COMPARATIVE STUDY OF WET DIGESTION METHODS OF SAMPLE PREPARATION FOR ELEMENTAL ANALYSIS.

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented in any previous application for a higher degree. All quotations are indicated and the sources of information are specifically acknowledged by means of references.

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THESIS RECOMMENDATION

We hereby recommend that the thesis prepared by Mr. V.O. Ajibola entitled, "Comparative study of wet digestion methods of sample preparation for elemental analysis" be accepted in partial fulfilment of the requirements for the degree of M.Sc. ANALYTICAL CHEMISTRY.

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DEDICATION

This work is dedicated to my parents,
my sisters and their families for
their love and support in all forms.

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A B S T R A C T

Nine wet digestion methods were used on two guava samples and eight tomatoe samples for the purpose of elemental determination. These samples "belong to the group of fruity vegetables. Acid and acid mixtures were used to digest the samples; these include HNO_3., HNO_3/H_2SO_4, , $HNO_3/HC10_4$, $HN0_3/HC10_4/H_2S04$, $HN0_3/NH_4\ N0_3$, and HCl/HNO_3. Selenium was used as a catalyst in one of the procedures. Eleven metals were determined. These are sodium, potassium, calcium, magnesium, iron, zinc, copper, manganese, lead, cobalt, and cadmium. Atomic Absorption spectrophotometry and flame photometry were used to determine the metals.

The reliability of each digestion method, for the determination of each, metal in each sample was assessed, by calculating the mean and standard deviation, and by performing the multiple range tests on the result of each method.

It was found that, no one single method of digestion could release all the metals of interest quantitatively and reliably from each sample. A minimum of three digestion methods for each sample was found tt be needed for the determination of these metals, each method being reliable for a group of metals. Some methods such as these involving the use of perchloric acid in the digestion mixture and those using nitric acid alone were found to lead to partial or total losses in certain trace elements like Co, Cd and Pb. The three most valuable digestion methods that are recommended for the types of biological samples studied are method A (H_2SO_4/HNO_3), method B (modification of A), method F (HNO_3/NH_4N0_3).

CHAPTER ONE

I N T R O D U C T I O N

1.0 DISTRUPTION OF SAMPLE MATERIALS

The ever growing interest in trace metals, whether as elements essential to normal biological development or as a potential source of danger to health, has resulted in the publication of numerous papers dealing with methods for the determination of such traces in matrices of organic matter (1). Trace elements occur in biological materials in varied forms, ranging from contaminants in inorganic form, having no relation to the organic matrix (e.g. through interactions with the environment during growth, storage or curing), to those combined with the matrix of the material or closely associated with it (e.g. magnesium in chlorophyll or cobalt in vitamin B_{12}). However, the inorganic content of most foods is only a minor constituent and in most instances it is necessary to remove all, or most, of the organic matter before the final determination of the elements. This requires the destruction of the organic matter by oxidation, with the metals finally being brought into solution in their ionic forms.

The requirements for the destruction of the organic matter in metallic element determination are:-

i) Oxidation of the organic component of the material in a reasonable time period without hazard and without need for undue attention or special apparatus.

ii) Adequate recovery of metal. This can be accomplished by little or no volatilization, and or negligible losses to the vessels employed in the destruction procedure. There should also be little or no reactions leading to incomplete solution of the metal or to formation of species interfering in subsequent estimation study by the methods such as Atomic Absorption spectrophotometry, Flame photometry etc.

iii) There should be no contamination of the analyte by the material of the vessel.

iv) Applicability to a wide range of materials.

No method has been reported to be wholly ideal, and in practice, losses of metals and some contamination of the analyte occur in some degree. There is also the problem of incomplete oxidation of the material, due to some resistant organic components and problems due to the matrix of the organic material in releasing the metals for subsequent determinations. The direct determination of metals, by atomic-absorption spectrophotometry, without prior destruction of organic matter have been reported by Gegiou and Botsivali, (2). In the above example, the flame was used to destroy the organic matter, but the matrix problems remained. The choice of method to be used depends to some extent on the element, or elements, to be determined and on the nature of the organic material.

The methods available for the destruction of organic materials fall into three groups:-

i) Methods involving gaseous oxidants, usually air or oxygen (ie dry ashing).

ii) Methods involving the use of liquid oxidants, or oxidants in solution (ie wet digestion) (3).

iii) Methods involving solid oxidants, which are made active by fusion.

The most important destruction methods include the first two methods, ie wet digestion and dry ashing. Neither of these two general methods is so superior, that it eliminates the other from consideration. However, it is probably fair to say that wet oxidation, in one form or another is more reliable than dry ashing for most metals and for a wider variety of organic materials, particularly when the sample is small (4)

1.1. DRY ASHING

This is particularly valuable for trace metal determinations, when its ability to handle large samples with minimum of added reagents is most desirable (3). In dry ashing, the organic matter is decomposed at a high temperature in the presence of atmospheric oxygen. In a typical procedure, the dry sample is weighed into a clean silica or platinum dish, and placed in a muffle furnace, usually at 500°C, until ashing is complete. After cooling, the ash is taken up in dilute mineral acid (5). There is general agreement that the most important factor to be controlled is the temperature as recoveries fall as the temperature rises (3). Some elements, e.g. zinc, lead, iron, chromium are prone to volatilization in presence of halogens (6). Alkaline metal, become volatile above 450°C(3). In addition to volatilization there is also the risk that insoluble silicates of the metals may be formed (6).

Dry ashing is relatively simple. It can accomodate large samples and does not require the addition of large amounts of potentially contaminating reagents. However, its principal disadvantage is the serious losses of trace elements that can occur because of (i) volatilization, (ii) retention on the walls of the ashing dish, (iii) retention in the acid - insoluble fraction of the ash. Loss due to volatility is especially serious for mercury and selenium (5).

Retention by the wall of the crucible has been found to be a problem, especially with cobalt, copper, iron, manganese when using silica dishes (5). These losses are usually attributed to the formation of complex silicates. This may arise through combination with the silica wall of the crucible and/or the siliceous residue of the sample itself (7)

To avoid losses through volatilization and wall retention, many workers use ashing aids, such as magnesium oxide, magenesium nitrate,

nitric acid, or sulphuric acid, which is added to the sample before or during ashing. These ashing acids act in various ways. HNO_3 acts as an oxidizing agent, speeding up the destruction of organics or allowing lower temperatures to be used. H_2SO_4 can convert chlorides into less volatile sulphates. $Mg(NO_3)_2$ has a double action. It decomposes to nitrogen oxides, which hasten oxidation, and MgO which dilutes the ash and reduces the total area of contact between the sample ash and container walls, thus reducing losses due to retention (5).

It is therefore evident that inspite of its advantages and apparent simplicity, dry ashing cannot be universally recommended for solution preparation and its choice will be governed by the elements required and the type of material (7).

1.2. WET - DIGESTION

Oxidation procedures which employ liquid oxidants are probably the most versatile of all procedures. They tolerate large variations in sample size and can be used prior to the determination of a wide range of elements.

They are characterized by the comparatively low temperatures developed, and large quantities of oxidizing agents added. The low temperature is usually advantageous in that losses by volatilization or reaction of the sample with the container are much less likely (3). Most often, the mixture is heated to 100 - 200^{o}C to aid the digestion process. Wet - digestion is fast, but more attention may be required. Risk of atmospheric contamination is less than in dry oxidation. The major disadvantage is the possibility of contamination from the large excess of reagents employed (5).

The oxidations can be carried out in either open or closed vessels. The best known closed oxidation system is the sealed procedure (3). This method has the disadvantage of explosion due to development of high pressure in the tube.

The vast majority of wet oxidation of organic matter are carried out in vessels open to the atmosphere. Kjeldahl flasks are commonly used for such digestions, the long neck giving some degree of refluxing.

Nearly all wet oxidation procedures involve the use of one or more of three strong acids - nitric, sulphuric and perchloric - although many involve the addition of other agents also.

1.2.1. OXIDATION WITH NITRIC ACID

Nitric acid is the most commonly used oxidant for the wet- digestion of organic matter. It reacts readily with many types of material, particularly those containing alcoholic - OH groups (3). The normal concentrated acid (sp. gr.1.42) boils at about 120°C. This enables it to be removed readily when reaction is complete, but also greatly reduces its effectiveness in oxidation reactions in open systems. The decomposition can be catalysed by the presence of certain compounds such as silver nitrate, selenium dioxide and ammonium vanadate. In spite of these improvements to this basic procedure, the decomposition of organic matter with nitric acid alone remains unsatisfactory for most materials, and is therefore seldom used (8). Metallic elements, in general, form soluble salts with nitric acid but not all organic matrices are decomposed by this acid alone (6). However, HNO_3 is most commonly used in conjunction with sulphuric or perchloric acids (8).

1.2.2. OXIDATION WITH SULPHURIC ACID

Dilute sulphuric acid has virtually no oxidizing power, other than that of the hydrogen ions which can be reduced to hydrogen gas by metals dissolving in the acid. The concentrated acid (sp. gr. 1.84) will, when warm or hot, oxidize many elements, being itself reduced to sulphur dioxide, elemental sulphur or hydrogen sulphide. The destruction of organic matter by hot oxidation with concentrated sulphuric acid is a widely used practice,

inspite of the slowness of the reaction, and tendency to form indestructible charred residues (8).

As complete destruction of many organic materials by heating with H_2SO_4 alone is slow, one attempts to speed up the reaction either by raising the working temperature above the boiling point of the concentrated acid (330°C), or by adding a catalyst such as copper sulphate, mecuric sulphate and selenium oxide or both (8). The working temperature can be raised by using sealed tubes or addition of sodium or potassium sulphate to the mixture. Digestion with sulphuric acid often results to loss of hydride - forming metals (34).

1.2.3. OXIDATION WITH NITRIC ACID PLUS SULPHURIC ACID

The mixture of nitric and sulphuric acids is very valuable for the destruction of organic matter and is the most impotant and versatile of the wet oxidation mixtures (8). This mixture oxidizes most organic samples, although some may require long digestion time and a few cannot be successfully decomposed. Carbohydrates are most easily destroyed than proteins and fats. In general, plant materials are oxidized more easily than animal material (4). Destruction of organic matter with sulphuric - nitric acid at atmospheric pressure usually proceeds without dangerously violent reactions.

Concentrated sulphuric acid (sp. gr. 1.84) having high boiling point (330°C) serves to raise the temperature of the oxidation mixture, thereby increasing the oxidizing power of the nitric acid. Sulphuric acid is also valuable in degrading the complex organic matter and so rendering them susceptible to attack by the oxidizing agent (3). It is necessary to stress, the necessity for constantly maintaining oxidizing conditions. Whenever charring takes place, reduction occurs, and appreciable losses

might therefore be expected if the digest is heated vigorously to fuming (9). Analysts avoid this danger by keeping the temperature relatively low with frequent addition of nitric acid.

A disadvantage in the use of sulphuric acid is the relative insolubility of many metal sulphates. In digestion of materials containing large quantities of, for example calcium, the formation of a bulky precipitate of calcium sulphate can very readily cause the loss of small amounts of lead by co-precipitation (3). This however limits the choice of this digestion method, but in certain cases the solubility product,(K_{sp}) values of these compounds are not exceeded at trace levels (5). This method also leads to production of resistant compounds e.g. sulphonic acid (33).

The decomposition is usually considered to be finished when the solution becomes clear. It is then allowed to cool, a little, water is added to hydrolyse the nitrosylsulphuric acid which may interfere in subsequent determination of trace elements. Gould (10) added urea to the final solution to destroy the oxides of nitrogen remaining in the solution.

This method is considered to be particularly suitable for the determination of metals such as Cu, Fe, Zn, Mn, Al, Pb and Co (4).

1.2.4. OXIDATION WITH PERCHLORIC ACID

Perchloric acid is an extremely valuable and efficient oxidizing agent for destruction of organic matter, yet its use is widely regarded with suspicion. It has many advantages for this work. It is stable; it has a very high oxidation potential; as the water azeotrope (72% w/w perchloric acid), boils at 203°C; it is fully miscible with water and other strong acids; when cold or dilute it has no oxidizing power, and majority of metal perchlorates are highly soluble in water (3).

Because of the danger of explosions with organic substances, perchloric acid is rarely used on its own for destruction of organic matter. Mixtures with sulphuric and/or nitric acid are normally used, which not only serve to dilute the perchloric acid, but also to ensure that easily oxidizable compounds are broken down by reaction with the other acid, first at low temperatures, before the perchloric acid begins to exert its oxidizing power above 160°C. Compounds containing hydroxy groups react with $HClO_4$ to give unstable perchlorate esters that decompose explosively (8). Perchloric acid digest should not be heated to dryness as even inorganic perchlorates are explosive (6).

The production of hydrochloric acid during the reduction of perchloric acid raises the possibility of losses of some elements through formation of volatile chlorides (3).

1.2.5. OXIDATION WITH PERCHLORIC PLUS NITRIC ACIDS

An important point of this method is that by the ommission of H_2SO_4, the higher temperature and dehydrating conditions associated with the presence of this acid cannot easily arise. This according to some workers, tend to reduce the hazards sometimes connected with $HClO_4$ combustions. On the other hand there is considerable risk, that in the absence of H_2SO_4 the digest may boil dry, thus increasing hazards.

The method has been found to be particularly suitable for the destruction of protein and carbohydrate material (11). Its use for samples consisting almost entirely of fat is not recommended, because of the unstable perchlorate esters formed, which decompose explosively.

The method is speedy in most instances and is easy to control. The temperature used are lower than in most of the wet- digestion procedures (120 - 140°C), and losses by volatilization are expected to be small.

Some types of organic materials, notably those containing heterocyclic nitrogen are not completely destroyed (8). This is a fault that the method shares with some other wet - combustion procedures, and for such materials dry ashing is recommended.

1.2.6. OXIDATION WITH PERCHLORIC, SULPHURIC AND NITRIC ACIDS

This method is particularly valuable in oxidation of stubborn materials. One of its disadvanges is the presence of sulphuric acid during recovery of elements such as lead, especially when the material contain large amounts of calcium. The combination of these acids would seem to be most effective when they are present in the proportions 1:1:3, i.e. perchloric - Sulphuric - nitric respectively. It has been reported that the destruction of organic matter by this method can be accelerated markedly by addition of catalyst such as selenium oxide (8).

1.2.7. OXIDATION WITH NITRIC ACID - AMMONIUM NITRATE

The method was originally devised for determination of Ca, Mg, Na, K and S all on one sample (11). It can also be applied when Cu, Fe, and similar elements are to be determined in samples in which the amount of organic matter is small and easily decomposed. The initial digestion is followed by expulsion of the nitric acid and continued oxidation in a melt of ammonium nitrate. When reaction is complete, the ammonium nitrate is expelled by stronger heating.

Oxidation is steady and rapid, and any residue can easily be freed from excess oxidizing agent by evaporation and the elements are obtained in form suitable for determination.

Occasionally substances are encountered which are very difficult to oxidize completely by the wet - digestion methods, or which contain compounds which interfere with the oxidation of the element. With acid

(i.e. HNO_3/H_2SO_4), digested tobacco, Gross (12) experienced difficulty which was attributed to the presence of pyridine. This difficulty might occur in materials rich in protein. The effectiveness of nitric and perchloric acid mixtures for wet oxidizing organic materials was evaluated by Martinie and Schilt (13). After wet oxidation, each sample was screened for organic residue by proton magnetic resonance, spectrophotometry, ultra-violet and carbon microanalysis. Most samples were found to exhibit some U.V. absorption and about one half retained measurable carboneous matter in solution. Compounds containing N-methyl, S-methyl, C-methyl and pyridyl groups proved to be resistant to wet oxidation. Most nitrogen containing samples were also found to yield precipitate of ammonium perchrorate on cooling. The identity was confirmed by elemental analysis and infra-red spectra.

In the most difficult cases, no combination of H_2SO_4 with HNO_3 or $HClO_4$ or both is effective in producing a colorless digest, and nothing is gained by addition of hydrogen peroxide, ammonium oxalate or other similar oxidants (1)

1.3 RECOVERY OF ELEMENTS

When organic matter is to be destroyed as a preliminary step to the determination of traces of metals the choice of method will depend on:-

a) The nature of the organic material and

b) The metal that is subsequently to be determined and the analytical method to be used for its determination (11).

Analytical techniques are available for the determination of inorganic substances, and most have been applied successfully. No one technique, however, will solve all of the problems likely to be encountered (6). The selection of technique to be used often depends on factors such as equipment available, number of samples to be analysed, staff, space and time considerations. T

The aim of this section is to survey analytical methods used in destruction of organic matter and techniques used for the determinations of the trace metals.

The elements commonly found in food samples can be conviniently divided into three groups viz:-

i) Essential bulk elements

ii) Essential trace elements and

iii) Non-essential trace elements.

1.3.1. ESSENTIAL BULK ELEMENTS

The elements which are necessary for biological function or for good health but without which life can still exist at some level are known as essential elements (17). These elements include calcium, sodium, potassium, and magnesium.

Sodium and ptassium are present in large quantities in organic matrices of biological samples. In many determinations it is not necessary to carry out an oxidation of the organic material, a simple extraction

being quite sufficient (3). Wet oxidation with nitric, sulphuric and perchloric acid mixtures have been used, with success prior to flame photometric determination. All essential bulk metals including sulphur have been determined after using nitric acid - ammonium nitrate mixture in destroying the organic material by Milton and co-workers (14). Destruction of organic matter in plant material using nitric - perchloric acid mixture was found to be rapid and the metals left in a condition in which they are soluble in dilute acids by Gieseking and co-workers (15). The same acid mixture was used by Gerritz (16), on feed concentrates and grasses. He reported that, the method permits a saving of time on the part of the analyst and a saving of equipment and reagents in determination of calcium. Gerritz also reported the possibility of determining magnesium since it does not form insoluble perchlorate salts. More perchloric acid was required to prevent the precipitation of potassium perchlorate.

Wet oxidation of organic matter before determination of bulk metals, should be without complication, although it is probably desirable to avoid the use of sulphuric acid so as to remove any possibility of the formation of insoluble alkaline earth sulphates (3).

1.3.2. ESSENTIAL TRACE METALS

This group of metals occur in minute amounts in living organisms. The essential trace metals include; iron, manganese, zinc, cobalt, and copper. They are needed in different concetrations in metabolism, and become toxic when the optimum concentrations tolerated by the organism is exceeded (17). Various wet digestion methods have been used to destroy the organic matter containing these metals prior to their determinations by either spectrophotometric or atomic- absorption methods.

Gorsuch (18) carried out survey experiments with cocoa as organic

material, in the study of the recovery of Fe, Zn, Co and Cu. From the results obtained, H_2SO_4/HNO_3 method of wet digestion gave good recoveries for Fe, Zn and Co. Losses were observed from $HNO_3/HClO_4$, and $HNO_3/H_2SO_4/HClO_4$ methods of digestions. For Cu however, recoveries were good and comparable for all three methods. Losses were also reported for Fe, Co, Zn, Mn when a biological sample was digested with nitric acid alone (8). The use of $H_2SO_4/HNO_3/HClO_4$ was recommended by Jackson (19), for the determination of Fe in cornstarch and dried spinarch. Wet digestion methods using nitric acid alone, and in usual mixtures with sulphuric and perchloric acids have been described (20). The use of HNO_3/H_2SO_4 to determine Mn in some teas using a tintometer have been reported (21). As reported, the method gave a consistent result with low standard deviations and high recoveries. Wet oxidation methods using acid mixtures have been found excellent for the determination of Zn (3). Many workers prefer to use atomic - absorption methods for the determination of zinc in foods following the acid oxidation of the organic matter (6). Allan (22) reported good recoveries of Zn and small standard deviations when $HNO_3/HClO_4$ was used to digest apple and grass samples.

1.3.3. NON-ESSENTIAL TRACE ELEMENTS

These are elements that, when accumulated in the body of living organisms, cause physiological disorders which may enventually lead to death. Some of these elements include: mercury, cadmium, and lead. Various digestion methods have been used to destroy organic matter prior to the determination of these metals.

Because of the volatility of most mercury salts digestion of organic matter for determination of mercury is often carried out in closed systems or an officient refluxing assembly using HNO_3/H_2SO_4 mixtures (6).

Knauver and Milliman (23), reported that H_2SO_4/HNO_3 mixture gave good results when digestion was carried out in a closed system. Gorsuch (18), also reported that the presence of sulphuric acid in digestion mixtures increased the recovery of mercury. Also in his survey, he found the presence of H_2SO_4, causing low recovery of lead content this he explained was due to co-precipitation of lead sulphate with calcium sulphate. Hoover (24), reported that addition of a strontium salt, reduces the loss of lead. Spectroscopic, electrochemical, and atomic absorption techniques have all been used for the determination of lead. Gorsuch (18), in his survey, reported that HNO_3/H_2SO_4 mixture gave good recovery for cadmium while losses were observed from $HNO_3/HClO_4$ and $HNO_3/H_2SO_4/HClO_4$ mixtures. Atomic absorption, anodic - stripping voltammetry and palarography have been used for the final determination of cadmium.

From the fact that even with all these digestion methods, organic components of the original sample might not be completely destroyed and also the fact that some metals are lost when certain digestion methods are used, an attempt was made to choose a good method for the distruption of organic matter in vegetable Fruits for elemental analysis. This method should be applicable to all samples analysed and those that fall into such group, it should also be rapid, keep most of elements in solution and in a form which they could be determined. Finally the method should be reproducible. Reproducibility of each method was determined by statistical analysis of results obtained from each digestion method used. Statistical methods used include calculation of Standard deviation and multiple range tests.

1.4. DESCRIPTION OF OPTICAL METHODS USED IN METAL DETERMINATIONS

Various methods have been used for the determination of metals after the destruction of organic matter in the sample. For all these methods

it is important that the elements desired are in concentrated form, and devoid of as many sources of interferences as possible. In the present study, atomic absorption spectrophotometry was used for the determination of the trace elements of interest i.e. iron, lead, cadmium, zinc, manganese, copper and cobalt. Flame photometry was used for the determination of bulk metals i.e. calcium, potassium, and sodium except for magnesium which was determined by atomic absorption spectrophotometry.

These two methods were used because of the ease in preparing the test solutions for subsequent elemental determinations, little or no interference from other metals, and sensitivity to part per million (ppm) levels.

1.4.1 ATOMIC ABSORPTION SPECTROPHOTOMETER.

Atomic absorption spectrometry has proved to be one of the most widely used and successful techniques for trace elemental analysis. It is quantitative, sensitive, specific, simple, rapid and relatively inexpensive. It can be used to determine most of the metals in the periodic table, based on the absorption of their free atoms. When the solution containing a metallic salt is aspirated into the flame e.g. air - acetylene flame, a vapour which contains atoms of the metal in their ground states may be formed. If radiation of resonance wavelength of the metal being determined is passed through the flame containing these atoms, part of the light would be absorbed, and the amount of radiation absorbed is proportional to the number of absorbing atoms. This is given by the Beer - Lamberts law:-

$$A = \log I_o/I_t = \varepsilon C l$$

Where I_o = Intensity of incident radiation.

I_t = Intensity of transmitted radiation.

ε = molar absorptivity

C = concentration of solution.

l = path length

A = Absorbance.

1.4.2. FLAME PHOTOMETER

Flame photometry is concerned with the emission of characteristic radiation in flames by individual elements and the correlation of the emission intensity with the concentration of these elements as it should also obey Beer - Lambert's law. Like any other optical method, determination of metal concentrations is made relative to some standards, in which case construction of calibration curve is necessary. It is used in determining sodium, potassium, lithium and calcium mainly. The solution containing the metallic salt is drawn into a non-luminous flame, ionizes and emits radiation of a characteristic wavelength. The emitted light is isolated into the characteristic wave band by an optical filter before passing on to energize the photocell, the output of which is then applied to deflect the galvanometer.

CHAPTER TWO

E X P E R I M E N T A L

2.1. MATERIALS: SAMPLE COLLECTION, DESCRIPTION AND PREPARATION

The two guava samples analysed were supplied by Dr. J.Y. Olayemi, from his backyard garden situated in Area A, at the Ahmadu Bello University, Zaria. The guava samples were from two different trees, not more than 10m apart. These guava samples, though different in their sizes, mineral contents, and appearance were said to have the same botanical names. The tomatoe samples were kindly supplied by Dr. Duro Olarewaju of the Horticulture Section of the Institute for Agricultural Research (I.A.R.), Samaru of the Ahmadu Bello University, Zaria. The guava samples were collected in the month of August, while the tomatoe samples in the month of October of the same year.

2.1.1. SAMPLE DESCRIPTION

Sample

GS_1 — Local or English name: Guava
Botanical name: Psidium guajava (large)
Location: Area A.

GS_2 — Local or English name: Guava
Botanical name: Psidium guajava (small)
Location: Area A.

T_1 — Local or English name: Tomatoe
Botanical name: Lycopersicon Esculentum.
Variety name: Cirio 56
Location: I.A.R.

T_2 Local or English name: Tomatoe

Botanical name: Lycopersicon Esculentum.

Variety name: Pevabo.

Location: I.A.R.

T_3 Local or English name: Tomatoe

Botanical name: Lycopersicon Esculentum.

Variety name: Harvester

Location: I.A.R.

T_4 Local or English name: Tomatoe

Botanical name: Lycopersicon Esculentum.

Variety name: Ronita

Location: I.A.R.

T_5 Local or English name: Tomatoe

Botanical name: Lycopersicon Esculentum.

Variety name: Ife 1

Location I.A.R.

T_6 English or Local name: Tomatoe

Botanical name: Lycopersicon Esculentum.

Variety name: Gamad.

Location: I.A.R.

T_7 English or Local name: Tomatoe

Botanical name: Lycopersicon Esculentum.

Variety name: Marzanino.

Location: I.A.R.

T_8 English or Local name: Tomatoe

Botanical name: Lycopersicon Esculentum.

Variety name: La Bonita.

Location: I.A.R.

2.1.2. PREPARATION OF SAMPLE

Before the actual analysis, it is necessary to get the sample in a suitable form. This ensures homogeneity of the sample, and creates a bigger surface area on which the reaction occurs. This has the advantage of making the actual experiment fast and reducing the sources of error due to the non-homogeneity of the analyte in the sample.

The fresh samples were washed with distilled water, and cut into some clean petridishes. These were then transfered into an air oven, set at about 60°C as recommended by Oyenuga (25). Drying was complete within 2 - 3 days. The dried sample were ground to powder using a mortar, and stored in air tight screw-capped bottles.

2.2. DETERMINATION OF MOISTURE CONTENT

The method generally used for the determination of moisture content in plant materials is measurement of the loss in weight due to drying at a temperature just above 100°C (7). The important factor in thermal drying is the maintenance of a differential between the vapour pressure of the substance to be dried and that of the atmosphere of the drying chamber (26). Other methods have been described for the determination of moisture in foods. One of these methods (27) involves reflux distillation of the sample with a solvent immiscible with water and having a higher boiling point and a lower specific gravity than water e.g. heptane, toluene etc.

PROCEDURE: A petridish was heated to dryness, cooled in a descicator and weighed. The fresh sample was cut, and spread in the container and weighed rapidly. The petridish and its content was transferred to the air oven operating at 105°C. The sample was dried to a constant weight.

CALCULATION

$$\text{Moisture (\%)} = \frac{\text{loss in weight on drying (g)}}{\text{Initial Sample wt. (g)}} \times 100$$

Results for the moisture content of all samples are given in appendix A.

2.3. WET DIGESTION METHODS USED.

The procedures used for digesting the samples are as follows:

2.3.1. METHOD A, (11)

About 1g of the dried sample was accurately weighed into 100cm^3 Kjeldahl flask and a mixture of 8cm^3 concentrated sulphuric acid (sp. gr. 1.84) and 10cm^3 of nitric acid (sp. gr. 1.42) were added. The mixture warmed cautiously until the reaction subsided. The mixture was then heated to a temperature of 120°C, until the solution just began to darken. This took about 45 min. The mixture was cooled and 5cm^3 of concentrated nitric acid was added, and the mixture was again heated over the heating mantle at about 120°C until the solution was clear. The mixture was not allowed to char. The solution was cooled and 10cm^3 of distilled deionized water was added followed by gentle boiling to fuming. Digestion was completed in 2 hrs., and the volume left after digestion was reduced to about three-quarter of the initial volume. The solution was then cooled and transferred quantitatively to a 50cm^3 volumetric flask and made to mark with distilled deionized water.

2.3.2. METHOD B, (4)

About 1g of the dried sample was accurately weighed into 100cm^3 Kjeldahl flask. The sample was soaked in 10cm^3 of 1:2 water-nitric acid for about 15hrs., after which 10cm^3 of concentrated sulphuric acid (sp. gr. 1.84) was added at such a rate as not to cause excessive frothing.

The mixture was heated at a temperature of about 120°C over a heating mantle, until the liquid darkened appreciably. 5cm^3 of concentrated nitric acid (sp. gr. 1.42) was added to the solution. Heating was continued for about 50min. when the solution became clear. 10cm^3 of distilled deionized water was added, and heating was continued until the final volume of the solution was about three-quarters of the initial volume. The solution was cooled and transferred to 50cm^3 volumetric flask and made to mark with distilled deionized water.

2.3.4. METHOD C_2 (16).

About 1g of the dried sample was accurately weighed into 100cm^3 Kjeldahl flask. 10cm^3 of distilled deionized water was added, followed by 10cm^3 of concentrated nitric acid (sp. gr. 1.42). The mixture was swirled and transferred on to a heating mantle. The contents were boiled at about 100°C for about 60min when the sample passed into a colloidal solution. The solution was cooled and 5cm^3 of 72% w/w percloric acid was added. The flask and the contents were again heated over a heating mantle. Heating was continued at a temperature of about 140°C, for about 20min, when dense white fumes of perchloric acid were noticed. The solution was allowed to cool, and later transferred quantitatively into a 50cm^3 volumetric flask, and made to mark with distilled deionized water.

2.3.5. METHOD D_2 (11).

About 1g of the dried sample was accurately weighed into 100cm^3 Kjeldahl flask. 10cm^3 of concentrated nitric acid (sp. gr. 1.42), followed by 5cm^3 of concentrated sulphuric acid (sp. gr. 1.84) were added. The mixture was swirled gently and transferred to a heating mantle. The sample was digested for about 45min at a temperature of about 120°C. The solution was cooled, and 5cm^3 of 72% w/w perchloric acid was added.

Heating was continued at about 140°C for about 20min after the appearance of white fumes. The solution was set aside to cool. The digest was diluted with about 10cm^3 of distilled deionized water and boiled for 10min. It was transferred quantitatively to a 50cm^3 volumetric flask, and made to mark with distilled deionized water.

2.3.6. METHOD E, (28)

About 1g of the dried sample was accurately weighed into 100cm^3 Kjeldahl flask. About 100mg. of selenium powder was also weighed into the Kjeldahl flask, and mixed thoroughly. 5cm^3 of distilled deionized water was added, followed by 15cm^3 of (1:1) concentrated nitric acid (sp. gr. 1.42), and concentrated sulphuric acid (sp. gr. 1.84) slowly and intermittently taking care that the mixture at no time froth appreciably. The content was swirled from time to time. A further 5cm^3 of concentrated nitric acid was added. The mixture was heated at about 120°C for 30min. The solution was allowed to cool, and 5cm^3 of 72% w/w perchloric acid was added. Heating was continued at about 140°C for 20min. The solution was again allowed to cool, and transferred quantitatively into a 50cm^3 volumetric flask and made to mark with distilled deionized water.

2.3.7. METHOD F, (11)

About 1g of the dried sample was accurately weighed and transferred into a 100cm^3 Kjeldahl flask. 10cm^3 of concentrated nitric acid (sp. gr. 1.42) was added and the mixture was heated at a temperature of about 100°C for 1hr. 10cm^3 of the oxidising agent (a solution containing 50g of ammonium nitrate in 25g of nitric acid.). It was heated at the same temperature for 30min. to expel water, so that oxidation proceeded in a melt of ammonium nitrate. More reagent was added (5cm^3 of oxidizing reagent) from time to time until no browning of the solution was observed

time, until no browning of the solution was observed and a clear melt was obtained. When the oxidation was complete, the clear melt was heated more strongly to volatilize the excess ammonium nitrate. The flask was held over a free flame to expel salt subliming on the side. The residue was dissolved in $2cm^3$ of concentrated hydrochloric acid (sp. gr. 1.18) and evaporated to almost dryness. The residue was fused as to remove all nitric acid. It was dissolved in about $10cm^3$ of 1M HCl and evaporated to a small bulk to ensure conversion of my metaphosphate to orthophosphate. It was evaporated to dryness, and the residue was dissolved in $15cm^3$ of warm distilled deionized water and a few drops of 1M HCl. The solution was transferred quantitatively to a $50cm^3$ volumetric flast and made to mark with distilled deionized water.

2.3.8. METHOD G, (29)

About 1g of the dried sample was accurately weighed into $100cm^3$ Kjeldahl flask and to it was added $16cm^3$ of concentrated nitric acid (sp. gr. 1.42). The flask was allowed to stand for one minute to allow any vigorous reaction to subside. The mixture was transferred on to a heating mantle, and digested at about 100^oC for one hour. The solution was cooled and $5cm^3$ of concentrated nitric acid (sp. gr. 1.42) was again added. Heating was continued at the same temperature for about 45min. About $10cm^3$ of distilled deionized water was added to the solution and heating was done to fuming, within about 10min. The solution was cooled and transferred quantitatively in to a $50cm^3$ volumetric flask and made to mark with distilled deionized water.

2.3.9. METHOD H_2 (30).

About 1g of the dried sample was weighed accurately into $100cm^3$ Kjeldahl flask. $10cm^3$ of concentrated nitric acid (sp. gr. 1.42), was

added, followed by $2cm^3$ of concentrated hydrochloric acid (sp. gr. 1.18). After mixing, it was set aside for about 20min., and then heated at about $80^{\circ}C$ for 40min. After the brown fumes of nitrogen oxides had disappeared, the temperature was increased to about $120^{\circ}C$, to allow for gentle boiling. The solution was cooled, and $5cm^3$ of concentrated nitric acid was added. Heating was continued until the digest was almost dry (about $5cm^3$). $10cm^3$ of distilled deionized water was added and the solution was brought just to boiling. It was allowed to cool, and transferred quantitatively to a $50cm^3$ volumetric flask, and made to mark with distilled deionized water.

2.3.10. METHOD I, (MODIFICATION OF METHOD G)

About 1g of the dried sample was accurately weighed into $100cm^3$ Kjeldah flask. $15cm^3$ of concentrated nitric acid (sp. gr. 1.42) was added and it was set aside for about 15hrs. to allow proper soaking. The mixture was transferred on to a heating mantle, and was digested at about $100^{\circ}C$ for 1hr. The solution was allowed to cool, and $5cm^3$ of concentrated nitric acid was added. Heating was continued at the same temperature for about 45min. About $10cm^3$ of distilled deionized water was added to the solution which was heated to fuming for about 10min. The solution was allowed to cool, and transferred quantitatively into a $50cm^3$ volumetric flask and made to mark with distilled deionized water.

Replicates for each method of digestion was prepared. Measurements of Absorption were carried out with SP1900 PYE Unicam Atomic Absorption spectrophotometer or the Evans Electroselenium flame photometer as the case may be.

2.3.11. PREPARATION OF BLANK SOLUTIONS

Blank solutions were prepared for each method that was used. This

was to correct the errors from contamination due to the large excess of reagents that was used in the oxidation procedures.

The blank solutions were prepared by using equal volumes of reagents, minus sample for each digestion method. These were heated in 100cm^3 Kjeldahl flask under the same experimental conditions as the sample (i.e. temperature and digestion time).

2.4. OBSERVATIONS

The observations made while carrying out the digestion of the samples are in tables 2.1 and 2.2.

Remarks on each method are given in table 2.3.

TABLE 2.1.

OBSERVATIONS DURING DIGESTION OF SAMPLES GS_1 AND GS_2

DIGESTION METHODS

A	B	C	D	E	F	G & I	H
A clear faint yellow solution obtained. No precipitate was formed	Solution obtained was similar to that from method A.	A clear solution was obtained, but on cooling crystalline white precipitate soluble in water was formed.	An almost colorless digest solution was obtained. Precipitate was formed on cooling but was soluble in water	The solution obtained was similar to that from method, D.	A clear yellow solution was obtained along with a brown precipitate which was soluble in hot concentrated nitric acid (sp. gr.1.42)	A yellow solution with white precipitate insoluble in water was obtained	A light yellow solution was obtained with white precipitate that was insoluble in hot concentrated nitric acid (sp.gr.1.42)

TABLE 2.2.

OBSERVATION DURING DIGESTION OF SAMPLES T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8.

DIGESTION METHODS

	B	C	D	E	F	G & I	H
A clear solution was obtained without precipitate	Similar to method A.	A clear yellow solution was obtained with formation of crystalline precipitate on cooling. Precipitate was soluble in water.	An almost colorless solution was obtained. Precipitate soluble in water was formed on cooling.	Solution obtained similar to method D.	A clear yellow solution without precipitate was obtained.	A yellow solution, with white fatty precipitate was obtained. Precipitate was insoluble in (1 + 1) HCl.	Yell solu with fatt prec was The prec was in h conc nitr (sp.

TABLE 2.3

METHOD	R E M A R K S
A	Digestion with H_2SO_4 - HNO_3 mixture was in most cases speedy and easily controlled. Because of the high boiling point of H_2SO_4. It allowed for an increase in the oxidation temperature used, thus increasing the oxidizing power of nitric acid. However, the sample might be charred (i.e. a reducing condition) due to the dehydrating power of sulphuric acid. Presence of sulphuric acid might lead to co-precipitation of heavy metals on insoluble sulphates of alkaline Earthmetals.
B	Same as in method A.
C	Digestion with HNO_3 - $HClO_4$ was speedy in most instances and easily controlled. The temperature used was low and perchlorates are generally easily soluble. Losses of heavy metals using this method might be significant, due to formation of volatile perchlorates.
D	Digestion with HNO_3 - H_2SO_4 - $HClO_4$ was also speedy, but prior digestion with HNO_3 - H_2SO_4 was necessary due to fear of explosion. It has the ability to destroy many substances (e.g. fat). The presence of sulphuric acid creating a dehydrating condition necessary for perchloric acid oxidation. It also helped in minimizing the loss of certain metals. The presence of sulphuric acid might also cause losses of metals such as lead from calcium rich samples.
E	The oxidation was the fastest of all the methods used due to the presence of selenium powder as catalyst. The reaction was vigorous and recovery of metals was simila to method D.

TABLE 2.3 CONT'D

METHOD	R E M A R K S
F	The oxidation was rapid compared to when HNO_3 was used alone. The residue could easily be freed from excess reagent by volatilization and elements were obtained in form suitable for determination. Losses in some heavy metals might be anticipitated.
G	Digestion with nitric acid alone was slow, the organic matter was not completely destroyed and losses of volatile compounds of Fe, Co, Zn and Mn were expected as previously reported (31). Low digestion temperature used in this method due to low boiling point of nitric acid (120°C for HNO_3, sp. gr. 1.42) could have led to the incomplete oxidation of organic matter such as Fat and protein.
H	Digestion with HNO_3-HCl for the determination of metals is not common, however the method was fast, but led to loss of certain metals due to the formation of their volatile chlorides. This method is said to be valuable in the determination of phosphorus and mercury (8)
I	Soaking of organic matter in concentrated nitric acid (sp.gr. 1.42) made subsequent digestion faster. Losses of volatile compounds of certain metals might also be encountered as in method G

2.5. PREPARATION OF STANDARD SOLUTIONS

The use of optical methods of analysis often requires the use of standard solutions, prepared by dissolving known amount of metal salt in water. The metal concentration in the sample is determined relative to this standard solution. A calibration curve is constructed, from the absorbance readings of different concentrations of this standard solution, from which the amount of metal present in the sample solution is read off. The most volatile inorganic salt of a metal is often used, especially in atomic absorption spectrophotometry and flame photometry. This is to ensure maximum vaporization of the metal salt, which inturn affects the extent of atomization and absorbance in case of atomic absorption and emission in case of flame photometry.

Standard curve for each metal analysed is given in Appendix B.

MATERIALS:

All metal salts used for the preparation of calibration curves were of analytical reagent grade (ANALAR), obtained from British Drug House (BDH).

2.5.1 SODIUM

Sodium was determined with Evans Electroselenium Flame photometer. Air pressure of about 1.2 atmospheres was used. The air - butane flame was used.

A stock solution was prepared by dissolving 2.5420g of sodium chloride in $100Cm^3$ of distilled deionized water. This was then diluted to $1000Cm^3$ with distilled deionized water. This solution contained 1mg per $1Cm^3$ of sodium, (i.e. 1000ppm).

Appropriate dilutions of the stock standard solution were made to give 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 (ppm) of sodium.

A calibration curve was constructed and the metal concentration in the sample solution was determined.

2.5.2 POTASSIUM

Potassium was determined using the Evans Electro selenium flame photometer. The air pressure was adjusted to about 1.2 atmospheres. The air -- butane flame was used.

A stock solution was prepared by dissolving 1.9068g of potassium chloride in $100Cm^3$ of distilled deionized water. This was then diluted to $1000Cm^3$ with distilled deionized water. This solution contained 1mg per $1Cm^3$ of potassium (i.e. 1000 ppm).

Appropriate dilutions of the stock standard solution were made to give 0.0, 2.5, 7.5, 10.0, 15.0, 20.0(ppm). of potassium.

A calibration curve was constructed from where the metal concentration in the sample solution was determined.

2.5.3. CALCIUM

Calcium was determined using the Evans Electroselenium flame photometer. The air pressure was adjusted to about 1.2 atmospheres. The air -- butane flame was used.

A stock solution was prepared by dissolving 2.7693g of calcium chloride in $100Cm^3$ of distilled deionized water. Concentrated hydrochloric acid (sp.gr. 1.18) was added dropwise to effect complete solution. This was then diluted to $1000Cm^3$ with distilled deionized water. This solution contained 1mg per $1Cm^3$ of calcium, (1000ppm).

Appropriate dilutions of the stock standard solution were made to give 0.0, 10.0, 30.0, 40.0, 50.0, 60.0 70.0(ppm).

A calibration curve was constructed, from where the metal concentration in the sample was determined.

2.5.4. MAGNESIUM

Magnesium was determined using the PYE Unicam Atomic Absorption spectrophotometer, SP1900. The 285.3nm resonance line of Magnesium was used. The air - acetylene flame was used.

A stock solution was prepared by dissolving 10.0135g of Magnesium sulphate heptahydrate, $MgSO_4.H_2O$, in 200Cm^3 of distilled deionized water. About 1.5Cm^3 of concentrated nitric acid (sp. gr. 1.42) was added. This was then diluted to 1000Cm^3 with distilled deionized water. This solution contained 1mg per 1Cm^3 of Magnesium, (i.e. 1000ppm).

Appropriate dilutions of the stock standard solution were made to give 0.0, 0.10, 0.20, 0.40, 0.60, 0.80, 1.00(ppm) of magnesium.

A calibration curve was constructed from where the metal concentration in the sample solution was determined.

2.5.5. ZINC

Zinc was determined, using the SP1900 Atomic Absorption spectrophotometer. The 213.9nm resonance of zinc was used. Airacetylene flame was used.

A stock solution was prepared by dissolving 1g of zinc metal, in 20Cm^3 of 1:1 hydrochloric acid-water mixture. This was then diluted to 1000Cm^3 with distilled deionized water. The solution contained 1mg per 1Cm^3 of zinc (i.e. 1000ppm).

Appropriate dilutions of the stock standard solution were made to give 0.0, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0(ppm) of zinc.

A calibration curve was constructed from where the metal concentration in the sample solution was determined.

2.5.6 CADMIUM

Cadmium was determined at 229.0nm resonance line, using SP1900 Atomic Absorption spectrophotometer. The air-acetylene flame was used.

A stock solution was prepared by dissolving 2.1032g of Cadmium nitrate in 100Cm^3 of distilled deionized water. This solution was then diluted to 1000Cm^3 with distilled deionized water. This solution contained 1mg per 1Cm^3 of Cadmium (1000ppm).

Appropriate dilutions of the stock standard solutionss were made, to give 0.0, 0.2, 0.4, 0.5, 1.0, 1.5, 2.0(ppm).

A calibration curve was constructed from which the metal concentration in the sample solution was determined.

2.5.7. COBALT

Cobalt was determined at 241.0nm resonance line, using the SP1900 Atomic Absorption spectrophotometer. The air-acetylene flame was used.

A stock solution was prepared by dissolving 4.9379g of cobaltous nitrate hexahydrate in 20Cm^3 of 3M hydrochloric acid. This was then diluted to 1000Cm^3 with distilled deionized water. This solution contained 1mg per Cm^3 of Cobalt (i.e. 1000ppm).

Appropriate dilutions of the stock standard solution were made to give 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0(ppm).

A calibration curve was constructed from which the metal concentration in the sample solution was determined.

2.5.8. IRON

Iron was determined at 248.3nm resonance line using SP1900 Atomic Absorption spectrophotometer. Air-acetylene flame was used.

A stock solution was prepared by dissolving 4.8400g of ferric chloride hexahydrate in 20Cm^3 of 3M hydrochloric acid. This was then diluted to 1000Cm^3 with distilled deionized water to give 1mg per 1Cm^3 of iron i.e. (1000ppm).

Appropriate dilutions of the stock standard solution were made to give 0.0, 1.0, 2.0, 3.0, 4.0, 5.0(ppm).

A calibration curve was constructed, from where the metal concentration in the sample solution was determined.

2.5.9. COPPER

Copper was determined at 324.8nm resonance line, using SP1900 Atomic Absorption spectrophotometer. Air -acetylene flame was used.

A stock solution was prepared by dissolving 3.7980g of cupric nitrate in $20Cm^3$ of 3M hydrochloric acid. This was diluted to $1000Cm^3$ with distilled deionized water, to give 1mg per $1Cm^3$ of copper, (i.e. 1000ppm).

Appropriate dilutions of the stock standard solution were made to give 0.0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0(ppm).

A calibration curve was constructed from where the metal concentration in sample solution was determined.

2.5.10. MANGANESE

Manganese was determined at 279.5nm resonance line, using SP190C Atomic Absorption spectrophotometer. Air-acetylene flame was used.

A stock solution was prepared by dissolving 4.0600g of manganous sulphate tetrahydrate in $20Cm^3$ of distilled deionized water. About $1.5Cm^3$ of concentrated nitic acid (sp. gr. 1.42) was added. This was diluted to $1000Cm^3$ with distilled deionized water to give 1mg. per $1Cm^3$ of manganese, (i.e. 1000ppm).

Appropriate dilutions of the stock standard solutions were made to give 0.0, 0.2, 0.5, 1.0, 2.0(ppm).

A calibration curve was constructed, and metal concentration from the sample was determined.

2.5.11 LEAD

Lead was determined at 213.9nm resonance line, using SP1900 Atomic Absorption spectrophotometer. Air-Acetylene flame was used.

A stock solution was prepared by dissolving 1.5980g of lead nitrate in $20Cm^3$ of 3m hydrochloric acid. This was diluted to $1000Cm^3$ with distilled deionized water, to give 1mg per $1Cm^3$ of lead.

Appropriate dilutions of the stock standard solution were made to give 0.0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0(ppm).

A calibration curve was constructed, from where the metal concentration of the sample solution was determined.

2.6. STANDARDIZATION OF STOCK SOLUTIONS

The standardization of a solution is knowing the exact amount of ions or materials present in that solution. This aspect is important because estimation of metal ions are made relative to the standard solutions prepared from the stock solution. The exact concentration of the standard should also be known. In very accurate determinations, the amount of salt weighed should agree very closely with that determined from this process. This also gives the percentage purity of a salt.

METHOD

Direct titration of the diluted stock solution with the sodium salt of Ethylenediaminetetracetic acid, (EDTA) was used (32). For potassium and sodium, back titration was used. Though it gave reasonable result, it is said to be unreliable.

CHAPTER THREE

3.0. Results and Discussion.

It should have been pleasant, if one general method could be found that is suitable for the destruction of organic matter in all biological materials prior to the determination of as many metals as possible. This is however not possible, at least for now, as shown by the results obtained by Osipitan(38), and the present study. The mean values and standard deviations for each method of digestion of the various biological samples analyzed are shown in tables 3.1 to 3.10.

In this section, attempts have been made to find the most reliable of all the wet digestion methods used, in making available for determination, most of the metals of interest, from each sample. The most suitable and best methods were determined on the following basis:

i) Must be highly reproducible i.e. small standard deviation.

ii) Should have the highest mean of all the values obtained from different digestion methods.

iii) Should preferably have the lowest reagent blank absorption reading.

iv) Should be fast and safe.

For the purpose of finding a most suitable method for each metal, from each sample calculations of the mean values of some metals in the samples and standard deviations were done. Multiple range tests were also performed by means of a computer.

TABLE 3.1: RESULTS OF ELEMENTAL ANALYSIS OF GS_1.

CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

Sample	Na	K	Mg	Ca	Zn	Cd	Pb	Mn	Fe	Co
A	1.02 ±0.09	503.33 ±7.53	21.67 ±2.58	52.83 ±2.14	3.18 ±0.24	0.45 ±0.00	5.92 ±0.38	0.40 ±0.00	3.18 ±0.14	2.15 ±0.16
B	1.33 ±0.05	409.17 ±14.63	14.17 ±2.04	52.58 ±1.62	3.48 ±0.38	0.90 ±0.06	6.20 ±0.28	0.45 ±0.03	3.02 ±0.12	1.48 ±0.04
C	1.22 ±0.04	555.00 ±5.48	20.50 ±0.00	37.42 ±2.73	3.67 ±0.33	0.00 ±0.00	0.00 ±0.00	0.07 ±0.03	1.50 ±0.17	0.00 ±0.00
D	0.87 ±0.05	517.50 ±18.09	23.33 ±2.58	39.67 ±1.72	14.15 ±0.08	0.25 ±0.00	3.58 ±0.24	0.46 ±0.02	2.53 ±0.08	1.70 ±0.19
E	1.25 ±0.06	403.33 ±16.02	16.25 ±1.37	38.83 ±1.21	2.65 ±0.27	0.69 ±0.04	4.40 ±0.11	0.39 ±0.00	2.72 ±0.12	1.47 ±0.05
F	3.50 ±0.06	685.00 ±29.49	23.75 ±1.37	54.50 ±1.22	3.72 ±0.04	0.00 ±0.00	0.00 ±0.00	0.08 ±0.02	1.53 ±0.21	0.00 ±0.00
G	1.00 ±0.00	573.33 ±26.01	20.00 ±0.00	57.00 ±0.63	4.20 ±0.27	0.00 ±0.00	0.00 ±0.00	0.11 ±0.02	1.43 ±0.05	0.00 ±0.00
H	2.73 ±0.83	353.33 ± 8.13	20.00 ±0.00	56.83 ±0.75	3.67 ±0.20	0.00 ±0.00	0.00 ±0.00	0.15 ±0.03	1.47 ±0.05	0.00 ±0.00
I	2.78 ±0.49	407.83 ±7.36	19.58 ±1.02	57.17 ±0.75	2.06 ±0.27	0.00 ±0.00	0.00 ±0.00	0.14 ±0.02	1.53 ±0.18	0.00 ±0.00

N = Six determinations.

TABLE 3.2: RESULTS OF ELEMENTAL ANALYSIS OF GS_2.
CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

Dig. Meth.	Na	K	Mg	Ca	Zn	Cd	Pb	Mn	Fe	Co
A	1.23 ±0.04	398.33 ± 4.08	32.92 ±4.08	48.83 ±1.17	0.37 ±0.05	0.61 ±0.04	4.95 ±0.18	0.50 ±0.01	2.48 ±0.13	1.63 ±0.16
B	1.13 ±0.07	381.00 ±13.72	30.83 ±1.29	47.67 ±1.03	0.87 ±0.03	0.66 ±0.02	6.23 ±0.26	0.65 ±0.01	2.35 ±0.16	1.87 ±0.12
C	1.39 ±0.06	353.33 ±5.16	28.50 ±1.37	38.70 ±0.45	0.42 ±0.04	0.08 ±0.02	0.00 ±0.00	0.39 ±0.02	0.90 ±0.12	0.00 ±0.00
D	1.12 ±0.03	340.00 ±6.32	30.42 ±1.02	45.50 ±0.84	0.60 ±0.06	0.42 ±0.02	2.45 ±0.17	0.51 ±0.00	1.69 ±0.15	1.12 ±0.12
E	1.20 ±0.04	437.00 ±8.37	28.00 ±3.26	41.90 ±1.95	0.79 ±0.05	0.59 ±0.02	4.12 ±0.15	0.53 ±0.01	2.28 ±0.16	1.46 ±0.06
F	2.83 ±0.14	473.33 ±7.53	26.50 ±1.37	51.33 ±0.82	0.45 ±0.08	0.00 ±0.00	0.00 ±0.00	0.43 ±0.03	0.65 ±0.09	0.00 ±0.00
G	2.09 ±0.05	341.67 ±7.53	28.33 ±1.29	49.83 ±0.41	0.47 ±0.07	0.00 ±0.00	0.00 ±0.00	0.42 ±0.02	0.85 ±0.05	0.00 ±0.00
H	1.58 ±0.09	330.00 ±5.48	30.00 ±0.00	51.33 ±0.52	0.37 ±0.02	0.00 ±0.00	0.00 ±0.00	0.41 ±0.02	0.78 ±0.06	0.00 ±0.00
I	1.23 ±0.05	467.50 ±6.12	26.67 ±2.04	51.87 ±0.50	0.24 ±0.06	0.00 ±0.00	0.00 ±0.00	0.41 ±0.02	0.79 ±0.08	0.00 ±0.00

N = Six determinations

TABLE 3.3: RESULTS OF ELEMENTAL ANALYSIS OF T_1
CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

Dig. Meth.	Na	K	Mg	Ca	Zn	Cd	Pb	Cu	Fe	Co
A	3.30 ±0.10	650.00 ±10.00	40.00 ±0.00	35.67 ±0.58	0.63 ±0.03	0.61 ±0.05	4.83 ±0.14	0.25 ±0.05	2.20 ±0.10	2.13 ±0.06
B	3.33 ±0.12	641.67 ±7.34	41.67 ±1.44	35.33 ±0.58	0.64 ±0.01	0.58 ±0.03	5.30 ±0.35	0.18 ±0.03	2.07 ±0.06	1.88 ±0.06
C	2.43 ±0.06	610.00 ±8.66	41.67 ±1.44	26.00 ±0.87	0.56 ±0.01	0.09 ±0.04	0.48 ±0.12	0.27 ±0.03	1.72 ±0.08	0.25 ±0.00
D	2.07 ±0.06	615.00 ±10.00	42.50 ±2.50	26.50 ±0.87	0.59 ±0.03	0.44 ±0.01	2.70 ±0.05	0.13 ±0.03	2.13 ±0.15	1.42 ±0.03
E	1.83 ±0.21	623.33 ±7.64	39.17 ±1.44	23.00 ±0.87	0.61 ±0.01	0.58 ±0.03	4.20 ±0.26	0.17 ±0.03	2.67 ±0.15	1.80 ±0.09
F	4.27 ±0.06	636.67 ±20.21	41.67 ±3.82	42.00 ±1.73	0.61 ±0.00	0.13 ±0.00	0.80 ±0.17	0.28 ±0.06	1.07 ±0.15	0.25 ±0.09
G	3.57 ±0.15	626.67 ±5.77	39.17 ±1.43	40.33 ±0.58	0.50 ±0.05	0.05 ±0.01	0.32 ±0.10	0.20 ±0.00	1.77 ±0.15	0.13 ±0.03
H	3.33 ±0.12	616.67 ±12.58	40.83 ±1.44	37.00 ±2.64	0.50 ±0.00	0.00 ±0.00	0.25 ±0.15	0.17 ±0.06	1.67 ±0.15	0.10 ±0.09
I	3.63 ±0.06	620.00 ±8.66	41.67 ±1.43	38.33 ±1.44	0.53 ±0.02	0.04 ±0.02	0.25 ±0.10	0.20 ±0.00	1.73 ±0.21	0.00 ±0.00

N = Six determinations

TABLE 3.4: RESULTS OF ELEMENTAL ANALYSIS OF T_2

CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

Dig. Meth.	Na	K	Mg	Ca	Zn	Cd	Pb	Cu	Fe	Co
A	3.07 ±0.21	755.00 ±8.66	40.83 ±1.43	41.00 ±0.00	0.59 ±0.03	0.53 ±0.02	4.45 ±0.33	0.37 ±0.06	3.13 ±0.06	2.15 ±0.30
B	3.00 ±0.20	753.33 ±5.77	42.50 ±0.00	39.17 ±1.44	0.65 ±0.02	0.56 ±0.04	5.08 ±0.69	0.33 ±0.05	3.07 ±0.12	2.03 ±0.06
C	1.90 ±0.20	733.33 ±5.77	41.67 ±1.44	28.00 ±0.87	0.58 ±0.02	0.00 ±0.00	0.57 ±0.29	0.22 ±0.03	1.73 ±0.12	0.15 ±0.00
D	1.93 ±0.06	770.00 ±8.66	40.00 ±0.00	24.00 ±1.50	0.60 ±0.00	0.27 ±0.03	2.55 ±0.15	0.18 ±0.03	2.67 ±0.15	1.50 ±0.09
E	1.73 ±0.15	726.67 ±2.89	40.00 ±0.00	21.00 ±0.86	0.53 ±0.11	0.38 ±0.08	3.10 ±0.20	0.27 ±0.06	2.65 ±0.09	1.62 ±0.06
F	3.80 ±0.10	760.00 ±15.00	37.50 ±0.00	41.67 ±1.15	0.58 ±0.02	0.00 ±0.00	0.65 ±0.09	0.30 ±0.05	1.70 ±0.26	0.00 ±0.00
G	3.13 ±0.12	755.00 ±8.66	40.00 ±0.00	42.33 ±1.15	0.55 ±0.02	0.00 ±0.00	0.37 ±0.03	0.17 ±0.06	1.80 ±0.10	0.15 ±0.00
H	3.40 ±0.20	763.33 ±10.41	39.17 ±1.40	43.33 ±0.58	0.65 ±0.09	0.00 ±0.00	0.27 ±0.08	0.20 ±0.00	1.80 ±0.20	0.00 ±0.00
I	3.07 ±0.12	765.00 ±10.00	42.50 ±2.50	43.67 ±0.58	0.56 ±0.01	0.00 ±0.00	0.22 ±0.06	0.13 ±0.03	1.67 ±0.15	0.10 ±0.09

N = Six determinations

TABLE 3.5: RESULTS OF ELEMENTAL ANALYSIS OF T_3
CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

Dig. Meth.	Na	K	Mg	Ca	Zn	Cd	Pb	Cu	Fe	Co
A	3.17	753.33	42.50	39.00	5.97	0.43	6.40	0.40	2.73	2.33
	±0.15	±10.41	±0.00	±0.86	±0.12	±0.01	±0.46	±0.10	±0.12	±0.12
B	2.93	753.33	42.50	37.00	5.93	0.54	6.10	0.38	2.97	2.45
	±0.06	±11.55	±2.50	±0.87	±0.15	±0.04	±0.45	±0.03	±0.15	±0.09
C	2.53	740.00	40.83	26.50	6.07	0.00	0.59	0.22	1.53	0.63
	±0.15	±10.00	±1.44	±0.87	±0.06	±0.00	±0.17	±0.03	±0.15	±0.06
D	2.17	726.67	42.50	22.67	6.10	0.38	3.75	0.32	1.90	1.65
	±0.06	±2.89	±0.00	±0.29	±0.10	±0.03	±0.15	±0.06	±0.20	±0.09
E	2.43	660.00	41.67	23.50	6.00	0.43	4.70	0.30	2.13	1.60
	±0.06	±15.00	±1.44	±0.87	±0.26	±0.03	±0.36	±0.00	±0.23	±0.09
F	5.10	753.33	37.60	40.17	6.13	0.00	1.05	0.33	1.40	0.10
	±0.10	±5.77	±0.00	±1.44	±0.15	±0.00	±0.13	±0.08	±0.17	±0.09
G	4.43	745.00	40.00	43.00	6.07	0.00	0.55	0.30	1.43	0.20
	±0.12	±5.00	±0.00	±1.73	±0.06	±0.00	±0.13	±0.05	±0.06	±0.09
H	5.00	748.33	39.17	42.66	6.17	0.00	0.17	0.30	1.53	0.20
	±0.10	±2.89	±1.44	±1.53	±0.06	±0.00	±0.08	±0.05	±0.15	±0.09
I	4.23	753.33	42.50	41.00	6.10	0.04	0.27	0.22	1.63	0.18
	±0.25	±11.55	±1.50	±1.73	±0.17	±0.02	±0.08	±0.03	±0.21	±0.10

N = Six determinations

TABLE 3.6: RESULTS OF ELEMENTAL ANALYSIS OF T_4
CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

	Na	K	Mg	Ca	Zn	Cd	Pb	Cu	Fe	Co
	3.07	761.67	42.50	37.67	0.65	0.52	5.70	0.27	2.23	2.03
	±0.11	±10.41	±0.00	±1.44	±0.04	±0.04	±0.18	±0.03	±0.15	±0.06
	3.00	773.33	42.50	35.33	0.69	0.60	6.77	0.30	2.13	2.23
	±0.17	±12.58	±2.50	±0.58	±0.02	±0.01	±0.23	±0.00	±0.06	±0.25
	2.27	743.33	40.83	26.00	0.58	0.00	1.07	0.22	1.33	0.70
C	±0.12	±7.64	±1.44	±2.29	±0.03	±0.00	±0.06	±0.03	±0.15	±0.10
	2.10	726.67	42.50	24.00	0.60	0.30	4.63	0.28	2.30	1.80
D	±0.10	±7.64	±0.00	±1.50	±0.00	±0.04	±0.12	±0.06	±0.17	±0.09
	2.63	660.00	43.33	22.93	0.62	0.43	5.80	0.33	2.35	2.13
E	±0.12	±13.23	±1.40	±0.92	±0.02	±0.02	±0.26	±0.03	±0.18	±0.06
	4.57	780.00	41.50	36.50	0.63	0.00	1.03	0.20	1.73	0.38
F	±0.06	±5.00	±1.00	±0.87	±0.02	±0.00	±0.15	±0.00	±0.12	±0.22
	4.50	770.00	40.83	37.90	0.58	0.00	0.23	0.27	1.50	0.50
G	±0.20	±8.66	±0.58	±1.68	±0.02	±0.00	±0.03	±0.03	±0.10	±0.17
	4.70	768.33	41.67	38.40	0.60	0.00	0.25	0.26	1.60	0.37
H	±0.20	±16.07	±0.76	±0.95	±0.01	±0.00	±0.00	±0.06	±0.20	±0.12
	4.43	778.33	42.50	36.67	0.61	0.00	0.22	0.27	1.50	0.37
I	±0.21	±2.89	±1.32	±0.58	±0.03	±0.00	±0.12	±0.03	±0.40	±0.12

N = Six determinations.

TABLE 3.7: RESULTS OF ELEMENTAL ANALYSIS OF T_5

CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

Bio. Meth.	Na	K	Mg	Ca	Zn	Cd	Pb	Cu	Fe	Co
A	3.07 ±0.11	626.67 ±7.64	37.00 ±1.00	41.33 ±1.53	0.63 ±0.02	0.56 ±0.05	5.72 ±0.10	0.38 ±0.06	2.80 ±0.17	2.23 ±0.15
B	3.03 ±0.15	618.33 ±10.41	36.67 ±0.29	39.33 ±1.15	0.65 ±0.01	0.58 ±0.06	6.05 ±0.28	0.35 ±0.05	2.87 ±0.21	2.20 ±0.00
C	2.00 ±0.10	580.00 ±13.23	37.17 ±0.29	23.33 ±2.56	0.62 ±0.07	0.04 ±0.01	0.50 ±0.10	0.32 ±0.06	1.67 ±0.15	0.25 ±0.09
D	2.10 ±0.17	578.33 ±5.77	37.50 ±0.00	21.17 ±1.15	0.65 ±0.02	0.28 ±0.01	4.17 ±0.46	0.28 ±0.03	2.40 ±0.43	1.50 ±0.00
E	2.47 ±0.12	520.00 ±20.00	40.50 ±0.50	24.00 ±1.50	0.67 ±0.02	0.38 ±0.02	5.30 ±0.26	0.38 ±0.08	2.60 ±0.09	1.93 ±0.14
F	4.33 ±0.16	608.33 ±7.64	40.33 ±0.58	40.50 ±1.73	0.57 ±0.30	0.07 ±0.03	0.43 ±0.12	0.33 ±0.03	1.30 ±0.20	0.20 ±0.09
G	4.60 ±0.10	670.00 ±10.00	36.67 ±0.76	40.67 ±0.58	0.52 ±0.04	0.04 ±0.01	0.28 ±0.06	0.38 ±0.03	1.60 ±0.20	0.10 ±0.09
H	1.77 ±0.12	658.33 ±7.64	37.17 ±0.29	42.33 ±1.15	0.61 ±0.01	0.02 ±0.00	0.22 ±0.12	0.32 ±0.06	1.73 ±0.15	0.00 ±0.00
I	4.50 ±0.17	646.67 ±5.77	39.17 ±1.44	41.00 ±1.73	0.50 ±0.01	0.02 ±0.00	0.40 ±0.09	0.32 ±0.08	1.60 ±0.10	0.00 ±0.00

N = Six determinations

TABLE 3.8: RESULTS OF ELEMENTAL ANALYSIS OF T_6

CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

Dig. Meth.	Na	K	Mg	Ca	Zn	Cd	Pb	Cu	Fe	Co
A	3.15 ±0.21	658.33 ±14.43	41.17 ±0.58	35.67 ±0.58	0.61 ±0.00	1.98 ±0.12	6.35 ±0.38	0.35 ±0.05	3.07 ±0.58	1.98 ±0.12
B	2.93 ±0.06	638.33 ±12.58	42.33 ±0.29	36.10 ±1.15	0.63 ±0.02	1.95 ±0.08	6.12 ±0.33	0.37 ±0.03	2.67 ±0.58	1.95 ±0.09
C	2.10 ±0.10	572.50 ±16.39	40.83 ±0.76	26.00 ±0.87	0.63 ±0.01	0.43 ±0.15	0.33 ±0.08	0.32 ±0.03	1.70 ±0.10	0.43 ±0.15
D	1.97 ±0.12	598.33 ± 2.89	40.00 ±0.00	24.50 ±0.87	0.69 ±0.01	1.50 ±0.17	4.12 ±0.12	0.30 ±0.00	2.42 ±0.18	1.50 ±0.17
E	2.27 ±0.12	550.00 ±25.00	40.83 ±0.76	26.00 ±0.86	0.68 ±0.02	1.65 ±0.09	5.92 ±0.10	0.30 ±0.05	2.60 ±0.17	1.65 ±0.09
F	4.10 ±0.17	605.00 ±10.00	39.17 ±0.58	39.50 ±0.87	0.60 ±0.00	0.35 ±0.23	0.67 ±0.08	0.28 ±0.06	1.95 ±0.15	0.35 ±0.23
G	4.20 ±0.17	651.67 ±12.58	41.17 ±0.77	40.33 ±0.58	0.60 ±0.03	0.00 ±0.00	0.27 ±0.08	0.32 ±0.06	2.05 ±0.23	0.00 ±0.00
H	4.07 ±0.21	636.67 ±15.28	40.17 ±0.29	41.00 ±0.00	0.62 ±0.01	0.00 ±0.00	0.33 ±0.08	0.32 ±0.08	2.07 ±0.18	0.00 ±0.00
I	4.00 ±0.10	640.00 ±10.00	40.67 ±1.15	39.83 ±1.26	0.58 ±0.02	0.00 ±0.00	0.28 ±0.12	0.27 ±0.03	1.84 ±0.20	0.00 ±0.00

N = Six determinations

TABLE 3.9: RESULTS OF ELEMENTAL ANALYSIS OF T_7

CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

Mix. Meth.	Na	K	Mg	Ca	Mn	Cd	Pb	Cu	Fe	Co
A	3.37 ±0.15	751.67 ±7.64	40.00 ±0.00	42.33 ±1.15	0.59 ±0.02	2.20 ±0.00	4.93 ±0.32	0.27 ±0.08	2.40 ±0.30	2.20 ±0.00
B	3.10 ±0.17	768.33 ±16.07	41.67 ±0.29	39.83 ±1.26	0.63 ±0.02	2.10 ±0.10	5.17 ±0.15	0.30 ±0.05	2.30 ±0.09	2.19 ±0.10
C	2.33 ±0.12	716.67 ±10.41	41.00 ±1.00	23.83 ±1.25	0.61 ±0.01	0.77 ±0.06	0.65 ±0.17	0.25 ±0.05	2.05 ±0.17	0.77 ±0.06
D	2.17 ±0.06	740.00 ±13.23	40.83 ±1.44	24.50 ±0.87	0.62 ±0.03	1.65 ±0.17	2.68 ±0.03	0.32 ±0.03	2.30 ±0.08	1.65 ±0.17
E	2.40 ±0.10	700.83 ±8.78	41.00 ±0.87	23.50 ±0.86	0.58 ±0.01	1.90 ±0.17	3.53 ±0.28	0.32 ±0.03	2.25 ±0.00	1.90 ±0.17
F	4.20 ±0.10	750.00 ±10.00	41.00 ±0.87	41.33 ±1.53	0.60 ±0.01	0.40 ±0.10	0.45 ±0.13	0.30 ±0.05	2.20 ±0.09	0.40 ±0.10
G	4.30 ±0.17	770.00 ±8.66	38.83 ±1.26	39.83 ±2.02	0.56 ±0.02	0.05 ±0.00	0.33 ±0.08	0.25 ±0.00	2.13 ±0.10	0.00 ±0.00
H	4.27 ±0.15	753.33 ±18.93	40.33 ±0.58	42.33 ±1.15	0.60 ±0.00	0.12 ±0.06	0.32 ±0.06	0.32 ±0.06	2.18 ±0.12	0.12 ±0.06
I	4.10 ±0.20	761.00 ±12.77	40.50 ±0.87	41.33 ±0.58	0.60 ±0.02	0.10 ±0.05	0.33 ±0.08	0.33 ±0.03	1.90 ±0.17	0.07 ±0.03

N = Six determinations

TABLE 3.10: RESULTS OF ELEMENTAL ANALYSIS OF T_8
CONCENTRATION OF ELEMENT MEAN WITH S.D(ppm)

Dig. Meth.	Mn	K	Mg	Ca	Zn	Cd	Pb	Cu	Fe	Co
A	2.97	643.33	41.17	39.50	0.59	0.52	4.83	0.23	2.23	2.27
	±0.21	±11.55	±1.26	±1.80	±0.04	±0.02	±0.31	±0.03	±0.15	±0.23
B	3.07	631.67	41.33	40.33	0.64	0.54	5.47	0.18	2.03	1.98
	±0.12	±23.63	±1.26	±0.58	±0.01	±0.02	±0.35	±0.03	±0.21	±0.12
C	2.27	585.83	41.00	28.83	0.59	0.04	0.57	0.23	1.67	0.17
	±0.21	±10.10	±1.00	±3.51	±0.03	±0.01	±0.06	±0.10	±0.23	±0.08
D	2.13	591.67	40.17	25.50	0.54	0.41	2.68	0.23	1.77	1.50
	±0.12	±15.28	±0.29	±1.50	±0.02	±0.04	±0.03	±0.03	±0.06	±0.13
E	2.33	620.00	39.33	22.83	0.57	0.51	3.85	0.28	1.93	1.80
	±0.21	±13.23	±1.15	±2.02	±0.04	±0.02	±0.33	±0.08	±0.06	±0.09
F	3.93	633.33	40.00	39.50	0.63	0.02	0.62	0.33	1.67	0.22
	±0.25	±5.77	±1.50	±1.80	±0.02	±0.02	±0.12	±0.12	±0.23	±0.06
G	3.60	611.67	40.50	41.33	0.61	0.03	0.27	0.25	1.66	0.13
	±0.20	±11.55	±0.50	±1.53	±0.03	±0.00	±0.03	±0.05	±0.18	±0.03
H	3.23	598.33	41.60	40.17	0.62	0.02	0.23	0.25	1.67	0.12
	±0.21	±2.89	±0.66	±2.75	±0.03	±0.01	±0.21	±0.05	±0.23	±0.10
I	3.43	613.33	41.33	42.00	0.63	0.01	0.28	0.28	1.73	0.22
	±0.06	±14.43	±1.26	±1.73	±0.06	±0.00	±0.03	±0.10	±0.35	±0.10

N = Six determinations

3.1 STATISCAL TREATMENT OF DATA

3.1.1. Standard deviation (36)

This is a simple statistical process. It shows how reproducible a set of measurements is. It can be expressed in percentage or the unit of measurement, and is given by:-

$$S_D = \sqrt{\frac{(xi - \bar{x})^2}{N - 1}}$$

S_D = Standard deviation

$\bar{x}$ = Mean of all Individual measurements

xi = Any measurement in the set

N = Number of measurements.

The standard deviations for the methods in determining each metal are given in tables 3.1 to 3.10.

3.1.2. Multiple range tests (35,37).

The objective of using these methods is to help arrange the digestion methods into comparable subsets. A computer, Cyber 72, was used for this purpose.

The multiple range test is a systematic procedure for comparing all possible pairs of groups means. The groups are divided into homogenous subsets, where the difference in means of any two groups in a subset is not significant at some prescribed level.

The procedure is based on the test:-

$$(\bar{x}_i - \bar{x}_j) \qquad R(\text{alpha}, g, f) * S_{\bar{x}}$$

Where R(alpha, g, f) is a range based on a significance level (alpha), the number of groups in the subset (g), and the degrees of freedom (f). $S_{\bar{x}}$ is the standard error in the combined subset.

A significance level, alpha may also be considered as an error rate i.e. declaring two means to be unequal when they are infact equal.

Four out of the seven available tests are used, and they differ in how they define error rate.

Pre - comparison definition of error rate:

$$\text{Alpha} = \frac{\text{number of errors}}{\text{number of comparisons}}$$

Experimentally, error rate is defined as:-

$$\text{Alpha} = \frac{\text{number of experiment with error}}{\text{number of experiments.}}$$

The multiple range tests used for treating the results include:-

i) Least significant difference, (LSD) procedure.

ii) Modified least significant difference, (MODLSD) procedure.

iii) Duncan's procedure.

iv) Student - Newman - Keuls, (SNK) procedure.

The LSD is essentially a student's t - test between group means. It holds the pre- comparison error rate to alpha but is usually not recommended, since as the number of groups increases, so does the experiment wise error rate. LSD should not be used if the analysis of variance is not significant. LSD is exact for unequal group sizes.

The MODLSD uses a single range value for testing all differences, which is derived (as in LSD) from student's t - test. MODLSD however, uses a third definition of error rate given as:

$$\text{Alpha} = \frac{\text{number of errors}}{\text{number of experiments}}$$

and transforms t, accordingly MODLSD is exact for unequal group sizes.

Ducan procedure attempts to avoid the difficulty in LSD by using a different range value for subsets of different sizes. The larger the potential subset, the larger the difference in means must be, in order to be declared significant. The Duncan procedure also uses the concept of a special protection level rather than a significant level: the probability of finding a significant difference given that the two groups are infact equal, is less than or equal to the specified significance level. Duncan procedure may be used whether or not the analysis of variance is significant. Duncan procedure is only approximate if group sizes are equal.

SNK is very similar to Duncan, in that different range values are used for different size subsets. The SNK holds the experimentwise error rate to alpha for each stage of testing, procedure for tests involving the same number of means. The alpha is neither experimentwise nor pre-comparison. SNK is only approximate if the group sizes are equal.

The results of the treatment of the data using these procedures are shown in tables 3.11 to 3.14. The digestion methods included in the subsets considered to be best by each multiple range test procedure are tabulated. The tests were performed using alpha = 0.05, for each procedure. The order of how severe these procedures are in comparing data is as follows:-

$$\text{LSD} \rhd \text{Duncan} \rhd \text{SNK} \rhd \text{MODLSD} \quad (37)$$

TABLE 3.11: RECOMMENDED DIGESTION METHOD(S) FOR EACH METALLIC ELEMENT BY LSD PROCEDURE

	Na	K	Ca	Mg	Zn	Co	Cu	Pb	Fe	Cd	Mn
GS_1	H, I, F	F	H, G, I	D, F	D, G	A	-	B	A	B	A, D
GS_2	F	I, F	I,F, H	D, B, A	B	B	-	A	B, A	B	B
T_1	F	F, B, A	G, F	A,H,F,I B, C, D	E, F, A B	A	A, C, F	B	E	B, E, A	-
T_2	F	F,H.D,I	G, H, I	A,C,B,I	C, F, A D, B.	B, A	F, B, A	B	B, A	A, B	-
T_3	H, F	C, G, H B,F,I,A.	H, G	C,E,F,A B, D, I	All Methods	A, B	E, F, B, A	B, A	B	B	-
T_4	G, F, H	H, G, B, I, F	F,I,A,G, H	F, B, H C,I,D,E	A, B	A, E, B	D, B, E	B	B, A D, E	B	-
T_5	G, H	I, H, G	F, G, I, A, H	F, E	C, A. B, D, E.	B, A	C,H,I,F B,A,E,G	A, B	E, A, B	A, B	-
T_6	I, H, F G	H, B, I G, A	F, I, G, H, A	A, C, G, B	D	B, A	D,E,C,G, H, A, B	B, A	A	B	-
T_7	I, F, H,	F,A,I,H, G, B	F,I,A,H	H, I, D C,E,F,B	F, C, D, B	B, A	A,B,F,D, E, H, I	A, B	H,F,E,B, D, A	B	-
T_8	F	B, F, A	A, F, H, B, G, I	All Methods	C, A, G, H,I,F,B	A	A,C,D,G H,E,I,F	B	E, B, A	E, A, B	-

(-) means metal was not detected.

TABLE 3.12: RECOMMENDED DIGESTION METHOD(S) FOR EACH METALLIC ELEMENT BY MODLSD PROCEDURE

	Na	K	Ca	Mg	Zn	Co	Cu	Pb	Fe	Cd	Mn
GS_1	[illegible], I, F	F	F, H, G, I	A, E, F	F, D, G	A	-	A	A, B	B	A, D,
GS_2	[illegible]	I, F	G, I, F, H	H,D,B, A	E, B	B	-	B	E, B, A	B	B
T_1	[illegible]	H,C,I,E, C,F,B,A.	I, G, F	All Methods	C, D, E F, A, B	A	B,G,I,A, C, F.	A, B	B, A, E	B, E,A	-
T_2	C, [illegible], F	B,A,G,F C,H,D,I	A, F, G H, I	H,D,E,G A,C,B,F	H	B, A	A	A, B	B, A	A, B	-
T_3	E, F	I,C,G,H, B, F, A.	A, F, I, H, G	All Methods	All Methods	A, B	E,G,H,D, F, B, A	B, A	A, B	B	-
T_4	I,G,[illegible],H	A, H, G, B, I	B, F, I G, K	All Methods	I,E,F,A B	A, E, B	A,G,H,I D, B, E	B	B,A,D,E	B	-
T_5	[illegible], I, G H	B, A, I H, G,	B,F,G,I A, H	I, F, E	H,C,A,B, D,E	E, B, A	All Methods	A, B	D,E,A,B	A, B	-
T_6	I, [illegible], F, G	H, B, I, G, A	All Methods	I,E,A,G, G, B	E, D	E, B, A	All Methods	E, B, A	E, B, A	A, B	-
T_7	I,[illegible],[illegible], G	D,F,A,I, H, G, B	B, G, F, I, A, H	A,H,I,D, C,E,F,B.	I, A, H, F,C,D,B	E, B, A	All Methods	A, B	C,G,H,F, E,B,D,A.	A, B	-
T_8	G, [illegible]	G, I, E B, F, A.	A, F, H, B, G, I	All Methods	E,C,A,G A,I,F,B	B, A	All Methods		All Methods	E, A,B	

(-) means metal was not detected.

TABLE 3.14: RECOMMENDED DIGESTION METHOD(S) FOR EACH METALLIC ELEMENT BY DUNCAN'S PROCEDURE'S

	Na	K	Ca	Mg	Zn	Co	Cu	Pb	Fe	Cd	Mn
GS_1	H, I, F	F	H, G, I	B, F	B, G	A	-	B	A	B	A, D
GS_2	F	I, F	I, F, H.	D, B, A	B	B	-	A	B, A	B	B
T_1	F	F, B, A.	G, F	All Methods	D, E, F, A, B	A	A, C, F	B	E	B, E, A.	-
T_2	F	F,H,D,I	G, H, I	A, C, B, I	I, C, F, A,D,B,H	B, A	F, E, A	B	B, A	A, B	-
T_3	H, F	C,G,H,B, F, I, A	H, G	All Methods	All Methods	A, B	E, G, H, D,I,E,A.	B, A	A, B	B	-
T_4	I,G,F,H	A,H,G,B I, F	F,I,B,G H	F,B,H,C I,D,E.	A, B	A, E, B	A,G,H,I, D, E, E	B	B, A, D, E	B	-
T_5	G, H	I, H, G	F,G,I,A H	F, E	H, C, A B, D, E	B, A	All Methods	A, B	E, A, B	A, B	-
T_6	I,H,F,G	H, B, I G	All Methods	A, C, G, B	D	B, A	F,D,E,C, G,H,A,B	B, A	A	B	-
T_7	I,F,H,G.	F, A, I H, G, B	F,I,A,H	A,H,I,D C,E,F,B	A, H, F,C,D,B	B, A	All Methods	A, B	G,H,F,E, B, D, A.	B	-
T_8	F	E,B,F,A	A, F, H, B, G, I.	All Methods	C, A, G, H,I,F,E	A	A,C,E,G, H, I, F	B	E, B, A	E, A, B	-

(-) means metal was not detected.

TABLE 3.13: RECOMMENDED DIGESTION METHOD(S) FOR EACH METALLIC ELEMENT BY S.N.K. PROCEDURE

	Na	K	Ca	Mg	Zn	Co	Cu	Pb	Fe	Cd	Mn
GS_1	H, I, F	F	H, G, I	A, D, F	D, F	A	-	B, A	A	B	A, D
GS_2	F	I, F	I, F, H	H,D,B,A	B	B	-	A	B, A	B	B
T_1	F	E,G,F,B, A	G, F	All Methods	D, E, F	A	G,I,A,C, F	B	E	B, E, A	-
T_2	F	B,A,G,F, H, D, J	F,G,H,I	D,E,G,A, G, B, I	H	B, A,	E, F, B, A	B	B, A	A, B	-
T_3	H, F	C,G,H,B, F, I, A	F,I,H,C	All Methods	All Methods	A, B	E,G,H,D, F, B, A	B, A	A, B	B	-
T_4	I,G,F,H	A,H,G,B, I, F	B,F,I,A G, H	All Methods	A, B	A, E, B	A, G, H I,D,B,E	B	B, A, D	B	-
T_5	I, G, H	I, H, G	B,F,G,I, A, H	I, F, E	H, C, A, B, D, E	B, A	All Methods	A, B	D, E, A, B	A, B	-
T_6	I, H, F	H, B, I, G,A	All Methods	I,E,A,C G,B,	D	B, A	All Methods	B,A	A	B	-
T_7	I, F, H, G	D, F, A, I,H,G,B	B,G,F,I, A, H	A,H,I,D, G,E,F,B	I,A,H,F C, D, B	B, A	All Methods	A, B	C, G, H, F,E,B,D,A.	B	-
T_8	F	G,I,E,B, F, A	A,F,H,B, G,I	All Methods	E, C, A, G,H,I,F	A	All Methods	B	All Methods	E, A, B	-

( ) means metal was not detected

3.2. DISCUSSION

For the purpose of discussion, frequency tables have been constructed from Tables 3.11 to 3.14, as shown in Tables 3.15 to 3.18. These tables are constructed on the basis of the number of elements in a sample which a particular digestion method is capable of determining reliably i.e. as recommended by the multiple range tests. Results from this treatment are different for each procedure. The LSD procedure which is the most commonly used of all the procedures, is used for discussing the data obtained. This is because the LSD is the most severe in comparing data, and the simplicity of the procedure. The other methods MODLSD, Duncan and SNK, appear to be more liberal in their comparisons, more methods of digestion are recommended for each metal determined. However, the order of how reliable these methods are still remains the same for all the procedures. Therefore the procedure that is strictest in its comparison may be considered as the best, since it narrows down the number of recommended digestion methods.

Sample GS_1

For sample GS_2, five digestion methods are recommended for reliably determining all the metals. Method A is recommended for Co, Fe, Mn, method B, for Pb and Cd, method D for Mg, Zn, M, method F for Na, K, Mg, and method I for Na and Ca. On the basis of the Standard deviation and mean values in table 3.1, method F is more suitable for the determination of Na and Mg than methods I and D respectively. Also, method D is more suitable for the determination of Mn than method A. For sample GS_1 therefore methods A and D determined the essential trace metals (Fe, Co, Mn, Zn), while methods F and I determined the bulk essential metals (Na, K, Ca, Mg).

TABLE 3.15: Reliability test for the digestion methods showing number of metals for which a method is recommended by the LSD treatment

	Digestion methods								
Sample	A	B	C	D	E	F	G	H	I
GS_1	3	2	0	3	0	3	2	2	2
GS_2	3	6	0	1	0	3	0	1	2
T_1	6	5	2	1	3	6	1	1	1
T_2	6	7	2	2	0	4	1	2	3
T_3	7	8	3	2	2	5	3	4	3
T_4	4	8	1	3	4	4	3	4	3
T_5	7	6	2	1	4	3	4	4	3
T_6	7	6	2	2	1	2	5	4	3
T_7	6	8	2	3	3	7	2	6	5
T_8	8	7	3	2	4	6	4	4	4

TABLE 3.16: Reliability test for the digestion methods showing number of metals for which a method is recommended by the MCDLSD treatment

	Digestion methods								
Sample	A	B	C	D	E	F	G	H	I
GS_1	5	2	0	2	1	5	2	2	2
GS_2	3	7	0	1	3	3	1	2	2
T_1	8	7	4	2	5	6	4	2	4
T_2	8	7	2	2	2	4	4	5	3
T_3	8	8	3	4	3	6	5	6	3
T_4	6	9	1	3	5	4	5	5	6
T_5	8	8	2	3	5	4	4	4	5
T_6	8	8	3	3	7	3	5	4	5
T_7	9	9	4	5	4	7	5	7	6
T_8	9	9	4	3	6	7	7	5	6

TABLE 3.17: Reliability test for the digestion methods showing number of metals for which a method is recommended by the Duncan treatment

	Digestion methods								
Sample	A	B	C	D	E	F	G	H	I
GS_1	3	4	0	1	0	3	2	2	2
GS_2	3	6	0	1	0	3	0	1	2
T_1	6	5	2	2	3	6	2	1	1
T_2	6	7	2	2	0	4	1	3	4
T_3	7	8	3	3	3	5	5	6	3
T_4	5	9	1	3	4	4	4	5	5
T_5	7	6	2	2	4	3	4	5	3
T_6	7	7	3	3	2	3	5	4	3
T_7	8	8	3	4	3	7	4	7	5
T_8	8	7	3	2	4	6	4	4	4

TABLE 3.18: Reliability test for the digestion methods showing number of metals for which a method is recommended by the SNK treatment

Sample	Digestion methods								
	A	B	C	D	E	F	G	H	I
GS_1	5	2	0	3	0	4	1	2	2
GS_2	3	6	0	1	0	3	0	2	2
T_1	6	5	2	2	5	6	4	1	2
T_2	6	7	1	2	2	4	3	2	3
T_3	7	8	3	3	3	6	5	6	4
T_4	7	9	1	2	4	4	5	5	5
T_6	7	7	3	3	3	3	5	4	5
T_7	8	9	4	5	3	7	5	7	6
T_8	8	8	4	3	6	7	6	5	6

Method B determined Pb and Cd reliably. These methods are fast, except for method I where soaking for about 15 hrs prior to digestion was necessary. The methods are safe and did not produce any insoluble precipitate.

Sample GS_2

Method B was able to determine most of the trace metals (Mn, Zn, Co, Fe and Cd) and Mg in sample GS_2. Method F also, was able to determine the bulk metals Na, K, and Ca reliably and method A was able to determine Pb, Mg and Fe reliably. From table 3.2, method B has a higher mean value for Mg and Fe compared with method A, this means that method B was more suitable for these metals. Method A could be used specifically for Pb. Combination of methods A, B and F should determine all the metals analyzed reliably in sample GS_2. No precipitate was formed with methods A and B, but C precipitate which was soluble in concentrated HNO_3 was formed with method F. The methods are fast and have low reagent blank values.

Sample T_1

Methods A, B and F are recommended for the determination of all metals analyzed in sample T_1. Method A is recommended for K, Mg, Zn, Co, Cu and Cd. Method B is recommended for K, Mg, Zn, Pb, and Cd, and method F for Na, Ca, Mg, Zn and Cu. From table 3.3, method B, determined Mg, Pb and Zn most reliably and therefore could be used for the determination of these metals. Method A determined Co and Cd most reliably, while method F determined Na, K, Ca and Cu most reliably. Methods A, B, and F, if used should determine reliably, all the metals analyzed in sample T_1. These digestion methods are safe, fast and have low reagent blank values.

Sample T_2

Method B is recommended for the trace metals (Co, Fe, Cu, Cd, Pb, Zn) including Mg. Method A is recommended for five trace metals, (Zn, Co, Cu, Fe, Cd) and Mg, while method F is recommended for Na, K, and Zn. However, from table 3.4, method A shows the highers mean for Co, Cu, and Fe, while method B shows the highest mean for Mg, Zn, Cd, and Pb. Method I shows the highest mean for Ca. The combination of these methods, A (for Co, Cu, Fe), B (for Mg, Zn, Cd, Pb), F (for Na, K) and I (for Ca), should determine these metals reliably in sample T_2. These methods are fast, except for method I, where the sample was soaked in concentrated nitric acid for about 15hrs. before digestion. They also have low reagent blank values.

Sample T_3

Four digestion methods are recommended for this sample. Method A is recommended for the determination of K, Mg, Zn, Co, Cu, Pb and Fe. Method B is recommended for K, Mg, Zn, Co, Cu, Pb, Fe and Cd. Method F is recommended for Na, K, Mg, Cu and Zn. Method H appears to be specific for Ca. However, from results in table 3.5, method A determined Mg and Pb most reliably, method B determined Co, Cu, Fe and Cd most reliably and method F determined Na, K, and Zn most reliably, based on the results of the mean and standard deviation values. Combination of these four digestion methods should determine metals analyzed reliably in sample T_3. These methods are fast and safe. They have low reagent blank values.

Sample T_4

Method A is recommended for five metals, (Ca, Mg, Zn, Co, and Fe). Method B is recommended for eight metals, (K, Ca, Mg, Zn, Co, Cu, Fe, Pb), method E for Mg, Co, Cu, Fe and method F for Na, K, Ca, and Mg.

From table 3.6, Na, K and Ca were determined most reliably by method E compared to other recommended methods. Actually method H had the highest mean for Ca only. Method B determined Zn, Co, Cu, Pb, Fe and Cd reliably, while method A determined Mg reliably. Method E determined Fe and Cu reliably. Method B and E determined Cu equally well and can be used as alternatives to each other if Cu is to be determined in sample T_4. Of all the methods recommended for T_4, method E was the fastest and it gave the clearest solution, though a precipitate which was soluble in water was formed. All the methods have low reagent blank values.

Sample T_5

For this sample, method A is recommended for Mg, Cu, Zn, Co, Cu, Pb, Fe and Cd. Method B is recommended for Zn, Cu, Pb, Fe, Cd, method G for K, Na, Cu, method E for Mg, Zn, Cu and method H for Na, K, Ca and Cu. From table 3.7, and considering the mean and reproducibility of each method, Co was determined most reliably by method A. Method B determined Pb, Fe, and Cd most reliably. Method H was found most reliable for Na and Ca, while method G was most suitable for K and Cu. Combination of methods A, B, E, G, and H should determine these metals reliably in sample T_5. All the recommended methods are safe, the methods are fast, except for method G that was slow and that contained apparently indestructible fatty tissue.

Sample T_6

For this sample, method A is recommended for the determination of K, Cu, Mg, Co, Cu, Pb and Fe; method B for K, Mg, Co, Cu, Pb and Cd. Method G is recommended for Na, K, Ca, Mg and Cu. From table 3.8, method A was found most suitable for K, Ca, Co, Pb and Fe. Method B was found most reliable for Mg, Cu, and Cd, while method G was found to be specific for Na. Combination of these digestion methods should determine

all metals analyzed reliably in sample T_6. These methods apart from G, are fast without any insoluble components.

Sample T_7

Five digestion methods are recommended for this sample. Method A is recommended for K, Ca, Co, Cu, Pb and Fe. Method B is recommended for K, Mg, Zn, Co, Cu, Pb, Fe and Cd. Method H is recommended for Na, K, Ca, Mg, Cu, Fe, and method I for Na, K, Cu, Mg and Cu. Though method F is recommended for seven metals, but on the basis of the mean and standard deviation values in table 3.9, for these metals, method F cannot be recommended for the determination of these metals i.e. Na, K, Ca, Mg, Zn, Cu, and Fe. Method A was found to be the most reliable for Co and Fe. Method B was found most suitable for K, Mg, Zn, Cd, and method H for Na and Ca. Method I was specific for Cu. Combination of these methods should determine the metals reliably from sample T_7. All these methods are fast, safe and have low reagent blank values.

Sample T_8

For this sample, method A is recommended for K, Ca, Mg, Zn, Co, Fe, Cd, and method B for K, Ca, Mg, Zn, Pb, Fe and Cd. Method F is recommended for Na, K, Ca, Mg, Zn, Cu, and method I for Ca, Mg, Zn, and Cu. From the results in table 3.10, method A was most suitable for K, Co, Fe and method B for Mg, Zn, Pb and Cd. Method F was most suitable for Na and Cu, while method I was most suitable for Ca. These digestion methods have the highest mean for the metals they are recommended for, even when the standard deviation is substracted from the initial mean.

The other procedures of statistical treatment of results i.e. MODLSD, SNK and Duncan appear to be less severe in the treatment of data, compared with the LSD procedure, as shown in tables 3.15 to 3.18.

Using the MODLSD procedure, methods A and F are recommended for the determination of five metals each in sample GS_1. Method B is recommended for two. In sample GS_2, methods A and B are recommended for the determination of three and seven metals respectively. Method E which has no metal recommended to it by the LSD procedure has three metals recommended to it by MODLSD. For all tomatoe samples i.e. T_1 to T_8, more methods are recommended by MODLSD. The Duncan procedure is the next most severe. The results obtained from this treatment are similar to LSD procedure, but more methods are recommended for certain metals in some cases. The results obtained for sample GS_2 are the same for both the Duncan and LSD procedures, but they differ from the remaining two procedures. The results obtained from SNK procedure are similar to those obtained from MODLSD. The samples for which each method of digestion is recommended by multiple range tests are given in table 3.19. The methods that could determine at least five out of the eleven metals in a given sample are considered.

TABLE 3.19: Summary of Samples among those studied for which specific methods of digestion are Recommended by multiple range tests.

Procedure	Digestion methods								
	A	B	C	D	E	F	G	H	I
LSD	$T_1 - T_4$ $T_5 - T_8$	GS_2 $T_1 - T_4$ $T_5 - T_8$	N/S	N/S	N/S	T_1, T_3, T_7 T_8	T_6	T_7	T_5
MODLSD	GS_1 $T_1 - T_4$ $T_5 - T_8$	GS_2 $T_1 - T_4$ $T_5 - T_8$	N/S	T_7	T_1, T_4, T_5 T_6, T_8	GS_1 T_1, T_3, T_7 T_8	T_3, T_4 T_6, T_7, T_8	T_2, T_3, T_4 T_7, T_8	$T_4 - T_8$
DUNCAN	$T_1 - T_4$ $T_5 - T_8$	GS_2 $T_1 - T_4$ $T_5 - T_8$	N/S	N/S	N/S	$T_1, T_3,$ T_7, T_8	$T_3, T_6,$	$T_3 - T_5$ T_7	T_4, T_5
SNK	GS_1 $T_1 - T_4$ $T_5 - T_8$	GS_2 $T_1 - T_4$ $T_5 - T_8$	N/S	T_7	T_1, T_8	T_1, T_3 T_7, T_8	$T_3, T_4,$ T_7, T_8	$T_3 - T_8$	$T_4 - T_8$

N/S = Not Selected.

From the discussion so far, and from table 3.19, method A, B, and F proved to be the most versatile of all the digestion methods used for the determination of the group of metals studied. For convenience of further discussion, these metals are divided into three categories viz:- essential bulk, essential trace and non-essential trace metals.

It would be noticed from tables 3.11 to 3.14, that method F was the best for the determination of the bulk metals i.e. Na, K, Mg and Ca in almost all the samples. This method uses a mixture of NH_4NO_3 and HNO_3. This result is consistent with the results obtained by milton and Hoskins (14), who recommended the method for the determination of bulk metals in biological samples that are not too rich in organic matter. Other alternative methods include those that use nitric acid alone or in combination with hydrochloric acid, i.e. methods G, H and I. These methods including F lead to the formation of the metal nitrates except for I which may contain some chlorides. The nitrates of these metals are very soluble, as shown in the solubility table given by Stark and Wallace (39). The chlorides of these metals are also soluble but not as soluble as the nitrates. The nitrates are not volatile at the temperature used for these digestion methods. The methods involving the use of perchloric acid i.e. methods C, D and E led to losses of the bulk metals especially Calcium. This may be due to the formation of volatile perchlorates of these metals. Gerritz (16) however, reported that the use of HNO_3-$HClO_4$ was reliable for the determination of calcium in feed concentrates and some grasses. Perchloric acid however, did not affect the recovery of Magnesium in most of the samples. This may be due to the fact that magnesium perchlorate is soluble and stable to heat below 250°C (40). No precipitate of calcium sulphate was observed when sulphuric acid was included in the digestion mixture. This is probably

because Ca was not present in a concentration that could cause precipitation of calcium sulphate. In other words, the solubility product of $CaSO_4$ was not exceeded.

The essential trace metals i.e. Fe, Zn, Mn, Co and Cu were determined most reliably by methods A and B, for all the samples. These two methods use sulphuric acid together with nitric acid. These metals may be present in different forms in the organic matrices. Depending on the forms in which they are in the matrix, they may or may not be easily released for subsequent determination. The use of sulphuric acid in these methods may help the digestion mixture by: (i) degrading the organic matter, (ii) creating dehydrating conditions necessary for oxidation, and (iii) increasing the oxidation temperature. In the methods where nitric acid was used alone, partial or total losses in Co, Mn and Fe were observed. This may be due to the formation of organometallic derivatives of these metals, which are either volatile or resistant to further oxidation by HNO_3 at the temperature used i.e. 100°C. With method H (HNO_3/HCl), for instance, $FeCl_3$ may be formed. This may then combine with nitric acid to form $Fe(NO_3)_3Cl$ which is volatile. This compound is prepared under laboratory conditions by heating $FeCl_3$ and HNO_3 together (40). It is doubtful if the nitrates of the essential trace metals are formed, when methods G, I and H are used. This is because the nitrates of these metals are soluble and not volatile at the digestion temperatures. The explanation to the inability of digestion methods G, H and I to determination these metals reliably, may be the formation of volatile or indestructible organic derivatives.

Again methods A and B determined the non-essential trace elements, Pb and Cd most reliably. One would expect the precipitation of $PbSO_4$ from solution, but this was not observed for all the samples studied. Lead sulphate is only sparingly soluble in acidic medium, and it tends to co-precipitate with $CaSO_4$ in calcium rich materials. Losses of lead was observed for the remaining digestion methods. The reasons are similar to those given for essential trace metals. Most cadmium salts are volatile, and they can be lost as volatile nitro -- complexes. Losses of Cd was reported by Gorsuch (18), when H_2SO_4 was not present in the digestion mixture used to destroy organic matter in cocoa samples.

3.3. CONCLUSIONS AND RECOMMENDATIONS

The results obtained from the treatments of data in tables 3.11 to 3.18 have shown that no single method of wet digestion is able to release all the metals studied in a sample quantitatively and reliably. Various methods have to be used. The choice of methods to be used for digestion depends on the type of material to be digested and the metals to be determined. The results also show that losses of lead may not be due to co-precipitation of $PbSO_4$ on $CaSO_4$ alone, but also by other means like volatilization or formation of resistant organolead compound. Perchloric acid, though a powerful oxidizing agent, led to losses of many metals. From table 3.19, methods involving the use of perchloric acid i.e. C, D and E are the least recommended by all multiple range tests performed on the data.

From results obtained from this study, it is recommended that, more than one digestion method should be used in the determination of metallic elements from biological samples. For vegetable samples such as those analyzed, methods A, B and F are most reliable for the determination of metals studied. Methods A and B could be used for trace metals while method F is suitable for the bulk metals.

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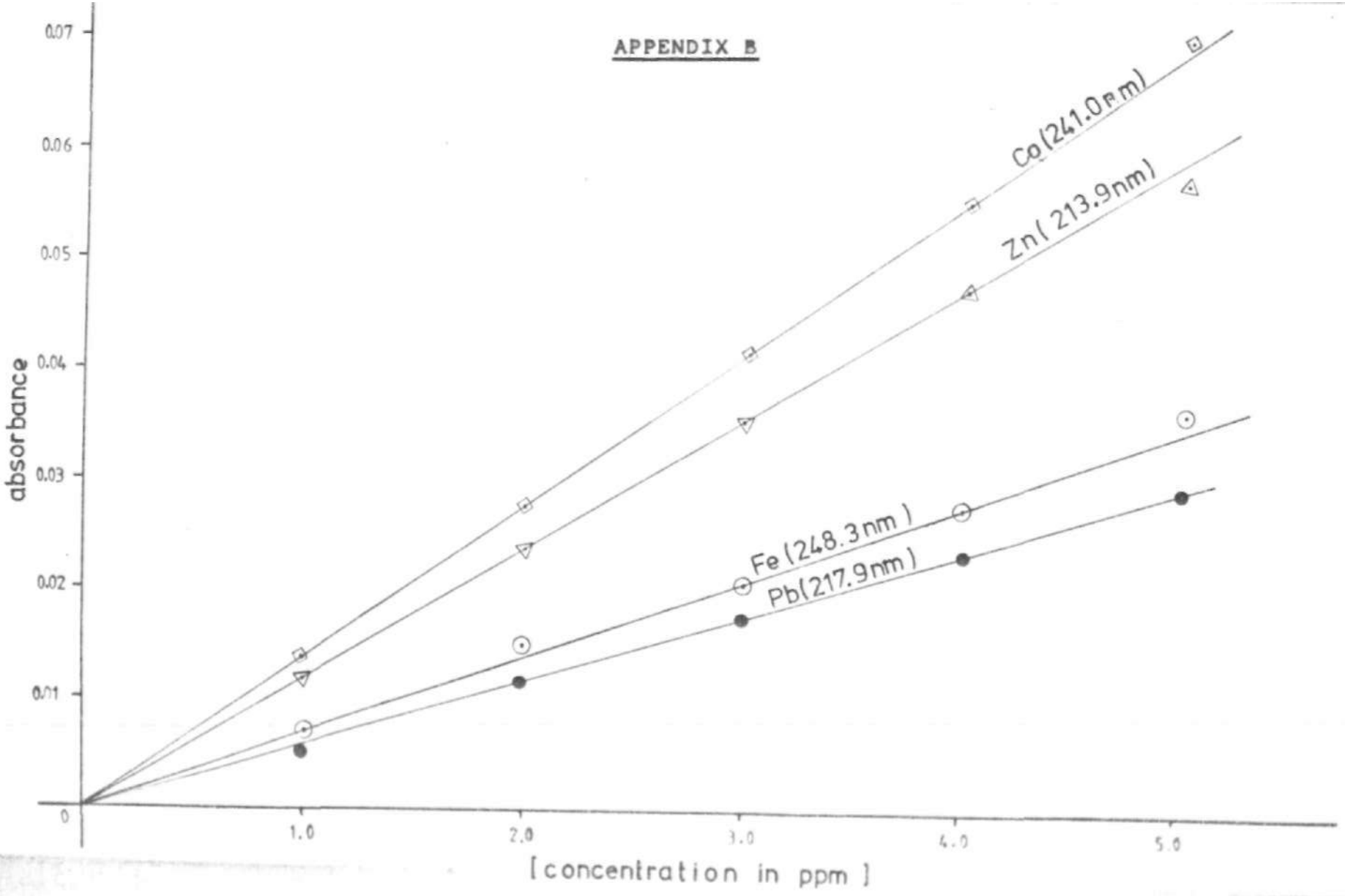
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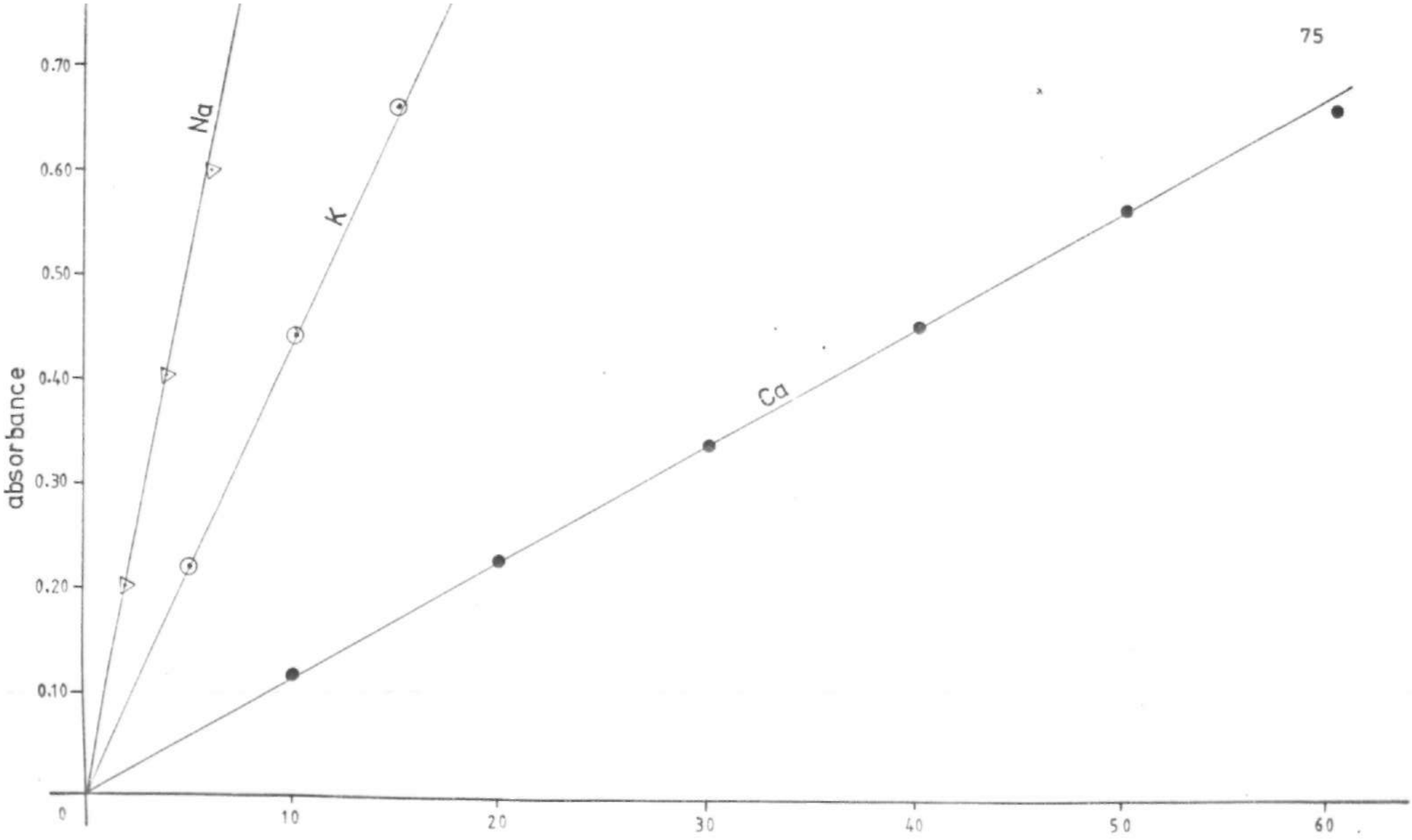
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SAMPLE	MOISTURE CONTENT AND STANDARD DEVIATION IN PERCENTAGE
GS_1	66.87 ±0.34
GS_2	64.55 ±0.54
T_1	92.69 ±0.27
T_2	93.53 ±0.46
T_3	92.68 ±0.62
T_4	93.19 ±0.25
T_5	91.35 ±0.12
T_6	92.82 ±0.21
T_7	92.61 ±0.31
T_8	92.44 ±0.16

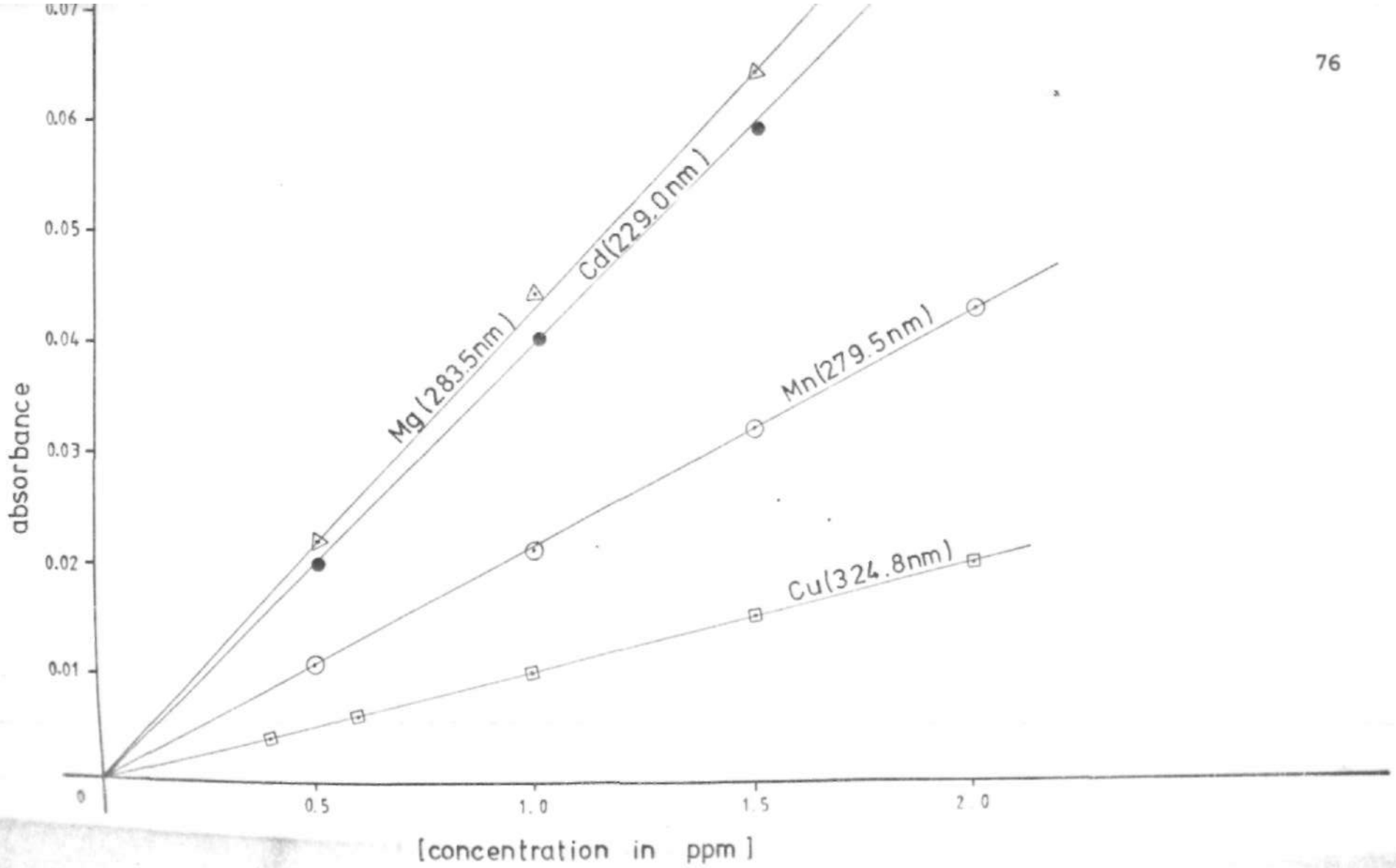
APPENDIX B



Calibration curves for: Cobalt (Co), Zinc (Zn), Iron (Fe), Lead (Pb).



Calibration curves for: Calcium (Ca), Sodium (Na) and Potassium (K).



Calibration curves for: Magnesium(Mg), Manganese(Mn), Cadmium(Cd) and Copper(Cu)