FACTORS IN THE DETERMINATION OF CALCIUM, COPPER, IRON, SODIUM AND PHOSPHORUS IN SOME ECOLOGICAL MATERIALS.

By

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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.

MR. JOB .A. [signature]
CANDIDATE

Date: 30/3/83

DEDICATION

This work is fondly dedicated to my kind and loving parents and to my family in general.

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ABSTRACT

The effects of heating temperature, time and acid-mixture in the determination of calcium, Copper, Iron, Sodium and phosphorus in some soil and plant samples by wet-digestion using Atomic absorption spectrophotometer, flame photometer and UV spectrophotometer have been studied. The values of Iron and Sodium obtained tend to increase with increase in temperature, while calcium and phosphorus show a decrease with increase in the digestion temperature. The copper values appear essentially independent of the heating temperature within the range studied. A comparative analysis by dry ashing at two temperatures agrees with the above trends.

Perchloric-nitric acid mixture gave the highest values for calcium and phosporus while the highest values of sodium were obtained using nitric-sulphuric acid mixture at the same temperature. The values of copper and Iron obtained were not significantly affected by the change in acid mixture. The values of sodium obtained by Atomic Absorption in the presence of an ionization buffer were comparable to those obtained by the flame emission technique.

It is suggested that the use of the same digest solution to determine several minerals, as is often done, may lead to errors, and optimum conditions should be determined for each mineral in accurate analysis.

TABLE OF CONTENTS

Table of Contents (cont'd)

Table of Contents (cont'd) Page

Table of Contents (cont'd) PAGE

CHAPTER IV

LIST OF TABLES

LIST OF FIGURES

APPENDIX

CHAPTER I

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

1.1 RESEARCH AIMS AND OBJECTIVES:

This project is designed to study factors which influence the levels of minerals such as calcium, copper, iron, sodium and phosphorus recovered from some ecological materials. An essential preliminary step in the determination of mineral elements in ecological material is the decomposition of the organic matter, either by wet digestion, dry ashing or fusion. Although, the choice of procedure is still largely a matter of personal preference, for some minerals (e.g. Phosphorus, mercury and Arsenic), wet digestion has been found (3) more suitable to minimize volatilization losses. Efforts have been directed towards reducing defficiencies of the conventional digestion procedures. However, the new digestion methods often show no advantage in precision or accuracy over the conventional method, but are known to be easy, fast, safe and require virtually no equipment (43).

Apparently, many of the published procedures are rather vague about the heating conditions during wet digestion stage. For instance, the requirement that "digestion mixtures be heated initially at moderate heat, with a latter increase or until white fumes of sulphuric acid are evolved" (1) appear inadequate.

Therefore, the factors involved in wet digestion need to be clearly specified for various classes of ecological materials. In the course of this work, we intend to examine the effects of heating time, temperature and the nature of acid mixture on the levels of calcium, copper, iron, sodium and phosphorus recovered from both plant and soil samples.

1.2 OCCURENCE AND PROPERTIES OF THE MINERALS.

1.2.1 CALCIUM.

The element, calcium, is widely distributed in minerals and in the sea. It occurs in substantial deposit such as dolomite, $CaCO_3.MgCO_3$ and calcium is the third most abundand metal terrestrially (13, 39). The calcium content in chalk and limestone soils may be 30% or more. In most non-calcareous soils, the forms of calcium are more variable but it is still an important soil element. Certain types of soils have significant amounts of calcium phosphate or sulphate and many contain calcium in feldspars, amphibolites and various clay minerals. In very acid soils however, the levels may be relatively low (1). In vegetation, calcium is essential for apical and root tip development and accumulates in cell walls as calcium pectate. It is one of the dominant elements in the skeletal structures of many animal groups.

Some waters are relatively rich in calcium and tests for hardness (which also include magnesium) are essential for water treatment. The concentrations of calcium usually encountered (1) in soils range from 0.1 - 2.0%, while in plant materials they lie between 0.3 to 2.5%.

1.2.2 COPPER

Copper is widely distributed in nature as metal, in sulphides, arsenides, chlorides and carbonates. It is extracted by oxidative roasting and smelting or by microbial-assisted leaching followed by electro-deposition from sulphate solutions. Copper is generally high in soils derived from igneous rocks and tends to be lower in extreme acid and alkaline soil. The concentration ranges normally encountered are 5 - 100 $\mu g\ g^{-1}$ and 2.5 - 25 $\mu g\ g^{-1}$ in soils and plant materials respectively (1).

Copper is attacked by halogens but is unaffected by non-oxidizing and complexing dilute acids in the absence of air. Copper readily dissolves in nitric acid and sulphuric acid in the presence of oxygen. It is also soluble in ammonia or potassium cyanide (13).

1.2.3 IRON

Iron is the second most abundant metal, after aluminium and the fourth most abundant element in the earth's crust. The core of the earth is believed to consist mainly of iron and nickel and the occurence of many iron meteorites suggests that it is abundant throughout the solar system; hence iron is a major component of most soils. The predominant iron minerals are the oxides such as the hematite, Fe_2O_3; magnetite, Fe_3O_4; but iron is also present in many other minerals notably limonite FeO(OH); carbonates e.g. siderite, $FeCO_3$; micas, amphibolites and clay (1, 3, 13, 38). The concentration ranges normally encountered are (1), 0.5 to 10% and 40 - 500 $\mu g\ g^{-1}$ in soils and plant materials respectively.

Iron dissolves readily in dilute mineral acids. With non-oxidizing acids and in the absence of air, Iron(II) is obtained but with air present or warm dilute nitric acid, some of the Iron goes to Iron(III). Very strongly oxidizing media such as concentrated nitric acid or acids containing dichromate passivate Iron. Air-free water and dilute air-free hydroxides have little effects on the metal, but hot concentrated sodium hydroxide attacks it (13).

1.2.4 SODIUM.

Although sodium is present in certain minerals such as feldspars and amphibolites,, the levels in non-saline soils are relatively low. It occurs principally as halide forming extensive beds by the evaporation of enclosed bodies of salt water subsequently burried by other sedimentary deposits (1,38). The concentration ranges normally encountered (excluding saline samples) are, 0.02 - 1% and 0.02 - 0.3% in soils and plant materials respectively (1).

1.2.5 PHOSPHORUS.

Phosphorus occurs in various orthophosphate minerals notably fluorapatite, $3Ca(PO_4)_2.CaF,Cl)_2$. Phosphorus resembles nitrogen in the soil in that a study of both organic and mineral forms is necessary to understand its significant for soil fertility. Much attention has been paid to the various mineral forms in soil which range from complex calcium phosphates (including apatite) particularly in neutral and basic soils to the hydroxy phosphates of aluminium and Iron which are more frequent in acid soil, (1, 3). An additional complication is the tendency for labile phosphorus to become fixed as insoluble phosphate which limits its availability to plants. Therefore the determination of "available" phosphorus is not straight forward.

Organic phosphorus accounts for well over half the soil total and include phytates, nucleic acids and phospholipids derived from plant materials. The major source of phosporus in water courses, lakes and even sea, is the build up of phosphates from excessive use of fertilizers, detergents and various industrial processes. The concentration ranges normally encountered are 0.01 - 0.2% and 0.05 to 0.3% in soils and plant materials respectively (1).

1.3 WET DIGESTION AND DRY ASHING.

One of the major steps in mineral analysis that must be carefully selected by the analyst is that of the best method for the decomposition of the samples, for few of these will be water soluble. It is a selection that must be properly made, because it sets the course of the subsequent analytical work and can save many unnecessary steps (1, 2, 3). However, no general rule regarding the most advantageous choice can be given, because the choice is governed by both the nature of the sample and the nature of the analytical work which is to follow. Obviously, the method must;

effectively decompose the sample, though, depending upon the ultimate goal of the analysis effective decomposition does not necessarily imply complete decomposition;

ensure that the introduction of large quantities of materials which may be troublesome in subsequent stages of the analysis is avoided;

ensure that the decomposition medium would not seriously attack the vessel, with the introduction of potentially troublesome material into the analysis.

The method should not result in the loss of constituents by volatilization when the determination of these constituents is to be followed, and should be reasonably fast and safe as well as enabling the handling of many samples for analysis.

1.3.1 WET DIGESTION.

The wet digestion method involves the use of acids, both oxidizing (nitric acid, perchloric acid, hot concentrated sulphuric acid), and non-oxidizing (hydrochloric acid, hydrofluoric acid, phosphoric acid, hydrobromic acid, dilute sulphuric acid and dilute perchloric acid). Relatively little use is made of sulphuric acid alone to decompose ecological materials, chiefly because of the formation of insoluble sulphates which are often very difficult to redissolve. However, as a mixture with other adids, it finds many uses.

Its high boiling point (about 340°C for the 96% m/m acid) enables it to expel most other volatile compounds including fluorides and its dehydrating property prevents the drying off of the digestion mixture (3). The decomposition of sulphides is the most common application of strongly oxidizing nitric acid, however, it is generally ineffective with oxides and because of its low boiling (86°C), excess nitric is easily expelled.

The almost universal solubility of perchlorates (with notable and useful exception of K, Rb and Cs perchlorates), favours the use of perchloric acid for wet oxidation and it has replaced sulphuric acid in popularity, even though the latter is often more efficient (3, 4). It is one of the strongest acids known, being a very powerful oxidant when hot and concentrated. Mixture of hot concentrated sulphuric and perchloric acids have an even greater oxidizing power. However, because of this great oxidizing power, it has not found much use in the decomposition of samples containing hydroxyl group due to the exploisive nature of the reaction.

The use of wet digestion method has been strongly recommended because it has several advantages. The method, for instance, has been found (1, 3) to be fairly

rapid, easily adopted for routine analysis and it is possible to determine many elements in a single digest solution. Wet digestion at a pre-selected temperature has been found to minimize the mineral losses resulting from volatilization especially when the analysis of elements such as phosphorus, Arsenic, mercury and others is to follow. The method also prevents the introduction of large quantities of materials which may be troublesome in subsequent stages of the analysis because, the excess acids can usually be more readily removed. The attack by the decomposition medium on the vessel with the introduction of potentially troublesome materials into the analysis is minimized because the attack on the vessel is usually negligible due to the lower temperature usually employed as compared to dry ashing.

However, there are several disadvantages of the wet digestion procedure. The procedure is less suitable for the determination of trace mineral elements, since large sample weights require large volumes of acid for complete oxidation. This may give rise to a high reagent blank. The use of various acid mixture and the possibility of precipitation cannot be ignored. For instance, it has been found (1) that potassium may precipitate as perchlorate and calcium as sulphate.

In addition, the precipitation of insoluble substances such as sulphates and phosphates and the attendant chemical interactions such as adsorption and co-precipitation of minerals of interest cannot be over-emphasized. Finally, but perhaps the one which causes most concern to the analyst, is the danger of explosion which may result from the incorporation of strongly oxidizing acids such as perchloric acid. However, since the effective decomposition of organic component of most ecological materials requires the inclusion of at least an oxidizing acid, efforts have been made (1, 2, 3) to reduce or eliminate the possibility of explosion where such acids are involved. Some of these precautions include; working on a small scale as much as possible; not digesting materials high in fat or oily substances or anything containing hydroxy groups with any strong oxidizing acid. Finally, materials containing large amount of organic matter should be pre-heated first by igniting before the acid digestion procedure, especially if such an additional step will not adversely affect the subsequent analysis to follow.

1.3.2 DRY ASHING.

The method involves complete combustion of all the organic matter followed by the dissolution of the mineral constituents using hydrochloric acid. The combustion is preferably carried out in a muffle furance which allows greater control than is possible using a gas burner (1). The method has many advocates and is attractive because large samples can readily be handled and reagent blanks are generally low so it is advantageous for trace element work. It is relatively simple, safe, fast and demands less attention as compared to wet oxidation. However, the dry ashing method also has several set backs. For instance, low recoveries of specific elements either as a result of volatilization losses or retention mechanism has been reported by many workers (1). The literature contains contradictions regarding alleged losses mainly because the factors controlling losses are not always assessed. These factors include; the temperature, time of ignition, sample composition, and the structure of the crucible. Volatilization losses are aggravated by violent deflagration with local overheating which must be avoided. Samples should preferably be placed in the muffle cold and the temperature allowed to rise slowly.

The use of oxidative additives such as nitric acid (to facilitate oxidation) or magnesium nitrate (to raise base status) should be cut to a minimum. However, for the estimation of non-metals generally, it is necessary to raise the base status of the sample and so inhibit the formation of volatile acidic constituents of the element concerned. Thus, for phosphorus and sulphur a magnesium salt is often added while for chlorine and boron calcium oxide is required. For some metals such as lead, volotilization losses have been found (1) to occur according to the composition of the sample, however, this could be reduced by ashing at the minimum temperature required to ensure a reasonable complete combustion. But for certain metals notably arsenic, mercury and selenium, this method is completely unsuitable. Losses by retention have long been suspected and are usually attributed to the formation of complex silicates through combination with the silica wall of the crucible and/or the siliceous residue of the sample itself. It seems trace elements, particularly copper and zinc together with iron and manganese, are most likely to be affected in this way (1, 3). On the other hand, some workers have reported (1) unexpected increases for specific elements including iron, aluminium and boron

and has been attributed to contamination from the lining of the muffle furnance. It is therefore evident that in spite of its advantages and apparent simplicity, dry ashing cannot be universally recommended for solution preparation and the choice will be governed by the element required and the type of material to be analysed.

1.4 AVAILABLE DETERMINATION METHODS.

1.4.1 CALCIUM

Several procedures are available for the estimation of calcium. These include: gravimetric, titrimetric, colorimetric, flame emission photometric and atomic absorption photometric as well as neutron activation methods. Atomic absorption spectroscopy is a relatively new analytical tool, however, much work has been done on the determination of calcium in soil and plant extracts using this method. Constant and increasing need for quick and accurate analytical results has led (3), to the introduction of this method in the field of elemental analysis.

The chief source of difficulty in the use of AAS for the determination of calcium is interferences from other elements or ions. These include, interferences from cations such as magnesium, titanium, aluminium, iron,

and anions such as sulphates, phosphates and aluminate. Although interferences from cations can be readily overcome, those from anions particularly that from phosphate has been a major source of error and a subject of much concern. Several procedures have been suggested for overcoming most of these interferences. These methods include: The use of a releasing agent such as strontium and lanthanium; the standard addition method as a background solution to compensate for interference. Others include, the removal of these interfering substances by ion-exchange resin and the use of organic solvent such as aspiration in the presence of a 2% glycerol solution. Although, most of these procedures have been found (1, 3) useful, the additional steps required made the determination of calcium considerably slower and more tedious. A more complex method was used by Pinta and Bove (3) in which a family of curves is established and the result for calcium determined from a curve representing the known level of phosphate in the sample.

1.4.2 COPPER.

There are various well-established methods for the determination of copper. These include: gravimetric, colorimetric, volumetric, polarographic, electrodeposition

and atomic spectrophotometric methods (5, 17, 18). The atomic absorption procedure is straightforward and almost free from interference but since the amount of copper is normally low in natural organic products, a concentrated sample solution may sometimes have to be prepared, while water sample could be concentrated by initial boiling to reduce a known amount to a certain predetermined volume. Polarography is a sensitive technique, but it is usually necessary to include a separation stage to eleminate interferences by other ions. The volumetric as well as gravimetric methods can be carried out in many ways.

1.4.3 IRON.

Iron has been quantitatively estimated using various methods such as gravimetry, volumetry, colorimetry, polarography as well as atomic absorption spectrophotometry. Atomic absorption spectrophotometry is a well established technique for determining iron, however, iron differs from most other metallic elements that can be determined by AAS in that a considerable range of analytical lines is available. The line to be used therefore, depends upon the concentration of iron to be determined (3). Reported sensitivities range from approximately 0.05 ppm at the 248.33 nm line to about 30 ppm at the 382.44nm line. In

addition to the wide choice of analytical lines available, the iron spectrum is extremely complex and in many instances the observed sensitivity is markedly dependent on the lamp current and the spectral band width used.

Because of the complexity of the iron spectrum, linear calibration graphs are obtained only with instrument with high resolution. Great care is therefore required in order to ensure that either the measured absorbances are sufficiently low to ensure linearity or correction for curvature is made particularly when using the standard addition method for which departure from linearity may not be readily apparent. Interferences in the determination of iron are minimized by the use of an oxidizing air-acetylene flame or the dinitrogen-oxide-acetylene flame, although there is some loss of sensitivity when the latter is used (3). The elements most likely to cause interference in the determination of iron appear to be silicon, chromium, nickel, and tungsten. Some interference from aluminium and boron has been noted (3). However, it appears to be generally accepted that the use of an oxidizing flame overcomes or reduces the effects of interfering ions; and comparison of results by AAS with those obtained by other methods such as colorimetry on the same samples indicate generally good agreement.

1.4.4 SODIUM

The gavimetric technique for sodium uses uranyl zinc acetate, but it is not very suitable owing to the low levels of sodium in most organic materials (1). Atomic absorption is also suitable for sodium but it does not have any marked advantage over emission and the equipment is initially more expensive. In addition, the use of ionization buffer like addition at potassium solution has been suggested to suppress the ionization of sodium during the aspiration. (1)

The flame emission method has been extensively employed in the determination of sodium with the necessary precautions to prevent interference mainly from other alkaline and the alkali earth metals. The relatively coal air-coal gas or propane-butene of the "EEL" instrument excites fewer elements than the high temperature flames of other instruments. Cationic interferences are therefore, less with an "EEL" instrument. However, a filter instrument is not sufficiently selective for accurate determination of sodium and potassium because emission due to cations such as iron, manganese and others are known (25), to pass through the filter. It has been found (24) that the use of narrow wavelength band filters largely eliminates emission from calcium and strontium.

1.5 FLAME EMISSION, ATOMIC ABSORPTION AND COLORIMETRIC TECHNIQUES.

1.5.1 INTRODUCTION.

The development of flame techniques using emission spectra and more recently absorption spectra have made sensitive and highly specific methods available to the analyst. Many elements in particular the alkali and alkaline earth elements which were formally difficult to determine, can now be estimated in a few minutes provided certain precautions are taken regarding interferences and flame conditions. Atomic absorption has now almost replaced direct emission for the analysis of most elements except perhaps sodium and potassium. However, flame emission is considered first below since many features of its development have led to their use in atomic absorption.

1.5.2 FLAME EMISSION

PRINCIPLE:

When atoms of an element are heated in a flame some of the heat energy is absorbed by a few of the atoms which become excited, hence, there is a transition by one or more electron from the ground state to higher energy levels. On reverting to the ground state, the electrons lose this energy which is emitted as electromagnetic radiation. The wavelength of the emitted

radiation is governed by the energy change involved in the transition. For each element, there are certain permitted shifts giving rise to a series of lines, each series being characteristic of the element. The intensity of any one line is governed principally by flame temperature other atomic species present and the number of atoms of the element in the flame at any one instant. If operating conditions are kept constant the intensity of radiation will be a measure of this number. This is the basis of quantitative flame photometry.

INSTRUMENTS:

Instruments used for flame emission consist of three main parts: the burner-sampler system: a means of isolating the required emission line and the detection-measuring system. The design of the aspiration system has been an important factor in the success of flame instruments. The most widely used is the nebulizer-spray chamber system. In this, the flow of oxidizing gas through a tube draws the solution through a concentric linear capillary. The solution leaving the inlet jet is partly disintegrated into a fine spray by the gas stream. This process is called nebulization and the term 'atomization' which was formally used is now restricted to the reduction of elements to the atomic state. Only

a small proportion of the solution passes into the flame and the remainder condenses in the spray chamber and is drained away. Both the size of this proportion and the over all rate of aspiration are related factors which must be controlled to achieve maximum sensitivity. The emission line can be isolated by a filter, prism or granting. Simple flame photometers employ a filter, but for sensitive work, a spectrophotometer is desirable.

FLAME CONDITION.

There are great differences in the case with which various elements are excited in a flame e.g. sodium and potassium are more readily excited than calcium and magnesium. For emission in particular, the available energy is therefore important. Metals such as calcium which tend to form heat stable oxides in the flame are better determined at the higher temperatures obtained with air or oxy-acetylene flame. It is essential to maintain stable flame conditions. Small changes in temperature can lead to considerable instrumental instability. The flame and the burner temperatures should be allowed to come to equilibrium by aspirating the solvent into the flame for a few minutes before taking any reading. The flow rates of the fuel and

oxidant gases are extremely important in this respect and should be carefully set at the beginning and maintained throughout the run to achieve maximum sensitivity and stability. The height of the burner is a contributing factor which must be regulated. However, many flame and nebuliser error sources can be controlled by internal standardization in which an element not being determined is added to every solution including standards (1)

INTERFERENCES:

A number of interferences occur which are associated with atomization conditions. These can usually be reduced by careful control of the flame conditions or solution preparation. The more important classes of interferences are given below (1).

Radiation effects: resulting from band spectra of other elements or molecules and also from an intense adjacent emission line.

Self absorption: this is a related effect whereby unexcited atoms re-absorb emitted radiation resulting in reduced sensitivity.

Formation of refractory molecules: this reduces the number of atoms available for excitation. An example

of this is the interference of aluminium and phosphate on calcium emission. It may be suppressed (1), by the addition of lanthanum or strontium as a releasing agent.

Ionization: this occurs most readily with the alkali metals in hotter flames and is significant because ionized atoms do not emit radiation at the wavelength normally selected for the analysis. The effect increases with temperature and is greater for a metal present in low concentrations. However when other more ionizing species are present, these furnish electrons which tend to inhibit ionization. An example of this is the enhancement of alkali metals by other metals.

In general the spectra line chosen for the determination of an element is that which gives the greatest sensitivity and will usually be the most intense line. However, if there are interferences with this line, it may be necessary to choose another. Organic solvents such as iso-propanol may be used to enhance the sensitivity.

1.5.3 ATOMIC ABSORPTION.

Atomic absorption instruments are basically spectrophotometers with a burner compartment instead of a cell compartment. They consist of a source of

radication, burner plus sampler compartment, monochromator and a detection and measurement system.

PRINCIPLE:

The use of flame absorption spectra instead of emission spectra was first proposed and developed by Walsh (1955). Atomic absorption spectra are formed by the absorption of radiation of certain wavelengths by atoms whose electrons are in the ground state. On absorbing this energy, the atoms become axcited. The extent of absorption is dependent on the number of atoms in the ground state in the path of the radiation beam at any one time and can thus be used as a quantitative method of determining this number.

In practice, a solution of the element is sprayed into a relatively cool flame in which the atoms tend to remain in the ground state. Radiation of a characteristic wavelength from a hollow cathode discharge lamp is passed through the flame and the decrease in intensity is measured using a monochromator and detector system. This decrease is related (directly proportional) to the concentration of the element in solution. Light sources such as tungsten filament and hydrogen discharge lamps give a continuous spectra and so are unsuitable for atomic absorption work. The usual sourceis a hollow cathode lamp. This is a discharge lamp which has a

hollow cylindrical cathode made from material which contains a substantial proportion of the element to be measured. Therefore, the radiation produced corresponds to be emission spectrum of the element and so the required line may be readily isolated by the monochromator.

After the elimination of flame and chemical interferences the most important causes of error in atomic absorption are nebuliser blockages, changes in air and fuel flow rate, wavelength drifting off peak, very low acetylene cylinder pressure, and hollow cathode lamp drift. Changes in burner height over a period may also cause difficulty. The problem of wavelength drift is sometimes accentuated by the heat absorbed by the monochromator compartment when the burner is first switched on. In such cases, the instrument should be allowed to stabilize. Some models avoid this difficulty by using an external burner or having a water cooled enclosure around the burner.

1.5.4 COLORIMETRY AND SPECTROPHOTOMETRY.

The absorption of electromagnetic radiation by chemical substances and the measurement of this absorption is the basis of colorimetry and spectrophotometry (1). Visible spectrophotometry extends from approximately 380 to 750 nm and is concerned with light absorption

by solution which the eye sees as coloured. Instruments used in colorimetry and spectrophotometry consist of four main parts, namely; a source of radiation, a means of isolating the required wavelength, compartments with suitable sample container and a means of detecting and measuring the intensity of radiation. For wavelengths in the visible range, a source of white light such as a tungsten filament bulb is used and is often fed by a stable voltage supply to avoid fluctuation in light intensity. The common sources of ultra-violet radiation are hydrogen, deuterium or mercury vapour discharge lamps. Several methods for selecting the wavelength to give maximum optical density can be used. In simpler instruments, filters are used to give a waveband covering the peak absorption as shown below.

The band width of the coloured filters is fairly wide but this can be reduced by the use of optical interference filters. Cells of various path lengths enable solutions with a wide range of colour intensities to be measured. For any particular run of sample, it is important for the reference and sample cells to be well matched. If a matched pair is however, not available the two cells should be interchanged and a mean of the two readings obtained. The simplest instruments use the eye as detector but most now have a photosensitive detector.

TABLE 1

FILTER SELECTION GUIDE

SOLUTION COLOUR	FILTER COLOUR	WAVELENGTH RANGE (nm)
Blue	Red	680 - 800
Turquoise	Orange	600 - 650
Green	Yellow	580 - 590
Violet-red	Green	530 - 550
Orange-red	Turquoise	480 - 500
Yellow-Orange	Blue	450 - 470
Yellow	Purple-blue	400 - 420

Other detector means include the barrier-layer, and the phototube (or photomultiplier). Not all instruments are direct reading. Some employ the null point principle in which the current from the detector-amplifier system is balanced by a potentiometer. Flunctuations in the intensity of the incident light will affect readings although voltage-stabilizers will minimize this problem. Alternatively, a double beam instrument can be used, in which the light beam is split after leaving the monochromator so that it can pass through both the reference and sample cells.

Few constituents of interest to the ecological analyst contain sufficient intrinsic colour to be measured directly; exceptions include chlorophyl, and carotenoids. For most constituents it is much more usual for the colour to be developed by reaction with a chromogenic reagent. The colour intensity usually increases with the concentration of the constituent being determined but in a number of cases an inverse relationship occurs. To be suitable for colorimetry, the reaction should ideally be: stoichiometric, fairly simple, and free from interference; give a colour which is stable with time and relatively insensitive to pH and temperature changes. It should not be critically affected by slight changes in the wavelength of the incident light and should produce a reasonably intense colour at low concentrations. It should also be specific for the constituent being measured; conform to the

Beer-Lambert law over a wide range of concentrations, and sufficiently reproducable to allow the use of permenent standards. However, these conditions are never fully achieved in practice.

Optical density measurements are made by comparing the sample with a reference solution with measurement done under the same instrument conditions. Construction of a calibration curve from the standard reading provides the simplest method of converting sample reading into concentrations. The graph being linear if the Beer-Lambert relationship is followed.

1.5.5 SPECTROPHOTOMETRY IN THE DETERMINATION OF PHOSPHORUS.

Although titrimetric, gravimetric, polarographic as well as neutron activation methods are available for phosphorus, colorimetry is almost always used for its determination as phosphates (1). Two chromogenic systems are favoured, in one of which 'molybdenum blue' is produced while the other method is that which depends upon the formation of the yellow vanadomolybdophosphoric acid. The colour formed by the reduction of the molybdophosphoric acid is more intense than the yellow complex and hence the method is thus more sensitive, although there is at the same time a loss of colour stability and an increase need for a more rigorous

control of operating conditions (1, 32, 36). The yellow vanadomolybdate color has a broad absorption spectrum but a wavelength under 440 nm is recommended (1), for the low concentration samples often encountered in ecological studies. A preliminatry separation of the phosphorus is sometimes made to eliminate the interference of silicate, arsenate and molybdate (which form similar heteropoly complexes) and of elements such as copper, nickel and chromium which form colored solutions (36). A compromise may sometime be necessary since the interference effects of the ions especially iron(II), falls off at higher wavelength.

It has been suggested (36) that there is the formation of two blue complexes; that which forms at an acidity of about 1M and has an absorbance maximum at 380nm, is called "Heteropoly blue" to distinguish it from "molybdenum blue" which forms at lower acidities and which has an absorption maximum at 650 - 700 nm.

The most important variable is probably the reduction stage and many different reducing agents have been recommended. They have varying effects on color, stability and intensity and also the response to interfering substances. Both hydrazine sulphate and ascorbic acid have been used as the reducing agents and the addition of potassium antimonyl tartrate to ascorbic acid was later found (36) to give increased

sensitivity when applied to the determination of phosphorus in natural water. Ascorbic acid, eventhough fairly sensitive is less convenient because it is preferably added as a solid and required a heating stage for maximum sensitivity. On the other hand, stannous chloride which appears to be the most sensitive reductant has a fairly critical development time while metol (P-methylamino-phenol sulphate), which gives a relatively stable color is less sensitive.

A further modification involves the extraction of the molybdophosphoric acid into an organic solvent such as isobutyl alcohol before reduction to heteropoly blue. This is one means of eliminating the interference of many other ions and to concentrate the phosphorus. Other important factors affecting the method include the concentration of the reagents and the overall acidity. This must be carefully controlled since at low acidity the molybdate itself will give a color in the absence of phosphate. Silicates and arsenate give similar colors to phosphate. Interference from arsenic(V) has been prevented (8), by its reduction with a mixed reducing agent ($Na_2S_2O_3$ and $NaHSO_4$); Iron(III) will depress the color when present in excess of 10mg l^{-1}, however,

the interference from vanadate and Iron(II) can be eliminated (7) by first passing the solution through a semimicro silver reduction. The interference from silica may be prevented by maintaining a final concentration of 0.76 M sulphuric acid.

CHAPTER II

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

2.1 APPARATUS.

50_{cm}^3 conical flasks (pyrex), electric hot plate (B & T model), stop-watch, pipette, pipette filler, burrette, atomic absorption spectrophotometer (AAS) (Unicam SP 1900) with digital readout, flame photometer (corning EEL) and the Unicam SP 500 spectrophotometer.

2.2 REAGENTS.

Unless otherwise stated, all the reagents used, were of analytical grade.

Hydrochloric acid (36% m/m)
Nitric acid (70% m/m)
Perchloric acid (70% m/m)
Sulphuric acid (98% m/m)
Copper sulphate (pentahydrate) (98.8% m/m)
Ferrous ammonium sulphate (98.5% m/m)
Potassium dihydrogen phosphate (97% m/m)
Sodium chloride (99.5% m/m)
Calcium carbonate (Precipitated) (98% m/m)
Ammonium molybdate (99% m/m)
Stannou Chloride
Lanthanum nitrate
Potassium Chloride (94.6% m/m)

2.3 STANDARDIZATION OF REAGENTS.

In trace metal analysis, it is usually necessary to standardize the reagents, especially those to be used in preparing the calibration curves because, a slight change in the percentage purity (or composition) in the chemical as stated on the reagent bottle can introduce a large error in the results. Thus, although most of the reagents used in the course of this work were of analytical grade, they were standardized before use.

2.3.1 STANDARDIZATION OF CALCIUM CARBONATE.

Eriochrome Black T indicator:

Was prepared by dissolving 0.200g of the dyestuff in 15cm^3 of triethanolamine and 5cm^3 of absolute ethanol.

EDTA Solution, 0.01M.

Was prepared by dissolving 1.861g of A.R. Sodium dihydrogen ethylenediamine tetraacetic acid in de-ionized water and diluted to 500cm^3 in a volumetric flask.

Buffer Solution of pH 10.

Was prepared using ammonium chloride (17.5g) in 142cm^3 of concentrate ammonia solution (S.G. 0.88) and diluted to 250cm^3 with distilled water.

Magnesium-EDTA Complex Na_2MgY . 0.1M.

Was prepared by mixing equivalent amounts of 0.4M solutions of EDTA and Magnesium sulphate. The solution was then neutralised to a pH between 8 and 9 using ammonium hydroxide solution.

Calcium Carbonate Solution, 0.01M.

To 0.500g of A.R. calcium carbonate was added 6M hydrochloric acid dropwise until effervescence ceased and the solution was clear. This was followed by the addition of sodium hydroxide solution till the solution was almost neutralised and diluting to $500cm^3$ using distilled water.

Procedure.

$25.0cm^3$ of the 0.01M calcium solution in a $250cm^3$ conical flask was diluted to $50cm^3$ using distilled water, followed by addition of $2cm^3$ of the buffer solution, $1cm^3$ of 0.1M Mg-EDTA and 3 drops of Erio T indicator. This was titrated with the EDTA solution until the colour changed from wine red to clear blue. The percentage purity of the calcium carbonate was found to be 98.4%.

2.3.2 STANDARDIZATION OF COPPER SULPHATE.

Indicator: Fast sulphon Black F.

0.5% aqueous solution of fast sulphon Black F was prepared by dissolving 1g of the dyestuff in 200cm^3 distilled water.

EDTA-Solution, 0.05M.

Was prepared by dissolving 18.613g of disodium-EDTA salt in 1 dm^3 aqueous solution.

Copper Sulphate Solution: 0.05M

Was prepared by dissolving 3.11g of A.R. pentahydrate copper(II) sulphate in distilled water and diluted to 250cm^3 in a volumetric flask.

Procedure:

25 cm^3 of the copper solution was further diluted with an equal volume of distilled water, followed by addition of 5cm^3 concentrated ammonium hydroxide solution and 5 drops of the indicator solution. This was titrated with the standard EDTA solution until the color changed blue to dark green. The percentage purity of the copper sulphate was found to be 98.6%.

2.3.3. STANDARDIZATION OF FERROUS AMMONIUM SULPHATE.

Reagents:

Ferrous ammonium sulphate: 0.02M

Was prepared by dissolving 7.8428g of ferrous ammonium sulphate (hydrated) in 1 dm^3 solution using 200cm^3 of an 0.5m sulphuric acid.

Potassium Permanganate solution: 0.02M

To 0.32g of A.R. potassium permangate was added $100cm^3$ distilled water and heated to boiling for 30 minutes, covering the beaker with a clock glass. This was set aside to cool and filtered to give a solution of 0.1M potassium permanganate from which the working solution (0.02M) was prepared.

Procedure:

$25.0cm^3$ of the ferrous solution was titrated with the standard potassium permanganate from burette until the first pink color appeared. The percentage purity of the ferrous salt was found to be 99.2%.

STANDARDIZATION OF SODIUM CHLORIDE.

Sodium Chloride: 0.217M

Was prepared by dissolving 12.7072g of dried sodium chloride in water and diluting to 1 dm^3 using distilled water.

Solution A.

Prepared by dissolving 9.0g of crystallised Uranyl acetate and 6.0g of glacial acetic acid and diluting to $100cm^3$ with distilled water using a volumetric flask.

Solution B.

Was prepared by dissolving 60.0g of Magnesium acetate and 6.0g of glacial acetic acid in distilled water and diluting to $100cm^3$.

Reagent Solution:

Was prepared by heating solutions A and B separately until all the salts dissolved and then mixed at 70^oC, allowed to cool and the mixture maintained at about 20^oC in a water bath for 2 hours which was then filtered.

Procedure.

$5cm^3$ of the sodium chloride solution in a vessel was treated with excess of the reagent solution. The mixture was immersed in a water bath maintained at 20 ± 1^oC and stirred vigorously for 60 minutes. The precipitate was then immediately filtered through a weighed sintered glass filtering crucible using suction. The precipitate was washed 4 times with 95% ethanol saturated with sodium magnesium Uranyl acetate. The precipitate was finally dried at 70 ± 1^oC for 30 minutes and weighed as sodium Uranyl - magnesium acetate complex, $NaMg(UO)_3(C_2H_3O_2)_9.6.5H_2O$. The percentage purity of the sodium chloride was determined as 99.8%.

2.3.5 STANDARDIZATION OF POTASSIUM DIHYDROGEN PHOSPHATE.

Phosphate Solution ≡ 0.2676g PO_4^{3-}

Was prepared by dissolving 0.40g (accurately weighed) of A.R. anhydrous potassium dihydrogen phosphate in 100cm^3 distilled water.

Magnesia Mixture.

Was prepared by dissolving 25.0g of magnesium chloride (hexahydrate) and 50.00g of ammonium chloride in 250cm^3 of distilled water. To this, excess ammonia solution was added and allowed to stand overnight and filtered. To the filtrate, about 3cm^3 concentrated hydrochloric acid was added and diluted to 500cm^3.

Procedure.

50cm^3 of the phosphate solution was slightly acidified by addition of 3cm^3 concentrated hydrochloric acid and 3 drops of methyl red indicator. To this, 25cm^3 of the magnesia mixture was added followed by pure concentrated ammonia solution with stirring until the indicator turned yellow. 5cm^3 excess of the concentrated ammonia solution was then added.

This solution was allowed to stand overnight in a cool place and the precipitate formed filtered through a clean weighed sintered glass, and washed with dilute

(0.8m) ammonia solution until a few cm^3 of the filtrate, when acidified with dilute nitric acid and tested with aqueous solution of silver nitrate gave a negative result for chloride. The precipitate was then finally washed with $10cm^3$ of 95% ethanol to remove the adhering water followed by further washing using anhydrous ether and later allowed to stand in a desicator to dry. The percentage purity of the potassium dihydrogen phosphate was determined as 96.8%.

2.4 COLLECTION AND PREPARATION OF SAMPLES.

TABLE 2

2.4.1 SAMPLES AND SOURCES

SAMPLE DESIGNATION	SAMPLE DESCRIPTION	SOURCE
A	Vigna Unquiculata (Ife Brown)	Institude for Agricultural Research Samaru - Zaria.
B	Triticum aestivum (Indus 66 1980-81)	Institute for Agricultural Research Samaru - Zaria.
C	Arachis hypogaea (m.25 .68)	"
D	Arachis hypogaea (MK.374)	"
E	Soil (fresh)	Botanical garden A.B.U. Zaria
F	Soil (fresh)	Area B41 garden A.B.U., Zaria.
G	Zea mays (fresh roots)	Grown on Soil Sample F, Area B41 garden.
H	Zea mays (fresh stems)	Grown on Soil Sample F, Area B41 garden.
I	Zea mays (fresh leaves)	Grown on Soil Sample F, Area B41 Garden.
M	Soil (fresh)	Area B41 garden, A.B.U. Zaria.
J	Zea mays (fresh roots)	Grown on soil Sample M, Area B41 garden
K	Zea mays (fresh stems)	Grown on soil Sample M, Area B41 garden.
L	Zea mays (fresh leaves)	Grown on soil Sample M, Area B41 garden.

2.4.2 SAMPLE PREPARATION.

Method.

Both the soil and the plant samples (except A, B, C and D) were dried for 36 hours in an air-circulation oven maintained at 105°C (for soil) and 80°C (for plant materials respectively). The samples were then thoroughly mixed and ground using a mortar. The fine samples were then kept in clean bottles separately for subsequent treatment.

2.5 DETERMINATION OF % DRY MASS.

Determination of water content is important in soil and plant studies and many methods have been developed for this purpose; Some of which are intended for use in the field, but most determinations are carried out in the laboratory (1). Since moisture estimations are often used for dry weight correction of other analytical data, it is generally more convenient to express the results as percentage dry matter.

Thermal drying at between 100 and 110°C in an air-circulation oven to constant weight is by far the most common technique in use for drying soil and at 80°C for most plant materials. However, heating for too

long periods at higher temperatures (say over 200°C) may lead to losses in weight due to volatilization, oxidation or partial breakdown of organic matter. For some purposes, these factors are critical and a true assessment of the moisture value is not possible without describing the nature of the material.

However, because of the simplicity of this method, it is by far the most common method usually employed inspite of its draw backs, and has given satisfactory results under controlled conditions, but to make the results more meaningful, both the time of drying and temperature should be specified with the results.

Method.

5g of each of the samples was accurately weighed out into a clean-weighed beaker. Using an air-circulating oven, maintained at 105°C (for soil) and 80°C (for plant) the samples were dried for 36 hours and set in a desicator to cool and later re-weighed. Three determinations were performed at the same time for each of the samples and the average result obtained.

Calculation.

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

2.6 PREPARATION OF SOLUTIONS FOR CALIBRATION.

2.6.1 Reagents.

Hydrochloric acid, 3M.

Was prepared by diluting 250cm^3 of concentrated hydrochloric acid (36% w/w) to 1 dm^3 with distilled water.

Lanthanum nitrate, 5000 ppm.

Was prepared by dissolving 15.5864g of lanthanum nitrate in 200cm^3 of distilled water, warmed to dissolve and the resulting solution transfered to 1 dm^3 volumetric flask and made to the mark using distilled water.

Ammonium molybdate Sulphuric acid reagent.

Was prepared by dissolving 25g of ammonium molybdate (hydrated) in about 200cm^3 of distilled water in a beaker and warmed slightly to dissolve. To 400cm^3 distilled water, 280cm^3 of concentrated sulphuric acid (98% m/m) was carefully added with mixing and cooling. The molybdate solution was filtered into the acid mixture with thorough mixing and the solution made to 1 dm^3 using distilled water. The reagent was stored in the dark.

Stannous Chloride reagent.

Was prepared by dissolving 0.5g stannous chloride (hydrated) in 250cm^3 (2% v/v) hydrochloric acid. (Prepared immediately before use).

2.6.2 STANDARD CALCIUM CARBONATE, A.

Was prepared by transfering 2.497g of calcium carbonate (oven-dried) to a 1 dm^3 volumetric flask using 100cm^3 of the 3M hydrochloric acid. The mixture was diluted to the mark with distilled water (1cm^3 ≡ 1mg Ca).

The standard working solutions were then prepared as follows: 10cm^3 of solution A was diluted to 100cm^3 (1cm^3 ≡ 100 µg Ca) to give solution B. To each of six 100cm^3 volumetric flasks, 0, 5, 10, 15, 20 and 25cm^3 respectively of B was accurately transfered. 20cm^3 of the lanthanum solution (5000 ppm) was also added to each of the flasks and the solution diluted to volume. These solutions contained 0, 5, 10, 15, 20 and 25 ppm Ca respectively.

2.6.3 STANDARD COPPER SOLUTIONS, A.

3.930g of pentahydrate copper(II) sulphate was dissolved in 100cm^3 distilled water and warmed slightly to dissolve. The solution was then transfered to a 1 dm^3

volumetric flask and diluted to the mark ($1cm^3$ ≡ 1000ppm Cu). For standard copper working solution, B; $10cm^3$ of the stock solution was diluted to $100cm^3$ ($1cm^3$ ≡ 100 ppm Cu).

To each of six $100cm^3$ volumetric flasks, 0.0, 0.2, 0.4, 0.6, 0.8 and $1.0cm^3$ respectively of solution B was added and diluted to volume using distilled water. These solutions contained 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ppm Cu respectively.

2.6.4 STANDARD IRON STOCK SOLUTION, A (1000 ppm).

Was prepared by dissolving 7.02g of hydrated ferrous ammonium sulphate in $50cm^3$ of distilled water containing $1cm^3$ concentrated sulphuric acid (98% m/m). The mixture was then transfered into a 1 dm^3 volumetric flask and made to volume.

For standard Iron working solution, B. (100 ppm), $10cm^3$ of A was transfered into a $100cm^3$ volumetric flask and diluted to the mark. To each of six $100cm^3$ volumetric flasks, 5, 10, 15, 20 25 and $30cm^3$ respectivily of solution B was added and diluted to volume using distilled water. The solutions contained 5, 10, 15, 20, 25 and 30 ppm Fe respectively.

2.6.5 STANDARD SODIUM STOCK SOLUTION, A. (100 ppm)

This was prepared by dissolving 2.542g of oven dried sodium chloride in distilled water and made to mark in a 1 dm^3 volumetric flask. Standard working solutions were prepared as follows: $10cm^3$ of solution A was transfered into a $100cm^3$ volumetric flask and made to volume using distilled water. To each of $100cm^3$ volumetric flasks 0, 1.0, 2.0, 3.0, 4.0 and $5.0cm^3$ respectively of solution B was added and the volume made up using distilled water. These solutions contained 0, 1, 2, 3, 4 and 5 ppm Na respectively.

2.6.6 STANDARD PHOSPHORUS STOCK SOLUTION, A.

Was prepared by dissolving 0.4393g oven-dried potassium dihydrogen phosphate in $100cm^3$ distilled water. The resulting solution was transfered into a 1 dm^3 volumetric flask and made to the mark, ($1cm^3$ ≡ 0.1mg P). Standard working solutions were prepared as follows: $2cm^3$ of solution A was accurately transfered into a $100cm^3$ volumetric flask and made to volume using distilled water, ($1cm^3$ ≡ 0.002mg P.). To each of eight $25cm^3$ volumetric flasks, 0, 2, 4, 6, 8, 10, 12 and $15cm^3$ respectively of solution B was added, followed by addition

of $2cm^3$ of the ammonium molybdate reagent and $2cm^3$ of the stannous chloride reagent with thorough mixing and the volume made up using distilled water. These solutions contained 0.0, 0.004, 0.008, 0.012, 0.016, 0.020, 0.024 and 0.03 mg P. respectively.

2.7 WET DIGESTION OF SAMPLES.

2.7.1 Perchloric-nitric-sulphuric acid mixture (1).

About 0.25g of the sample was accurately weighed into a $50cm^3$ clean dry conical flask. To this, $2.5cm^3$ concentrated nitric acid (70% m/m), $1cm^3$ concentrated perchloric acid (60% m/m) and $0.25cm^3$ concentrated sulphuric acid (98% m/m) were added and swirled gently to mix. The mixture was then heated initially slowly at moderate heat until most of the brown fumes were evolved. The heating was later increased and continued until white fumes of sulphuric acid were evolved. The digestion flasks were cooled and the contents quantitatively transfered into $25cm^3$ volumetric flasks, followed by addition of $4cm^3$ of the lanthanum solution and the volume made to the mark using distilled water.

Appropriate dilutions of this stock sample solution were made (where necessary), for the analysis of the various elements. Blank solutions were prepared in the same way.

2.7.2 MODIFICATION OF THE LITERATURE METHOD.

2.7.2a Using Perchloric-nitric-sulphuric acid mixture.

Using the same quantity of the sample (0.25g), and the same amounts of the various acids, the digestions were performed at 132 ± 1°C, 100 ± 1°C, 70 ± 1°C varying the length of heating between 10 - 60 minutes at each temperature.

2.7.2b Using Perchloric-nitric acid mixture.

The digestion was performed using 0.25g of the sample, 2.5cm^3 conc. nitric acid and 1cm^3 of the perchloric acid, and heating at 132°C between 10 - 60 minutes.

2.7.2c Using Nitric-Sulphuric acid mixture.

The digestion was carried out using 0.25g of the sample, 2.5cm^3 of concentrated nitric acid and 0.25cm^3 concentrated sulphuric acid, and heating at 132°C varying the period of digestion between 10 - 60 minutes.

2.8 DETERMINATION OF THE MINERALS.

Instrumental setting:

The atomic absorption spectrophotometer was set up with the following instrumental operating conditions.

TABLE 3

AAS OPERATING PARAMETERS.

ELEMENTS	WAVELENGTHS	FLAME	SUITABLE RANGE (ppm)
Calcium	422.7	Air-acetylene	0 - 40
Copper	324.7	Air-acetylene	0 - 5
Iron	248.3	Air-acetylene	0 - 20
Sodium	589.0	Air-acetylene	0 - 5

2.8.1 DETERMINATION OF CALCIUM.

The atomic absorption spectrophotometer was set up with the calcium/magnesium hollow cathode lamp for 15 minutes before use to stabilize. Distilled water was then sprayed into the flame to wash the suction tract and the instrument brought to zero reading. The calcium standard solutions were then aspirated using the resonance line of 422.7nm and air-acetylene flame. This was followed by the aspiration of the sample solutions as well as the blank solutions (for background correction); the instrument being washed thoroughly using distilled water between each spraying. A mean of four readings for each standard solution was then plotted against the concentration. The mineral contents (ppm) of the samples were determined (after blank corrections) from the standard curve.

DETERMINATION OF IRON, COPPER AND SODIUM.

Iron, copper and sodium were determined as for calcium using the appropriate instrumental operating conditions for each of the elements.

2.8.2 DETERMINATION OF SODIUM USING FLAME PHOTOMETER.

(a) Procedure:

The same standard solutions were used as in atomic absorption spectrophotometer. The gas pressure was brought to 10 atm. and other settings were made following the recommendations in the manual. The calibration was prepared using the standard solutions after setting to 100% absorption with 5ppm Na solution and finally to zero with distilled water. Both the sample and the blank solutions (for background corrections) were aspirated with thorough water-washing after each aspiration. The calibration curve obtained by plotting the concentration (in ppm) against absorption of the standard solution was used to determine the sodium contents.

(b) DETERMINATION OF SODIUM IN THE PRESENCE OF AN IONIZATION BUFFER.

Method.

The acid digestion procedure was followed using the perchloric-nitric-sulphuric acid mixture and heating for 40 minutes. However, instead of making up the digest solution using distilled water, $5cm^3$ of a 5000 ppm potassium solution was added (to make a final 100 ppm K),

before making up the solution to mark with distilled water. The resulting solution was then aspirated using both the flame photometer and the atomic absorption spectrophotometer under the same conditions as previously stated.

2.8.3 DETERMINATION OF PHOSPHORUS USING UV SPECTROSCOPY.

Procedure.

$5cm^3$ of the sample (digest) solution was quantitatively transfered into a $25cm^3$ volumetric flask and diluted until the flask was about two-third full. $2cm^3$ of the ammonium molybdate reagent was added and thoroughly mixed, followed by addition of $2cm^3$ of the stannous chloride reagent and mixed. The volume was finally made to the mark with distilled water. The optical density of the resulting blue solution was then measured after leaving it to stand for 5 minutes, using an ultraviolet Unicam SP 500 spectrophotometer at 700nm.

The ammount of phosphorus was determined using a calibration curve obtained from the standards. Blank determinations were then carried out in the same way and subtracted where necessary.

2.9 DRY ASHING METHOD.

Procedure

Prior to chemical analysis by both atomic absorption and flame emission spectroscopy, 0.25g portions of the soil sample (sample E), were accurately weighed out into four silica crucibles. The weighed samples were placed in an oven in the cold and the temperature gradually increased to 400°C. The samples were then ashed by heating in a muffle furnace maintained at 400°C for a period of 6 hours. The ashed samples were digested in exactly $20cm^3$ of a 1:1 (v/v) constant boiling HCl: water solution for 3 hours on a hot plate maintained at 80 - 90°C. The solutions were then filtered into polyethylene bottles. To the filtrated, $4cm^3$ of the 500ppm lanthanum solution was added and the volume finally made to $25cm^3$.

The sample solutions were then aspirated as before to determine the mineral contents of the sample using AAS (for Ca, Cu and Fe), and the flame photometer for Na. The ashing was repeated at 600°C and the minerals determined as before.

CHAPTER III

3.0 RESULTS AND DISCUSSION

The digestion methods using three types of acid mixtures at different temperatures are coded as follows:

1. Nitric-sulphuric-perchloric mixed acid digestion at 132 ± 1°C.

2. Nitric-sulphuric-perchloric mixed acid digestion at 100 ± 1°C.

3. Nitric-sulphuric-perchloric mixed acid digestion at 70 ± 1°C.

4. Nitric-perchloric mixed acid digestion at 132 ± 1°C.

5. Nitric-sulphuric mixed acid digestion at 132 ± 1°C.

3.1 % MOISTURE CONTENTS

Table 4 lists some of the samples used in this study and gives the percentage moisture contents of the samples. The observed percentage of 70.24(H) and 65.70 (I) compare favourably with the value of 72.4% for the total moisture of Zea mays (41) found

elsewhere. A value of 12.57% has also been obtained (42) for the moisture content of maize seed. The other samples used in the analysis were already dried and ground from their sources.

TABLE 4

% MOISTURE CONTENTS

SAMPLES	SAMPLE DESCRIPTION	% MOISTURE CONTENTS
E	Botanical soil	12.40
F	Farm soil	13.20
G	Zea mays (Roots)	48.75
H	Zea mays (stems)	70.24
I	Zea mays (leaves)	65.70

TABLE 5

ESTIMATED STANDARD DEVIATIONS (ppm) FOR
ALL WED DIGESTION PROCEFURES USING SAMPLE F.

ELEMENTS	WET DIGESTION 1	2	3	4	5
Ca	0.67	0.69	0.67	0.62	0.72
Cu	0.02	0.02	0.01	0.02	0.01
Fe	0.89	0.88	1.02	0.60	1.01
Na	0.01	0.01	0.01	0.01	0.01
P	0.1	0.08	0.10	0.10	0.1

3.2 ESTIMATED STANDARD DEVIATIONS OF ANALYSIS.

In order to estimate how much spread would be expected using the various variations of wet digestion procedure, trial runs of 12 determinations for each variation were measured using sample F. The estimated standard deviations for the 12 determinations at the three temperatures (1 to 3) and varying the acid mixtures (1, 4, 5) are shown in table 5. The observed deviations for all procedures are sufficiently low to suggest good reproducibility of the methods. Each result in all subsequent tables is a mean of at least three close determinations.

3.3 CALCIUM CONTENTS.

The calcium contents (ppm) of the samples analysed are given in appendix 1. and illustrated for sample E in figure 1. As can be seen from appendix 1 and figure 1, the amounts of calcium obtained from the samples decrease with increase in the digestion temperature and without any much initial increase with the heating time. In fact, there is a slight but perceptible decrease with heating time. Thus, digestion at 132°C gave the lowest calcium values for any of the samples compared to digestion at 100°C and 70°C. This may be attributed to some volatilization losses which tend to increase with the digestion temperature as well as with prolonged heating.

In general, the perchloric-nitric acid mixture gave the highest values of calcium while the lowest results were obtained for the nitric-sulphuric-perchloric acid mixture at 132°C. The values using nitric-sulphuric are intermediate between the two at the same temperature. This is probably due to the Co-precipitation or adsorption of calcium with the insoluble sulphates formed as a result of including sulphuric acid in the mixture. It is also probable that slight losses are increased due to formation of some chloro-complexes of the metal ion with the inclusion of perchloric acid but with the former factor being the predominant one.

Of all the methods used, the dry-ashing method gave the lowest calcium contents (see table 8). This would tend to support the previous suggestion that lower values of calcium are obtained at high temperatures during wet digestion as a result of volatilization losses. A closer examination of the two sets of results shows that the values of calcium obtained after dry-ashing are closest to those obtained at 132°C using the nitric-sulphuric-perchloric acid mixture. This would tend to indicate that the nature of losses are not the same in the liquid and solid states, as much

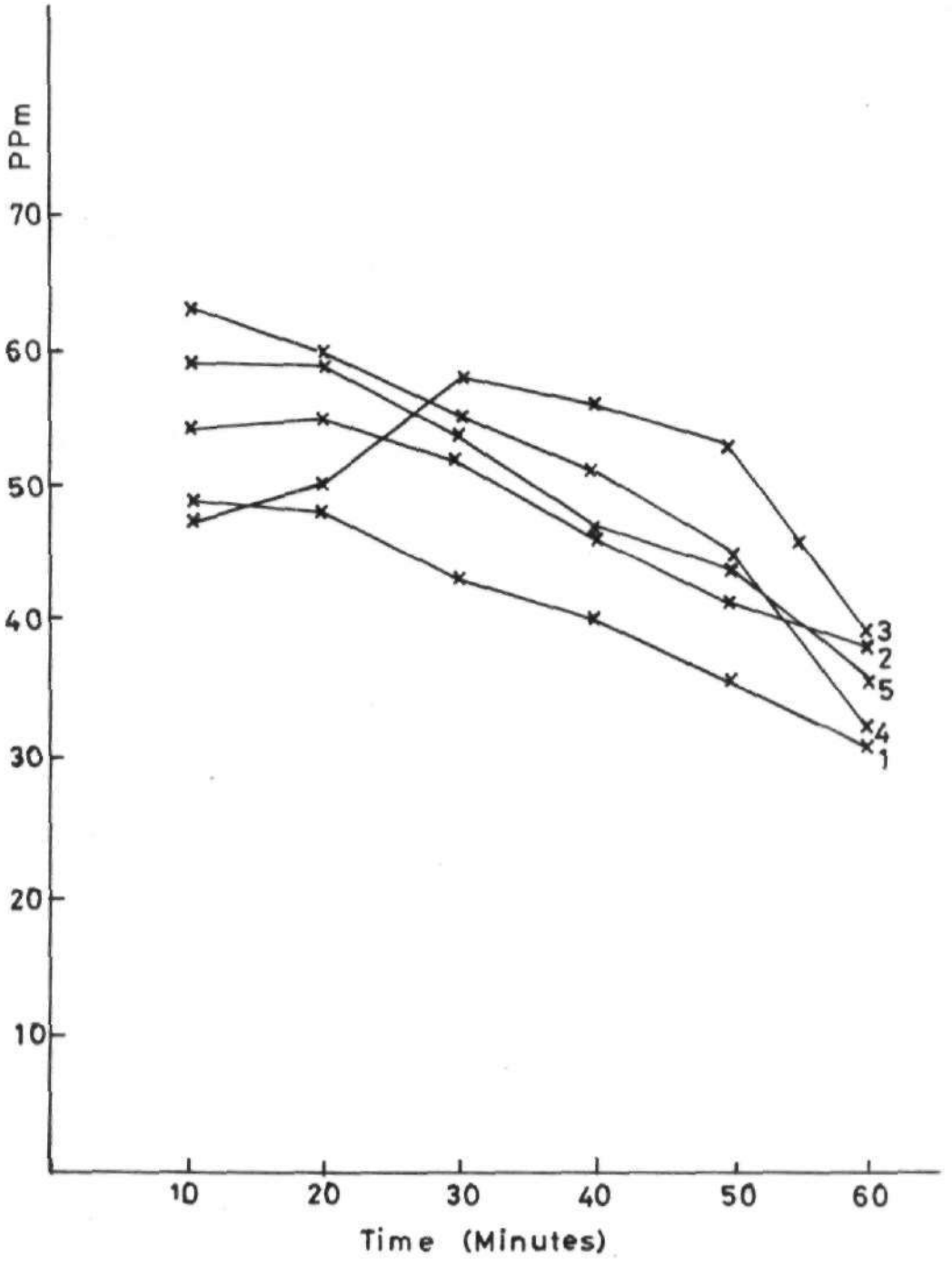

Fig. I Variation of Calcium contents in Sample E with heating time, temperature and nature of acid mixture;

(1) HNO_3 + H_2SO_4 + $HClO_4$ at 132°C

(2) HNO_3 + H_2SO_4 + $HClO_4$ at 100°C

(3) HNO_3 + H_2SO_4 + $HClO_4$ at 70°C

(4) HNO_3 + $HClO_4$ at 132°C

(5) HNO_3 + H_2SO_4 at 132°C

lower values would be anticipated at the higher ashing temperatures of 400 and 600°C. However, the calcium contents of 60 - 68 ppm in soil and 39 - 125 ppm in the plant materials compared favourably with the values 50 to 200 ppm and 30 to 250 ppm reported in the literature (1) for soil and plant materials respectively.

3.4 COPPER CONTENTS.

The copper contents (in ppm) of the samples analysed are given in appendix 2 and the trends illustrated for sample F in figure II. The amounts of copper obtained at the three temperatures were not significantly different. Between 10 - 20 minutes of digestion time appears sufficient to recover copper from the samples. It would appear that volatilization losses are not important for copper determination. It might well be that copper is not tightly bound to the organic matrix.

The copper contents obtained using the three different acid mixtures at the same temperature (132°C), are also not significantly different. Any of the three acid mixtures may therefore be used for wet oxidation of the samples. Maxwell (1968), in his work on "rock and mineral analysis" also found (3) that copper did not show any noticeable response to the nature of acid.

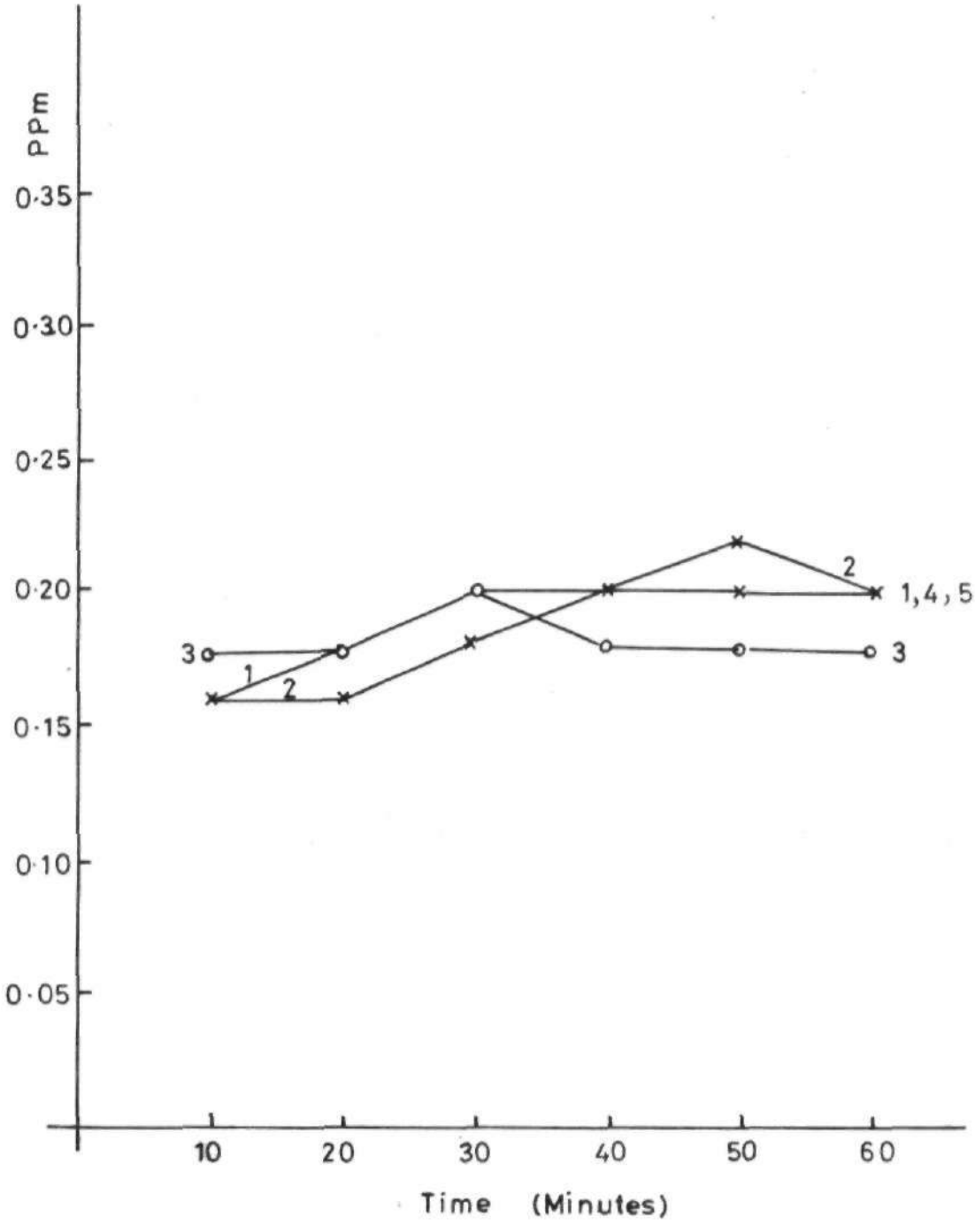

Fig. II Variation of Copper contents with heating time, temperature and nature of acid mixture for Sample F.

The results obtained using the dry ashing method (table 8) and wet digestion (appendix 2) for copper are essentially the same. This would imply that either wet digestion or dry ashing may be used for the determination of copper.

3.5 IRON CONTENTS.

The Iron contents (in ppm) of the samples are given in appendix 3 and illustrated for sample E in figure III. The total iron contents obtained increase generally with increase in heating temperature. There is also an initial increase in the iron contents with heating time up to a certain limit. Thus, the highest iron contents are obtained at 132°C with the lowest values at 70°C while the values at 100°C are intermediate between the values at the two temperatures. The trend appears opposite to that observed for calcium and volatilization losses are probably not important for iron. This might also imply that iron is tightly bound to the organic matrix and higher temperatures are required to dislodge it from the matrix.

The effect of the nature of acid mixture is not quite pronounced, with the three acid mixtures apparently equally good. The values of iron obtained from sample F by dry ashing (table 7), are very close to those

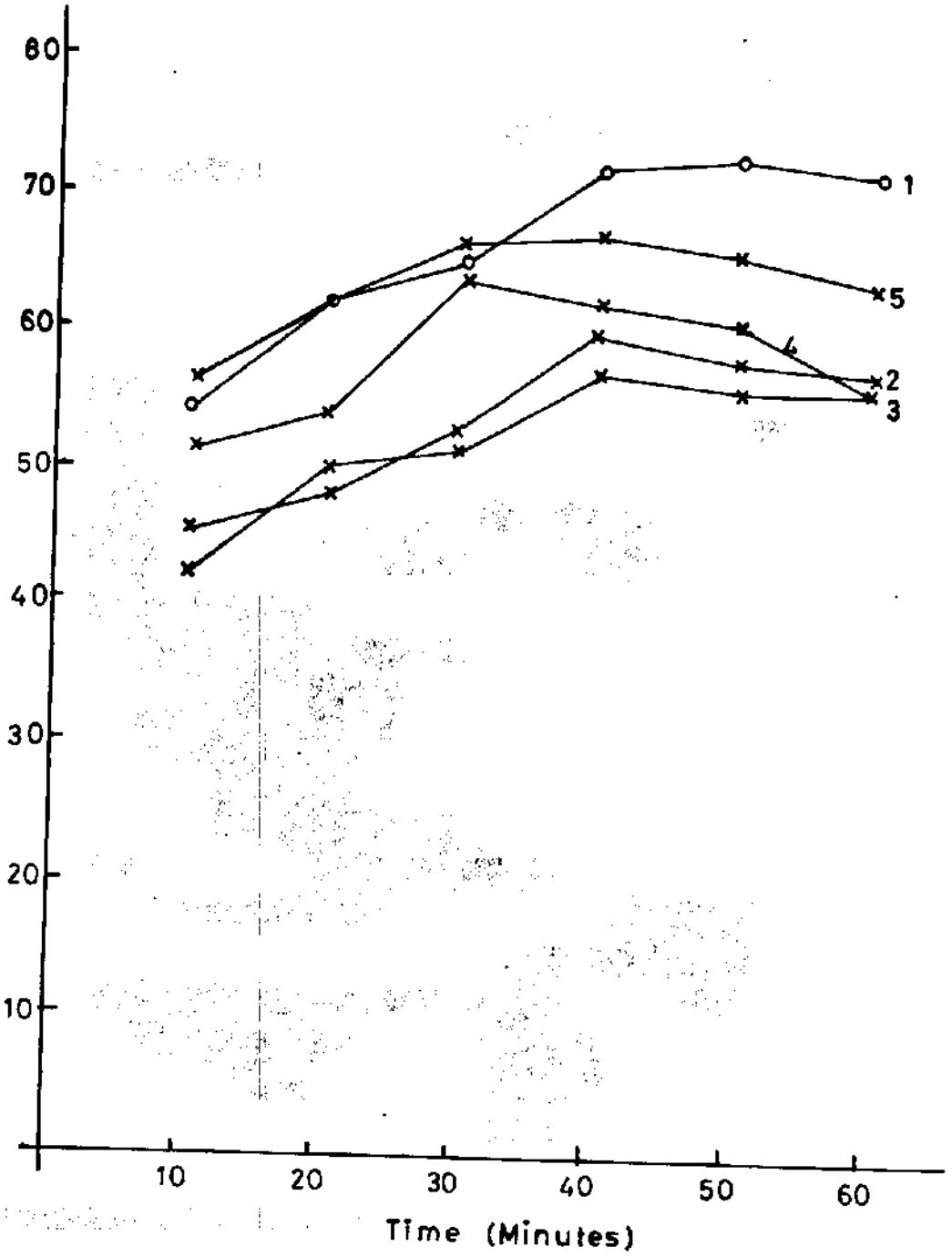

Fig. III Variation of Iron contents with heating time, temperature and nature of acid mixture for Sample E.

obtained at 132°C. This is in agreement with the earlier suggestion that high temperatures are probably necessary to dislodge iron from the organic matrix and that retention and volatilization losses are not important. The iron contents of the analysed soil samples (52 to 72 ppm) are at the low end of the range of (50 to 1000 ppm) that has been observed (1) in soil samples. This may be a reflection of the parent rocks from which the soil samples were derived. In plant samples, our range of 1.2 to 3.8 ppm is in agreement with the range of 0.4 to 5.0 ppm often found (1) in plant materials.

3.6 SODIUM CONTENTS.

The sodium contents (in ppm) of the samples analysed are given in appendix 4 and the trend illustrated for sample C in figure IV. The sodium contents obtained increase with increase in digestion temperature. There is also an initial increase with heating time up to a certain limit. This would imply that, like iron, sodium is probably tightly bound to the organic matrix and high temperatures are required to dislodge it from the matrix. However, once dislodged, prolonged heating at high temperatures should be avoided, as lower values are obtained with prolonged heating (fig. IV). The effects of nature of acid mixture are not well-pronounced, however, there

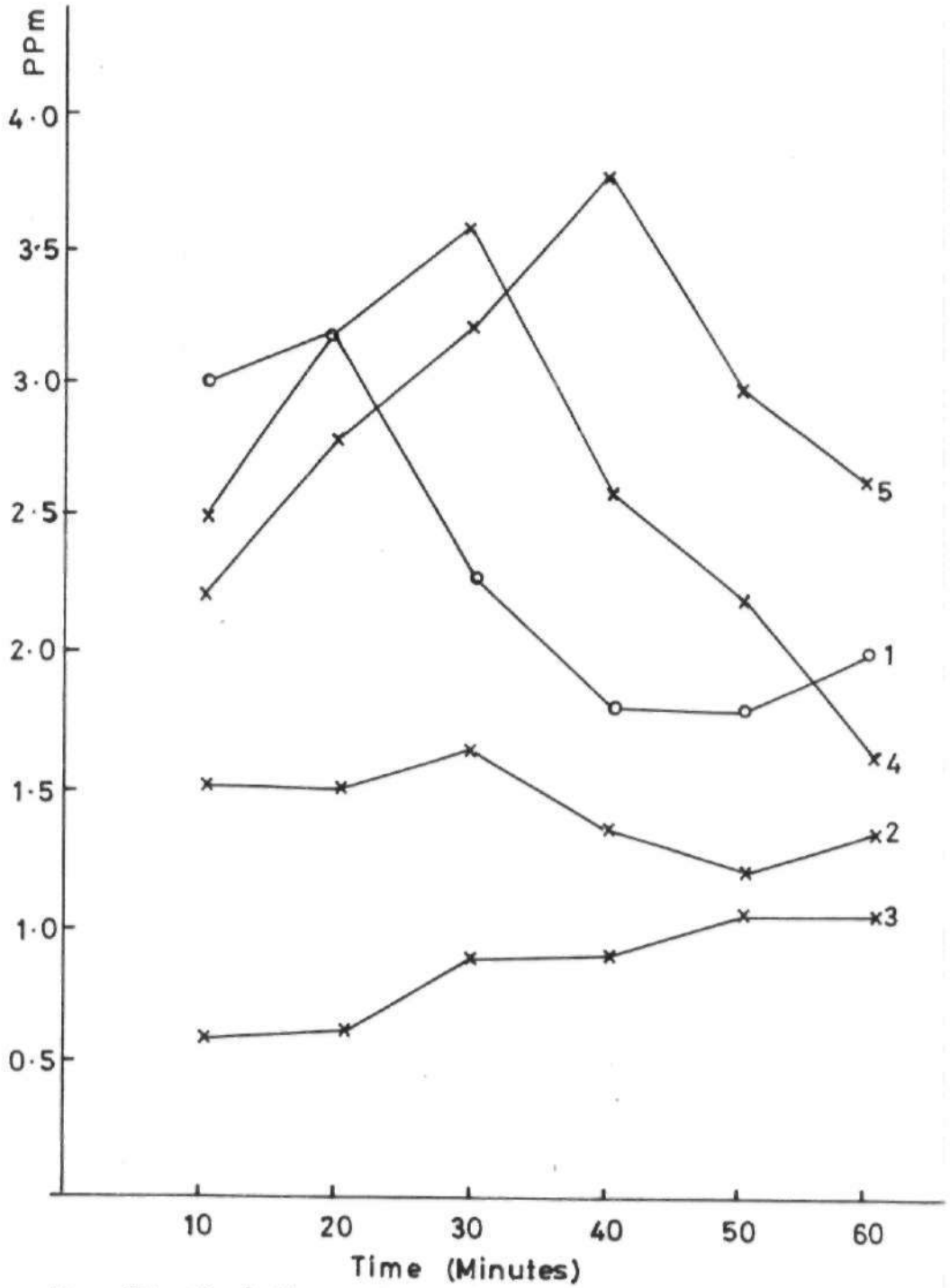

Fig. IV Variation of Sodium contents with heating time, temperature and nature of acid mixture for Sample C.

appear to be slight increase in the sodium contents obtained when the nitric-sulphuric acid mixture is used. This might be due to the slight decrease in volatilization losses in the absence of perchloric acid in the mixture.

The contents of sodium obtained by dry ashing (table 7), are generally lower than those obtained by wet digestion. Probably volatilization losses are important at the enhanced temperature of ashing. The sodium values of 1.4 to 3.8 ppm obtained in this analysis are within the range of 2 to 30ppm often found (1) in plant materials. In the soil samples, the rangeof 2.0 to 3.4 ppm obtained is also in agreement with the range of 2.0 - 100 ppm normally encountered (1) in soil samples.

3.6.1 COMPARISON OF FLAME EMISSION AND ATOMIC ABSORPTION SPECTROSCOPY IN SODIUM DETERMINATION.

The sodium contents of some of the samples analysed using both the atomic absorption and flame emission techniques are given in table 6. The sodium contents obtained using the atomic absorption spectrophotometer in the absence of an ionization buffer are lower than those obtained with the flame photometer. This would suggest that using the high temperature instruments such as the AAS in the determination of sodium might lead to a fraction of the sodium atoms ionizing in the flame.

Addition of an ionization buffer (lanthanum nitrate) led to sodium values by the AAS method which were very comparable to those by the flame emission photometer. It would appear that the AAS method may be used in place of the flame emission if an appropriate ionization buffer is used.

TABLE 6

COMPARISON OF AAS AND FLAME PHOTOMETER IN THE DETERMINATION OF SODIUM.

SAMPLES	AAS	FP	AAS WITH IONIZATION BUFFER
A	2.1	2.5	2.3
B	1.3	1.5	1.4
C	2.6	3.2	3.1
D	3.5	4.5	4.2

3.7 PHOSPHORUS CONTENTS

The phosporus contents of the samples analysed are given in appendix 5 and the trend illustrated for sample I in figure V. From the results, it would appear that phosporus determination requires low temperature for wet digestion. Thus, the highest phosphorus contents were generally obtained at 70°C followed by those at 100 and 132°C in that order. Although the chemistry of the reactions involved in wet digestion is still not well understood, it would appear that the reactions go to completion after a time interval which depends on the material being digested, the heating temperature, and the nature of acid mixture (43). Beyond this time interval, lower values of phosphorus areobtained. This is in agreement with other results (1, 3, 43), that volatilization losses are important for phosphorus. This would indicate that the experimental conditions during the digestion of the samples for the determination of phosphorus should be specified in order to obtain accurate results. The initial slope (fig. V) of the curve shown is probably due to the resistance of the organic matter in the sample matrix to decomposition.

The perchloric-nitric acid mixture gave higher values of phosphorus than the other acid mixtures. This might probably be due to the formation of insoluble sulphates with the inclusion of sulphuric acid in the other mixtures and hence the attendant co-precipitation or adsorption of phosphorus. On the other hand perchloric acid has been strongly recommended (3) for wet digestions because of the universal solubility of the resulting perchlorates. Thus, the low phosphorus contents obtain using sulphuric-nitric acid mixture may not be totally due to volatilization losses but due to some other chemical losses. The phosphorus value obtained (table 9) following the literature (1) digestion procedure is significantly lower than those obtained using the various modified procedures. This is in agreement with the suggestion (43), that for accurate results, the experimental conditions should be explicitly stated.

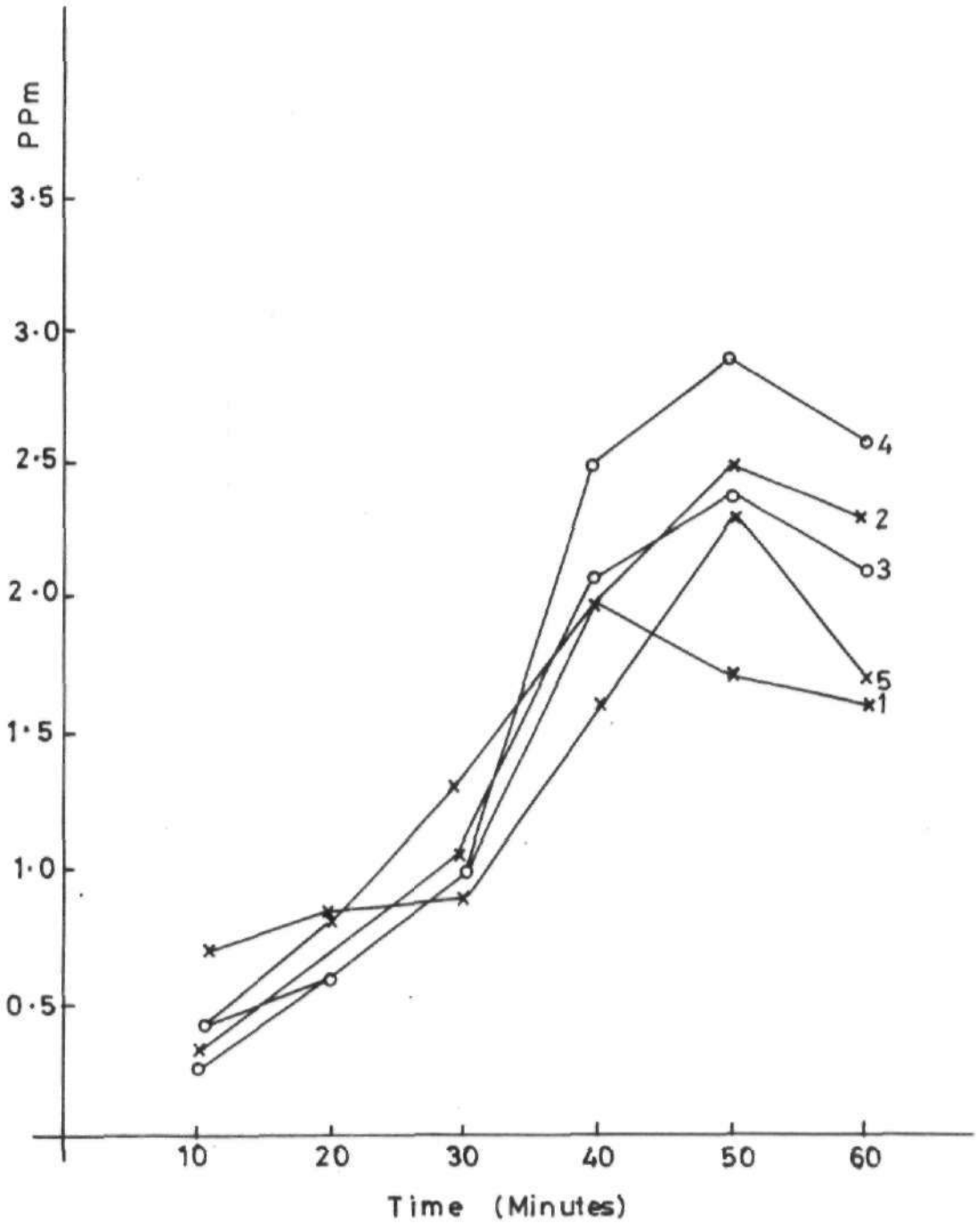

Fig. V Variation of Phosphorus contents with heating time, temperature and nature of acid mixture for Sample I.

TABLE 7

ANALYSIS OF SAMPLE F USING DRY ASHING METHOD

ELEMENTS	400°C	600°C
Calcium	16.0	14.0
Copper	0.2	0.2
Iron	54.0	58.0
Sodium	1.7	1.8

TABLE 8

COMPARISON OF WET DIGESTION AND DRY ASHING METHODS USING SAMPLE F.

ELEMENTS	DRY ASHING		WET DIGESTION*				
	400°C	600°C	1	2	3	4	5
Ca	16.0	14.0	26.0	38.0	39.0	68.0	66.0
Cu	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Fe	54.0	58.0	60.0	50.0	33.4	52.8	56.0
Na	1.7	1.8	2.3	2.2	2.0	2.7	2.6
P	-	-	1.5	1.7	2.2	2.6	2.4

*The values (in ppm) quoted for the respective digestion methods, are the maximum values obtained using the different digestion procedures.

TABLE 9

MINERAL CONTENTS OF SAMPLE E USING THE LITERATURE DIGESTION PROCEDURE WITHOUT ANY MODIFICATION.

ELEMENTS	MINERAL CONTENTS (ppm)*
Calcium	26.0
Copper	0.2
Iron	60.0
Sodium	2.2
Phosphorus	1.3

*Each result is an average of at least 4 determinations.

3.8 THE LITERATURE DIGESTION PROCEDURE

The values obtained for calcium and phosphorus in following the literature digestion procedure (1) are significantly lower than the maximum values obtained by the different digestion modifications, while those of copper iron and sodium showed only some slight differences. Thus, it would appear that the need for exact specification of experimental conditions during the wet digestion is more important for calcium and phosphorus than for copper, iron and sodium in this sample. This is in agreement with the suggestion (43) that the use of a single digestion solution for the determination of several minerals may lead to some experimental errors.

CHAPTER IV

4.1 SUMMARY AND CONCLUSION

The AOAC methods (1980), for example require (44) that wet digestion for the determination of calcium, copper, iron, magnesium, manganese, potassium and zinc from the same digest solution be done at an unspecified temperature and an undefined length of time. We consider this inadequate and liable to errors. As can be seen from this study, the level of metals determined may sometime depend on the temperature and length of heating. Calcium and phosphorus values obtained tend to decrease with increase in temperature, while iron and sodium tend to increase, and copper is essentially unaffected by temperature variation, at least, up to 600°C used in this study. It was even not possible to obtain iron at 70°C from samples (H and I).

Although some losses of calcium have been reported in the literature in the presence of sulphuric acid due to formation of insoluble sulphates, we report here losses of calcium which like the well documented losses of phosphorus (1, 3, 43), appear due to volatilization. The perchloric-nitric acid gave the highest values for calcium and phosphorus at 132°C, indicating possible

losses due to formation of insoluble sulphates and possibly co-precipitation in the presence of sulphuric acid. The sulphuric-nitric acid mixture at 132°C gave the highest values for sodium, while copper and iron values obtained were essentially unaffected by variation in the acid mixture used.

The trends observed for wet digestion of the samples are in agreement with the results obtained for the minerals by dry-ashing using sample F (soil). Thus for both iron and copper dry-ashing at 400 and 600°C gives values which are similar to those obtained by wet digestion. The values of calcium obtained by dry-ashing are significantly lower than those by wet digestion, and would tend to agree with the suggestion that for calcium in these samples volatilization losses are important at high temperatures. Sodium at 400 and 600°C shows some slight decrease, and it would appear there is a critical temperature between 70 and 400°C beyond which sodium solutions from these samples should not be prepared either by wet digestion or dry-ashing for determination.

From the above, it may be concluded that the use of the same digest solution to determine several minerals in these and related samples may lead to errors as the

various minerals tend to respond differently to changes in acid digestion mixture, temperature and length of heating during wet digestion. Both wet digestion and dry-ashing may be used for the preparation of solutions for the determination of copper and iron without significant differences in the values of the minerals obtained. Calcium and sodium will show some significant differences between the two methods and adequate care should be excercised in using either method for preparation of sample solutions.

4.2 SUGESTIONS FOR FURTHER STUDIES.

It is suggested that the effects of heating time, temperature, nature of acid mixture and other factors be examined using known calcium compounds and for a wider variety of ecological materials. For accurate and precise analysis it is essential the conditions of wet digestion and dry-ashing be clearly stipulated.

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APPENDIX I

CALCIUM CONTENTS (IN ppm) OF SAMPLES ANALYSED.

CODES	1	2	3	4	5
		SAMPLE A			
TIME (MIN.)					
10	22.5	22.5	18.0	43.5	36.5
20	20.5	22.0	20.5	40.5	36.0
30	21.0	21.5	24.0	40.0	35.0
40	20.0	22.0	24.0	38.0	35.0
50	19.5	21.0	22.0	35.5	32.5
60	18.0	20.0	20.5	33.0	30.0
		SAMPLE B			
10	10.0	11.5	12.5	18.5	15.0
20	8.5	11.0	13.0	18.0	14.0
30	8.5	10.5	12.5	16.0	14.0
40	9.0	10.0	10.5	14.5	14.0
50	8.2	8.5	11.0	14.0	13.0
60	7.0	6.0	10.0	12.0	10.0
		SAMPLE C			
10	7.0	6.0	8.0	12.5	10.0
20	6.5	8.5	9.0	10.0	10.0
30	6.4	8.0	8.5	10.5	9.0
40	6.4	6.5	9.5	8.0	6.5
50	6.4	6.5	8.0	7.0	7.0
60	5.5	6.0	7.5	6.0	6.0

Appendix 1 (cont'd)

CODES	1	2	3	4	5
		SAMPLE E			
TIME (MIN.)					
10	49.0	54.0	46.5	62.5	59.0
20	47.5	54.5	50.0	60.0	59.0
30	42.5	52.0	58.0	55.0	54.0
40	40.0	45.5	56.0	50.5	47.5
50	34.5	40.5	48.0	45.0	44.0
60	30.5	38.0	39.0	32.0	36.5
		SAMPLE F			
10	26.0	38.0	39.0	68.0	66.0
20	26.0	36.5	38.0	66.0	65.0
30	21.0	37.0	37.5	61.0	60.0
40	20.0	36.0	37.5	61.0	55.0
50	19.0	36.0	37.5	56.5	55.0
60	18.5	34.0	36.5	54.0	50.5
		SAMPLE G			
10	19.2	37.5	39.0	30.5	26.5
20	16.0	37.0	37.5	30.5	25.0
30	16.5	36.0	37.9	28.0	23.0
40	15.0	36.5	37.0	20.5	18.5
50	14.5	36.5	37.0	20.0	16.0
60	14.5	36.0	36.0	18.5	16.0
		SAMPLE H			
10	28.5	41.0	39.0	40.0	35.5
20	29.0	39.0	40.0	41.5	35.0
30	29.0	40.0	41.0	38.0	32.0
40	29.5	39.0	40.0	36.5	30.5
50	29.5	38.0	39.0	31.0	29.0
60	29.0	38.0	39.0	28.0	30.0

Appendix 1 (cont'd)

CODES	1	2	3	4	5
		SAMPLE I			
TIME (MIN.)					
10	51.0	50.5	50.2	125.0	110.0
20	72.5	51.2	50.5	119.0	96.0
30	77.0	51.2	51.2	122.0	108.0
40	74.5	51.2	51.6	121.0	108.0
50	70.0	51.4	51.4	105.0	99.0
60	66.5	50.5	51.1	102.0	96.0
		SAMPLE J			
10	20.0	22.5	24.5	26.5	19.5
20	21.0	26.0	25.0	21.0	19.0
30	18.5	23.0	28.0	20.0	15.5
40	15.0	20.0	24.5	18.0	15.0
50	15.0	20.5	22.0	18.5	15.0
60	14.0	20.0	20.5	16.0	12.0
		SAMPLE K			
10	15.0	14.0	15.5	24.5	13.5
20	11.0	16.0	17.5	23.0	13.5
30	15.5	16.5	16.0	19.0	10.5
40	10.0	15.0	16.5	18.5	9.5
50	9.5	12.0	14.5	16.0	10.0
60	9.0	12.0	14.0	17.0	9.5
		SAMPLE L			
10	23.5	28.5	24.5	36.4	20.5
20	23.5	30.0	30.0	34.0	18.0
30	24.8	28.0	32.0	32.5	18.5
40	25.0	27.5	28.5	32.0	17.5
50	24.8	26.0	28.0	30.0	16.0
60	20.0	24.0	28.0	29.5	16.0

Appendix I (cont'd)

CODES	1	2	3	4	5
		SAMPLE M			
TIME (MIN.)					
10	45.8	48.5	42.5	60.5	39.5
20	62.0	50.5	48.0	58.5	38.0
30	65.5	50.0	56.0	54.5	34.5
40	64.0	45.0	52.0	52.0	30.0
50	60.0	42.0	50.0	44.5	28.0
60	56.5	40.5	45.5	41;5	29.0

APPENDIX 2

COPPER CONTENTS (IN ppm) OF SAMPLES ANALYSED.

CODES	1	2	3	4	5
		SAMPLE A			
TIME (MIN.)					
10	0.16	0.14	0.16	0.18	0.15
20	0.20	0.18	0.23	0.20	0.18
30	0.18	0.16	0.23	0.18	0.18
40	0.16	0.16	0.20	0.16	0.17
50	0.16	0.18	0.20	0.17	0.17
60	0.16	0.18	0.18	0.16	0.17
		SAMPLE B			
10	0.10	0.10	0.08	0.10	0.10
20	0.10	0.10	0.10	0.12	0.10
30	0.08	0.16	0.12	0.12	0.12
40	0.10	0.16	0.14	0.12	0.12
50	0.08	0.10	0.14	0.14	0.10
60	0.08	0.10	0.14	0.12	0.08
		SAMPLE C			
10	0.18	0.10	0.18	0.20	0.16
20	0.20	0.10	0.20	0.20	0.18
30	0.18	0.16	0.16	0.24	0.22
40	0.23	0.16	0.16	0.20	0.25
50	0.16	0.16	0.16	0.15	0.22
60	0.16	0.16	0.16	0.16	0.18

Appendix 2 (cont'd)

CODES	1	2	3	4	5
		SAMPLE E			
TIME (MIN.)					
10	0.24	0.20	0.16	0.22	0.24
20	0.24	0.21	0.20	0.24	0.26
30	0.30	0.26	0.25	0.28	0.32
40	0.20	0.28	0.26	0.30	0.30
50	0.20	0.28	0.28	0.24	0.25
60	0.15	0.20	0.28	0.18	0.20
		SAMPLE F			
10	0.16	0.16	0.18	0.16	0.16
20	0.18	0.16	0.18	0.16	0.16
30	0.20	0.18	0.20	0.20	0.20
40	0.20	0.20	0.18	0.20	0.22
50	0.22	0.22	0.18	0.20	0.20
60	0.20	0.20	0.18	0.20	0.20
		SAMPLE G			
10	0.15	0.16	0.16	0.18	0.14
20	0.18	0.16	0.18	0.20	0.16
30	0.18	0.18	0.18	0.20	0.17
40	0.20	0.20	0.18	0.18	0.20
50	0.20	0.16	0.18	0.16	0.18
60	0.18	0.18	0.18	0.16	0.18
		SAMPLE H			
10	0.14	0.18	0.18	0.18	0.14
20	0.14	0.16	0.18	0.20	0.16
30	0.16	0.18	0.20	0.20	0.16
40	0.14	0.18	0.20	0.18	0.14
50	0.16	0.18	0.20	0.18	0.16
60	0.16	0.18	0.20	0.18	0.16

Apendix 2 (Cont'd)

CODES	1	2	3	4	5
		SAMPLE I			
TIME (MIN.)					
10	0.14	0.16	0.23	0.18	0.18
20	0.16	0.20	0.20	0.18	0.18
30	0.18	0.18	0.23	0.20	0.18
40	0.18	0.18	0.20	0.20	0.18
50	0.18	0.18	0.26	0.20	0.18
60	0.16	0.18	0.23	0.18	0.16

APPENDIX 3

IRON CONTENTS (IN ppm) OF SAMPLESANALYSED

CODES	1	2	3	4	5
		SAMPLE A			
TIME (MIN.)					
10	1.4	1.0	0.8	1.2	1.2
20	1.4	1.0	1.2	1.2	1.4
30	1.8	1.2	1.4	1.4	1.4
40	1.6	1.2	1.4	1.4	1.6
50	1.4	1.0	1.2	1.4	1.4
60	1.2	1.2	1.0	1.2	1.4
		SAMPLE B			
10	1.2	0.8	0.6	1.4	1.0
20	1.6	0.8	1.0	2.0	1.4
30	1.8	1.2	1.0	2.2	1.8
40	2.4	1.4	1.0	2.4	2.2
50	2.2	1.4	1.2	2.2	2.6
60	2.4	1.4	1.2	2.0	2.6
		SAMPLE C			
10	1.2	0.5	0.4	1.0	0.9
20	1.2	0.8	0.6	1.2	1.0
30	1.0	1.0	0.8	1.2	1.2
40	0.8	0.8	1.0	1.0	1.0
50	0.8	0.8	1.2	0.8	0.9
60	0.8	1.0	1.2	0.7	0.8

Appendix 3 (cont'd)

CODES	1	2	3	4	5
		SAMPLE E			
TIME (MIN.)					
10	54.0	44.5	42.0	51.0	56.0
20	62.0	47.5	50.0	54.0	62.0
30	65.0	53.0	51.0	64.0	66.2
40	72.0	60.0	57.0	62.0	66.5
50	73.5	58.0	56.0	60.5	66.0
60	72.2	57.0	56.0	56.0	64.0
		SAMPLE F			
10	48.5	40.0	26.4	45.0	50.2
20	48.5	44.0	33.0	48.0	52.4
30	53.0	48.0	32.0	49.0	53.2
40	57.0	50.0	31.0	52.8	56.0
50	54.0	49.6	33.2	52.8	54.0
60	60.0	49.4	33.4	50.0	50.1
		SAMPLE G			
10	42.0	26.0	12.4	43.5	40.0
20	44.0	26.0	21.6	45.0	42.0
30	46.0	30.0	23.6	48.0	44.5
40	47.0	32.0	23.2	48.0	50.0
50	50.0	31.0	24.0	49.0	53.5
60	50.0	30.4	27.8	48.0	52.0

Appendix 3 (cont'd)

CODES	1	2*	3*	4	5
		SAMPLE H			
TIME (MIN.)					
10	2.4	*	*	2.7	2.2
20	2.6			2.8	2.4
30	2.8			2.8	2.7
40	3.0			2.6	3.2
50	2.8			2.4	3.0
60	2.6			2.4	2.9
		SAMPLE I			
10	1.4			2.0	1.8
20	2.4			2.2	2.4
30	3.8	*	*	2.6	2.9
40	4.0			2.4	3.6
50	4.2			2.4	3.8
60	3.6			1.8	3.8
		SAMPLE J			
10	36.0	36.0	19.0	35.0	43.2
20	43.0	42.0	25.0	43.6	45.0
30	48.4	44.0	34.0	44.0	50.0
40	48.6	44.0	38.0	45.0	56.4
50	58.0	45.0	38.4	44.0	56.0
60	56.4	45.0	40.0	44.0	52.6

Appendix 3 (cont'd)

CODES	1	2	3	4	5
		SAMPLE K			
TIME (MIN.)					
10	33.0	29.5	22.0	35.0	31.5
20	35.0	32.0	24.5	37.0	33.0
30	37.0	35,5	29.0	42.0	36.5
40	40.0	36.0	32.0	48.0	42.5
50	50.0	38.5	37.0	47.5	52.5
60	50.0	40.0	38.0	48.0	52.0
		SAMPLE L			
10	2.2	1.9	1.2	2.5	2.0
20	2.4	2.0	1.5	3.0	2.2
30	2.8	2.3	2.0	3.5	2.7
40	3.4	2.7	2.5	3.8	3.5
50	3.6	3.2	2.9	3.8	4.2
60	3.7	3.3	3.0	3.7	4.1
		SAMPLE M			
10	9.4	8.2	5.2	8.8	8.0
20	12.6	10.0	6.0	10.2	10.2
30	13.0	9.7	7.2	9.6	12.0
40	12.4	9.6	8.0	7,8	11.0
50	9.2	8.2	7.8	7.2	10.8
60	9.0	8.6	7.8	7.2	10.0

*It was not possible to obtain any iron at these temperatures for these samples.

APPENDIX 4

SODIUM CONTENTS (IN ppm) OF SAMPLES ANALYSED

CODES	1	2	3	4	5
		SAMPLE A			
TIME (MIN.)					
10	2.3	1.8	0.9	2.5	2.2
20	2.5	2.1	0.9	2.8	2.6
30	3.2	2.1	1.1	2.2	3.4
40	2.5	2.4	1.2	2.2	3.6
50	2.3	2.4	1.4	2.1	3.0
60	2.3	2.4	1.4	2.0	2.4
		SAMPLE B			
10	1.7	1.0	0.6	2.0	1.8
20	2.0	1.2	0,7	2.4	2.6
30	1.7	1.3	0.8	2.2	3.0
40	1.5	1.3	0.9	1.6	2.4
50	1.5	1.4	1.0	1.6	1.8
60	1.3	1.5	1.0	1.4	1.8
		SAMPLE C			
10	2.4	1.5	1.2	2.5	2.2
20	3.0	1.5	1.2	3.2	2.8
30	3.2	1.7	1.8	3.6	3.2
40	2.3	1.4	1.8	2.6	3.8
50	1.8	1.2	2.1	2.2	3.0
60	1.8	1.4	2.1	1.6	2.6

Appendix 4 (Cont'd)

CODES	1	2	3	4	5
		SAMPLE E			
TIME (MIN.)					
10	2.5	1.4	1.2	2.0	2.3
20	2.8	2.1	1.7	2.2	2.6
30	3.0	2.0	1.8	2.1	2.5
40	3.4	1.9	1.9	2.2	2.4
50	3.2	1.6	1.7	1.9	2.3
60	3.3	1.5	1.6	1.6	2.2
		SAMPLE F			
10	2.3	2.0	1.6	2.7	2.6
20	2.4	2.2	1.9	3.2	2.9
30	2.4	1.9	2.0	2.8	3.4
40	2.1	2.0	2.0	2.6	3.3
50	2.3	2.0	1.8	2.8	2.6
60	2.3	1.9	2.0	2.9	2.8
		SAMPLE G			
10	1.8	1.7	1.7	2.0	1.9
20	1.9	1.9	1.8	2.1	2.0
30	2.1	1.9	1.7	2.0	2.2
40	2.0	1.8	1.7	1.9	2.0
50	1.9	1.8	1.7	2.0	2.0
60	1.9	1.9	1.7	1.8	1.9

Appendix 4 (cont'd)

CODES	1	2	3	4	5
		SAMPLE H			
TIME (MIN.)					
10	0.9	0.5	0.6	1.0	0.8
20	1.0	0.6	0.6	1.0	0.9
30	1.0	0.6	0.6	1.1	1.2
40	0.9	0.6	0.7	1.0	1.0
50	1.0	0.6	0.7	0.9	1.0
60	0.9	0.5	0.7	0.8	1.9
		SAMPLE I			
10	1.6	0.8	0.9	1.1	0.8
20	1.8	0.9	1.2	1.1	0.9
30	1.6	1.0	1.2	1.0	1.0
40	1.6	1.1	1.1	1.1	0.9
50	1.8	1.3	1.2	1.2	0.9
60	1.7	1.2	1.0	1.2	0.8
		SAMPLE J			
10	1.8	1.6	1.5	2.0	2.0
20	1.9	2.1	2.0	2.3	2.4
30	2.2	2.0	1.5	2.0	2.6
40	2.2	2.1	1.5	1.8	2.3
50	2.1	2.0	1.3	1.7	2.3
60	2.1	2.0	1.2	1.6	2.1

Appendix 4 (cont'd)

CODES	1	2	3	4	5
		SAMPLE K			
TIME (MIN.)					
10	2.0	2.0	1.8	2.3	2.3
20	2.5	2.2	2.2	2.4	2.5
30	2.4	2.0	2.1	2.7	2.2
40	1.8	1.9	2.0	2.2	1.7
50	1.6	1.7	1.9	2.0	1.5
60	1.6	1.5	1.8	1.9	1.4
		SAMPLE L			
10	1.6	2.3	2.3	2.4	1.8
20	2.6	2.5	2.4	2.5	2.0
30	2.4	2.2	2.7	2.5	2.6
40	2.2	1.7	2.2	2.4	2.6
50	2.1	1.5	2.0	2.0	2.2
60	2.0	1.4	1.8	2.0	2.1
		SAMPLE M			
10	2.0	1.6	2.1	2.2	1.9
20	2.0	1.9	2.3	2.3	2.1
30	2.2	2.0	2.1	2.1	2.4
40	2.2	1.7	2.0	1.8	2.0
50	1.7	1.5	1.9	1.5	1.6
60	1.6	1.5	1.7	1.5	1.6

APPENDIX 5

PHOSPHORUS CONTENTS (IN ppm) OF THE SAMPLES ANALYSED

CODES	1	2	3	4	5
		SAMPLE E			
TIME (MIN.)					
10	0.2	0.8	0.5	0.7	1.2
20	0.3	0.8	0.7	1.3	1.5
3.0	0.6	0.8	0.9	3.6	2.0
40	1.1	1.3	1.2	4.4	2.4
50	1.7	1.7	2.0	4.0	3.3
60	1.6	1.2	2.0	3.4	3.1
		SAMPLE F			
10	0.4	0.4	0.8	0.6	0.5
20	0.7	0.6	1.0	1.0	0.6
30	0.9	1.4	1.4	1.4	1.2
40	1.3	1.6	1.8	2.1	1.6
50	1.5	1.7	2.2	2.6	2.4
60	1.4	1.6	2.2	2.4	2.2
		SAMPLE G			
10	0.4	0.3	0.4	0.4	0.6
20	0.6	0.4	0.6	0.6	0.8
30	0.8	1.1	1.2	1.0	1.3
40	2.0	2.1	1.9	1.0	2.0
50	1.4	2.8	2.7	3.3	2.6
60	1.2	2.6	2.8	3.1	2.3

Appendix 5 (cont'd)

CODES	1	2	3	4	5
		SAMPLE H			
TIME (MIN.)					
10	0.4	0.4	0.3	0.4	0.4
20	0.6	0.4	0.4	1.0	0.6
30	0.7	1.0	0.8	1.3	1.0
40	1.1	1.4	1.4	1.9	1.4
50	1.0	1.3	1.7	1.4	1.2
60	0.9	1.1	1.7	1.3	1.0
		SAMPLE I			
10	0.4	0.3	0.3	0.4	0.7
20	0.8	0.7	0.6	0.6	0.8
30	1.4	1.1	1.2	1.0	0.9
40	2.2	2.0	2.1	2.5	1.6
50	1.7	2.5	2.4	2.9	2.3
60	1.6	2.3	2.4	2.6	1.9
		SAMPLE J			
10	0.3	0.4	0.4	0.4	0.5
20	0.4	0.7	0.6	0.5	0.8
30	1.3	1.3	0.9	1.2	1.2
40	2.0	1.3	1.4	1.6	1.6
50	2.5	2.4	1.6	1.6	1.4
60	2.0	2.2	2.0	1.3	1.1

Appendix 5 (cont'd)

CODES	1	2	3	4	5
		SAMPLE K			
TIME (MIN.)					
10	0.3	0.3	0.2	0.7	0.4
20	0.4	0.4	0.4	1.0	0.9
30	0.6	0.9	1.0	1.5	1.4
40	2.0	1.6	1.7	2.2	2.0
50	2.0	2.5	2.4	2.6	2.2
60	1.8	2.3	2.6	2.2	1.8
		SAMPLE M			
10	0.5	0.5	0.5	0.4	0.6
20	0.9	0.8	0.6	0.8	0.8
30	1.2	1.3	1.0	1.0	1.3
40	1.4	1.8	1.5	3.6	1.7
50	1.5	1.9	1.9	2.9	1.1
60	1.5	1.8	2.0	2.6	0.9