

ANALYSIS OF SOME TRACE METALS IN
SOME RAW AND PROCESSED FOODS

BY

JAMES ITODO EJEH (B.Sc. ZARIA)

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D E C L A R A T I O N

I declare that this thesis is a record of my own research findings. It has not been presented in any previous application for a higher degree.

JAMES ITODO EJEH

Date: _____

THESIS APPROVAL

This thesis by James Itodo Ejeh meets the regulations governing the award of the degree of master of Science of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.



Professor G.H. OGBADU
Internal Examiner/Supervisor
Department of Biochemistry,
Ahmadu Bello University,
Zaria - Nigeria.


External Examiner

20/8/91

DATE

DATE

Professor A.I. UKOHA
Internal Examiner/Supervisor
Biochemistry Department,
Ahmadu Bello University,
Zaria - Nigeria.



Dean, Postgraduate School
Ahmadu Bello University,
Zaria - Nigeria.

DATE

14/11/91

DATE

DEDICATION

TO MY LATE MOTHER

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A B S T R A C T

Fresh tomato, puree and ketchup samples were collected from a vegetable and fruit processing company in Bauchi State. Similarly, groundnut, groundnut cake, crude and refined oil samples were collected from three groundnut processing companies two in Kano (A & B), and one, (c) in Zaria.

The atomic absorption spectrophotometric (AAS) technique was used for the analysis of copper (Cu), iron (Fe) Zinc (Zn) manganese (Mn), and lead (Pb) levels of the samples.

The results indicate that amongst the metals analysed in the samples from the tomato processing company, iron was found to be highest with a level of 230ppm obtained from the puree. The Cu levels in the fresh tomato, puree and ketchup differ significantly ($P < 0,05$, ANOVA). There was a statistical difference in the levels of Fe in these samples also. The levels of Zn in the fresh tomato, puree and ketchup also differed significantly. The levels of Mn in these samples differ significantly. There was statistical difference in the levels of Pb in these samples from the tomato processing company.

The results obtained from the analysis of groundnut samples from the three companies (A, B & C) indicate that in each of the companies, iron level is highest amongst the metals. The mean values are 109.3 and 98.8ppm in samples from factories A & B respectively; and these were obtained from groundnut cakes. The highest mean value obtained in factory C was 81.9ppm and it was obtained from groundnuts.

The levels of Cu in the samples from factory A differ significantly. So also are those of Fe, Zn, Mn and Pb in the samples from the factory A, The levels of Cu in the samples from factory B are statistically significant. Those of Fe, Zn and Mn in the same samples from factory B differ statistically also. However, the Pb levels in samples from factory B are not statistically significant ($P > 0.05$). In factory C, the levels of each of the metals in the samples are statistically significant.

Results of Pb and Fe are high compared to the international standards permissible limits, for foods, while Cu, Zn and Mn are within their permissible limits.

Some suggestions have been made as to the effective control of trace metals in the environment without causing much harm to man.

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INTRODUCTION AND LITERATURE REVIEW1.1 INTRODUCTION

For several decades, national and international organizations have striven to improve the health of the people throughout the World. Although substantial progress has been made, much more needs to be done if the goal of health for all by the year 2000 is to be met. In particular, the impact on health of food - borne disease associated with contamination of food supply has not been well recognized by some national governments and the efforts made by international organizations have not prove too effective. To a significant extent, the ubiquitous nature of the problem has made it less visible than the more dramatic but less globally significant outbreaks of other diseases, which have received greater attention and resources than food safety. Indeed, the Declaration of Alma-Ata (WHO, 1978) only implicitly considered food safety as an essential component of primary health care, rather than recognizing it explicitly as a major component contributing significantly to disease prevention and health promotion.

Science and technology over the years have brought many benefits to mankind. Not the least has been a marked increase in the food supply and nutritional status, although much still remains to be done. These benefits have been accompanied by dangers of pollution. Public concern over contaminants such as pesticides, chemical additives and heavy metals (in the food area) has been aroused (Crosby, 1977) and the long-term risks to health must be assessed and balanced against the benefits

achieved. This assessment requires careful and concerted thought and action co-ordinated at international level.

Most countries have enacted pure food laws in one form or another over the years. Regulations usually take the form of maximum permissible limits for specified metals in individual foods or a general limit may be established for all foods with a list of permissible exceptions to the general rule. Such specific legislations applies in general to a strictly limited number of toxic elements e.g. arsenic and lead. (Ireilly, 1980) Additional protection for the consumer is available however under general pure food regulations which prohibit the selling of materials pure food regulations which prohibit the selling of matters (product) that are dangerous to public health (Crosby, 1977).

In the Netherlands, control of metallic contamination is based on prohibition of the use of machinery, utensils, containers, solders, etc that contain harmful metals (Crosby, 1977).

Many International Organizations are at present engaged on a programme for the establishment of uniform food standard. It is hoped that the adoption of agreed standard will facilitate the development of International trade as well as promote fair practices in the food manufacturing industry; ultimately for the benefit of the consumer. Pre-eminent amongst these bodies is the Codex Alimentarius Commission, established jointly by the Food and Agricultural Organization and the World Health Organization (WHO) of the United Nations in 1962 (Crosby, 1977).

Other International bodies working towards the harmonisation of foods standards include the European Economic Community (EEC) and various organizations with a particular commodity interest e.g. International Association for General Chemistry standards and International Commission for uniform methods of Sugar Analysis. In addition, the work of these bodies provide a basis for those developing nations without established food laws of their own. Concern has been expressed in many quarters over the level of heavy metals in the environment (Waldron, 1975). There has been much speculation that children may absorb more lead than adults thus placing them at greater risk. Although adults are thought to absorb about 10% of ingested lead, Alexander et al, (1972) reported an average absorption of 53% in 8 children.

The high productivity of greenhouse borders is maintained by considerable fertiliser, pesticide and herbicide application as well as by intensive farming methods (Zurera-Cosano et al, 1989). The use and environmental consequences of metal-based pesticides containing such heavy metals as Hg, Pb, Cd, Sn, Ni and Cu have been widely questioned over recent years (Zurera-Cosano et al, 1989). Vegetables absorb these metals from the soil as well as from deposits on the parts of the vegetable exposed to the air from the polluted environment. Obviously, in greenhouse crops, the possibility of environmental contamination from the air and from rain, which in the case of lead are the two major routes of contamination, is limited (Bauchauer, 1973; Havre and Underdal, 1976; Zurera et al 1987). The absorption of metals through the soil is

influenced by different factors such as pH of the soil (Street et al, 1977), the organic matter content (Kaakkola and Ylaranta, 1976) and the interaction of other metals such as Se (Lisk, 1972; Francis and Rush, 1973; Cary, 1981).

A number of elements present in trace amounts in food are known to be essential in human nutrition (Nielsen and Sandstead 1974; Jones, 1977; Buss, 1983; Merta, 1983). Cereals, especially when unrefined provide a significant if not the major source of Fe, Zn, Cu, Se, Mn, Cr and Ni in the diet (Jones, 1977) Buss, 1983). For example, UK diet studies (Buss, 1983) have shown that cereal foods, principally bread contribute on average 19% of the zinc, 26% of the copper, 50% of the selenium and 78% of the manganese. The manganese figure would have been much higher were it not that in the UK, tea contributes about 5% of the manganese in the diet. Studies on the levels of various trace elements in wheat and milling products and bread derived therefrom have been carried out in the USA (Cserniejewski et al, 1964; Ishander and Morad, 1986), Australia (Mugford and Steele, 1980; Steele, 1981), Germany (Meuser et al, 1981) and Hungary (Sxalay and Kuranyi 1982). All of these studies involved copper, manganese and zinc, some iron (Cserniejewski et al, 1964; Waggle et al, 1967; Mugford and Steele, 1980; Steele, 1981; Ranhetra et al, 1985; Ishander Morad, 1986) selenium but only one nickel and chromium (Cook et al, 1970). All reached the conclusion amount of trace elements than refined products.

Cadmium, lead and mercury are three of the trace metals known to be toxic to human beings. Cadmium is a cumulative

poison (Jenning and Rainbow, 1979) associated with hypertension, osteomalacia especially among patients with low dietary calcium intake (Deluea, 1976) and itai-itai disease, a painful and disabling illness which occurred in Japan as the result of eating sea foods contaminated with cadmium (Tsuchiya, 1976). Lead poisoning disturbs the synthesis of heme in the body (Dresel and Falk, 1956) and it usually causes permanent brain damage in children (WHO 1972). The most harmful compounds of mercury are methyl mercury and ethyl mercury (Davison *et al*, 1981). Among the many problems caused by mercury poisoning are mental retardation, damage to the central nervous system, paralysis and lack of muscular coordination (WHO, 1974).

Iron deficiency is a problem in affluent as well as poor nations and much of this is caused by the poor availability of iron for absorption (Underwood, 1981; Venkatachalam 1968). Iron availability is dependent upon the chemical form of the iron and upon its interactions with various components (Hazell, 1985). For example, much of the iron added to fortify food is insoluble and unavailable for absorption (Lee and Clydesdale, 1978). Also, there are a variety of dietary constituents which can either enhance or inhibit iron availability, for example, protein (Klavins, 1962), vitamin C (Hallberg, 1981), and citric acid (Gillooly *et al*, 1983) have been shown to enhance iron availability, whereas fibre (Fernandez and Phillips, 1982) Phytate (Hussain and Patwardhan, 1959) and tannin (Disler *et al*, 1975) have been shown to inhibit iron availability. Fruit Juices that contain vitamin C, citric acid and other organic

acide have also been found to enhance iron availability (Rossander *et al.*, 1979).

However, there is less information on the effects of food processing on iron availability. Current research in this area has been reviewed (Lee and Clydesdale, 1978; Lee, 1982), but there are still many foods and processing methods that have not been examined. Many of these processes, especially those in which additives are also involved, might be expected to have a marked influence over iron chemistry and, hence iron availability.

Manganese is an essential diet required by mammals for normal growth and reproduction (underwood, 1977; Leach and Lilbam, 1978). A daily intake of 2.5 - 5.0mg has been recommended as adequate for adults and most normal diets contain sufficient levels of manganese (Iacon, 1983).

With regard to human consumption, it has been reported that people drinking well-water containing high levels of manganese have suffered loss of appetite, loss of memory or even mental disturbances (WHO, 1971). The maximum permissible level of manganese in water set by the WHO for a person weighing 70kg is 1.25mg per day.

OBJECTIVES OF THE STUDY

In recent years, concern has been expressed in many quarters over the level of heavy metals in the environment (Waldron, 1975). Nickel, when inhaled is carcinogenic while copper causes congestion of the nasal mucous (Waldron, 1975).

Combustion of petroleum is the main source of lead, chromium, nickel and copper into the environment, though other

sources like metal smelting, municipal refuse are also important (Anonymous, 1965). Day *et al*, (1975) have drawn attention to the high concentrations (about 1000ppm) of lead in urban street dust as opposed to dust from rural areas (85ppm), and its significance for the daily intake of children. The importance of air-borne lead pollution on the levels in blood for residents in a high density traffic area have been discussed by Waldron (1975). Some correlation between the increase in blood lead levels and traffic density was observed. Little information on the ultimate fate of lead in the environment is known but Wong *et al*, (1975) have demonstrated the transformation of certain inorganic and organic lead compounds into tetraethyl lead by a purely biological process, involving microorganisms in a manner analogous to mercury and arsenic.

A comprehensive study of the distribution of trace metals in the Nigerian environment does not appear to be available.

In view of the industrialisation and agricultural activities, this study has been undertaken to provide some preliminary data on some of the metal contaminations on which further systematic monitoring can be based.

This investigation has been undertaken to;

- i) Find the levels of some trace metals (copper, iron zinc, manganese and lead) in some raw and processed foods.
- ii) Determine any variations in the levels of the metals in the same food from different locations.

- iii) Determine the influence or effect of processing on the metal content of the food.
- iv) Make possible suggestions about control measures of the environmental pollution by trace metals.

In order to achieve these objectives, samples of fresh tomato and its products; groundnut and its products were collected from the northern parts of Nigeria.

It is not the purpose of this investigation to ascertain which factories had the highest, or which had the lowest trace metal content, and in order to avoid any possibility of factories being associated with the concept of "best" or "worse" with regard to heavy metal content, factory names have been omitted.

1.2 LITERATURE REVIEW

1.2.1 TRACE METALS

A large number of elements occur in living tissues in such small amounts that their precise concentrations could not be measured with the analytical methods formerly available. Such elements were therefore reported to be present in "trace" amounts and the term "trace elements" became widely accepted. This term, with its connotation of imprecision has continued in popular usage, although most of the trace elements present in biological materials can now be measured accurately and reliably.

No clear line of demarcation, fitting all elements in all circumstances, can be drawn between the trace metal and the major elements. Those elements that occur or function in living tissues in concentrations most conveniently expressed $\mu\text{g/g}$ or $\mu\text{g/litre}$ are generally considered as trace elements (WHO, 1973). These concentrations are lower by several orders of magnitude than those of such typical major elements as calcium phosphorus.

1.2.2 CLASSIFICATION OF TRACE ELEMENTS:

Trace metals are ubiquitous and electropositive, and according to Underwood (1977) can be divided into three groups, namely; essential, probably essential and non-essential. On the other hand, WHO (1973) is of the view that classification of the trace metals into essential, non-essential, and toxic groups can be inaccurate and misleading. All the essential elements

become toxic at sufficiently high intakes and the margin between levels that are beneficial and those that are harmful may be small. In some instances, e.g. selenium and fluorine in man and copper in sheep, the margin between toxicity and deficiency is very small (Underwood, 1977). However, it is usual to differentiate between those elements which are known to be essential for animal (human) life and those which display severe toxicological effect at extremely low levels and have no known beneficial function in living organisms. At present, less than 14 elements are believed to be essential for animal life viz, iron, iodine, copper, zinc, manganese, cobalt, molybdenum, selenium, chromium, nickel, tin, silicon, fluorine and vanadium (WHO, 1973). Trace metals such as Ca, Co, Cu, Fe, K, Zn, Mg, and Na are essential for plants (Valkovic, 1975).

1.2.3 FUNCTIONS OF TRACE ELEMENTS

Some trace metals when present in toxic concentrations in food materials can lead to the formation of stable bonds in some active sites of some enzymes (Giddings, 1973). This chemical affinity is the basis of metalotoxicity in man. The attachment to an enzyme, of this metal, impairs the normal metabolic roles of such enzyme (Giddings, 1973). Thus, the high affinity of lead for thiol and phosphate containing ligands in living systems, for example, inhibits the biosynthesis of heme, affecting membrane permeability leading to convulsions, behavioural disorders and death (Giddings, 1973). The most important mechanism of toxic action is thought to be the poisoning of enzymes. The more electronegative metals, notably copper, mercury and silver, have a great affinity for amine, imine and

sulphydryl groups which are doubtless reactive sites on many enzymes, and are readily chelated by organic molecules (Bowen, 1979).

The essential trace metals; iron, copper, zinc, cobalt, manganese, selenium molybdenum and chromium serve as essential components of a wide variety of metalloproteins, usually enzymes, which participate in important hydrolytic, oxidative or transfer biological processes. They are also necessary for the optimal functioning of the mammalian organs (e.g. growth, healing) and life itself. Criteria which a metal must satisfy before being termed essential have been reviewed by Cotzias and Faradori, (1969).

They are:

- i) The organisms can neither grow nor complete its life cycle in the absence of the elements.
- ii) The element has a direct influence on the organism and is involved in its metabolism.
- iii) The element cannot be replaced completely in any other element.

The indispensable feature of the most prominent of these trace metals (Fe, Zn, Cu, Mn, Mo, Cr, Co, Se) rests on the role as functional or structural components of crucial metalloenzymes and metalloproteins (Frieden, 1974). The most important oxidases and all oxygen carrying proteins are Cu and or Fe metalloproteins. Cytochrome oxidases, a Cu-hemoprotein, is probably the single most crucial metabolic enzyme in the mammalian cell (Bowen, 1966). Metalloenzymes have assumed even greater importance in aerobic cell biology since the realization (Bowen, 1979) that adaptation to oxygen required protection against two unavoidable by-products of oxygen reduction, superoxide and peroxide ions.

Kashim Ibra
Ahmadu Bello U.
Zaria - Nigeria.

These toxic intermediates are mitigated by a team of protective metalloenzymes, superoxide dismutase (mainly Cu-Zn, but also Mn, Fe enzymes) and the heme enzymes, peroxidase and catalase. There is enhanced interest in Zn-enzymes since the demonstration of Zn^{2+} as a vital constituent of DNA and RNA polymerases and reverse transcriptase (Underwood, 1977).

The metabolic functions of these elements are still not fully understood, but Bowen (1966) has classified them into electrochemical, catalytic, structural and miscellaneous groups. Examples of the electrochemical group are sodium potassium, calcium and manganese, which exist inside cells in the ionic state at concentrations that differ from those in the surrounding medium. They act as a store for available free energy for use in various cellular processes and they influence the solubility of protein molecules in particular. Catalytic functions arise through an association with enzyme systems and in many instances the metal is firmly bound into the protein structure. Bowen (1966) listed a number of important metal-activated and metalloenzyme systems in which the metal contents ranged from 0.05 to 6.5%. The structural role of metals is chiefly to neutralise negative charges of anions, free carboxylic acids, nucleic acids and peptic substances, (Crosby, 1977).

Among the trace metals, the transition metals, which happen to be the essential ones share two properties of great importance in biology; namely; their ability to exist in more than one relatively stable oxidation state, and their ability to form many complexes (Underwood, 1977).

Copper, cobalt, zinc, manganese, molybdenum, nickel and vanadium have been associated with enzyme systems; particularly those involved in oxidation processes. Cobalt is present in vitamin B₁₂, while chromium plays an important role in carbohydrate metabolism together with insulin (Hambridge, 1974). Manganese activates enzymes involved in the transfer of phosphate and hydroxyl groups as well as some dehydrogenation reactions.

1.2.4 SOURCES OF CONTAMINATION OF TRACE METALS

The non-essential metals which total about thirty are believed to be acquired by the animal body as environmental contaminants and also reflect the kind of environment in which the organism lived (Crosby, 1977).

Other elements e.g. arsenic, antimony, lead, cadmium and mercury, are known to be toxic at very low levels of intake and there are no known deficiency symptoms (Crosby, 1977). The effects of some elements are cumulative. Toxic elements are present in foods to a greater or lesser extent as contaminants as a result of increasing industrialisation contamination may arise from a number of different sources. Crops could contain various amounts of contaminants according to the nature of the soil, fertiliser and/or herbicide treatment and proximity to industrial activity. Subsequent harvesting, storage, processing and packaging (Especially canning) and domestic operations may also affect the level of contaminants in the food consumed. Not all such processes will increase the contamination; for example, rejection of outer leaves, washing and cooking (leaching of

soluble contaminants) may lead to reduction. The existence of such factors (Many of which apply also to nutrient elements) has stimulated a world-wide study of concentrations of metal in foods in order to assess both the safety and the adequacy of human diets. Food-processing equipment and containers have long been recognised as a source of chemical as well as microbiological contamination of food (Reilly, 1973). In modern times, the use of similar poorly glazed vessels to hold olives during pickling has resulted in lead poisoning in Yugoslavia (Analytical Methods Committee (AMC), 1960). Lead has always been a problem in food processing because the metal lends itself readily to the fabrication and repair of cooking and storage utensils. Amateur repair work on cooking and storage equipment in which solders containing high levels of lead are used to join breaks in metals or attached loose handles is another cause of lead contamination.

The problem of trace metal contamination of food during processing occurs frequently as a result of misuse of equipment or the overlooking of the consequences of using unsuitable metals in apparently insignificant ways.

The trace metal content of plants indirectly affects the dietary intake by both man and animals since the plants seem to provide the main source of minerals to man and his animals.

In the mid 1960s, it was found that some containers and utensils designed to come into contact with food were, in fact, coated with a tin-lead alloy of varying composition, rather than with pure tin. A typical example was a frying pan that was found to have 54% lead in the coating which caused considerable

concern (Chapman and Pindas, 1965).

Ogezin and adiuku-Brown (1987) observed a high concentration of lead, cadmium and zinc in a study. They also observed that the plant leaves which showed an optimum level may indicate that the soils in the mining districts may accumulate the analysed elements. These elements may be toxic especially in the immediate vicinity of the base metal mining district in Zurak where the concentration of the elements; lead (360ppm) zinc (522ppm) and cadmium (2.27ppm) are higher compared to the World Health Organisation (WHO) permissible limits for safe drinking water.

Aquatic insects play important role in the transport of metals in the aquatic environments and in many biological monitoring programme. Jop and Wojtan (1981) have observed that the animals assimilate trace metals in considerable amount even in the case of slight metal contamination of the aquatic environment.

Ejike *et al* (1987) in a study to find out the tissue metal contents of microbenthos of two city reservoirs in Joe Plateau in relation to their feeding functional groups, observed that except for the herbivorous grazers, the pattern of trace element accumulation and the sorption capacity did not follow the pattern of availability of trace metals in sediments of the reservoirs. The herbivorous grazers showed trace element accumulation in the pattern of Fe, Mn, Zn, Cu, Pb, which is consistent with the pattern of availability in sediments. They observed also that the organism's feeding habits relate most to that observed by macrobenthos. These groups of herbivores live permanently in the reservoir sediments and feed on the plant materials in the sediments. Similar observation has been reported by Brock (1983).

Holzinger (1977) has observed that the content of heavy metals in animals feeding on the sediments depends on the amount of the elements deposited on the sediment.

In a study, Thomas *et al.*, (1975) determined the levels of lead in samples of canned pineapples, fruit salad, loganberries, blackcurrants, cherries, apricot, pears, raspberries and tomatoes. They found that the lead content of the 168 samples was in the range of 0.02 to 8.16ppm. In an earlier work, Thomas *et al.*, (1972) determined the levels of lead and cadmium in Brussels sprouts, apples, pears, cabbages, potatoes, onions carrots, swedes, watercress, frozen vegetables, tomatoes, dried herbs etc. The lead content of the 231 samples was in the range of 0.01 - 3.85ppm while cadmium content ranged from 0.01 to 0.22ppm.

Kirkpatrick and Coffin (1975) used atomic absorption spectrophotometry to measure a number of trace metals e.g. chromium, cobalt, copper, iron, manganese, nickel and zinc in cured meats and eggs.

The body burden of lead is a function of exposure. For example, the American total burden is 121mg of lead with an organ concentration of 140ppm in the spleen. The body burden of Africans is 63mg, with 21ppm in the spleen (WPC, 1977).

Day *et al.*, (1975) have drawn attention to the high concentration (about 100ppm) of lead in Urban street dust as opposed to dust from rural areas (85ppm), and its importance for the daily intake of children. The significance of air-borne lead pollution on the levels in blood for residents in a high-density traffic areas have been discussed by Waldron (1975).

Atomic absorption spectrophotometry for the estimation of lead, iron, zinc and other metals in wines and spirits has been used successfully by Reilly (1973).

Samuel and Abdulkaila (1984) investigated the contributions of cadmium, lead and mercury introduced into beans ground on domestic Nigerian grinding stones using colorimetric methods. The highest additions of Cd, Pb and Hg were 0.14, 0.72 and 0.17ppm respectively. The corresponding lowest values were 0.01, 0.05 and 0.01ppm, respectively. These amounts introduced by the grinding stones are greater than the tolerable daily intakes recommended by the World Health Organisation.

Thomas and Smythe (1973) observed high values of Zn, Cu, Mn and Fe in plant materials. The values of Zn ranged from 25 - 174ppm, while that of Cu ranged from 5 - 92ppm, Mn ranged from 11-63ppm, while Fe ranged from 59-358ppm.

Michie and Dixon (1977) have recently drawn attention to the inhomogeneity of tea. Replicate analyses of packaged tea of lead content showed a wide variation and subsequent work established that this variation was due to particle of dust admixed with the tea leaves during processing. The dust had a lead content many times greater than the occurring naturally in the leaves. They found similar variations for iron, zinc and copper.

Osborne and Laal-Khoshah (1989) studied the iron, copper, zinc, manganese, chromium, nickel, selenium, protein and ash contents of two different bread grists and flour samples from the first to the fourth break and A to J reduction systems of a commercial flour mill. They observed that iron, zinc, manganese

and selenium levels followed the trend of ash and protein with the lowest from, A, B and C rolls and highest from either the fourth break or J roll. Copper and chromium levels were relatively homogeneous among the grist and mill streams while for nickel, there was only a slight increase through the break and reduction system.

Tran, et al., (1987) compared the manganese content of 33 samples of seeds from two species of lupin grown under field conditions in Victoria, Australia. Their results indicated that *Lupinus angustifolius* had a much lower manganese content (61ppm) than *L. albus* (137ppm). In *L. albus* 80% of the manganese content of the seed was concentrated in the endosperm, while in *L. angustifolius*, only 40% of the manganese was present in the endosperm, demonstrating the ability of this species to exclude manganese from the seed. Whole flour and spray-dried powder produced from *L. albus* seeds with a manganese content of 4479ppm contained 4479ppm and 4337ppm of manganese, respectively.

In contrast, protein isolate prepared from the same seeds contained only 176ppm. In order to avoid potential manganese toxicity problems from these foodstuff, *L. albus* seed containing high levels of manganese could be used for the production of protein isolate, while *L. angustifolius* could be used to produce all three products (Tran et al., 1987).

Compost utilisation as manure is becoming widespread during recent years, as a consequence of the rise in price of conventional fertilizers. In addition, the agricultural use of compost contributes to the disposal of waste which is a well known problem of modern times.

Nevertheless, addition of organic matter and nutrients to the soil by using compost has some important drawbacks; organic matter in compost has a high carbon nitrogen ratio, which could cause plants and soil microorganisms to compete for the soil nitrogen (Purves, 1973); Gonzalez vita and Martin Martinez, 1982); secondly, some undesirable materials present in compost, e.g. thin glass and plastics, are added to the soil. But the main problem of the use of compost as manure comes from its high content of heavy metals, causing an increase come from its high content of heavy metals, causing an increase in soil pollution. Consequently, the plant content of those elements also increases, toxicity symptoms can occur and significant amounts can be introduced into the food chain (Purves, 1972; Giordano *et al*, 1975).

Cabrere *et al*, (1989) compared the effect of adding annual doses of 14,000kg/ha of urban compost to a soil with the addition of 500kg/ha⁻¹ of 15-15-15 compound NPK fertilizer. Two rotation cycles of sorghum/wheat were carried out during the experiment and the crop yields were not significantly affected by the treatments. It was observed that compost added in the proportion used did not cause any statistically significant change in the initial level of available phosphorus in the soil, but caused an increase of the potassium potential.

Soil total contents of copper and zinc as well as available levels of both metals showed noticeable increases, but other metal were scarcely affected by the addition of compost. The results suggested that large doses could be an important cause of pollution of the soil with trace metals.

Zurera-Cosano et al., (1989) determined eight trace metals in some horticultural species cultivated in greenhouse borders by atomic absorption spectrophotometry to evaluate the contribution of these vegetable to the daily intake of cadmium and lead in Spain. The existence of significant differences ($P > 0.001$) in the levels of metallic concentration found among the vegetable species has been proved, and the greater capacity of concentration of trace metals, except copper, in green beans was observed. No statistically significant correlation ($P > 0.05$) between the concentration of the soil and that of corresponding vegetable species was found. The trace metal uptake from soil to plants is a function of the physical and chemical nature of the soil and is altered by innumerable environmental and human factors.

Havre and Underdal (1976) observed that the serial zones of the vegetables are the most important entry point for the metals.

Plants undoubtedly absorb trace metals from the soil through their roots. This absorption is influenced by, among other factors, the pH of the soil (Anderson and Nibon, 1974). The trace metal retaining capacity and availability of the soils could be attributed not only to differences in acidity, but also to the organic matter content (Jaakkola and Ylaranta, 1976). These authors showed that a large amount of organic matter in the soil limited the availability of cadmium to plants. Organic matter in the soils form the strongest metal complexes and hence retain the metal more firmly.

Soulis, et al., (1988) determined Fe, Cu, Mn, and Zn content in a number of processed and fresh Greek orange (citrus *Sinensis* var *valencia late*), lemon citrus lemon var *melini*),

grapefruit (*Citrus paradisi* var *dunkan*) and mandarin (*Citrus deliciosa* var *mediterranea*) juices by atomic absorption spectrophotometry. The values obtained were within limits similar to those for juices from the European countries. In Greece, a large quantity of fruit juices is consumed, especially during the summer months, and it is therefore of interest to know the metal content of such juices.

The presence of some metals in the juices may cause undesirable flavour changes (metallic flavours). A number of the metals are toxic and the sale of Beverages containing toxic substances is prohibited. The presence of some minerals is important for the appearance, shelf life and organoleptic characteristics of the juices (Amerine, 1974).

The nutritional value of fresh and processed fruit juices depends on their content of sugars, vitamins and minerals (Soulis *et al.*, 1988).

In recent years there has been a tendency for Governments to adopt legal limits for the levels of some wine constituents. Legal or suggested limits exist for Al, As, B, Br, Cd, Cr, Cu, F, Fe, Hg, Li, Ni, Pb, Se, Sn, and Zn (Amerine and Ough, 1980). According to Ough *et al.*, (1982), it is important to establish baselines for the composition of wines and to be able to detect any changes from the norms.

Lazos and Abxakis (1989) determined the concentration of fourteen metal ions in 113 commercial Greek wines by atomic absorption or emission spectrophotometry. The ions of K (1440ppm), Na(27.7ppm), Mg(101ppm) and Ca(31.9ppm) were found in greatest concentration. Among the others, mean Fe and Al concentration

were 3.51 and 2.35ppm respectively; Cu, Zn, and Pb were between 0.20 and 0.41ppm; and Li, Ni, Cr, Co and Cd were 0.10ppm.

It is known that many metals are normal constituents of grapes, and also that many of them can be added to grapes in fungicides and insecticides, fumes, dust, etc. A large proportion of the metals is, however, lost during alcoholic fermentation.

Grapes contain only traces of lead naturally, but they can be contaminated from fungicides, dust, car exhaust fumes and industrial pollution (Favretto *et al*, 1975). Some lead is lost during alcoholic fermentation and much is removed during the fining treatment with ferrocyanide. Cadmium, Lithium, Chromium and nickel are well of toxicological importance, but zinc, on the other hand, is important to yeast and human nutrition. A possible source of zinc may be zinc containing fungicides and insecticides (Lagos and Alexakis, 1989).

The most common precipitate found in wines is potassium hydrogen tartrate. To avoid this, the wine is stabilized by cooling or by ion exchange. The latter may result on some increase in sodium content. Other sources of sodium ions are various sodium salts. Efforts are made to minimize the sodium content because sodium intake is of importance to people on low sodium diets. Calcium ions can combine with oxalates and tartrates, which may precipitate after bottling. Magnesium is usually the third most abundant cation after potassium and calcium. Generally, it does not cause a stability problem in wines (Ough *et al*, 1982), although there are some indication that it may be of importance for tartrate stability and acid

teste (Amerine and Ough, 1980). According to Ough *et al.*, (1982) magnesium may be toxic to persons with kidney disfunction, but at a level above that which is normally found in wines. Iron is of importance to the wine maker because when it is present at 7-10ppm, it may cause cloudiness or colour change; the content depends upon iron levels in soil and dust, and contamination during harvesting, transportation and processing. Copper can also form precipitates, cloudiness occurs in wines containing 0.02-0.4ppm (Amerine and Ough, 1980). The amount of lead in wine has received extensive research due to its significance to public health and its exogenous origin Farretto *et al.*, 1975; Amerine and Ough, 1980; Danilatos and Salaha Moutsooulou, 1983).

1.2.5 METABOLISM OF TRACE-METALS:

The great majority of trace elements serve chiefly as key components of enzyme system or of protein with vital functions (Frienden, 1974). Enzymes in which metals are tightly incorporated are called metalloenzymes, since the metal is usually tightly embedded deep inside the structure of the protein. If the metal atom is removed, the protein usually loses its ability to function as an enzyme. The enzymes known to be activated by essential trace metals form a long and impressive list, e.g. phosphate transferases, decarboxylases, acyl transferases, reductases, oxidases, phosphomonoesterase, aminopolypeptidase, glycylglycine-dipeptidase and arginine desimidase.

1.2.6 METAL ACTIVATED ENZYMES

In these enzymes, the activating metal is loosely bonded and is readily lost on processing. The activating metal, most

often magnesium, is believed to act as a temporary link between enzymes and substrates during chemical reaction (Bowen, 1966). In many cases, the activating metal is not unique. For example, magnesium can frequently be replaced by divalent manganese, cobalt, iron or zinc, and rarely by aluminium, barium, calcium, cerium, chromium, lanthanides, lead, nickel (Schutte, 1964). In such cases the efficiencies of the different metals as activators differ widely, and it is clear that the fact that zirconium, say, can activate an enzyme in vitro is not evidence that it is essential in vivo. The concentration of the activating metal is also important, as some if not all metals inhibit enzyme action at high concentration. There is some evidence of synergism between pairs of elements in activating this class of enzyme, and enzymes often act most efficiently in the presence of traces of elements which are not essential for them to function. The number of metal - activated enzymes known is increasing so fast that no list can remain complete for long (Schutte, 1964). Here, the salient features of the commoner types, classified under their activating metal are only outlined.

Magnesium is the commonest enzyme activator. It is especially important in activating the large class of phosphate transferases, and a number of decarboxylases, but will also activate acyl transferases and one reductase.

Manganese resembles magnesium in activating a number of phosphate transferases and decarboxylases, notably those used in the Krebs cycle. It appears to remain in the divalent state as it does not activate many oxidases or reductases.

Iron^{II} activates a number of oxidases, notably those involving molecular oxygen, but the majority of iron enzymes are metalloenzymes and the same holds for copper, molybdenum and zinc.

Cobalt^{II} activates a few enzymes such as acid phosphomonoesterase, amino polypeptidase, glycylglycyl-dipeptidase and arginine desimidase.

1.27 METALLO-ENZYMES

The active metal is firmly bound in a constant stoichiometric ratio to protein in these enzymes. Frequently, the metal is chelated by, or attached to, a small molecule called a "prosthetic group". In view of their better defined chemical nature, metallo-enzymes have been more intensively studied than metal activated enzymes in recent years. It is now clear that metalloenzymes containing copper, iron, molybdenum and zinc are rather numerous and many more probably await discovery. Once an enzyme has been isolated, it can be analysed for metals and its molecular weight can be found. The valency of the metal can be found from electro-spin resonance (ESR) studies, and the type of binding may be inferred from more detailed ESR measurements on hyperfine structure, or dialysis, protein displacement or chemical techniques (Bowen, 1966).

There are several ways of classifying metallo-enzymes, but the simplest one, under individual metals, fails because several examples are known to contain more than one metal (Bowen, 1966). Here they are classified according to the prosthetic group involved, i.e.

Metalloprotein enzymes: These have no prosthetic group and the metal is bound directly to the protein, so that one function of the metal may be that of maintaining the spatial arrangement of the protein chain. They include many copper and zinc enzymes and some iron enzymes.

Metalloporphyrin enzymes: The metal is chelated by a porphyrin prosthetic group. Examples include many oxidases containing iron bound in a heme group, and a few enzymes containing cobalamin or its derivative 5, 6 dimethyl benzimidazol cobamide.

Metalloflavin enzymes: These contain a flavin prosthetic groups, but the mode of attachment of the metal is not known. They include many oxidases or dehydrogenases containing iron and/or copper, magnesium, manganese, molybdenum or zinc together with either FAD or FMN.

CHAPTER TWO

2.0 MATERIALS AND METHODS2.1 SAMPLE COLLECTION

At the tomato processing company, samples of fresh tomatoes, pures and ketchup were collected.

Similarly, samples were collected from the groundnut processing companies A, B and C.

The samples were raw groundnut, groundnut cake, crude and refined oils.

These samples were stored in plastic bottles which were refrigerated at 4°C for the period of the analysis.

The tomato processing factory derives its raw materials from farms within a 50km radius around the factory site. These raw materials are not stored but ready for use as soon as they are brought to the factory. The groundnut factories get their raw materials from the states in the Northern part of the country. The raw materials could be stored for as long as three months depending on the quantity available for use at a particular time.

2.2 WET DIGESTION OF SAMPLES FOR TRACE ELEMENT ANALYSIS

The method employed is that of the Analytical Methods Committee, (1960), which uses nitric and perchloric acids as the decomposition reagent for organic matters. These acids have the advantage of not producing insoluble sulphates which absorb a considerable proportion of trace metals.

APPARATUS:

1. Kjeldahl flasks (200ml)
2. Heating Mantles - Electrothermal, England

3. Volumetric flasks (25ml)
4. Measuring cylinders (10ml, 20ml)
5. Weighing balance - Mettler, top loading Type Pc 1140
6. A Pye Unicom SP 1900 atomic absorption spectrophotometer
7. Hollow cathode lamps for the various metals.

REAGENTS:

All reagents used were of analytical reagent grade unless otherwise stated.

1. Concentrated nitric acid (sp. gr. 1.42)
2. Perchloric acid (60% v/v)
3. Acid washing liquid for glassware: Prepared by diluting concentrated nitric acid 100 times with distilled, deionized water.

2.2.1 SOLID SAMPLES

2g of the dried ground samples was accurately weighed into a 200ml Kjeldahl flask. To this was added 25ml of concentrated nitric acid (HNO_3) and glass beads. The mixture was boiled slowly for 30 minutes under fume hood. This was cooled and 15ml of perchloric acid (HClO_4) added. The mixture was then boiled very gently for about one hour until it became colourless and dense white fumes evolved. The mixture was allowed to cool and quantitatively transferred to a 25ml volumetric flask and diluted to volume with distilled, deionized water.

2.2.2 SEMI-SOLID SAMPLES

The samples were prepared as recommended by Analytical Chemist Society (1973).

10g of the sample (well-mixed) was taken and 40ml of distilled, deionized water and 10ml of concentrated hydrochloric

acid were added. The mixture was heated to boiling and then simmered gently for about 5 minutes, cooled, transferred to a 100ml volumetric flask and made up to volume. 20ml was filtered for the analysis by atomic absorption spectrophotometer.

2.2.3 OIL SAMPLES

The samples were prepared as recommended by Analytical Chemists Society (1973).

5ml of the oil was added to 20ml of pentylacetate. The mixture was aspirated directly into the atomic absorption spectrophotometer.

A blank was prepared using the same amount of reagents. The digested samples and blank were then transferred into plastic reagent bottles (50ml) and stored in a refrigerator until required for use.

2.3 ANALYSIS OF TRACE ELEMENTS

2.3.1 ATOMIC ABSORPTION SPECTROPHOTOMETRY

The determination of the trace elements; copper, iron, zinc, manganese and lead in the digested samples was carried out by the method described by Osborne and Voogt (1978). The solution contained from the wet digestion was sprayed into the flame of an atomic absorption spectrophotometer and the absorption of each of the metals to be analysed was measured at a specific wavelength.

PRINCIPLES

In this technique, a sample is aspirated into a flame and atomised. A light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed. Absorption is more sensitive because it depends upon the presence of free unexcited atoms. In the usual flame,

the ratio of unexcited to the excited atoms at a given moment is very high. Because each metallic element has its own characteristic absorption wavelength, a source light composed of that element is employed, which makes the method free of spectra or radiation interferences. Thus, the amount absorbed in the flame is proportional to the concentration of the element in the sample.

To determine the concentration of a particular metal in an unknown samples, a calibration curve of concentration versus absorbance is usually prepared, using standard solutions prepared from the stock solution. Each determination was carried out in triplicate.

2.3.1.1 COPPER

Reagents:

1. Hydrochloric acid, 3M: prepared by diluting 260ml of concentrated hydrochloric acid (SP. gr. 1.18) to 1,000ml with distilled, deionized water.
2. Copper stock solution, (1000ppm) prepared by dissolving 9.981g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), analytical grade, in 25ml of 3M hydrochloric acid. This was diluted to 250ml with distilled, deionized water.
3. Copper working standard solution; 10ml of the stock solution was diluted to 100ml with distilled, deionized water, and 0.0, 0.25, 0.5, 1.00, 2.0, 3.0, 4.0 and 5.0ml of this solution were pipetted into separate 100ml volumetric flasks and made up to volume with distilled, deionized water. These gave working standard solutions containing 0.0, 0.25, 0.5, 1.0, 2.0, 3.0,

4.0 and 5.0ppm of copper respectively.

Method:

Copper was determined at 324.80nm with a maximum lamp current of 5mA and a slit setting of 0.15-0.20mm. The flame system was air-acetylene. The concentrations of copper in the sample solutions were read off from the calibration curve plotted from the readings of the standard solution.

Calculation:

The method employed is that of the American Water Works Association, American Public Health Association and Water Pollution Control Federation (1985).

$$\text{Metal Concentration (ppm)} = \frac{A \times B}{C}$$

Where:

A = Concentration of metal in digested solution, ppm.

B = Final volume of digested solution (ml)

C = Sample and size (g)

2.3.1.2 IRON

Reagents

1. Hydrochloric acid, 3M. Prepared as for copper
2. Iron stock standard solution (1000ppm). This was prepared by dissolving 2.158g of ferric ammonium sulphate ($\text{Fe}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$), analytical grade in 25ml of 3M hydrochloric acid and diluted to 250ml with distilled, deionized water.
3. Iron working standard solution: 10ml of the above stock solution was diluted to 100ml with distilled, deionized water

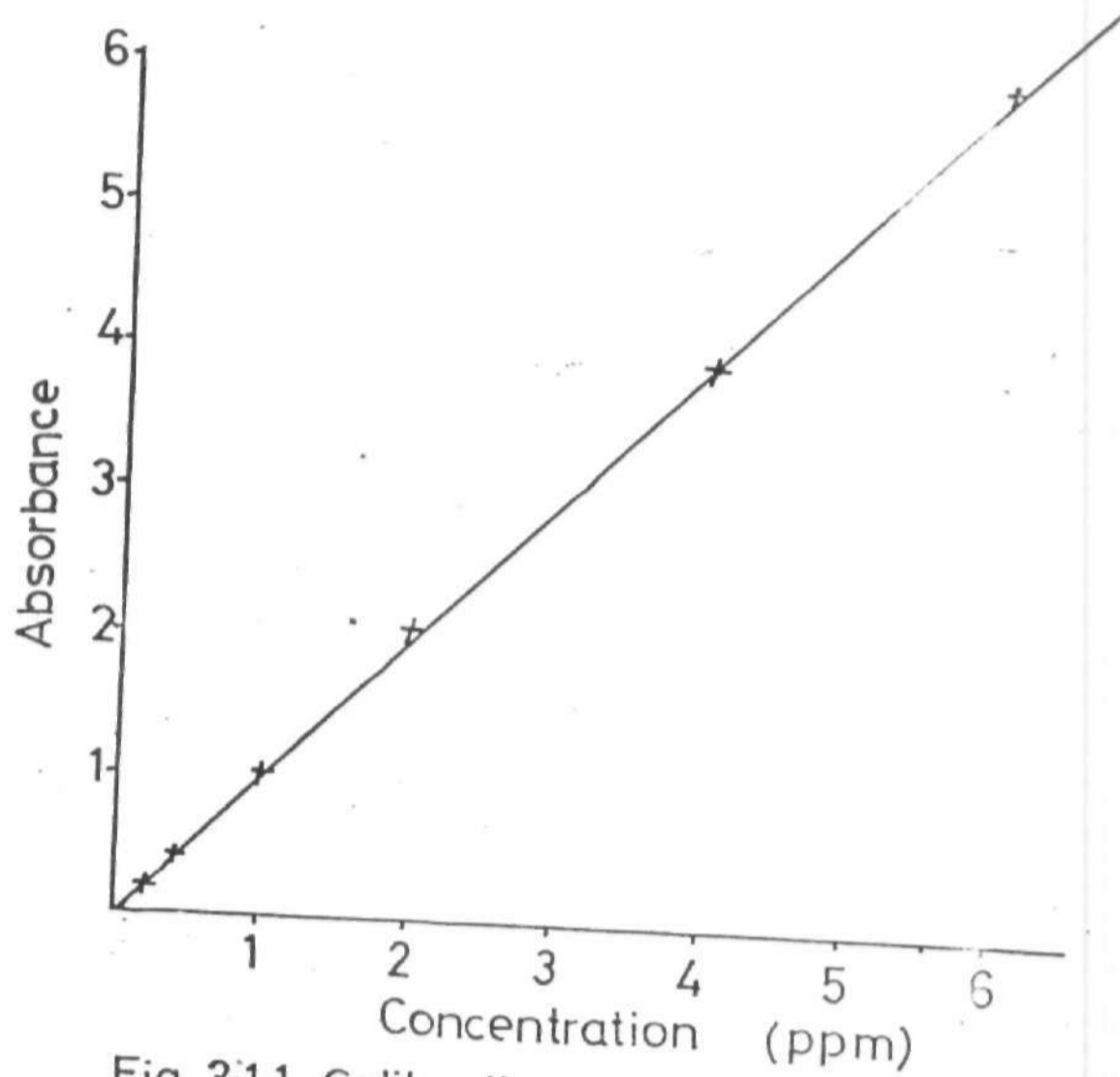


Fig 3.1.1 Calibration curve for copper determination

and 0.0, 0.5, 1.0, 2.0, 4.0 and 5.0ml were pipetted into separate 100ml volumetric flasks. These were made up to volume with distilled, deionized water and they gave working standard solutions containing 0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0ppm of iron respectively.

Method:

Iron was determined at 248.30nm with a maximum lamp current of 15mA and slit setting of 0.10-0.15mm. The flame system was air-acetylene. The concentration of iron in the sample solutions were read off from the calibration curve plotted from the readings of the standard solutions.

Calculation:

As for copper.

ZINC:

Reagents:

1. Hydrochloric acid, 3M. Prepared as for Copper.
2. Zinc stock standard solution (1000ppm). This was prepared by dissolving 1.10g of zinc sulphate ($ZnSO_4 \cdot 7H_2O$), analytical grade, in 25ml of 3M hydrochloric acid. This was diluted to 250ml with distilled, deionized water.
3. Zinc working standard solution: 10ml of the above stock solution was diluted to 100ml with distilled, deionized water, and 0.0, 0.1, 0.3, 0.4, 0.5, 0.75 and 1ml of this solution were pipetted into separate volumetric flasks and made up to volume with distilled, deionized water. These gave working standard solutions containing 0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0ppm of iron respectively.

Method:

Zinc was estimated at 213.9nm with a maximum lamp current of 10mA and slit setting of 0.10-0.15mm. The flame system was air-acetylene. The concentration of zinc in the sample solution were read off from the calibration curve plotted from the readings of the standard solutions.

Calculation:

As for Copper

MANGANESE:Reagents:

1. Hydrochloric acid, 3M. Prepared as for Copper
2. Manganese stock standard solution (1000ppm). This was prepared by dissolving 1.015g of Manganous sulphate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$), analytical grade in 25ml of 3M-hydrochloric acid and diluted to 250ml with distilled, deionized water.
3. Manganese working standard solution: 10ml of the above stock solution was diluted with distilled water and 0.1, 0.2, 0.5, 1.0, 1.5, 2.0 and 3.0ppm of manganese respectively.

Method:

Manganese was determined at 279.50nm with a maximum lamp current of 12mA and slit setting of 0.10-0.15mm. The flame system used was air-acetylene. The concentration of manganese in the sample solutions were read off from the calibration curve plotted from the readings of the standard solutions.

LEAD:Reagent

1. Hydrochloric acid, 3M. Prepared as for Copper
2. Lead stock standard solution (100ppm)

This was prepared by dissolving 1.5984g of lead nitrate ($\text{Pb}(\text{NO}_3)_2$), analytical grade.

3. Lead working standard solution: An intermediate 10ppm lead solution was prepared by diluting 1ml of the stock solution to 100ml with distilled, deionized water. 0.1, 0.4, 0.5, 3.0,

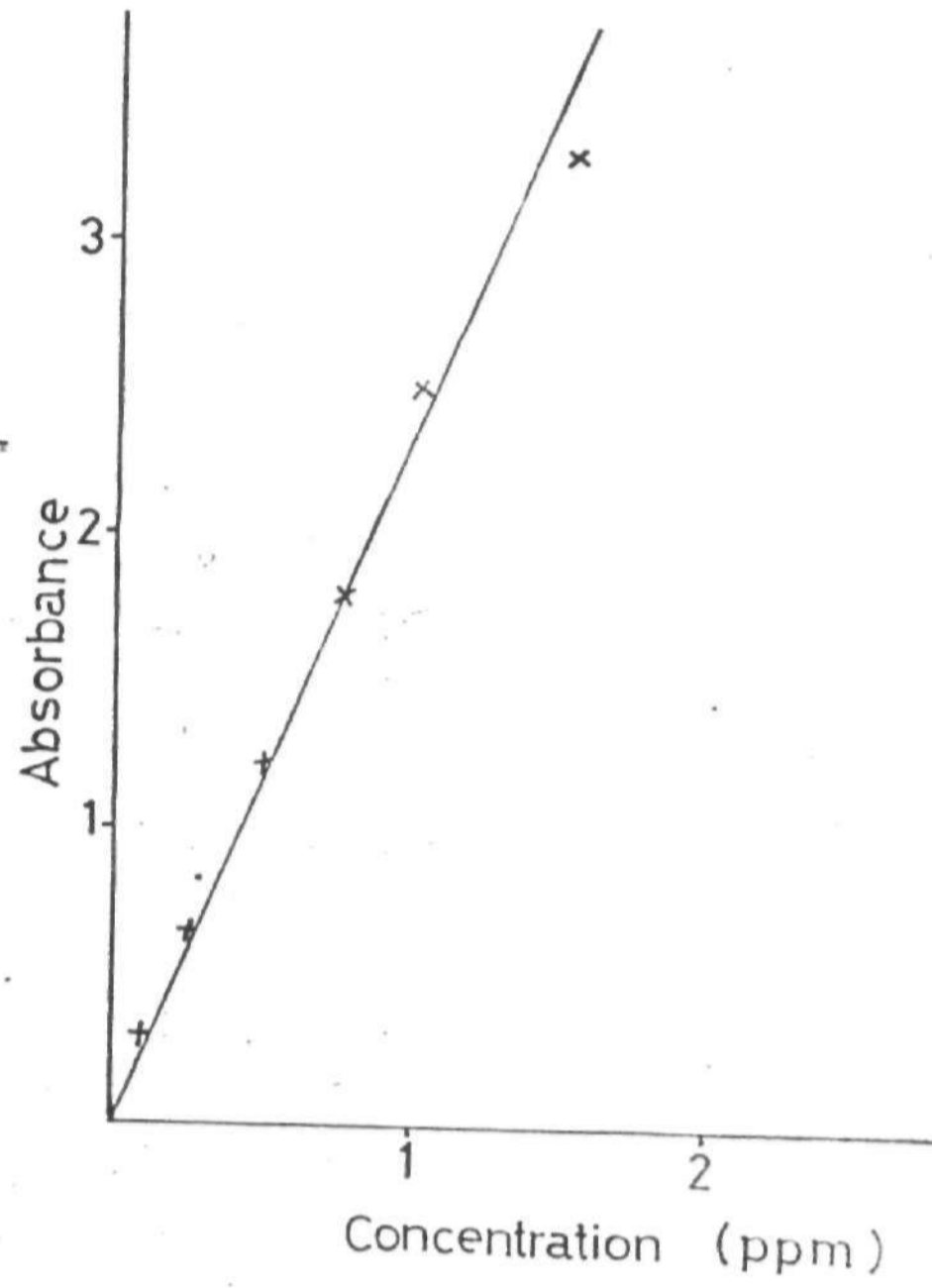


Fig 3-1-3 Calibration curve for det
zinc determination

and 0.4ppm lead standard solutions were prepared by diluting appropriate volumes of the intermediate solution.

Method:

Lead was determined at 283.3nm with a maximum lamp current of 6mA and slit setting of 0.10-0.15mm. The flame system used was air-acetylene. The concentration of lead in the samples were read off from the calibration curve plotted from the readings of the standard solutions.

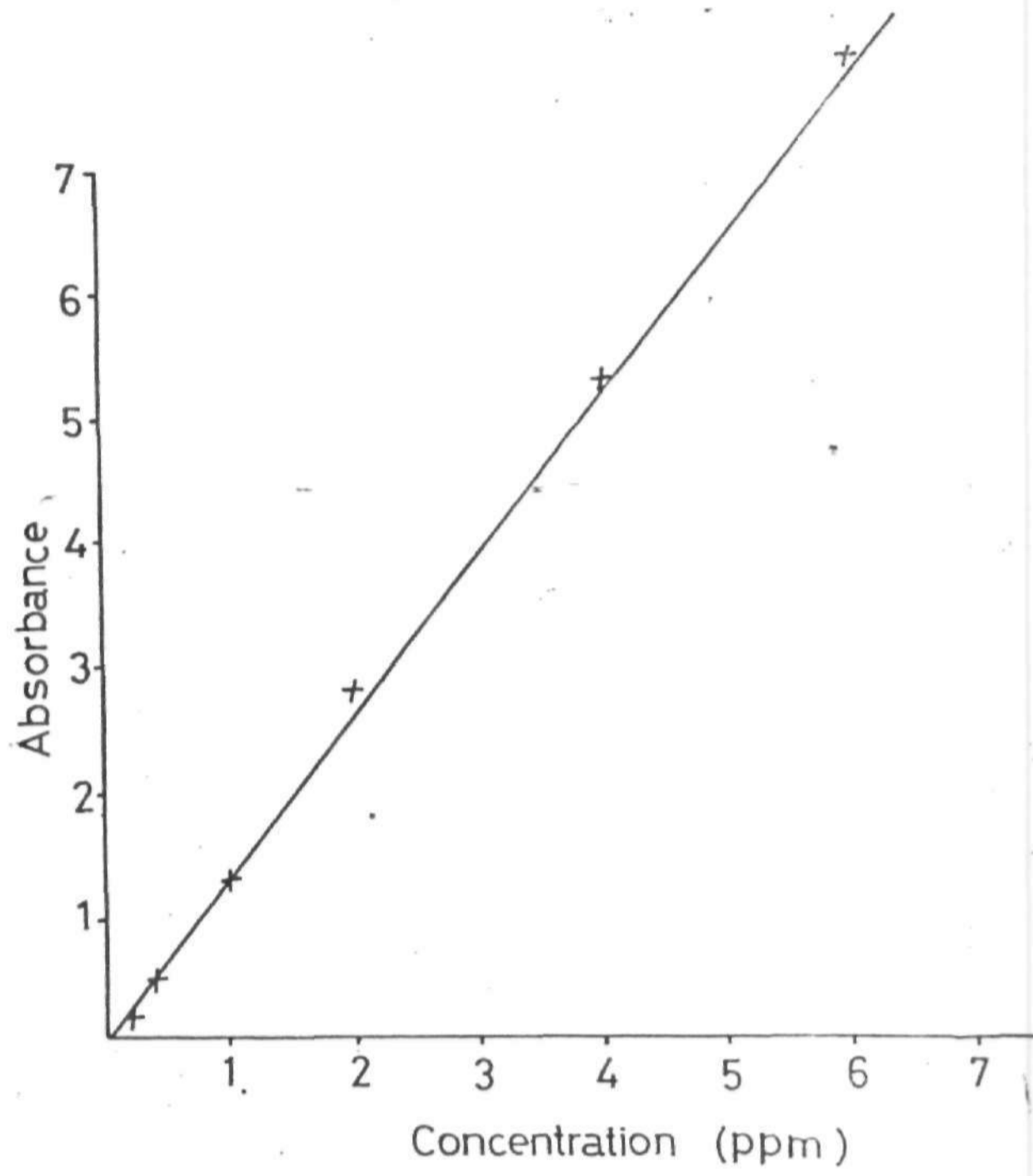


Fig 3-1-4 Calibration curve for manganese determination

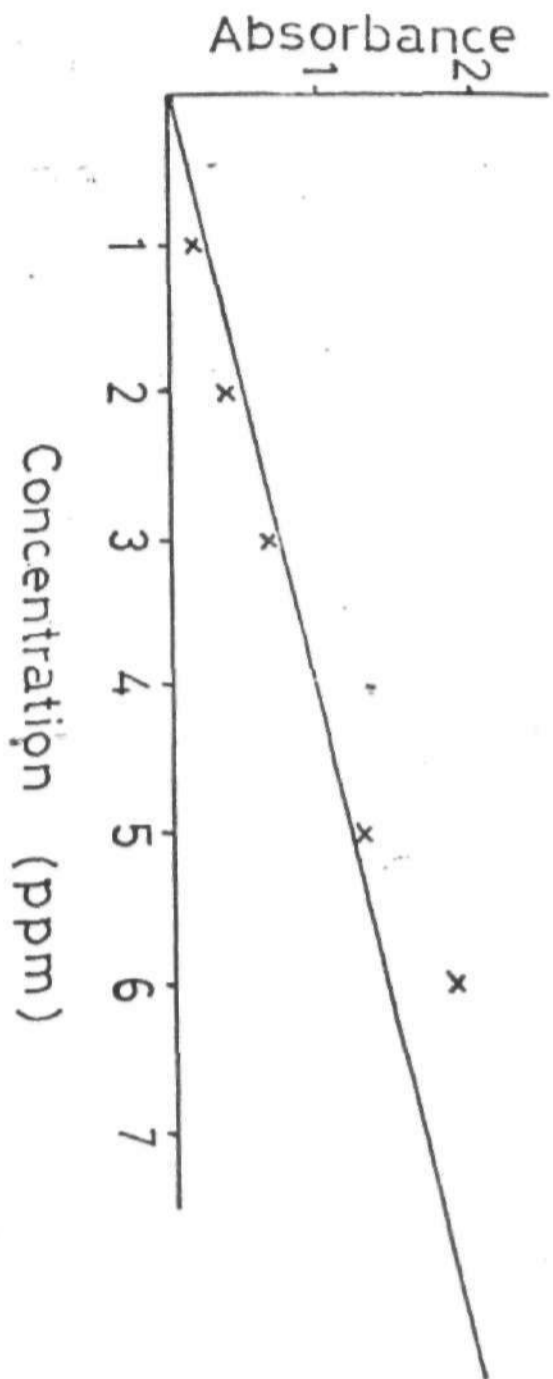


Fig 3.1.5 Calibration curve for lead determination

CHAPTER THREE

3.0 RESULTS3.1.1 COPPER CONTENT

The highest level of copper found in the samples from the tomato factory is 15.7ppm. (Table 3.1.1). It was obtained from puree. There were significant differences ($P = 0.05$) in the copper level of tomato. Those of puree also show statistical difference. The copper levels of the ketchup also differ significantly.

The copper content of the groundnut processing factory samples are lower than that of the samples from the tomato factory. The highest level of copper obtained is 13.9ppm. It was obtained from groundnut cake in factory B (Table 3.1.3).

The copper levels of groundnut differ statistically. The levels of copper in the groundnut cake also differ statistically. There are significant differences in the levels of copper in the crude oil. Those of the refined oil show statistical difference also.

3.1.2 IRON CONTENT

The highest level of iron, obtained in the samples from the tomato factory is 230ppm (Table 3.1.1). It was obtained from puree. The differences in the iron content of tomato are significant. The iron content of puree also differ significantly. There are significant differences in the levels of iron in the ketchup.

The iron content of the groundnut processing factory samples are lower than that obtained in samples from the tomato factory. The highest level of iron obtained is 109.3ppm (Table 3.1.2).

Table 3.1.1 Copper, Iron, Zinc, Manganese and Lead contents (ppm) of tomato, puree and ketchup from the tomato factory.

SAMPLES	Cu	Fe	Zn	Mn	Pb
TOMATO	13.5 ± 4.9	72.7 ± 7.6	97.8 ± 78.0	15.3 ± 4.2	18.7 ± 10.8
PUREE	15.7 ± 3.1	230.0 ± 190.0	30.1 ± 14.0	7.4 ± 2.4	80.7 ± 42.6
KETCHUP	3.1 ± 1.8	17.4 ± 5.9	10.6 ± 7.9	3.5 ± 1.0	0.8 ± 0.7

Each value represents the mean of the results of six determinations.

± = Standard deviation.

It was obtained from groundnut cake of factory A. The differences in the iron content of groundnut are significant. Those of the crude oil show statistical difference. The iron content of the refined oil differ statistically.

3.1.3 ZINC CONTENT

The highest level of zinc obtained in the samples from the tomato processing factory is 97.8ppm (Table 3.1.1). It was obtained from tomato. The differences in the zinc levels of tomato are significant. There are significant differences in the levels of zinc in the puree also. The differences in the levels of zinc in the Ketchup are significant.

The zinc levels of the samples from the groundnut processing factories (A, B, & C) are lower than those of the tomato factory. The highest level obtained is 55.7ppm (Table 3.1.3). It was obtained from the groundnut cake of levels of groundnut. There are also significant differences in the zinc levels of groundnut cake. Those of crude and refined oils show statistical difference also.

3.1.4 MANGANESE CONTENT

The highest level of manganese obtained in the samples from the tomato processing factory is 15.3ppm (Table 3.1.1). It was obtained from tomato. The differences in the manganese levels of tomato are significant. There are significant difference in the levels of manganese in the pure. The levels of manganese in the ketchup show statistical difference.

Table 3.1.2 Copper, Iron, Zinc, Manganese and Lead contents (ppm) of Groundnut, Groundnut cake, crude and refined oils from factory A.

SAMPLE	Cu	Fe	Zn	Mn	Pb
GROUNDNUT	8.9 ± 0.3	67.9 ± 19.2	51.9 ± 6.3	21.5 ± 3.7	9.5 ± 2.6
GROUNDNUT CAKE	5.4 ± 1.1	109.3 ± 28.8	37.8 ± 6.8	13.4 ± 0.2	6.6 ± 1.1
CRUDE OIL	1.2 ± 0.2	20.5 ± 0.2	7.7 ± 0.8	2.1 ± 0.3	74.6 ± 5.4
REFINED OIL	1.1 ± 0.1	8.4 ± 0.6	5.5 ± 1.3	1.6 ± 0.2	68.1 ± 10.1

Each value represents the mean of the results of three determinations.

± = Standard deviation.

The manganese levels of the samples from the groundnut processing factories (A, B, & C) are higher than those of the tomato factory. The highest level obtained is 21.5ppm (Table 3.1.2). It was obtained from groundnut in factory A. The differences in the manganese levels of groundnut are significant. The manganese levels of groundnut cake differ statistically. The manganese levels of the crude and refined oils, are also statistically different.

3.1.5 LEAD CONTENT

The highest level of lead obtained in the samples from the tomato factory is 80.7ppm (Table 3.1.1). It was obtained from puree. The differences in the lead levels of tomato are significant. The lead content of puree also differ significantly. There are significant differences in the levels of lead in the Ketchup.

The lead levels of the samples from the groundnut processing factories (A, B & C) are lower than those of the tomato factory. The highest level of 74.6ppm was obtained from crude oil in factory A (Table 3.1.2). The differences in the lead levels of groundnut are significant in factories A. & C while in factory B, the differences are not significant. The lead levels of groundnut cake differ significantly also in factories A & C while they do not differ significantly in factory B. The lead levels of crude and refined oils are statistically different in factories A and C, while there is no difference in factory B.

Table 3.1.3 Copper, Iron, Zinc, Manganese and Lead contents (ppm) of groundnut, groundnut cake, crude and refined oils from factory B.

SAMPLES	Cu	Fe	Zn	Mn	Pb
GROUNDNUT	11.8 ± 3.7	33.2 ± 1.7	46.7 ± 9.6	12.3 ± 1.4	5.1 ± 1.2
GROUNDNUT CAKE	13.9 ± 2.0	98.8 ± 29.7	55.7 ± 11.8	19.5 ± 3.4	6.1 ± 0.8
CRUDE OIL	1.0 ± 1.0	11.3 ± 0.3	5.8 ± 1.6	2.1 ± 0.0	63.7 ± 15.3
REFINED OIL	1.2 ± 0.1	10.3 ± 0.9	6.0 ± 1.5	1.9 ± 0.1	53.7 ± 13.3

Each value represents the mean of the results of three dt determinations.

± = Standard deviation.

Table 3.1.4 Copper, Iron, Zinc, Manganese and Lead contents (ppm) of groundnut, groundnut cake, crude and refined oils from factory C.

SAMPLES	Cu	Fe	Zn	Mn	Pb
GROUNDNUT	4.9 ± 0.1	81.9 ± 2.8	21.5 ± 1.1	14.3 ± 0.5	5.2 ± 0.2
GROUNDNUT CAKE	2.8 ± 0.1	0.7 ± 0.0	9.4 ± 0.3	13.1 ± 0.5	2.6 ± 0.5
CRUDE OIL	ND.	8.1 ± 0.3	4.6 ± 0.6	0.6 ± 0.0	60.1 ± 0.5
REFINED OIL	ND.	1.6 ± 0.0	1.2 ± 0.1	0.3 ± 0.0	22.8 ± 0.6

Each value represents the mean of the results of three determinations.

± = Standard deviation.

CHAPTER FOUR

4.0 DISCUSSION4.1.1 COPPER

The low level of copper in the samples may be explained by saying that the soil had low content of the trace metal. It has been observed by Street et al, (1977) that the absorption of metals through the soil is influenced by different factors such as the pH of the soil, the organic matter content (Jaakkola and Ylaranta, 1976) and the interaction of other metals such as selenium (Francis and Rush, 1973; Cary, 1981) Another probable reason for the low level of copper in the samples could be that the metal was not employed much in the construction of the factory machinery.

The observation in the tomato factory samples is not quite different from that of the groundnut factory samples. Generally, low level of copper was observed.

The levels of copper obtained when compared with the International Standard of 100ppm (Appendix III) are found to be low. This implies that no health hazard can be caused by the level of copper in the foods.. In the UK, South Africa and Canada, acceptable limits of 20, 20 and 50ppm respectively have been recommended (Reilly, 1980).

4.1.2 IRON

The level of iron were high. A lot of heat is needed during food processing. As a result, part of the machinery that are ungalvanized become heated up and the metal leak into the food as oxides. Leo and Clydesdate (1980) and Lee

(1982) have shown that heat treatment can either increase or decrease the solubility and availability of food iron. The high level of the metal in the puree could be as the effect of corrosive action of the organic acid content of the puree on the metals of the factory machinery to which the puree comes into contact. The level of 230ppm in the puree (Table 3.1.1) when compared to the permissible limit of 100ppm (Moore, 1974) can pose some health hazards to the consumers of these foods. However, much higher levels of iron has been determined in foods earlier. Crosby (1977) reported that black currents had 1300ppm of iron. This is to point out that there is a wide margin between permissible and toxicity levels. Reilly (1973) identified food processing equipment and containers in which foods are stored as sources of contamination of trace metals in foods.

4.1.3 ZINC

The levels in the tomato was higher compared to those of puree and ketchup. This agrees with the observation of Osborn and Leal-Khoshab (1989) that less refined products contain appreciably higher amounts of trace elements than refined products. Permissible limits of 100ppm in edible gelatin (Appendix III), 50ppm in the UK, 100ppm in galatin in South African and 100ppm in galatin in Canada have been recommended (Reilly, 1980). Since the levels are lower than the standard limits, there should be no fear of zinc toxicity in these foods.

4.1.4 MANGANESE

The level in tomato was higher compared to those of puree

and ketchup. Those of the groundnut samples are higher than those of the other samples from the groundnut factories. This agrees with the observation of Osborne and Laal-Khoshab (1989).

The very low level of manganese in these samples may be as a result of low occurrence in the soil. It could probably be as a result of alkaline nature of the soil. A condition that brings about the conversion of the exchangeable divalent manganese to the tetravalent ion which cannot be utilized by plants (Page, 1962).

4.1.5 LEAD

The levels of lead in the study were very high compared to the International Standard permissible limit of 5ppm (Appendix III). The high level of lead in these foods render them unsafe for human consumption. The recommended levels for the UK, South African and Canada are 2, 5 and 7ppm respectively (Reilly, 1980).

The high level of lead in the puree as compared to tomato may be explained by the action of organic acids in the juice on the metals of the factory machinery. Lead and its alloys are used very much in the factories. Chapman and Pindas (1985) observed that a frying pan was found to have 51% lead in the coating which caused considerable poisoning. The high levels of lead observed in the oils could be as a result of heating process that made the metal wear off as oxides into the oil.

4.1.6 GENERAL DISCUSSION

There is less information on the effects of food

processing on trace metal availability. All of the foods studies underwent heat treatment associated with their processing. It has been shown (Lee and Clydesdale, 1980; Lee, 1982) that heat treatment can either increase or decrease the solubility of food trace metals. Although, this may have been due to heat treatment itself, it might have been due to other factors frequently associated with food processing, in particular the use of certain additives.

Fruit juices contain the organic acids; citric, ascorbic and malic (Hazall and Johnson, 1987). Ascorbic acid as an antioxidant, citric acid as a sequestrant, and acetic and lactic acids as acidulants have the potential to influence the chemical form of food trace metals. They are added to foods to prevent microbial growth, rancidity due to lipid oxidation, and undesirable flavour, colour and textural changes; many of which are due to trace metal catalysis of lipid oxidation or formation of coloured-complexes (Faria, 1972; Howlett, 1972; Stockey, 1972).

4.1.7 CONCLUSION

It has been observed from this study that iron and lead were higher than their permissible limits. Zinc, copper and manganese were lower than their permissible limits.

It is indicative from the result that location and processing are factors to reckon with when considering heavy metal content of vegetable crops. Zurera-Casano *et al*, (1989) observed that vegetables absorb metals from the soil as well

as from deposits on the parts of the vegetables exposed to the air from the polluted environment. It is obvious that there is similarity in heavy metal content between the two groundnut processing factories from Kano. On the other hand, the heavy metal content of these two factories differ markedly from those of the factory in Zaria.

Processing can increase the heavy metal content of food when there is contamination from the fabrication material of the machinery to which the food comes into contact or from the liming of the walls of the container in which the food is packaged. A very good example of this is when tomato is processed into puree. The level of lead is increased drastically in the puree. A lot of heat is needed during food processing. As a result of that, the materials that are ungalvanized in the plant become heated up and these metals leak into the food as oxides. It may also be as a result of the use of food additives.

Varietal difference is another factor to look at when considering heavy metal content of vegetable crops. It is also evidenced from the results of this study that higher contents of the metals were found in the tomato products than the groundnut products. The trace metal accumulation varies even within one species according to the variety (Crews and Davies, 1985) and the part of the plant (Zurera *et al*, 1987).

4.1.8 SUGGESTIONS

Already, there are many sources from which these metals find their ways into the body; the water we drink the use of clay pots, ungalvanized cooking utensils, the air, just to

mention but a few.

In the light of the above, it is worth suggesting to the Federal Government of Nigeria to:

- i) Make the food processing factories take more seriously the quality control of the products. This quality control should be geared towards trace elements in foods.
- ii) In a situation where a person consumes a lot of the puree with the groundnut oil, the amount of heavy metal consumed by the person can be imagined. Either the food processing factories be made to carry out thorough quality control test on their products or/ and the consumers be enlightened on what they are consuming and the implications.
- iii) Research in trace metal in the area of foods should be encouraged and those already carried out should be traced and their findings made known to the processors.
- iv) This study is a very small aspect of the work that has to be done in the food area as regards trace metals; so more investigations should be embarked upon to have enough data to work with.
- v) It is true that standards of these trace elements in foods are available in the literature, but that does not prevent Nigeria from coming out with here just as some countries have done.

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APPENDIX I: Copper, Iron, Zinc, Manganese and Lead contents (ppm) of tomato, purée and Ketchup from the tomato factory (A).

SAMPLES	Cu	Fe	Zn	Mn	Pb
TOMATO	23.0, 11.2, 13.16	82.43, 62.5, 72.37	111.5, 57.24, 250.0	6.76, 13.16, 9.87	23.65, 6.58, 16.45
PUREE	11.9, 18.25, 20.07	35.71, 209.85, 228.1	14.2, 42.88, 47.45	5.4, 10.22, 10.77	128.85, 72.99, 72.9
KETCHUP	1.92, 2.13, 2.49	6.77, 15.63, 17.05	4.63, 6.04, 8.17	2.5, 2.34, 3.05	1.78, 0.71, 0.71

APPENDIX I (contd.) Copper, Iron, Zinc, Manganese and Lead contents (ppm) of tomato, puree and ketchup from the tomato factory (B).

SAMPLES	Cu	Fe	Zn	Mn	Pb
TOMATO	9.21, 11.18, 13.16	74.3, 65.79, 78.95	58.6, 39.47, 69.74	22.37, 13.82, 15.79	32.9, 6.58, 26.32
PUREE	12.87, 15.15, 51.51	30.8, 346.72, 529.20	14.0, 32.85, 29.2	5.06, 6.39, 6.75	8.52, 82.12, 118.61
KETCHUP	6.79, 2.84, 2.27	21.79, 22.73, 20.6	8.7, 26.28, 9.8	4.3, 4.83, 3.98	1.5, 0.0, 0.0

APPENDIX II Copper, Iron, Manganese and Lead contents (ppm) of Groundnut, Groundnut cake, Crude and Refined oils from Factory (A).

SAMPLES	Cu	Fe	Zn	Mn	Pb
GROUNDNUT	8.3, 9.4, 8.9	114.8, 44.4, 44.4	36.4, 59.6, 59.6	30.6, 17.0, 16.9	12.1, 13.2, 13.2
GROUNDNUT CAKE	3.9, 8.2, 4.2	47.1, 169.1, 111.8	21.2, 46.1, 46.1	13.9, 13.2, 13.2	9.2, 5.3, 5.3
CRUDE OIL	0.9, 0.9, 1.7	20.9, 20.1, 20.6	5.8, 8.8, 8.7	1.4, 2.4, 2.6	61.5, 79.2, 83.0
REFINED OIL	0.9, 1.1, 1.4	7.0, 8.9, 9.3	2.5, 7.2, 7.0	1.0, 1.8, 2.0	43.6, 77.9, 82.8

APPENDIX II (contd.) Copper, Iron, Zinc, Manganese and Lead contents (ppm) of Groundnut, Groundnut cake, Crude and Refined oils from factory (B).

SAMPLES	Cu	Fe	Zn	Mn	Pb
GROUNDNUT	20.3, 10.2, 5.1	34.3, 29.2, 36.2	23.4, 59.6, 57.1	14.6, 8.9, 13.3	7.6, 5.1, 2.5
GROUNDNUT CAKE	18.8, 11.5, 11.5	26.0, 135.2, 135.2	26.9, 70.2, 70.2	11.1, 23.7, 23.7	8.0, 5.1, 5.1
CRUDE OIL	0.8, 1.2, 1.1	12.0, 10.7, 11.1	2.0, 7.3, 8.1	2.1, 2.1, 2.1	26.4, 79.2, 83.0
REFINED OIL	1.4, 1.1, 1.3	12.3, 9.6, 8.9	2.5, 8.2, 7.5	1.6, 2.1, 2.0	30.0, 78.9, 79.1

APPENDIX II (contd.) Copper, Iron, Zinc, and Manganese contents (ppm) of Groundnut, Groundnut cake, Crude and Refined oils from Factory (C).

SAMPLES	Cu	Fe	Zn	Mn	Pb
GROUNDNUT	5.0, 5.1, 4.6	79.4, 77.5, 88.8	21.3, 19.4, 24.0	14.8, 13.1, 15.0	5.0, 5.0, 5.6
GROUNDNUT CAKE	2.7, 2.7, 3.0	0.7, 0.8, 0.7	9.2, 9.0, 10.0	14.3, 12.2, 12.8	3.5, 2.7, 1.6
CRUDE OIL	0.0, 0.0, 0.0	8.8, 7.5, 8.1	3.1, 5.6, 5.0	0.6, 0.6, 0.6	59.4, 59.6, 61.3
REFINED OIL	0.0, 0.0, 0.0	1.6, 1.6, 1.6	1.1, 1.1, 1.3	0.3, 0.3, 0.3	24.2, 21.8, 22.3

(Modified from Davis, 1966, of regulations specified in notes at end of Table)

TABLE 3.2 (continued)

GENERAL LIMITS IN PARTS PER MILLION

(a) Statutory—Lead 2 ppm.
—Arsenic 1 ppm.

(b) Recommended—Copper 20 ppm.
—Zinc 50 ppm.
—Tin 250 ppm (canned foods only)

SPECIFIC LIMITS IN PARTS PER MILLION

Foods	Lead	Statutory Arsenic	Fluorine	Recommended	
				Copper	Zinc
Agar	10				
Alcoholic cordials	1.0			7.0	
Alginate acid and alginates	10				
Apples	3.0				
Baking powder			15		
Beer	0.5				
Beer: black beer or black beer and m		0.5			
Beverages: alcoholic	1.0	0.2			
Beverages: non-alcoholic: prepared					
from cider	1	1			
Beverages: non-alcoholic: ready-to-					
drink	0.2	0.1			
Beverages: ready-to-drink					
Black 7% ⁴	10		0.1	2.0	5.0
Brandy	10		2		
Caramel	0.5				
Carrageen	5.0				
Chemicals	10				
Chemicals: excluding synthetic	10				
colourings		2.0			
Chemicals: for which arsenic limits are					
specified in the British Pharmacopoeia or the British Pharmacopoeia					
Codes, excluding synthetic colours		2.0*			
Chemical: for which lead limits are					
specified in the British Pharmacopoeia or the British Pharmacopoeia					
Codex ¹					
Codex ²					
Cider	4.0				
Cider: dried and roasted					
Cocktails	0.5				
Cocoa nib, mass and liquor	1.0				
Cocoa powder					
Coffee beans	5.0*				
Colours					
Colouring matters ³					
Curry powder	20		5.0		
Dextrose: anhydrous or monohydrate	20				
Fats	0.5				
Fats and clearing agents	0.5				
Fish: canned	5.0				
Fish paste: canned	5.0				
Flavourings	10				
Flour: self-raising: containing a far-					
measuric substance and an acidic					
phosphate					
Food prepared for babies and young	3.0				

SPECIFIC LIMITS IN PARTS PER MILLION

Foods	Lead	Statutory Arsenic	Fluorine	Recommended	
				Copper	Zinc
Frozen confections	0.5	0.5			
Fruit juices: concentrated	2.0				
Fruit juices: excluding lime or lemon	0.5				
Fruit juices: undiluted		0.5			
Gelatin: edible	5.0		2.0		
Geneva	0.5				
Gin	0.5				
Glucose: liquid or solid: sulphated ash					
content greater than 1 per cent	5.0				
Golden raising powder					
Herbs: dried	10		5.0		
Hop concentrates: excluding those for					
commercial brewing					
Hops: dried: excluding those for com-					
mmercial brewing					
Ice-cream	1.0				
Ice-cream: excluding water ices					
Iron: reduced iron: used in the pre-					
paration of flour					
Leithin	5.0		5.0*		
Lemon juice	2.0				
Lime juice	2.0				
Liqueurs	1.0				
Liquorice: dried extract		2.0			
Meat: canned	5.0				
Meat extract	5.0				
Meat paste: canned	5.0				
Milk beverages: ready-to-drink: pre-					
pared	1.0				
Molasses: edible	5.0				
Mustard	5.0				
Mustard: ground		5.0			
Oils: edible	20				
Oils: dehydrated	0.5				
Pears	10				
Pectin: liquid	3.0				
Pectin: solid	10		2.0		
Perry	5.0		3.0		
Phosphates: acidic: for use as food	0.5				
ingredients					
Proteins: hydrolysed	5.0				
Kum	0.5				
Scanned: products derived from sea-					
weed	10				
Soft drink concentrates: for use in the					
manufacture of soft drinks	2.5		0.5		
Soft drinks: concentrated	1.0		0.5		
Soft drinks: ready-to-drink	0.2				
Spices					
Spices: excluding ground	10		5.0		
Spices: ground	20				
Starch conversion products: sulphated					
ash content greater than 1 per cent	5.0				
Sugar: raw: for the manufacture of					
refined sugar	5.0				

SPECIFIC LIMITS IN PARTS PER MILLION

Foods	Lead	Statutory Arsenic	Fluorine	Recommended	
				Copper	Zinc
Sugar: white: refined: ash content less than 0.03 per cent	0.5				
Sugar and sugar syrups: sulphated ash content greater than 1 per cent	5.0			150	
Tea	10			20	
Tomato catsup				100 ^a	
Tomato juice	1.0			100 ^a	
Tomato juice beverages				100 ^a	
Tomato juice cocktails	1.0				
Tomato ketchup				20	
Tomato paste				100 ^a	
Tomato paste: total solids between 15 and 25 per cent	3.0				
Tomato paste: total solids greater than 25 per cent	5.0				
Tomato powder				100 ^a	
Tomato powder: total solids between 15 and 25 per cent	3.0 ^b				
Tomato powder: total solids greater than 25 per cent	5.0				
Tomato purée				100 ^a	
Tomato purée: total solids between 15 and 25 per cent	3.0				
Tomato purée: total solids more than 25 per cent	5.0				
Tomato relish				20	
Tomato sauce				20	
Vegetable juices: excluding tomato juice and tomato juice cocktail	0.5				
Vegetables: dehydrated or dried, excluding onions	5.0				
Water ices	0.5				
Whisky	0.5				
Wines	1			7.0	
Wines: excluding vintage port	1.0				
Yeast: brewers' yeast: for the manufacture of yeast products	10 ^c	5.0 ^d		120 ^e	
Yeast and yeast products					
Yeast and yeast products: excluding brewer's yeast for the manufacture of yeast products	7.0 ^f	2.0 ^g			

^a—Note, 20 ppm is the limit specified in the BP or BPC, whichever is the higher.
^b—the limit specified in the BP or the BPC.
^c—calculated on the fat-free substance.
^d—calculated on the dry fat-free substance.
^e—see also exceptions in The Colouring Matter in Food Regulations 1973.
^f—see The Miscellaneous Additives in Food Regulations 1974.
^g—calculated on dried tomato solids.
^h—Normally assumed 5 ppm applies to all tomato powders.
ⁱ—calculated on dry matter.
^j—see other categories of beverages.
^k—Iron powder max arsenic 10 ppm (The Bread and Flour (Amendment) Regulations 1972).

General notes The above limits should be considered in relation to the requirements of The Miscellaneous Additives in Food Regulations 1974. Also note ppm is replaced in modern regulations by mg/kg.

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