and sewage sludge application may, therefore, result in plant uptake of some of the heavy metals, and therefore resulting in their introduction into food chains [4].

The proposed banning of ocean dumping and the unfavourable economics of incineration have also stimulated the agricultural usage of sewage sludge.

Composting of sewage sludge has been proposed as an aid in sludge utilization since it presents several advantages over direct utilization. Among these advantages are the reduction in pathogen hazards and objectionable odours, decreased transportation costs due to decreased water content of compost and easier handling. However, the hazards of increased soil heavy metal burdens on plants and the food chains remains an issue of great concern [18].

Experience gained in Israel over a period of more than twenty five years has shown that good yields can be obtained by irrigation of agricultural land with secondary treated sewage effluents. However, the potential cumulative effects of toxic heavy metals present in sewage effluents continues to present a problem. The metals tend to accumulate in the top layers of the soil in various degrees of availability and with prolonged use of sewage effluents for irrigation, some metals are liable to build upto toxic levels and cause damage to sensitive plants. Even when no visible damage is inflicted, the toxic elements accumulated in plant tissue may be introduced into the food chains by way of animal feed and human food [1]. The background and human and environmental impact of some trace metals are discussed elsewhere [23].

In general sewage sludges contain higher levels of heavy metals than in sludge free soil. Sabey, B.R. et al [25] says that every metric tonne of a typical anaerobically digested sludge contains 2 to 10 Kg of potassium along with smaller quantities of other elements. Maina, D. M. [20] reported high levels of heavy metals in sludge than in sludge free soil. Therefore, repeated application of heavy dressings over a long time and the use of sludge from industrial areas may lead to damage of crops and animals consuming them.

The accumulation of heavy metals in the soil and their subsequent uptake by plants is of major concern when considering the application of sewage sludge. This is because accumulation in the edible parts represents a direct pathway for their incorporation into the food chains through consumption of produce with elevated concentrations of heavy metals. With the advent of increased ecological concern, measures for decreasing environmental pollution need to be established.

Plants uptake of different trace elements vary from plant to plant and with the element itself. Some plants are heavier trace element accumulators while others have mechanisms of regulating the amount of trace elements they take. The type of soil and its pH are some of the factors affecting metal uptake since some elements form complexes at certain pH values and hence rendering them unavailable to the plants. Some of the results obtained by various authors are discussed below.

1.2.1 Manganese

Soon Y. K. et al [28], using brome grass (bromus inermis leyess) observed that manganese concentration decreased with the application of sludge. This reduction was seen to be greater with high rate of calcium and aluminium sludges. Dilution with increased plant growth and soil pH were the two main factors seen to be affecting manganese concentrations in the grass. Total manganese uptake tended to be reduced by high application of iron sludge. Application of sludge did not have any effect on the manganese concentration in brome grass or corn grain (zea mays) from one year to the next.

Dowdy R. H. et al [8], also observed a decrease in manganese content in carrot tissue. This decrease was despite the increase of sludge loading to the soil. Reduced manganese uptake by young carrots and lettuce tissue was also observed where heavy application of organic farm yard manure was done. Availability of manganese is lowered by presence of organic matter.

Animals have been reported to carry a big manganese load without ill-effects. For example, the growth of rats is unaffected by manganese intakes as high as 1000-2000ppm although higher amounts interfere with phosphorous retention. Hens tolerate 1000ppm without ill-effects but 4800ppm is highly toxic to young chicks [26].

1.2.2 IRON

Some authors do not include iron in the group of trace elements because of its relatively high requirement by animals and its occurrence in substantial concentrations as part of the haemoglobin molecule. Other authors consider iron as a trace element due to the fact that it functions in the tissues as a component of several oxidative enzymes in a manner analogous to copper and other trace metals and occurs in many body tissues and fluids in lower concentration than those of several of the acknowledged trace elements [29].

Iron assimilation in man varies so widely with different individuals and different dietaries and the number of variables affecting absorption of this element is so great that it is difficult to translate physiological requirements into precise dietary needs.

The position is complicated further by the ability of the body to increase absorption efficiency during periods of iron deficiency. It is estimated that the total normal daily loss of iron lies between 0.5 and 1.0 mg/day. This is a considerable quantity when it is realised that no more than 1.0 - 1.5 mg/day is absorbed from normal diets [29].

Green vegetables have been classified as intermediate sources of iron. Plants like legumes and leaves which contain about 16 ppm of iron have been described as excellent sources.

Boiling of vegetables in water has been found to reduce the levels of iron by as much as 20% [29].

With regard to sludge-borne iron, Dowdy, R. H. *et al* [8] observed that the iron contents of the edible roots and tuber tissue were not affected by the rate of sludge application. This is possible due to the fact that iron occurs in the soils at high concentrations and relatively high availability.

II.2.3 COPPER

With regard to plant uptake of sludge-borne copper, various authors have investigated this using different plants. Soon *et al* [25] found out that copper concentration in brome grass increased with the rate of sludge application and tended to be greater in the second and third cuts than in the first.

Dowdy, R. H. et al [8] observed that potato accumulated increased amount of copper with increased addition of sewage sludge, ranging from 8.6 ppm for 420 tonnes/ha treatment. Carrots and radishes were found to very effectively exclude copper from the edible tissue. However, the increase of copper in the potato were described as low considering the 16% sludge/soil mix in which the potato grow. As for lettuce, copper levels increased with the increase of sludge application. The situation was different for corn leaves where no increase in copper level was observed.

Using composited sludge, copper concentration in lettuce was not significantly different due to sludge type although sludge contain 68% more copper than compost. This phenomenon was explained by the fact that when sewage sludge undergoes composting, the chemical species of metals often change and thus altering metal availability to the plant. Composited sludge may thus have a greater limiting capacity than uncomposited one which would also change metal availability [27].

Sludge-applied copper was not absorbed by barley from either acid or calcareous soil even if the sludge contained 610 ppm copper and an application of 830 μg/100g soil [8]. Patterson *et al* [24] also showed that soil addition of 134 tonne/ha sludge had no effect on copper uptake by oat plant.

Requirements of copper in human nutrition varies greatly especially with age. At early infancy it may be as low as 14 μg/kg body weight. Growing children have been estimated to retain very much larger amounts, namely 0.06 - 1.0 mg/kg body weight which indicates a requirement of 1.2 - 2.0 mg daily for five year old weighing 20 kg. Estimates of the average daily intakes of copper from various diets vary over a wide range. A typical high quality adult diets supply 2-3 mg Cu/day [29].

The magnitude of daily intake are principally determined by the type of food, the locality in which they are produced and the degree of contamination from processing and storage and from treatments with copper fungicides. Different classes of food contain different amount of copper in them [29].

In all animals, continued ingestion of copper in excess of requirements leads to some accumulation within the tissues especially in the liver. In some species, the liver has a remarkable ability to take up and retain very large amounts of ingested copper. Up to certain levels which vary greatly among individuals and species, the liver cells appear to store such copper without physiological harm. Above these levels, in sheep and cattle particularly, a catastrophic liberation of a high proportion of the liver copper into the blood may occur, with resultant extensive heamolysis and jaundice, followed by death. Little is known of the minimum levels of copper necessary to induce chronic poisoning in man, but it is estimated that ingestion of copper of the order of 10 times the level in normal human diets would be necessary before injurious effects would ensue. Wilson's disease in man is characterised by excessive concentrations of copper in the tissues, but it doesn't result from chronic ingestion of copper in amounts larger than normal [29].

1.2.4 ZINC

Zinc is one of the common trace elements found in sewage sludges. It is especially at high concentration in sludges from industrial areas.

Zinc uptake by plants is widely reported. Simeoni, L. A. et al [27] using lettuce observed that all application of sludge increased the zinc concentration in the plant at least 1.6 times more than application of compost. In oats, high application of sludge on acid soils produced zinc 1.1 times greater than obtained with the same rate of application of compost. Both lettuce and oats gave higher yields when grown on soil amended with sludge especially at high application rates. This was attributed to increased zinc accumulation in lettuce and the response of both crops to higher salinity of the sludge.

In an experiment by Dowdy, R. H. et al [8], zinc content increased from 23 to 103 ppm, 37 to 98 ppm and 24 to 53 ppm in carrot, raddish and potato tissue respectively, when 450 tonnes/ha sludge was added. Zinc content of pea fruit and corn grain was increased from 70 to 130 ppm and from 41 to 65 ppm respectively by addition of the same amount of sludge. The level in tomato went up from 9 to 31 ppm while the level in the edible lettuce tissue reached 225 ppm. All in all, zinc content in all tissues increased significantly with addition of sludge.

For barley seedlings grown on sewage sludge, Dowdy, R. H. et al [9] observed that uptake of sludge-borne zinc from acid soils increased linearly with increased rates of sludge application except for the incubated samples at the lowest rate of application.

Such a linearity could be useful for predicting zinc availability. Inspite of doubling the zinc uptake by addition of 30 tonnes/ha sludge, only 2.5% of the sewage-borne zinc was removed by.first cropping for the 15 and 30 tonnes/ha treatment.

Similar trends of increased zinc uptake with increased sludge application has been observed in green house studies. On the alkaline soil zinc uptake increased with increased rates of sludge addition, although the slope was less than for the acid soil [9].

Soon Y. K. et al [28] observed that the zinc concentration in brome grass increased with the rate of sludge application. Concentration in the second and third cuts was greater than in the first cut. In corn stover, the zinc concentration was increased by sludge application and the sludge sources did not differ in their effect on zinc concentration in corn stover in the first year of application. The concentration in corn grains increased slightly by high sludge application and remained relatively constant across the year.

Zinc toxicity in man has been reported as a result of consuming acid foods which were cooked in galvanized vessels. Little is known of tolerance of ruminants for zinc, but rats, pigs and poultry exhibit a high tolerance. Zinc intake equivalent to 2500 ppm are without discernible effects on rats over several generations, but twice this level growth is severely depressed with high mortality in young animals. Anaemia of the hypochronic and microcytic are also symptoms of zinc toxicity in rats. Defective development and mineralization of bones, reduced assimilation of several nutrients and lipotropic effect also imply zinc toxicity in rats [29].

A normal well balanced adult diet supplies an average of 10 - 15 mg zinc daily, with actual amount greatly influenced by the protein content because of the association of zinc with the proteins in the food. Among foods, green vegetables are classified as intermediate sources of zinc [29].

1.2.5 LEAD -

A question of practical significance to vegetable gardeners relates to the source of heavy metals such as lead. Lead contamination of garden vegetables may result from deposition of the metals as part of air particulates, originating from auto exhausts, industrial emissions or re-entrained dust or it may result from uptake of the metal from the soil. In the former case, it is expected that a certain amount of surface lead may be removed by washing and this was found to be true [14].

As for the sludge borne lead, Berthet et al [2], using selective extraction procedures observed very small availability of lead in sewage sludges and in the soils. This is also in accordance with the insignificant export via vegetables and leached waters. The observation was that, whatever the treatment was, the lead fell out particularly on the green parts of the vegetables and to lesser extent in the non-tuberous roots showing the predominance of atmospheric pollution. No appreciable migration to the reproductive and reserve organs was observed.

Application of sludge did not cause any apparent effect on lead concentration in bromegrass, corn grain or stover. There was no increase of lead in corn leaves and grains with greater metal loading [25]. This observation was also supported by Dowdy, R. H. et al [8] who reported the availability of lead in sludge amended soils as low.

Bannin et al [1] found out that the concentration of metals in Rhodes grass grown on sewage effluent irrigated fields was in general higher than in water irrigated fields except for lead which was the exception possibly due to effects of air-borne lead pollution of the plant canopy.

In a field where 230 kg/ha lead was added, the lead uptake was found to be insignificant and did not exceed 1.0 ppm in the roots and the tuber tissue. This low lead value contrasts with values of 20 to 25 ppm lead in raddish roots grown on soil containing 70 to 370 kg/ha. Hence during the sewage treatment and digestion, lead was probably precipitated and organic bound in a form unavailable for plant uptake. [8]. Lead uptake patterns for the acid soils by barley did not differ with the zinc. It increased linearly with the increase in sludge. However, most of the lead was retained in the roots. A 50% increase in barley lead content was observed when 30 tonnes/ha sludge was added to the acid soil. In contrast lead uptake from calcareous soil did not increase with sludge application of up to 30 tonne/ha probably due to insolubility of lead at high pH. Incubation increased the uptake of native lead from the soil [9].

A preliminary study reported by James R. P _et al_ [14] on fourteen vegetable gardens in Boston area showed some leafy vegetables with elevated lead content. A screening programme in Boston and New York showed a greater problem with lead in Boston than in New York. A composition of leafy vegetables grown on soils low in lead showed significantly high lead content in the vegetables from gardens with high levels of lead in the soil.

This is as in the table below:

Table 1.1

Lead in leafy vegetables - Effects of soil lead.

	PPM (Pb)	
	SOIL	VEGETABLE
Group I (High)	2200 (2)	16 (22)
Group II (Low)	150 (8)	6(12)

Number of samples are in parentheses.

None of the samples above was from gardens near heavy traffic. A number of vegetable species from single experimental plot having elevated soil lead showed considerably low lead contents in garden fruits than in the leafy or root vegetables. Lead content of root vegetables did not differ significantly from that of leafy vegetables. A comparison between beet roots and beet greens from the same garden, however, showed significantly higher levels of lead in leaves (20.0 ppm Pb) than in roots (13.0 ppm Pb). Tomato fruits had considerably lower lead content than did leafy vegetables from the same gardens.

This is illustrated by the table below.

Table 1.2

Lead in leafy vegetables and tomatoes.

	PPM (Pb)	
	LEAFY VEGETABLES	TOMATOES
Group I (High)	25(8)	<2(6)
Group II (Low)	7(6)	<2(2)

Number of samples are in parentheses.

Significant differences in lead content of different species of leafy vegetables on the same garden was also observed.

Table 1.3

Lead content of leafy vegetable species

Vegetable	PPM(Pb) Garden	 Greenhouse
Beetgreens	19.6	2.6
Chard	9.8	-
Lettuce	11.9	1.5
Collards	-	1.3
Mustard	5.3	0.6

For vegetables raised in the green house in filtered air, no significant differences in lead content were observed among beetgreens, lettuce, collards and mustard although the value for beetgreens were slightly higher.

Lead occurs in living tissues after being acquired and accumulated as environmental contaminants and its presence merely reflects the contact of the organism with its environment.

CHAPTER 2

2.1 EXPERIMENTAL PROCEDURES AND THEORY OF ANALYTICAL TECHNIQUE USED

The chemicals and reagents used were of analytical grade. They were:

1. Nitric acid
2. Perchloric acid (70%)
3. Ammonium hydroxide
5. Sodiumdiethyldithiocarbamate
6. Cellulose and Starch binders
7. Nitrates of manganese, iron, copper, zinc and lead
8. Millipore filter membranes
9. Acetone

2.1.1 Preparation of 2% Sodiumdiethyldiocarbamate (NADDTC)

5 grams of analytical grade sodiumdiethyldithio carbamate were accurately weighed. It was then put into a 250 ml volumetric flask and the flask filled to the mark with double distilled water. This gave 2% NADDTC (weight by volume).

2.1.2. Preparation of standard stock solutions

1000 ppm stock solutions were prepared by dissolving an accurately weighed amount of salt equivalent to its molecular weight divided by the atomic weight of the element into a 1000ml distilled water. These stock solutions were then diluted accordingly to give solutions of required concentrations. Mixed standards were also prepared by mixing the diluted solutions in beakers.

2.2 MATERIALS AND METHODS

Dry sewage sludge was obtained from Kariobangi treatment works in Nairobi city. The determination of total heavy metals was carried out using Energy Dispersive X-Ray fluorescence spectrometry (EDXRFS). The soil was obtained from the University of Nairobi botanical garden and the total heavy metal analysis was carried out as was done on sludge.

Both the sludge and soil were dried in the sun after which they were mixed thoroughly in plastic containers at different ratios based on their weights. The range varied from 0% sludge (pure soil) to 100% sludge (pure sludge). Each amendment was replicated thrice for each of spinach and kale grown on these soils, respectively. There were 30 pots for each type of vegetable. After germination of spinach and kale seeds, the plants were thinned to two plants per pot when they were about five centimeters tall.

The leaves, which are the only edible parts of the vegetables were sampled thrice at one month intervals by cutting the leaves with stainless steel blades. They were then washed with tap water and later rewashed twice using double distilled water after which they were put in sample containers and dried at a constant temperature of $65^{o}C$ for seventy two hours in accordance with Chakrabarti et al [4]. After drying they were ground and passed through a 0.5 mm sieve and stored ready for analysis.

Other vegetable samples were obtained from areas in Nairobi and Nakuru where sewage sludge had been applied for many years. From Kiambu, the vegetables were sampled from gardens amended with farmyard manure. The soil in the environment where the vegetables grew was also sampled at a depth of between 10 - 15 cm.

The drying of the soil samples was done at a constant temperature of $40^{o}C$ for seventy two hours. It was later ground, passed through a 0.5 mm sieve and thereafter crushed using an agate mortar up to particle size of less than 50 microns.

2.2.1 Sample preparation, procedure, and measurement.

2.2.1.1 Plant material

About 1.0 g plant material accurately weighed, was wet digested by use of nitric and perchloric acids. The dry plant powder was put into a round bottomed flask together with 30 ml of nitric acid and heated under reflux until the brown fumes of nitrogen oxides disappeared. After cooling, 10 ml of 70% perchloric acid was added and the solution heated again under the same conditions until the solution turned clear. The solution was then cooled and poured into a beaker and the volume adjusted to 100 ml. The pH was then adjusted by use of ammonia solution and dilute nitric acid to a value between 5 and 6 [13]. The precipitation of the metal ions in the solution was done by adding 10 ml of 2% sodiumdiethyldithiocarbamate (NADDTC) solution (weight/volume) and the precipitate formed left to stabilize for 30 minutes. Thereafter, it was filtered through a millipore filter paper of 0.45 microns pore size to give a thin sample.

2.2.1.2 Soil and sludge:

Fine powders of soil or sludge were mixed thoroughly with cellulose using a mechanical mixer so as to give a homogenous sample. Pellets of mass between 0.1 g and 0.2 g, accurately weighed, were then made using stainless steel dies at a pressure of 3 - 5 tonnes.

Cellulose was used for the purposes of binding the particles and also to dilute the sample. The term dilution factor is defined as below.

$$\text{Dilution factor (D)} = \frac{\text{Mass of sample + mass of cellulose}}{\text{mass of cellulose}}$$

2.2.1.3 Measurements

The spectrometer used for measurements consisted of Silicon-Lithium [Si(Li)] detector FWHM of 200ev at 5.9 KeV and a CANBERRA 40 multichannel analyser with on-line DEC 350 minicomputer. Measurement time for plant samples was 10,000 seconds while that of soil samples was 2,000 seconds. A radio isotope source Cadmium-109 was used as the source of primary radiation.

3.3 INSTRUMENTATION

3.3.1 Theory of X-Ray Fluorescence Spectrometry (XRFS)

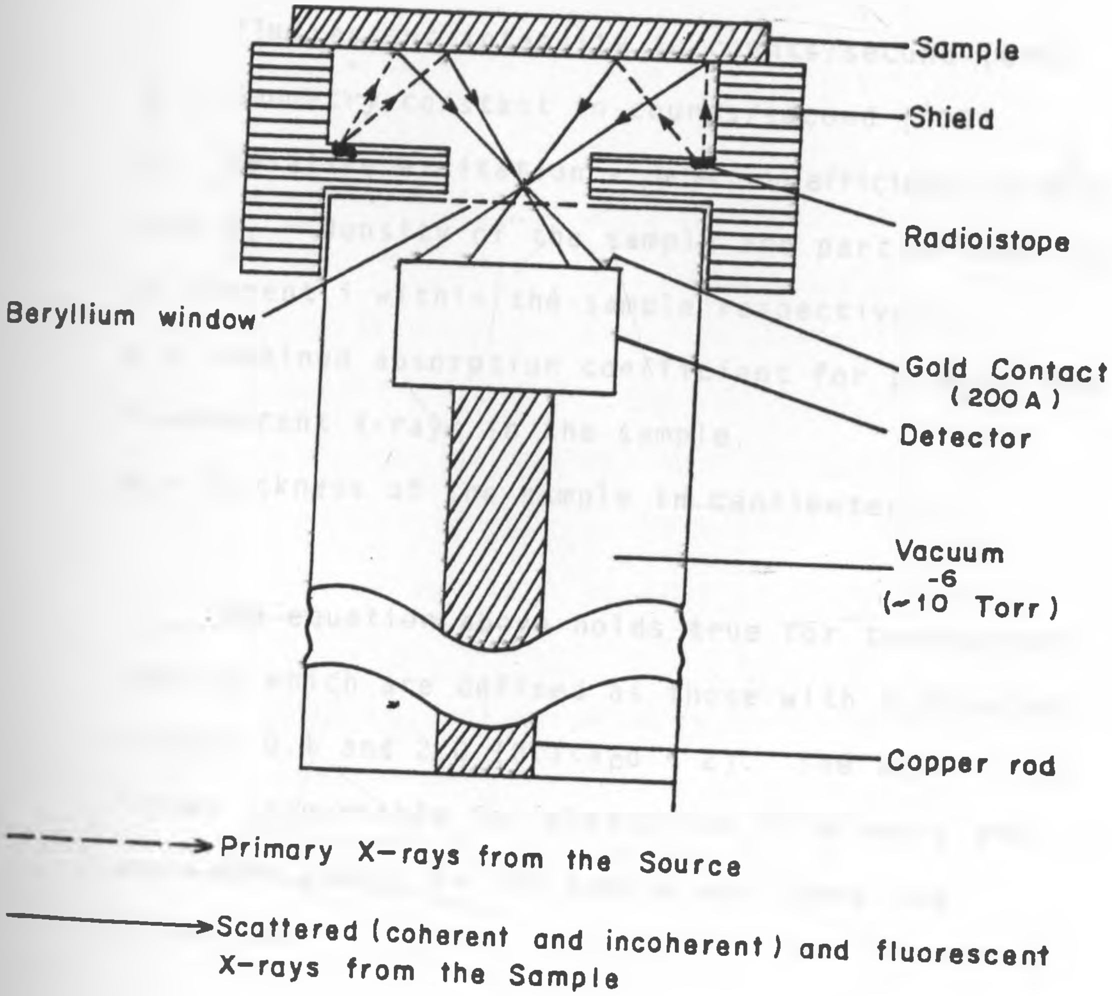
The X-Ray fluorescence spectrometer used consists of an X-Ray excitation source, a solid state silicon-lithium [Si(Li)] drifted detector, a pre-amplifier and a multichannel analyser (MCA).

The silicon-lithium drifted detector was operated at liquid nitrogen temperature (77K) so as to reduce thermal noise which tend to increase the background contribution to the energy photo peaks spectrum.

The excitation source used was a Cadmium-109 radioisotope of 0.37 GBq activity with half-life of 453 days. It produces characteristic silver (Ag) K- X-rays of energy 22.1 Kev by electron capture decay mode capable to excite elements from calcium to molybdenum in the K-series and silver (Ag) to uranium (U) in the L-series.

Primary radiation from the excitation source impringes a sample to produce characteristic X-Rays of the elements that constitute the sample through photoelectric absorption effect. The multichannel analyser was used to store and display the results of the detected X-Rays. The geometry adopted for sample excitation was to have the sample placed on top of the excitation source with the detector crystal located below the beryllium window to receive the characteristic X-Rays through a narrow collimator as shown in the figure below.

Figure 1: Schematic XRFA Unit



The equation used to relate measured intensities to concentration of the elements in the sample is that by fundamental parameters derived elsewhere [17]

$$I_i = G_o K_i (\rho d)_i \frac{[1 - \exp(-a\rho d)]}{a\rho d} \text{ -------- } 3.1$$

where

I_i = fluorescent intensity in counts/second (CPS)

G_o = Geometry constant in counts/second (CPS)

K_i = Relative excitation - detection efficiency in Cm^2/g

ρ and ρ_i = Density of the sample and partial density of element i within the sample respectively.

a = Combined absorption coefficient for primary and fluorescent X-rays in the sample.

d = Thickness of the sample in centimeters.

The equation above holds true for transparent samples which are defined as those with $a\rho d$ values between 0.1 and 2.0 ($0.1 < a\rho d < 2$). The $a\rho d$ is the factor responsible for absorption of primary and secondary X-rays in the sample and hence the expression

$\frac{1-\exp(-a\rho d)}{a\rho d}$ corrects for matrix absorption effects.

Equation 3.1 also holds true for quantitative analysis of transparent samples such as those prepared from soils and ores.

For thin sample, such as those prepared from digested material (plants, tissue and liquids) the equation reduces to:

$$I_i = G_o K_i (\rho d)_i \quad \text{-------------} \quad 3.2$$

Since $a\rho d <<< 1$, the absorption is considered minimal with intensity directly proportional to the concentration of element i

2.3.2 Determination of absorption correction Factors ($a\rho d$)

The determination of trace elements in light element matrices by X-ray fluorescence spectroscopy requires the use of appropriate techniques to compensate for matrix absorption effects. Matrix enhancement effects are usually minor or negligible [10].

Matrix absorption effects were determined experimentally for transparent uniform samples. Relative X-ray intensities were measured from a target located at a position adjacent to the back of the sample, with and without the sample. The combined fraction of the exciting and fluorescent radiation transmitted in the total sample thickness m (gcm^{-2}) is expressed as

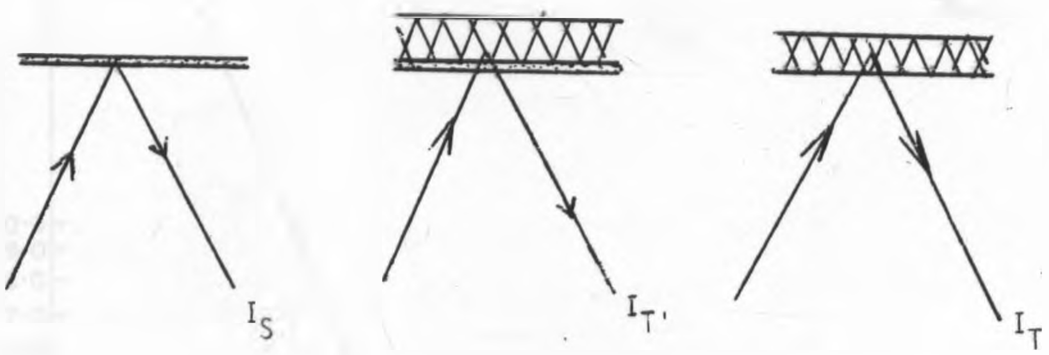
$$e^{-(\mu_{e/\rho} + \mu_{f/\rho})m} = \frac{I_{T'} - I_s}{I_T} \quad \text{-----------} \quad 3.3$$

Taking natural logarithms on both sides of the equation gives

$$\left(\mu_{e/\rho} + \mu_{f/\rho}\right)m = - \frac{I_{T'} - I_s}{I_T} \qquad \text{--------} \; 3.4$$

I_s, I_T and $I_{T'}$ are the intensities of X-rays plus background from the sample alone, the target alone and the sample plus target respectively. The values $\mu e/\rho$ and $\mu f/\rho$ are the total mass attenuation coefficients of the sample for the excitation and fluorescent radiations respectively.

Figure 2. Schematic experimental procedure used for the determination of matrix absorption effects.

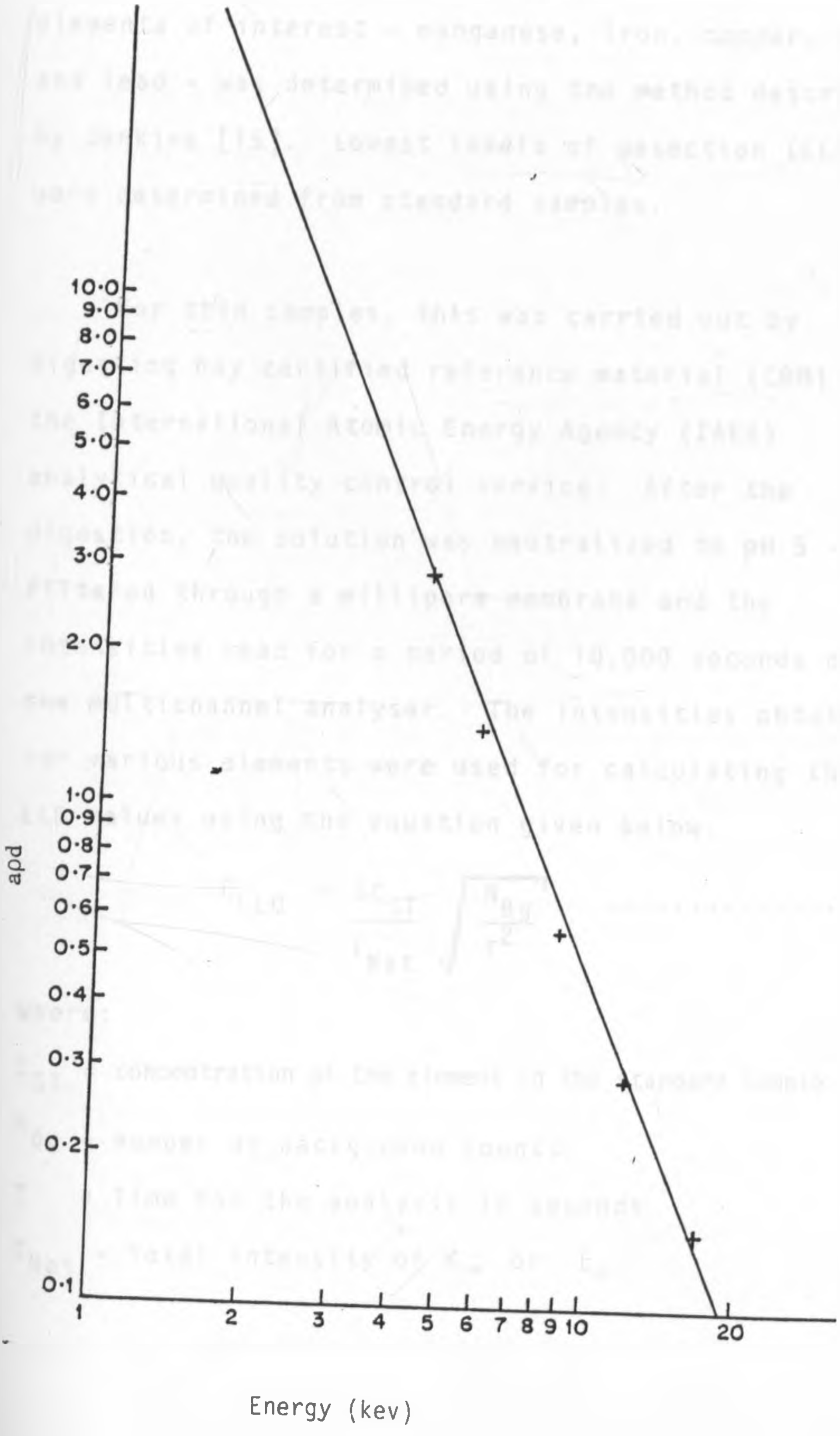


Sample alone Sample + Target Target alone

The measurement with the multielement target on top of a transparent sample yields attenuated characteristic X-rays of the elements in the target when excited by attenuated beam of primary X-rays from the sample [17].

After calculation of the absorption correction factors ($a\rho d$) of all the elements in the target, an absorption correction graph of $a\rho d$ values against the energy (Kev) was drawn on a log-log graph paper.

Figure 3: An absorption correction graph



2.3.3 Determination of sensitivities of the X-ray Fluorescence Spectrometer

The sensitivity of the XRF technique on the elements of interest - manganese, iron, copper, zinc and lead - was determined using the method described by Jenkins [15]. Lowest levels of detection (LLD) were determined from standard samples.

For thin samples, this was carried out by digesting hay certified reference material (CRM) from the International Atomic Energy Agency (IAEA) analytical quality control service. After the digestion, the solution was neutralized to pH 5 - 6, filtered through a millipore membrane and the intensities read for a period of 10,000 seconds on the multichannel analyser. The intensities obtained for various elements were used for calculating the LLD values using the equation given below.

$$C_{LLD} = \frac{3C_{ST}}{I_{Net}} \sqrt{\frac{N_{Bg}}{T^2}} \qquad \ldots\ldots\ldots\ldots\ldots 3.5$$

Where:

C_{ST} = concentration of the element in the standard sample

N_{Bg} = Number of background counts.

T = Time for the analysis in seconds

I_{Net} = Total intensity of K_α or L_α

For soils, the above determination was done by reading out intensities of transparent samples made from standard soil samples. The time used for the analysis was equal to 2,000 seconds.

The equation used was similar to the one used for thin samples except the new factor (Acorr = absorption correction) was introduced so as to take care of the absorption of the X-rays. And hence the equation becomes.

$$C_{LLD} = \frac{3C_{ST}}{I_{Net} \times A_{corr}} \sqrt{\frac{N_{Bg}}{T^2}} \quad \text{---------} \; 3.6$$

2.3.4 Calculation of relative errors

This was done using the residual errors given by the computer. Given that X1 and X2 are the residual errors of K_{α_1} and K_{α_2} or (L_{α_1} and L_{α_2}) respectively, the relative error was given by the equation below.

$$\frac{C\sqrt{(X_1^2 + X_2^2)}}{I} \quad \text{-------------} \; 3.7$$

where

C = Concentration of the element in the standard.

I = Intensity of secondary X-Rays in counts/second.

CHAPTER 3

1. RESULTS AND DISCUSSION

Two standard soil samples were analysed for the trace and major elements present. They were soil -7 from the International Atomic Energy Agency (IAEA) and soil standard SY-3 from the Canadian mines and geology analytical control section.

The analysis was done so as to establish the accuracy of the fundamental parameter method which was applied for the analysis of all the samples in the project. Also analysed was a hay Standard certified reference material (CRM) which was digested by using concentrated nitric and perchloric acid mixture and thereafter the ions precipitated by adding 2% NADDTC at a pH value between 5 and 6.

The results of the lowest limits of detection for both plant and soils are given below. Also given are the results for the three standard samples analysed.

Table 3.1: Lowest Limit of Detection in Plant Samples

Element	Detection Limit ($\mu g g^{-1}$)
Mn	1.1
Fe	1.3
Cu	0.4
Zn	0.4
Pb	0.2

Table 3.2: Lowest Limit of Detection in Soil Samples

Element	Detection Limit ($\mu g g^{-1}$)
Mn	26
Fe	34
Cu	6.5
Zn	6.6
Pb	7.8

Table 3.3: Composition of Standard soil - 7 (n=10)

ELEMENT	CERTIFIED VALUE	CERTIFIED RANGE	RESULTS OBTAINED BY FPM
Ca	16.3%	15.3 - 17.4%	18.2%
Ti	0.3%	0.26 - 0.37%	0.31%
Mn	631	604 - 650	620
Fe	2.57%	2.52 - 2.63%	2.83%
Cu	11	9 - 18	18.3
Zn	104	101 -113	109
Sr	108	103 - 114	105
Y	21	15 - 27	17
Zr	187	180 - 201	181
Nb	12	7 - 17	7
Pb	60	55 - 71	122

The concentrations not expressed in percentages are in μgg^{-1}

Table 3.4: Composition of standard soil SY-3 (n=8)

ELEMENT	CERTIFIED VALUE	RESULTS OBTAINED BY FPM
Ca	5.9% A	5.0%
Mn	0.26% A	0.24%
Fe	4.9% A	4.9%
Cu	17 B	23
Zn	250 A	216
Sr	300 A	268
Y	740 B	643
Zr	340 B	335
Nb	145 B	144
Pb	130 A	269

where; A refers to certified values and B refers to uncertified values.

The concentrations not expressed in percentages are in μgg^{-1}

Table 3.5: Composition of standard hay CRM* (n=8)

ELEMENT	CERTIFIED VALUE ($\mu g g^{-1}$)	CERTIFIED RANGE ($\mu g g^{-1}$)	RESULTS OBTAINED BY FPM ($\mu g g^{-1}$)
Mn	47	32 - 52	48
Fe	185	177 - 190	218
Cu	9.4	8.8 - 9.7	11.3
Zn	24	21 - 27	26
Pb	1.6	0.8 - 1.9	1.8

The results given in the three tables above show that values obtained by fundamental parameter method (FPM) for elements manganese, iron, copper, zinc and lead are in the same range with those given as certified values. In most cases these results are falling within the certified ranges. These results therefore show that the method is accurate and reliable for analysing transparent (soil and sludge) and thin (liquid) samples.

3.1 Results of samples from Kariobangi treatment works area in Nairobi and from Nakuru and Kiambu.

3.1.1 Manganese:

The mean level of manganese in these soils was found to be 1.35%. The concentration values ranged from 0.47% to 1.46%.

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* Certified Reference material from IAEA

Spinach plants contained manganese in the range of 145 to 1228 μgg^{-1} with an average value of 745 μgg^{-1}. The concentration in kale was in the range of 110 to 371 μgg^{-1} while the mean was 206 μgg^{-1}.

Underwood, E. J. [26] says that manganese deficiency has not yet been obtained in sheep or in man, although there is no reason to doubt that manganese is as essential for these species as it is for others. Rats were reported to have an intake of 1000 - 2000 ppm without any ill effects while hens can do with 1000 ppm intakes without any ill effects also.

Going by the above standards and the fact that few of the vegetable samples contained manganese above 1000 ppm in spinach and none of the kale samples went beyond 400 ppm, it may be safely concluded that eating vegetable products from this area pose no problem of manganese toxicity to man and other animals. This is of course based on the assumption that man has a similar tolerance and ability to accommodate high concentration of manganese in his body system.

3.1.2 Iron:

The concentration of iron in the soil was found to be highest among all elements of interest in this research. Its average concentration was 7.78% while the range was 5.77% to 8.91%.

High levels of iron were also detected in plants. Spinach samples contained an average iron concentration of 650 μgg^{-1} and all the values were in the range between 490 and 990 μgg^{-1}. Kale had an average iron content of 575 μgg^{-1} and the values ranged between 339 and 758 μgg^{-1}.

The concentration of iron in sludges and unamended soils did not show a great difference. It was therefore assumed that the participation of sludge amendment in iron uptake by plants is minor. As for animals, the high levels of iron in the ingested food is regulated in the body by its capacity to adjust during cases of iron deficiency. Boiling of vegetables reduces the levels of iron by as much as twenty percent. [26]

3.1.3 Copper:

Total copper content in the soil ranged between 30 μgg^{-1} and 154 μgg^{-1}. The mean value was 85 μgg^{-1} range.

In spinach leaves, all the copper concentration values obtained fell within 16.7 to 35.3 μgg^{-1} range.

The mean value was 24.8 μgg^{-1}. The concentration of copper in kale was similar to the one in spinach. In kale the range was between 16 and 33 μgg^{-1} while the mean value was 24.7 μgg^{-1}. It would seem therefore that the copper content in both vegetable plants grown on similar conditions tends to a constant value. This is emphasized by the fact that the ranges and means for both crops are almost identical.

The mean concentration values in spinach and kale were 24.8 and 24.7 μgg^{-1} respectively. These are high values compared to the value of 8.6 ppm obtained by Dowdy, R. H. et al [8]. Underwood, E.J. [29] recommends the maximum concentration in food at early infancy to be as low as 14 µg/kg body weight. Foods containing about 25.0 μgg^{-1} can therefore provide more than ten times the recommended value for infants and such can be dangerous. For growing children who require about 1.2 - 2.0 mg daily, diet from vegetables with copper content as high as the ones obtained in this work could easily lead to accumulation of injurious amounts of copper in the body. Consumption of green vegetables grown on sewage amended soils should therefore be discouraged due to high copper level among other reasons.

3.1.4 Zinc:

Zinc in the soil was observed to be in the range of 318 μgg^{-1} to 1130 μgg^{-1}. The average value of zinc in the farms was 620 μgg^{-1}.

The plant zinc level was observed to be within the range of 302 ppm to 1486 μgg^{-1} for spinach and 123 to 282 μgg^{-1} in the case of kale. The mean values for both plants were 835 ppm and 182 ppm respectively. In almost all cases, the zinc level in spinach was higher than the one in kale although both crops grew under the same soil and weather conditions. It would seem therefore that spinach accumulates much more zinc in its leafy tissues than does kale. In some cases the zinc concentration in the leafs of spinach was higher than the concentration in the soils on which it grew. However, the plants looked healthy and without any symptoms of deficiency or poisoning (toxicity).

Underwood [29] suggests that a normal well balanced adult diet supplies 10 - 15 mg zinc daily. Consumption of 100 g of spinach with an average concentration of 835 μg/g as given above would provide about 84 mg of zinc. 100 g of kale would provide about 18 mg zinc. Assuming that an average man consumes about this amount of vegetables, it seems then that these vegetables will always provide more zinc than the recommended value. Going from the fingures obtained, it seems not advisable to use any of the two vegetables from sludge areas in order to avoid zinc toxicity.

3.1.5 Lead:

Soil lead content was between 109 ppm and 432 ppm while the mean value obtained was 263 ppm.

Plant samples analysed did not contain high lead content as was the case for other elements. In spinach the average lead content in the leaves was 12.2 μgg^{-1} and the values obtained ranged from 7.7 to 15.9 μgg^{-1}. Kale had lower lead concentration in its leaves than spinach. The mean value obtained was 9.1 ppm while the range was between 6.4 and 10.8 μgg^{-1}. There were fewer samples of kale which had more than 10 μgg^{-1} of lead in the leafy tissues. As for the spinach leaf samples, concentrations above 10 μgg^{-1} were most common.

The lead monitored in this research can safely be said to exclude surface lead. This is because the samples were thoroughly washed with double distilled water before analysis. During the consumption of these vegetable products therefore, washing would not actually eliminate the lead inside the plant tissue.

Hansford *et al* [11] say that foods containing more than 10 ppm of lead are dangerous to human health. Of all the spinach samples, 70% of them contained lead quantities more than 10 ppm. Similarly above 60% of the kale samples contained more than 10 $\mu g g^{-1}$ lead. Considering the many physiological problems associated with lead toxicity, it would be advisable for the affected urban people to be discouraged from participating in vegetable gardening using the sludge from this area.

Table 3.6 Results of spinach and kale samples from Nairobi.(n=24)

ELEMENT	MEAN CONCENTRATION IN SOIL	MEAN CONCENTRATION AND RANGE IN SPINACH	MEAN CONCENTRATION AND RANGE IN KALE
Mn	13500	745(145 - 1228)	206(110 - 371)
Fe	77800	650(490 - 990)	575(339 - 758)
Cu	85	24.8(16.7 - 35.3)	24.7(16 - 33)
Zn	620	835(302 - 1486)	182(123 - 282)
Pb	263	12.2(7.7 - 15.9)	9.1(6.4 - 10.8)

All concentrations are expressed in $\mu g g^{-1}$

Table 3.7 Results of spinach and kale sampled from Nakuru treatment works area. (n=24)

ELEMENT	MEAN CONCENTRATION IN SOIL	MEAN CONCENTRATION AND RANGE IN SPINACH	CONCENTRATION AND RANGE IN KALE
Mn	2058	139(103 - 180)	22.3(16 - 35)
Fe	49400	207(132 - 292)	96.6(63 - 118)
Cu	17.2	8.4(5.4 - 9.4)	4.5(3.7 - 5.1)
Zn	333	62.3(35 - 151)	44.5(34 - 51)
Pb	57	1.9(0.3 - 9.7)	2.1(02 - 5.3)

All concentrations are expressed in μgg^{-1}

Table 3.8 Results of analysis of spinach and kale from Kiambu. (n=24)

ELEMENT	MEAN CONCENTRATION IN SOIL	MEAN CONCENTRATION AND RANGE IN SPINACH	MEAN CONCENTRATION AND RANGE IN KALE
Mn	7024	172(156 - 182)	62(52 - 83)
Fe	96760	481(449 - 538)	150(103 - 200)
Cu	29.3	5.7(4.5 - 7.2)	6.6(4.1 - 12.5)
Zn	259	78(39 - 107)	99(63 - 189)
Pb	48	0.6(0.4 - 0.7)	0.5(0.2 - 1.5)

All concentrations are expressed in $\mu g g^{-1}$

These results for samples obtained from Nairobi, Nakuru and Kiambu show that the vegetables from Nairobi have the highest concentrations of the five elements - manganese, iron, copper zinc and lead. This can be explained by the fact that the treatment plant in Nairobi handles even waste water from industrial area. Nairobi is also the most industrialized and populous urban centre in Kenya and the only treatment plant it has, handles all its industrial effluents and the waste water from homes of the 1.5 million residents. With all these flowing into the treatment plant, the resultant sludge is not surprisingly heavily loaded with trace elements.

The application of sludge in these farms has taken place for a period of not less than ten years continuously. A brief interview with the peasant farmers who are mainly vegetable growers disclosed that the application of sludge followed by irrigation with the sewage water during the dry seasons takes place at least twice a year. While the fact that addition of sludge improves the soil by addition organic matter and macro-elements phosphorus, nitrogen and potassium is not disputable, it is also important to know that the heavy metals added are not bio-degradable and will last in that soil for a very long time. The availability of these elements to the plants becomes more apparent as the sludge decomposes and future crops will always be carrying heavy metal loads which eventually will end up in the human bodies. All the results obtained from this study have indicated higher heavy metal levels in vegetables grown on sludge amended soils than those grown on unamended soils.

Samples from Kiambu which were grown on soils amended with farmyard manure gave low levels of heavy metals especially copper and lead. This can be attributed to low level of both elements in the soil and in animal wastes.

Sludge from Nakuru had very low trace element content compared to the Nairobi sludge. This is due to the fact that the Nakuru treatment plant is small and mainly handles domestic waste water from a smaller population.

Results from spinach and kale grown on soil amended with sewage sludge at different percentages indicates a general increase in kale manganese content from both fields while it gives a decreasing manganese content in spinach from field one and an increasing trend in field two. This consistent increase in the kale metal content is similar to what was observed by Soon, Y.K. et al (25) using bromegrass plant. They observed that the total manganese uptake tended to be increased by application of iron sludge and decreased by application of calcium sludge. The sludge used in this study had more iron (4.0%) than calcium (3.1%). As for spinach the decrease may be attributed to non-uniform mixing of soil and sludge since the decrease was not consistent. All in all the observation is that sludge amendments to soil increased the manganese content in both spinach and kale. Uptake of iron by the vegetables from both fields, did not give a consistent increasing or decreasing trend as the percentage of sludge was raised.

However, it seemed to be higher in plants grown pots with low percentage sludge. After 10% application, the uptake becomes low and almost stabilizes.

Copper in both spinach and kale increased with the addition of sludge. This was observed in both plots. However spinach copper content was higher than the one of kale at the same rate of soil treatment. This consistent increase of plant copper content was also observed by Dowdy R. H. et al [8] in potato. They observed that potato accumulated increasing amounts with increased addition of sludge. A similar increase was also observed in lettuce.

Zinc in both vegetable crops showed a similar trend with that one of copper. Vegetable zinc content increased regularly with the increase of sludge addition. Both plots did not differ on the trend. Also noted was that spinach proved to be better accumulator of zinc since plants under the same treatment had more zinc than in kale. Similar increase in plant zinc have been reported in oat, lettuce [27] carrots, raddish, potato, pea fruit and corn grain [8].

Lead uptake did not seem to be sensitive to the rate of sludge addition. It was particularly very low in even samples grown on pure sludge. The conclusion was that the sludge-borne lead is not immediately available to the plant. The contribution of automobile exhaust gases on the plant lead content was taken care of since the plants were not grown near a major highway. Also the fact that the samples were thoroughly washed means that all the surface lead was removed. The only source was then from the soil. Low availability of lead in sludge amended soil has been reported [2,8].

The results show an increase of uptake of these heavy metals with increase of the amount of sludge added. It therefore means that municipalities should adopt an integrated waste water and sludge management approach so that they can control the amount of sludge added to the soils and also the frequency of this exercise. Since most of these vegetables grown on sludge amended soils are fully consumed by the Urban population, the risk of poisoning of these people or emergence of an apidemic really exists. In Japan, there arose an epidemic known as "Itai-Itai" in a Japanese basin of Jintsu due to consumption of food crops with elevated cadmium content [3]. Sludge from the industrial areas should actually be analysed for these heavy metal content before any step is adopted on its agricultural use.

The other alternative of sludge use is to apply it to soils where plants which have been found to be non-accumulators of these trace elements are grown. Some plants, like rice do not accumulate heavy metals and hence sludge can be applied on rice fields to improve the soil quality.

3.2 Metal composition of vegetables grown on sludge amended soil in relation to sludge addition

The sludge addition in these experiments covered the whole range between zero percentage sludge to a hundred percent sludge. These rates are generally higher than the general field sewage sludge amendments to soil. As a result of these additions, the whole range of possible sludge application rates and its phytotoxicity was fully covered.

Among the elements studied in this study, copper, zinc and manganese showed a general increase in both lettuce and kale as the percentage of sludge was increased. Although the increases were not linear, the metal content in plants grown on amended soils at all rates was higher than those in the controls. This indicates that the sludge addition increased the plant available content of the heavy metals.

Iron content in plants did not seem to be influenced by the sludge addition. In most cases, plants grown on un-amended soils gave higher iron content than those grown on all other amended soils. Sludge addition acted in a way to suggest a depression in iron availability.

Lead concentration in both plants was low. None of the plants even those grown on undiluted sludge gave a value higher than 6.7 ppm. The sludge addition did not give any immediate increase in the plant lead content as compared to those plant sampled from areas where sludge had been on use for more than ten years. The content of lead actually varied with plant species than the soil media. The plants were not near any highway and hence the source of lead from automobiles was ruled out. Spinach accumulated heavier lead load than kale at all rates of applications. The results given below gives the relationship between percentage sludge increase to the soil, the total soil metal concentration and the plant metal concentrations. The percentage sludge is given on a weight basis.

Table 3.9: Manganese content in Spinach and Kale at different sludge amendment rates.(Concentration values are in μgg

Field one:

Percentage of sludge	0	10	20	30	40	50
Concentration in spinach	241	222	127	146	139	149
Concentration in kale	6.4	17.5	25.5	23.6	25.5	35
Total Conc. in soil	5713	5508	5364	4786	3710	3310

Field Two:

Percentage of sludge	0	20	40	60	80	100
Concentration in spinach	254	419	532	471	1006	1119
Concentration in kale	8.5	66	120	135	233	276

Table 3.10: Iron Content (Concentration values are in μgg^{-1})

Field One

Percentage sludge	0	10	20	30	40	50
Concentration in spinach	468	357	314	340	281	186
Concentration in kale	261	168	172	144	135	155
Total concentration in the soil	89210	89351	82340	79954	72452	58785

Field Two

Percentage sludge	0	20	40	60	80	100
Concentration in spinach	189	135	145	136	136	142
Concentration in kale	101	124	121	113	188	190

Table 3.11: Copper content. (concentration values are in μgg^{-1})

Field One

Percentage of sludge	0	10	20	30	40	50
Concentration in spinach	6.8	19.0	22.7	25.9	25.0	23.6
Concentration in kale	5.7	7.3	8.3	6.6	7.8	10.5
Total concentration in soil	23	28	38	102	85	97

Field Two

Percentage of sludge	0	20	40	60	80	100
Concentration in spinach	15.3	30.5	19.6	22.3	21.2	31.2
Concentration in kale	3.3	7.6	8.7	10.1	11.0	14.4

Table 3.12: Zinc content (concentration values are in μgg^{-1})

Field One.

Percentage sludge	0	10	20	30	40	50
Concentration in spinach	152	347	358	404	424	459
Concentration in kale	53	82	96	89	101	142
Total concentration in soil (μgg^{-1})	311	344	452	802	701	724

Field Two:

Percentage sludge	0	20	40	60	80	100
Concentration in spinach	137	1036	910	1089	926	1422
Concentration in kale	47	196	323	493	297	536

Table 3.13: Lead content

Field One

Percentage sludge	0	10	20	30	40	50
Concentration in spinach	0.6	3.4	1.3	1.5	0.7	0.9
Concentration in kale	0.8	0.5	0.4	0.5	0.5	0.3
Total concentration in soil (μgg^{-1})	48	93	154	137	185	248

Field Two

Percentage sludge	0	20	40	60	80	100
Concentration in spinach (μgg^{-1})	3.2	6.7	6.0	4.8	5.8	4.0
Concentration in kale (μgg^{-1})	4.1	4.6	5.4	3.2	4.5	3.0

Concentration values in the above tables are in all μgg^{-1}

The following are graphical representations of the relationship between the plants heavy metal content and the level of sludge added to the soil.

Figure 4 : Manganese in field I

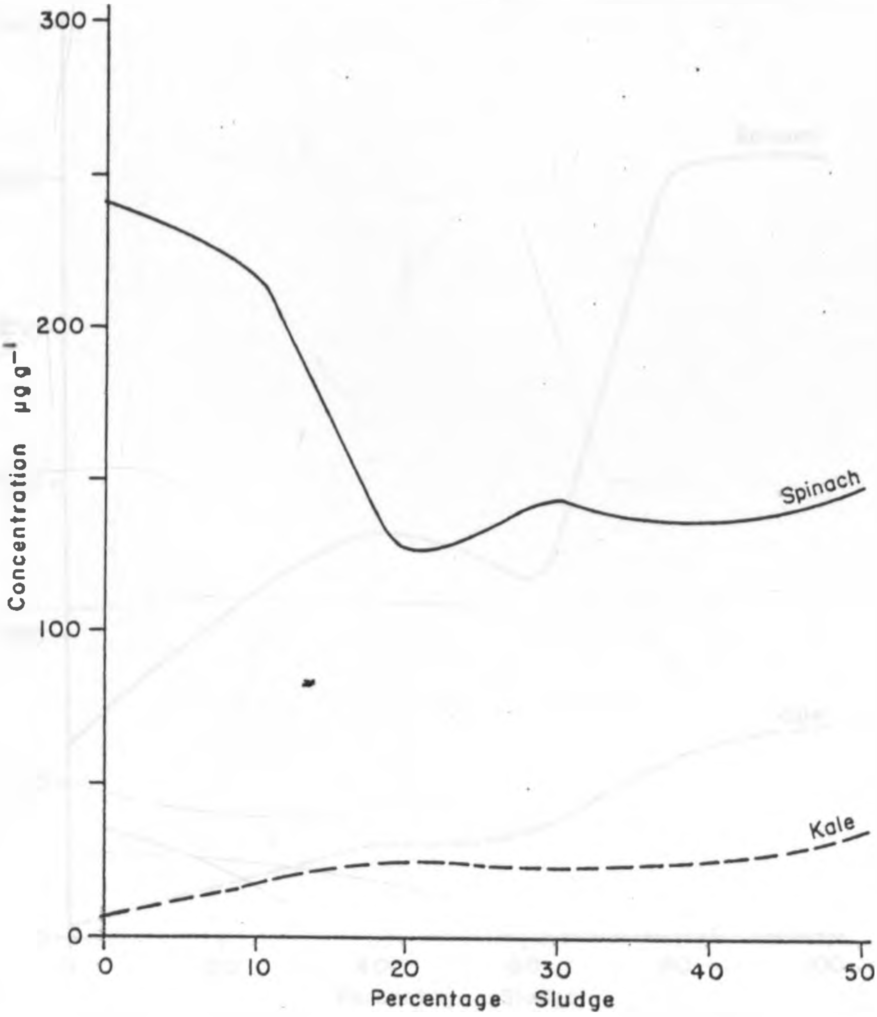


Figure 5 : Manganese in field 2

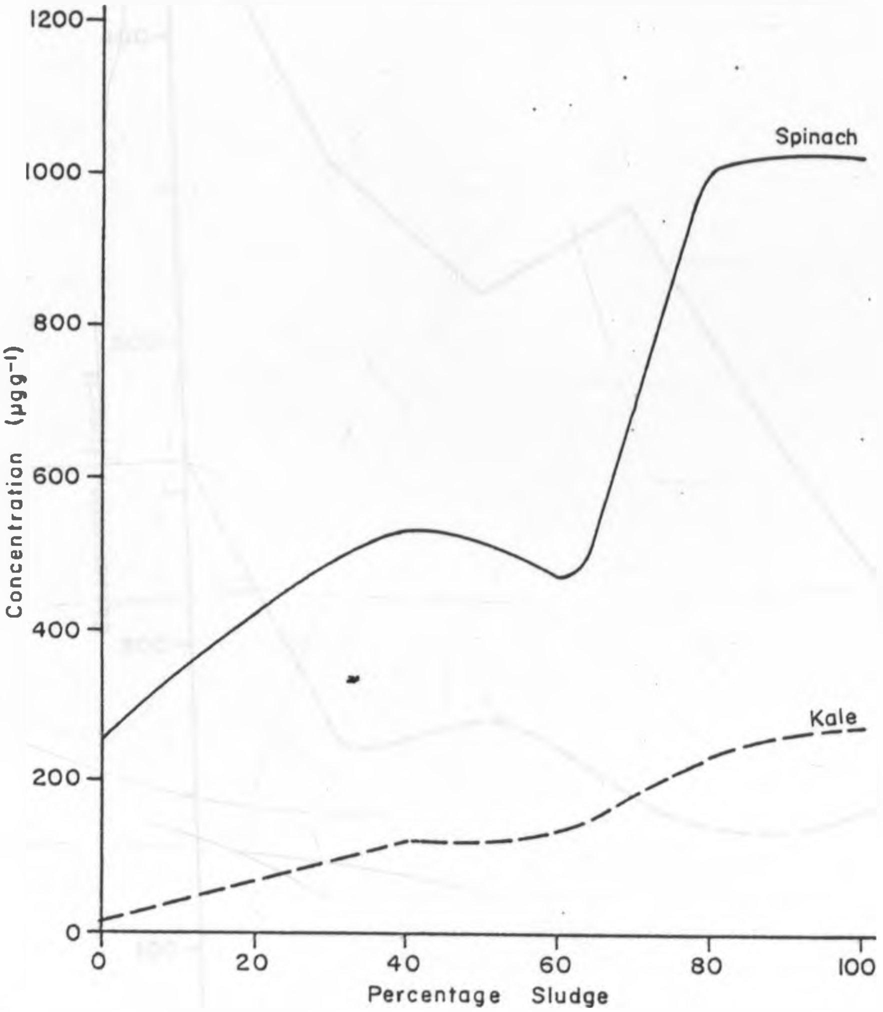


Figure 6 : Iron in field I

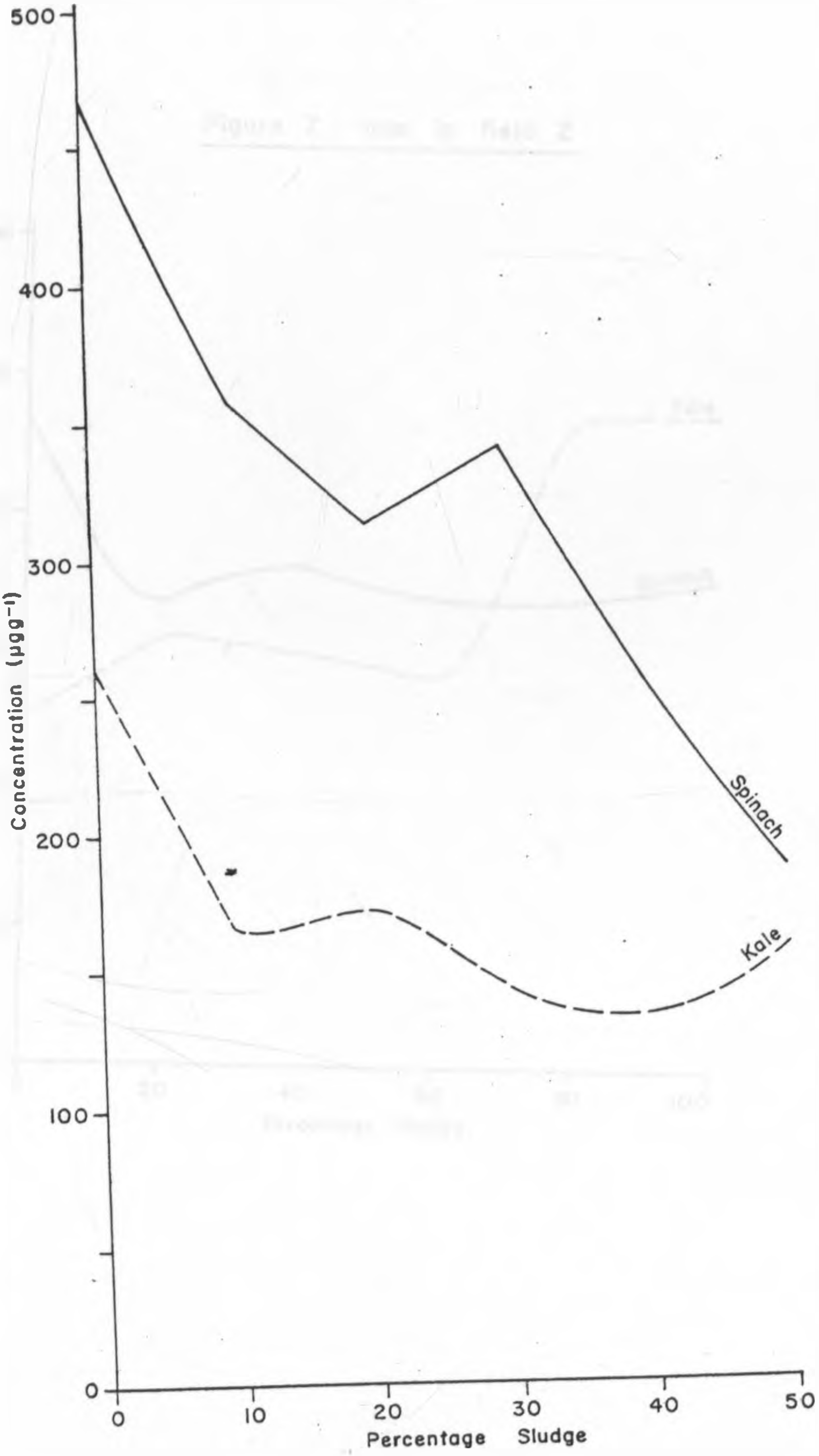


Figure 7 : Iron in field 2

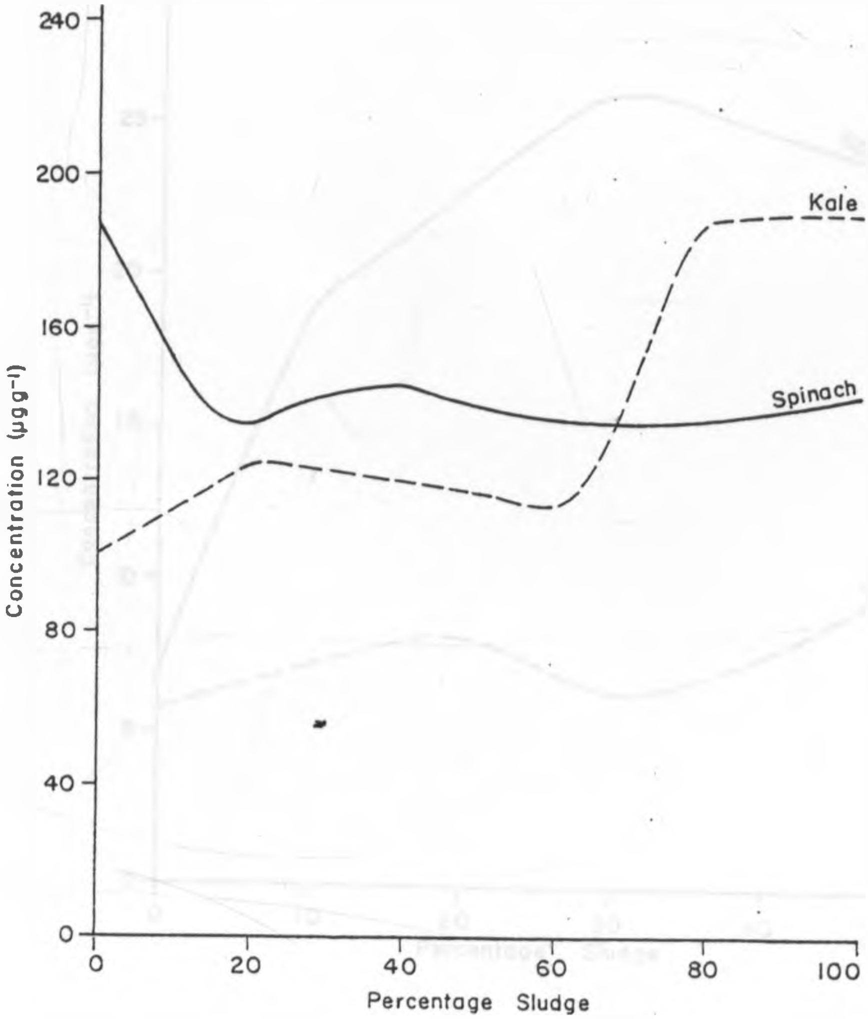


Figure 8 : Copper in field I

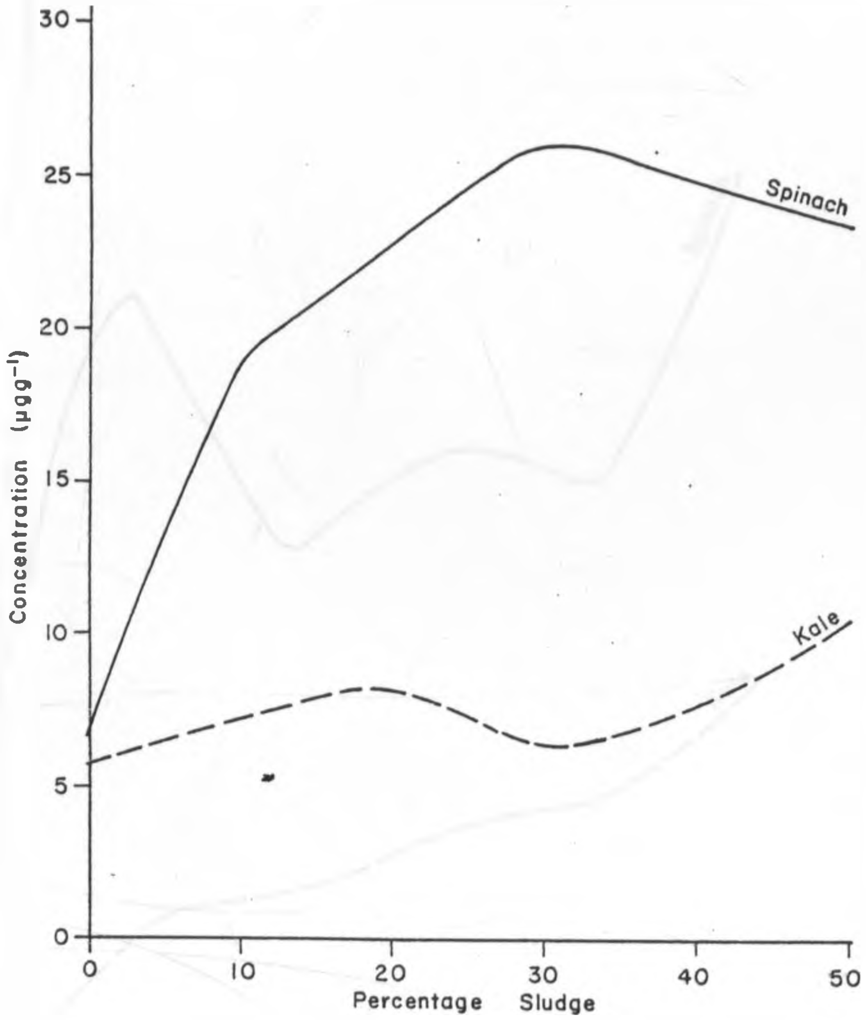


Figure 9 : Copper in field 2

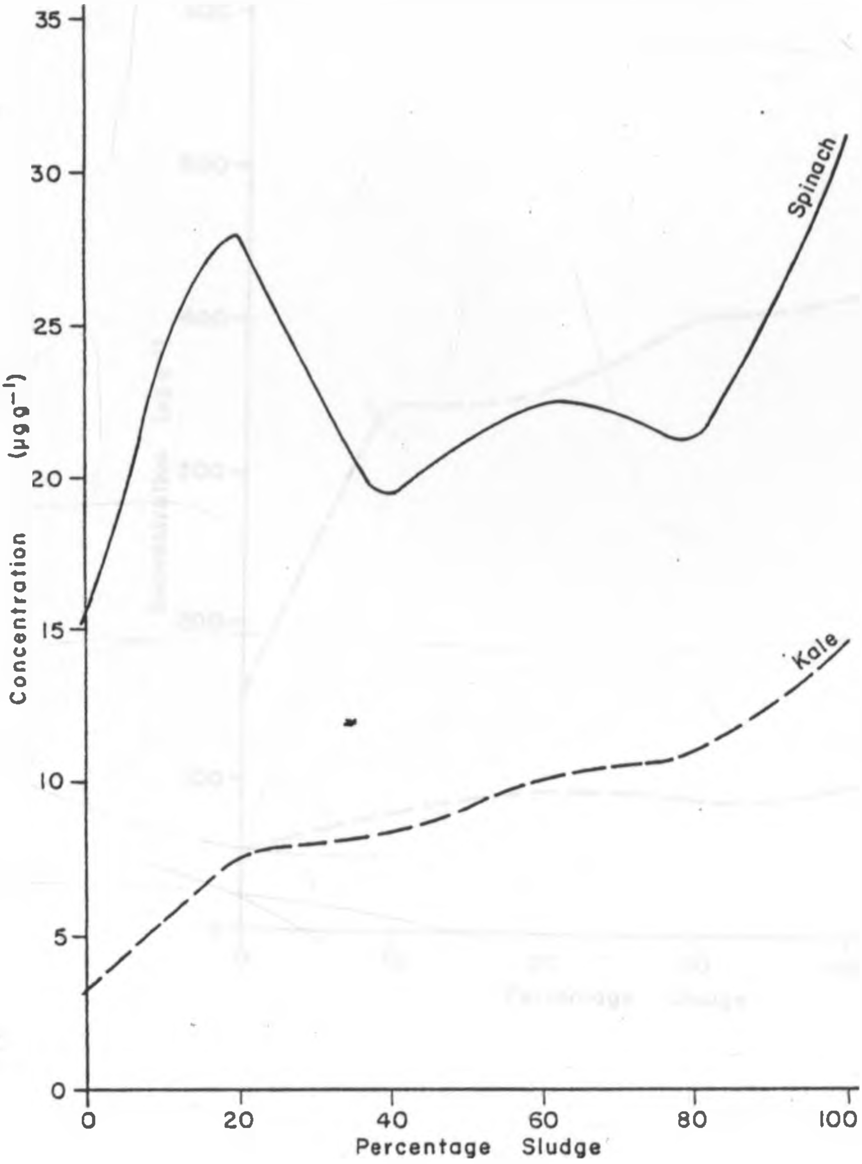


Figure 10 : Zinc in field 1

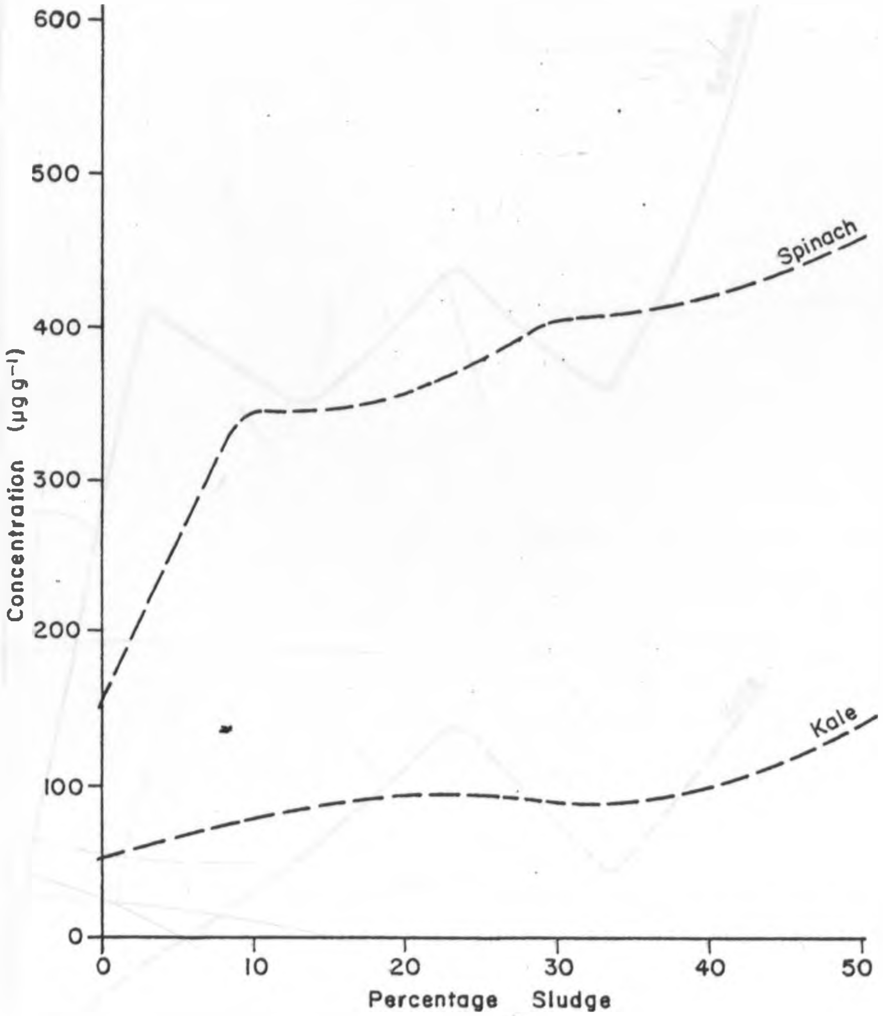


Figure 11 : Zinc in field 2

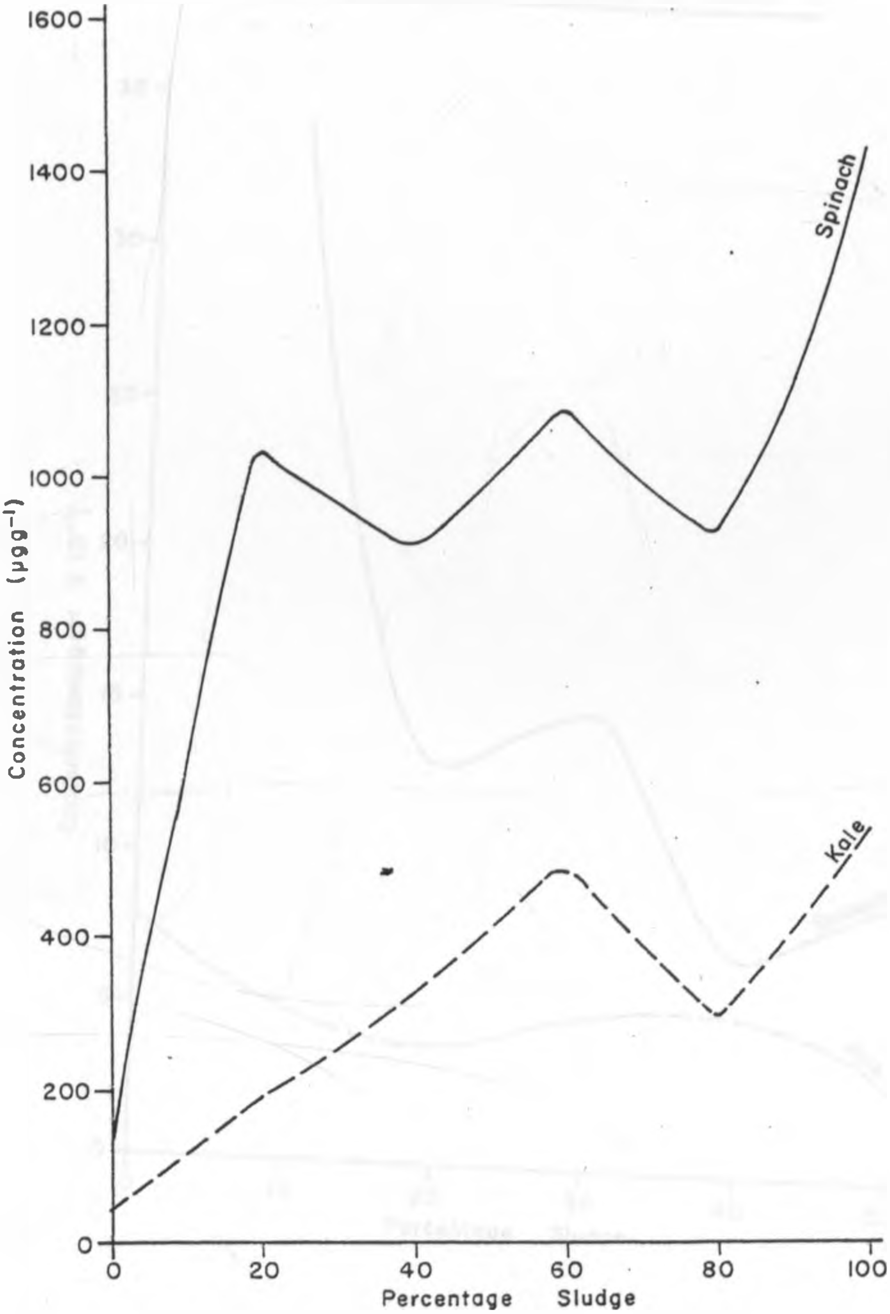


Figure 12 : Lead in field I

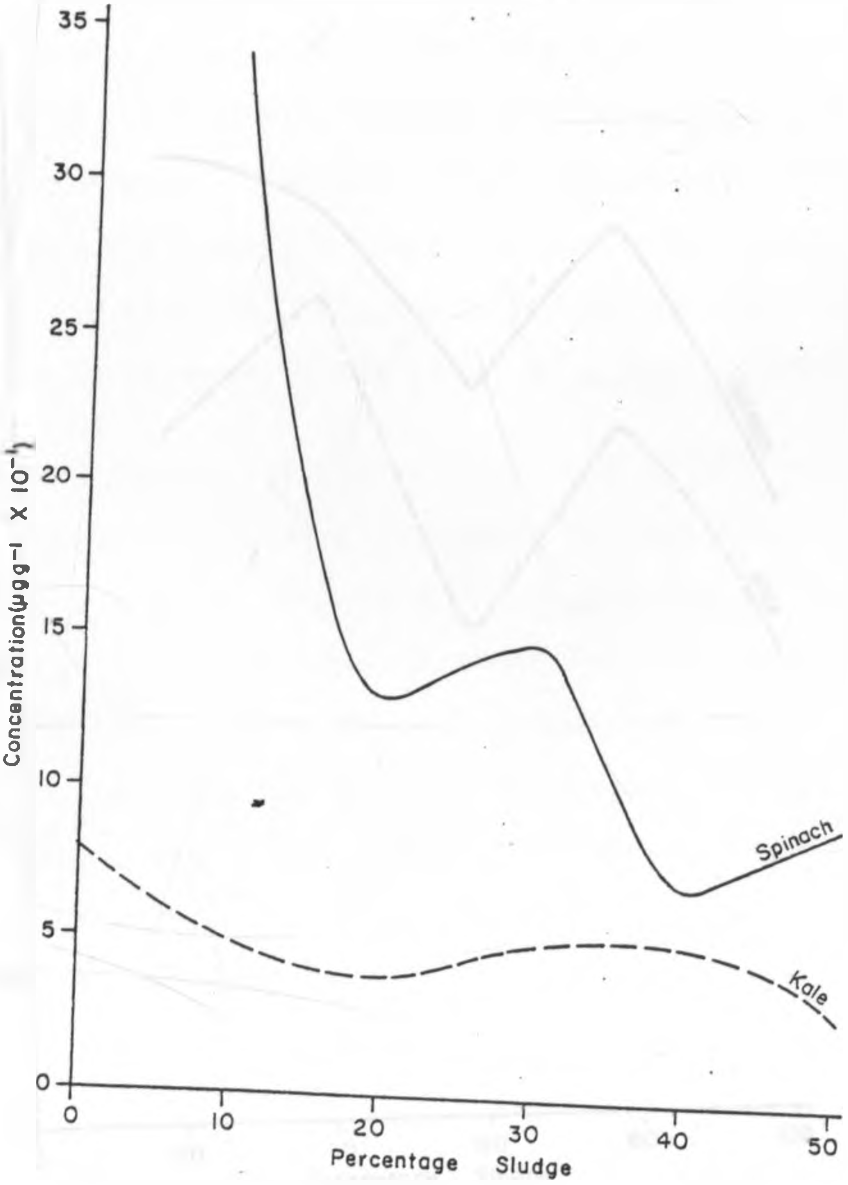
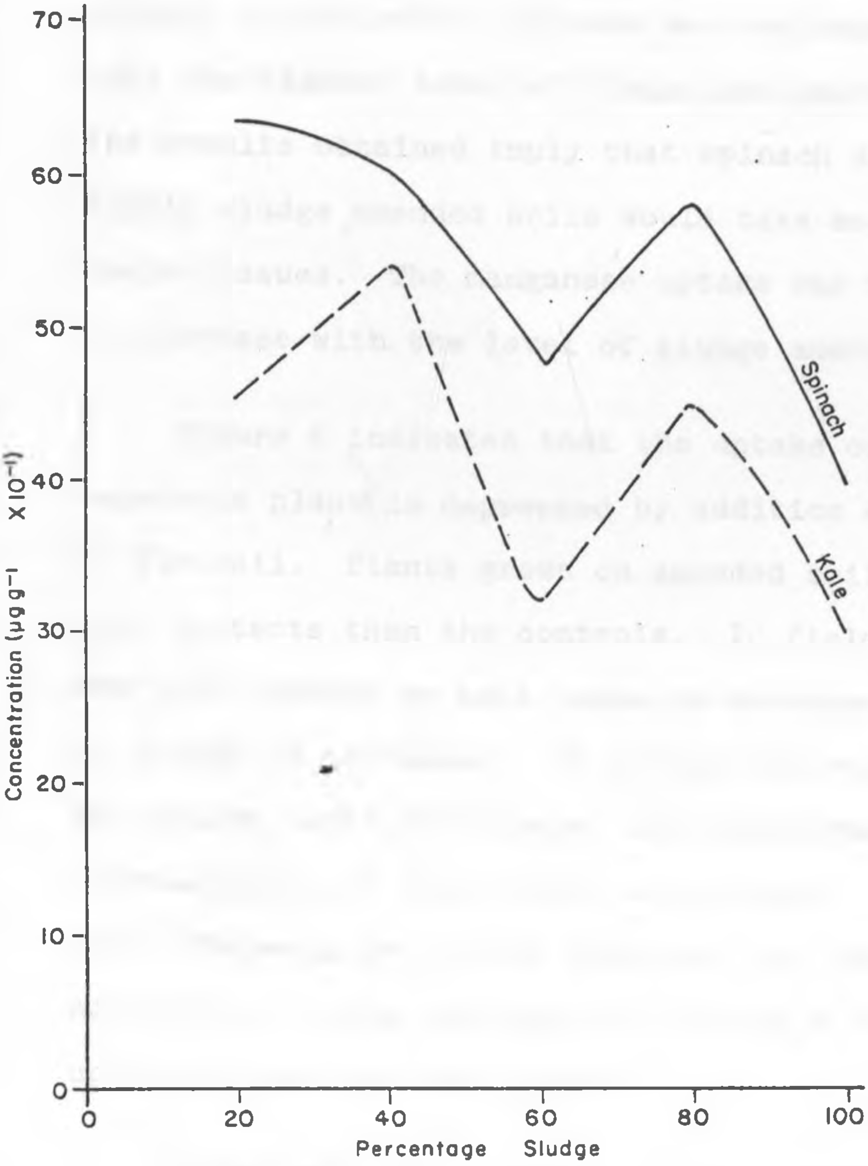


Figure 13: Lead in field 2



Both figures 4 and 5 show that the uptake of manganese by kale generally increased with the addition of sewage sludge. However, in spinach, figure 4 shows a decrease in the uptake as the sludge was added while figure 5 shows a general increase in the uptake. Although there occurred an inintial decrease in field one, upto 20% sludge, a consistent increase was envisaged thereafter upto the highest level of sludge application. In general, the results obtained imply that spinach and kale grown in highly sludge amended soils would take more manganese into their tissues. The manganese uptake was therefore seen to increase with the level of sludge amendment.

Figure 6 indicates that the uptake of iron by both vegetable plants is depressed by addition of the sludge to the soil. Plants grown on amended soils had lower iron contents than the controls. In field 2 (figure 7) the iron content in kale seems to increase as the amount of sludge is increased. An initial decrease was obtained in spinach (upto 20% sludge) and thereafter the concentration of iron almost stabilized. The results from the above two fields indicate that the mode of addition of sludge may also be playing a role in the uptake of iron by both plants.

Copper and zinc uptake by both vegetables gave very similar trends. At all cases, the uptake of both metals increased with the addition of sludge to the soil. This was more so for copper which increased almost linearly in kale.

(figures 8, 9). Uptake of both copper and zinc therefore increased as the amount of sludge was added to the soil.

Figures 12 and 13 show the relationship of concentration of lead in the plants with the amount of sludge added to the soil. The results indicate that addition of sludge does not result to an immediate increase in lead uptake by the plants. Figure 12 shows that addition of sludge results to a decrease in the uptake of lead by both spinach and kale. Figure 13 also shows a decreasing trend.

3.3 Plant metal uptake as related to the age of the plant.

The results of these experiments showed that the level of copper and zinc in the leaves of both spinach and kale increased with the age of the plants. For both plants, the levels of both elements were observed to increase from the first harvest through to the third. The first harvest was done thirty five days after transplanting while the second and third were done sixty five and ninety five days respectively after transplant.

Below are the tables for the concentrations obtained.

Table 3.14: Copper(n=8)

Sampling	Days after transplant	Concentration in spinach(μgg^{-1})	Concentration in kale(μgg^{-1})
First harvest	35	12.6	9.5
Second harvest	65	14.5	12.1
Third harvest	95	17.8	13.9

Table 3.15: Zinc (n=8)

Sampling	Days after transplant	Concentration in spinach (μgg^{-1})	Concentration in kale (μgg^{-1})
First harvest	35	143	347
Second harvest	65	147	386
Third harvest	95	182	402

Manganese, Iron and Lead.

Manganese, iron and lead gave decreasing trends in both vegetables.

The level of iron in spinach and kale during the first harvest were 306 and 219 μgg^{-1} respectively while in the third sampling the respective concentrations were 207 and 192 μg/g respectively.

Manganese levels during the first sampling in spinach and kale were 158 and 535 μgg^{-1}, respectively, while 120 and 531 μgg^{-1} were the respective concentrations obtained during the third harvest.

Lead in both vegetable plants was generally low. The values obtained in the third sampling were much lower than those obtained from the first sampling. In some cases, during the third sampling, the lead level was below the lower detection limit of the X-ray fluorescence spectrometer used. This suggests that the plants have a mechanism of ridding itself off the lead load.

However, lead was obtained within the range of upto 5.9 μgg^{-1} in the first sampling in spinach and up to 2.2 μgg^{-1} in the third sampling. The mean value during the first harvest was 3.8 μg/g while that one of third harvest was 1.8 μgg^{-1}.

Kale had mean lead value of 2.1 μgg^{-1} during the first harvest and 0.8 μgg^{-1} during the third harvest.

It is therefore observed that lead levels in the above two garden vegetables decrease with age.

3.4 Conclusions and Recommendations

The significant findings from this work are that the uptake of trace elements generally increase with increase of the sludge application rate. Spinach had higher increases in metal contents as compared to kale.

The vegetables from Kariobangi (Nairobi) had all the five trace elements in higher concentration than those from Nakuru and Kiambu. The analysis of the two sludges (Nairobi and Nakuru) show that the

Nairobi sludge has the trace elements in higher amounts. This is supported by the fact that Nairobi has a bigger industrial base than Nakuru. This results in the industrial effluents being more contaminated with essential and non-essential elements.

The recommendation is that, there is a need for control of discharge of industrial effluents into the sewerage systems if the resultant sludge is intended for disposal in agricultural land as a fertilizer. A successful program for sludge utilization in agriculture would require a sound understanding of the sludge composition and a knowledge of possible interactions between sludge and soil.

As the industrial activities go on increasing, the sludge heavy metal content will be expected to rise. Continued use of sludge in agriculture will pose even greater dangers to health. Further studies on the effects of these heavy metals on health in Kenya and especially in the urban areas is needed.

Since the combined amount of sludge produced in this country cannot make any significant impact on the total fertilizer requirements, the use of sewage-sludge in agriculture should be discouraged and alternative disposal methods sought.

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Below are tables of raw data of metal concentrations obtained after analysis of various soils and sludges. **CV = Certified Value; CR = Certified Range; OV = Obtained Value.**

Appendix 1: Metal concentrations of CRM soil - 7

Element	C_1	C_2	C_3	C_4	C_5	CV	CR	OV
Ca	17.0	19.3	18.1	18.2	18.3	16.3	15.7-17.4	18.2
Ti	0.31	0.29	0.31	0.30	0.32	0.30	0.26-0.37	0.31
Mn	618	623	549	640	670	631	604-650	620
Fe	2.61	2.92	2.74	2.95	2.95	2.57	2.52-2.63	2.83
Cu	7.2	25.8	19.7	19.4	19.4	11	9-13	18.3
Zn	117	106	111	106	105	104	101-113	109
Sr	111	101	103	106	106	108	103-114	105
Y	7.5	15.0	20.8	21.5	19.6	21.7	15.0-27	17
Zr	166	192	177	184	185	187	180-201	181
Nb	7.4	7.3	6.4	6.5	6.6	12	7-17	7.0
Pb	127	119	117	122	125	60	55-71	122

Concentrations of Ti and Fe are given in percentages while the rest are in $\mu g g^{-1}$

Appendix 3: Metal concentrations of sludge from Nairobi

	C_1	C_2	C_3	C_4	C_5	Average
Ca	3.1	2.7	3.6	2.9	3.1	3.1
Ti	0.28	0.22	0.29	0.23	0.25	0.25
Mn	0.21	0.20	0.20	0.21	-	0.21
Fe	3.81	4.00	4.5	-	3.8	4.0
Cu	181	225	242	190	204	208
Zn	0.17	0.18	0.20	0.17	0.17	0.18
Rb	29. 50	36.00	29.00	26.00	29.00	29.90
Sr	107	115	134	115	117	117.6
Zr	365	379	420	339	354	371.4
Nb	69.10	76.0	81.80	58.60	64.8	70.1
Pb	312	333	356	303	332	322.2

Concentrations of Ca, Ti, Mn, Fe and Zn are expressed in percentages while the rest are in $\mu g g^{-1}$

Appendix 4: Metal concentrations of sludge from Nakuru

Element	C_1	C_2	C_3	C_4	C_5	Average
Mn	956	1029	1076	978	926	993
Fe	37175	38851	32035	39452	42552	38013
Cu	99.50	95.80	115	87.2	91.0	97.70
Zn	1360	1420	1483	1331	1356	1390
Pb	84.0	87.4	94.3	78.6	83.2	85.5

All concentrations are expressed in $\mu g g^{-1}$.

Appendix 5: Metal concentrations of soils from Kariobangi works.

Element	1	2	3	4	5	6	7	8	9	10	11	12	Averag
Mn	1.42%	1.25%	1.45%	1.46%	1.38%	1.35%	1.15%	1.18%	0.869%	0.95%	0.47%	0.47%	1.12%
Fe	8.35%	7.99%	7.49%	7.66%	8.91%	8.85%	8.16%	8.22%	7.53%	8.27%	5.77%	6.12%	7.78%
Cu	102.4	122.1	46.2	47.2	106.9	117.9	29.3	36.4	66.7	48.3	153.5	144.3	85.1
Zn	789.5	775.7	360.4	355.4	440.2	460.6	318.2	348.4	1017.0	1130	730.0	710.9	619.7
Pb	268.5	277.0	255.8	271.7	108.6	338.2	167.8	203.9	218.2	223.1	386.0	431.6	262.5

Concentrations of Mn and Fe are given in percentage while the rest are in $\mu g g^{-1}$.

Appendix 6: Metal Concentrations of soil samples from Kiambu.

Metal	1	2	3	4	5	6	7	8	9	10	11	Average
Mn	0.72%	0.64	0.65%	0.66%	0.75%	0.73%	0.73%	0.71%	0.71%	0.67%	0.70%	0.70%
Fe	9.53%	8.41%	9.15%	9.29%	10.09%	9.70%	9.65%	10.26%	10.27%	9.44%	10.2%	9.64%
Cu	23.3	28.8	19.2	23.3	32.7	32.4	29.7	26.9	26.9	33.3	25.8	27.5
Zn	251	227	252	257	278	267	249	268	268	250	245	256
Pb	52.4	48.0	44.1	32.9	52.9	45.0	48.0	47.0	47.0	53.3	47.7	47.1

Concentrations of Cu, Zn and Pb are expressed in $\mu g g^{-1}$

Appendix 7: Metal Concentrations of soil samples from Nakuru

Element	1	2	3	4	5	6	7	8	Average
Mn	0.24%	0.22%	0.22%	0.21%	0.20%	0.20%	0.20%	0.21%	0.21%
Fe	5.61%	5.27%	4.99%	4.81%	5.05%	5.17%	4.63%	4.84%	5.05%
Cu	14.8	8.0	17.0	15.4	40.8	34.9	12.1	7.5	18.8
Zn	267	250	294	301	402	406	256	247	303
Pb	20.8	22.4	31.5	29.3	61.8	51.8	27.4	27.1	34.0

Concentrations of Cu, Zn and Pb are expressed in $\mu g g^{-1}$