METHODS FOR FLUORIDE ANALYSIS IN PLANTS, FOODS AND SOIL USING FLUORIDE ION SELECTIVE ELECTRODE.

BY

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

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

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DEDICATION

To my daughter WAIRIMU
and
my father NJENGA KIRUKA.

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ABSTRACT

In the recent past there has been an increasing interest in the determination of the fluoride content in foods. The fluoride analysis has been carried out using mainly the fluoride ion selective electrode. This interest has arisen due to the need to evaluate the total fluoride intake per day from both water and food and hence establish any correlation with incidences of dental fluorosis or caries. This has led to the development of various methods for the release of fluoride ions which may exist in inorganic or organic forms. The methods include ashing in the furnace, acid digestion, oxygen bomb, Schöniger oxygen flask combustion, pyrohydrolysis and direct diffusion. The latter method has gained popularity especially for low fluoride concentrations. Available literature shows different values of fluoride content even for the same foods and this may be attributed to methodology, accuracy and/or the processing of the foods.

These methods of fluoride analysis are discussed and compared in this work. Inorder to handle many samples an open ashing method has been developed. This involves ashing in an open flame using calcium hydroxide as a fixative followed by microdiffusion at 60°C for 20 hours. The results obtained by this method compared well with those obtained using Schöniger oxygen flask method. Direct diffusion (without ashing) gave much lower values than the ashing method.

The effect of aluminium and silicon on diffusion has been studied. In order to minimise interference, it was found necessary to use perchloric acid and low amounts of sample (<0.5g) for diffusion.

The use of different buffers in the measuring solution has also been investigated. It was found that TISAB II alone is not effective in presence of aluminium (>2mg) but 1.4M sodium citrate plus TISAB II buffer was effective even in presence of up to 50mg aluminium.

Fluoride content in different types of soils has been analysed. For mobile fluoride ammonium lactate was used as the extracting medium. Values obtained with ammonium lactate were compared with those obtained using either water, 1M hydrochloric acid or sodium citrate plus EDTA. Ammonium lactate was found to give higher values than either water or sodium citrate plus EDTA but it was selected for fluoride extraction because of it's wide use as extracting media for other common ions in the soil. The extraction time, pH of the extracting media and amount of the sample were found to be important factors in fluoride extraction while clay content, organic matter and soil pH play important roles in fluoride accumulation in the soil. Analysis of soils from different localities showed the fluoride concentration to range from 21-282 $\mu g\ g^{-1}$ fluoride. High concentrations were recorded for those soils collected near cement and diatomite industries.

Analysis of fluoride in plants and vegetables collected from various parts in Kenya has been done. It was found that

there was variation of fluoride content in the same type of vegetable grown at different places. For example, Solanum nigrum from Lake Bogoria contained 29.98 μg g^{-1} while that from Kiambu contained 3.82 μg g^{-1} fluoride. Vegetables of the same family grown in the same locality accumulated almost the same amount (Cucurbita sps 7.16 μg g^{-1} and Cucurbita pepo 7.32 μg g^{-1} fluoride).

Plants picked from the shores of Kenyan lakes were found to have higher fluoride concentrations for example Euperus laevigatus (from Lake Nakuru, 140 mg l^{-1}) contained 1049 μg g^{-1} fluoride, while those grown at a distance from the same lake especially Bequaertia robyns contained 32.06 μg g^{-1} fluoride. It was found that, up to 98% of fluoride in tea was acid labile and the amount of fluoride in tea was found to depend on the age of the tea plant. The older the tea plant, the higher the fluoride concentration.

ABBREVIATIONS

CDTA	-	Trans 1,2-cyclohexanediamino-tetraacetic acid.
EDTA	-	1,2-ethylenediamino-tetraacetic acid.
Tiron	-	Pyrocatechol-3,5-disulphonic salt.
HMDS	-	Hexamethyldisiloxane.
TMFS	-	Trimethylfluorosilane.
TISAB	-	Total ionic strength adjustment buffer.

CHAPTER I

1:0 INTRODUCTION

Fluorine ranks thirteenth in abundance in the earth's crust. It is found in such minerals as apatite (calcium fluorophosphate), fluorspar (calcium fluoride) and cryolite (sodium aluminium fluoride). Large scale production of fluorine started during the second world war when Uranium hexafluoride was required for atomic weapons. Recently it has been used in the production of fluorocarbons for use as propellant.

Fluorine compounds are emitted to the atmosphere through various industries for example, brick, phosphate fertilizer, steel, aluminium, cement and fluorite industries. Fluorine compounds are also found in plants, soil and water normally in low concentrations. Since fluorine compounds are present in all parts of the environment, it is not surprising that most foods contain some amount of fluoride. Chemically, fluorine is very reactive and this makes it to be physiologically active to an extent that even small concentrations can affect the enzymatic reactions and may also combine with organic or inorganic compounds in animals.

1:1 PHYSIOLOGICAL ASPECTS OF FLUORIDE

The World Health Organization (1970) suggested an optimal limit of 0.7-1.5 mg l^{-1} fluoride for the fluoride in drinking water. Below 0.7 mg l^{-1}, dental caries occur and above 1.5 mg l^{-1} dental fluorosis takes place. Fluoride is a bone seeking element and it is important for the maintenance of

normal skeletal structure [Zipkin et al 1962]. A survey carried out in the United States showed that, the incidence of osteoporosis was lower in naturally high fluoride areas than in localities of low fluoride areas [Bernstein et al 1966], whereas the incidence of aortic calcification was reduced in high fluoride areas. Fluoride has a high affinity for calcium, magnesium and manganese and this causes it to interfere with the activity of many enzymes and as a result may adversely affect the function of the endocrine processes. Long term intake of large amounts of fluoride causes skeletal fluorosis.

In Kenya, bone fluorosis has been observed among residents in Thindigwa coffee estate (Kiambu), Kenya Breweries (Nairobi), Magadi and Kerio Valley. The fluoride concentration of drinking water in these areas was found to be above 10 mg l^{-1}. High prevalence and severity of dental fluorosis in Kenyan communities have also been reported [Manji and Kapila 1983]. Work done on the determination of fluoride levels for surface water and borehole waters in Kenya shows that, the fluoride levels for surface waters is quite low 0.1-0.7 mg l^{-1} while levels of borehole waters vary widely 0.1-50 mg l^{-1}. The high levels are mainly found in the Rift Valley. However cases of dental and skeletal fluorosis have been reported even in areas where communities are supplied with river water with very low fluoride concentration [Njenga 1982].

1:2 FLUORIDE CONTENT IN FOODS

Man gets his fluoride from water, beverages and food. Fluoride content from the food varies considerably according to where and how it is grown and prepared. Climatic conditions, soil and fluoride emitting industries also affect the fluoride concentration [Perkin et al 1980]. Fluoride levels in most foods are below 1 $\mu g\ g^{-1}$ with exception of tea which accumulate 8-400 $\mu g\ g^{-1}$ fluoride [Waldbott 1978, Underwood 1962, Purves 1977]. About 90% of fluoride in tea is water soluble and one cup of tea may contain upto 0.5 mg l^{-1} fluoride. This means that, normal intake of tea will increase the fluoride intake per day. Fish and other sea foods are known to contain high fluoride concentration (approximately 250 $\mu g\ g^{-1}$). Spencer et al (1970) investigated the fluoride balance of adult men after eating food supplemented with fish protein concentrate and found that, the daily fluoride intake increased from 1.2 $\mu g\ g^{-1}$ to 4.7 $\mu g\ g^{-1}$ fluoride per day. Other types of meat contain 0.2-2.0 $\mu g g^{-1}$ fluoride while eggs and milk are usually poor sources of fluoride. Table 1:1 shows the fluoride concentration of different types of food reported by various workers.

TABLE 1:1 FLUORIDE CONTENT IN FOOD.

Type of Food	Fluoride ($\mu g\ g^{-1}$)	Reference
Plain flour	0.2-1.6	Sherlock (1984)
Self-rising flour	0.2-1.8	
Uncooked rice	<0.2-0.4	
Baking Powder	2.3-3.0	
Winter wheat	0.8-3.1	Becker and Bruce (1981)
Bananas	5.3-8.1	Akinos et al (1972)
Maize	12.4-17.6	
Coffee seeds	18.1-24.6	
Spinach	0.57	Taves (1983)
Purified salt	4.0	Kumpulainen and Koivistoinen (1977)
Crude sea salt	18-55	
Fish protein	1200	Sherlock (1984)
Fish concentrate	20-760	Ke et al (1969)
"	20	Moller (1982)
"	1050	Berg and Haug (1971)
Tuna fish	<0.2-2.0	Sherlock (1984)
"	3.52-4.0	Kumpulainen and Koivistoinen (1977)
Tilapia fish	15.0	Berg and Haug (1971)
Nile fish	7.0	
Meat (beef)	0.4-2.8	Sherlock (1984)
"	0.038	Taves (1983)
"	0.2-2.0	Waldbolt (1963)
Pork	0.2-3.0	
"	0.3-0.8	Sherlock (1984)
Milk	0.09-0.32	Kumpulainen and Koivistoinen (1977)
"	0.019	Taves (1983)
"	0.09-0.36	Nömmic (1953)
"	0.06-0.1	Kumpulainen and Koivistoinen (1977)
Tea	8-400	Waldbott (1978), Underwood (1962), Purves (1977)
"	836-1300	Venkateswarlu and Narayanarao (1957)

The amount of fluoride in plants varies according to where they are grown. Some plants are capable of accumulating fluoride as shown in table 1:2 depending on the nature of pollutant.

TABLE 1:2 EFFECT OF AIRBORNE FLUORIDE ON FOOD GROWN NEAR FLUORIDE EMITTING INDUSTRIES

Food	Source of Pollution	Normal Fluoride ($\mu g\ g^{-1}$)	Found Fluoride ($\mu g\ g^{-1}$)
	Aluminium factory		
Peach	"	0.21[a]	3.2-21.9[a]
Carrot	"	0.22-2.0[a]	5.0[a]
	Superphosphate industry		
Wheat grain	"	0.7-2.0	2.6
Pear	"	3.8[b]	10.7[b]
Plum	"	1.9	6.3
Apples	"	0.8	6.6
Corn	"	0.7	1.3
Cabbage leaves	"	0.15	9.6[a], 297
Lettuce	"	0.1-0.3	44.0
Potatoes (boiled)	"	0.4	7.7
Beans (cooked)	"	1.7	17.3

(a)-Sherlock (1984) (b)-Taves (1983) Others-Waldbott (1978)

Lettuce may therefore accumulate 400 times the normal value while carrot will accumulate 35 times the normal value when in an environment with high fluoride. Cabbage leaves and spinach have also been known to accumulate fluoride.

The fluoride content of fruits and vegetables is normally low and it seldom exceeds 0.2-0.4 $\mu g\ g^{-1}$ fluoride. Some roots are also known to contain high fluoride content for example cassava and yams [Murray 1986]. In cereals, fluoride tends to accumulate in the outer part of the grain with levels which are normally below 0.5 $\mu g\ g^{-1}$ fluoride [Becker and Bruce 1981] although higher levels have been reported in winter wheat from Sweden which contained 0.8-3.1 $\mu g\ g^{-1}$ fluoride.

In vegetation, the fluoride concentration varies among the species and the age of the plant. In most cases, old leaves are found to contain higher fluoride content than young leaves. High fluoride content may have some toxic and detrimental effect in plants such as necrosis and chlosis [Weinstein 1977], while reduction of growth may also occur [Murray 1984]. Best accumulators of fluoride are camellia, dichapetalum, tea, gastrolabium, acacia and palicourea.

The fluoride in plants is found to be of organic and inorganic form and the amount of the two forms varies according to the plant species. Until recently, there was no evidence that organic fluoride was metabolised by most plants. The only exceptions were some poisonous plants which synthesise fluorocitrate. These are found mainly in Africa, Australia and South America [Vickery and Vickery 1976]. However, it has now

been established that plants can convert atmospheric fluoride to an organically combined form partly volatile which escapes through the stomata. There are also plants which are capable of synthesising fluoroacetate and fluorocitrate from inorganic fluoride [Ming-Ho Yu and Miller 1970, Peters and Shorthouse 1967]. Lettuce has been found to form fluorocitrate from fluoroacetate and then defluorinate it producing inorganic fluoride which is easily released in water [Ward and Huskisson 1972]. Fluoro-oleic acid from toxicarium plant has also been identified as one of the organic fluorides in plants [Hall 1968].

1:3 FLUORIDE CONTENT IN SOIL

The fluoride content in the soil also varies from one place to the other depending on the type of the soil. Table 1:3 shows the fluoride content of soil samples from different countries [Smith 1983]. It is clear that, there is a great variation in fluoride content from one country to another.

TABLE 1:3 REPORTED FLUORIDE CONCENTRATION IN SOIL ($\mu g\ g^{-1}$).

Country	Range	Mean	Reference
Germany	80-1100	530	Smith (1983)
Japan	260-2500	370	
New Zealand	68-540	200	
Sweden (sandy soil)	43-198	90	
Sweden (clay soil)	248-657	450	
United States	10-7070	290	
Norway (sandy soil)		115	Eyde (1983)
Norway (clay soil)		288	

The availability of soil fluoride to plants depends on the form in which the fluoride occurs. For example, in alkaline calcium containing soils, the fluoride is bound as calcium fluoride which has a very low solubility and hence fluoride is bound strongly and very little is available for plants. Organic soils contain high fluoride content mainly from organisms but this is easily released in water. In clay soils, aluminium complexes the fluoride making it unavailable for plants while in saline soils, the dominance of sodium and the resultant greater solubility of fluoride can result in high levels of water soluble fluoride [Erashidi 1986, Smith 1984]. The fluoride content also depends on the pH of the soil and the organic matter [Morshina and Fanaskova 1985].

There are various processes that increase the fluoride content in soils. Volcanic eruptions contribute large amounts of fluoride to surface soils by way of ash depositions. Most of the fluoride is water extractable hence available for plants. Man also increases fluoride in the soil by addition of fertilizers. Long term experiments have shown that, extensive use of phosphate fertilizers can lead to an increase in the fluoride content of some crops like potatoes and leafy vegetables. Fluoride containing pesticides also have been found to increase the total fluoride in the soil [Becker and Bruce 1981, Oelschläger 1971].

The geology of Kenya is mainly characterised by various landscape formed by the process of faulting and volcanic eruption mainly known as the Rift Valley. Although most of the volcanic activity lies within the Rift Valley, some of the prominent volcanoes lie more than 100 km outside the Rift Valley for example Mt. Kenya, Kilimanjaro and Elgon. This indicates that most of the land surface in Kenya is covered by the volcanic ash and rocks.

Extensive work on fluoride content in water has been carried out by different researchers [Njenga 1982, Manji and Kapila 1983, Gitonga and Nair 1983]. Most of the rivers outside the rift valley lakes and boreholes contain low fluoride concentration. The underground water in the rift valley have passed through volcanic rocks and as a result, fluoride from the rocks passes into the water. Fluoride analysis on fishes from Kenyan lakes have also been carried out by Bergh and Huag (1971), Table 1:1. Kenyan food may be expected to contain

high fluoride content since it is grown in fluoride rich soils and hence may be a contributor to fluorosis.

1:4 ANALYTICAL METHODS

The success of an investigation of biological effects of fluoride in the body depends above all, on an accurate method for determining fluoride. The method should be applicable to the determination of fluoride in soils, plants, foods, animal tissue, body fluid and water. Many methods for fluoride analysis have been used with limiting amount of success.

For a long time, total fluoride determination has relied upon the classical Winter-Willard distillation method of the ashed sample followed by visual or colorimetric analysis. Recent methods which use ion selective electrode have been developed and found to be easier and reliable for the analysis of water samples [Njenga 1982]. Other methods which have been used for fluoride analysis are:- Chromatography, mass spectrometry, molecular absorption spectrometry, polarography radioactivation analysis and microwave plasma detection [Tsunoda et al 1977].

In plants, foods and biological samples, fluoride is found in two forms:-

(a) Inorganic fluoride which is acid and alkali labile,

(b) The organic fluoride which needs to be ashed with strong alkali or oxidising agent to free the fluoride.

As a result, the method for fluoride analysis depends on whether one is interested in total fluoride or in the labile fluoride. For the total fluoride, different methods have been used. For

example, ashing method using ashing and fusing agents like sodium hydroxide, magnesium chloride [Eyde 1982, Taves 1983], oxygen flask method [Moody et al 1980] and oxygen bomb [Venketeswarlu 1975].

For labile fluoride, acid digestion [Villa 1979, Stuart 1970] and diffusion [Hall 1968, Taves 1968] methods have been used. Total fluoride in the soil is analysed by fusion of the soil sample with a strong alkali or by pyrohydrolysis while for labile fluoride, different extraction media have been used.

The use of all the above methods by various investigators has given conflicting results as regards to the fluoride content in certain foods and biological samples as seen in table 1:1. The conflicting results may be due to interfering ions which might have not been taken care of or due to analytical errors.

1:5 OBJECTIVES.

In recent years, there has been an increasing concern in fluoride levels in the environment in areas where there is high prevalence of dental or skeletal fluorosis in Kenya. The main blame has been put on levels of fluoride in potable water. While this is largely true, there are cases of fluorosis even in low water fluoride areas.

Work has been done on fluoride content in water from rivers, boreholes and lakes but very little, if any, has been done in foods and plants. Therefore, the main objective of this work was to develop a method for fluoride analysis in foods and plants and then use the method to determine fluoride content in foods and plants.

In order to do so, a method for fluoride analysis in foods and plants which is reliable, cheap, accurate and reproducible was to be established. This would involve a detailed study of:-

1. The ashing and diffusion methods of the samples.
2. The effect of interfering ions, mainly aluminium, iron and silicon on diffusion.
3. The effect of interfering ions in the measuring solution.
4. Different buffers and their ability to remove interfering ions.

In order to determine factors that affect fluoride levels in foods and plants, it would be necessary to determine the labile fluoride in the soil. This was to be done by first developing a method for determining the labile fluoride in the soil.

Having developed the methods, a survey of the fluoride content in various foods, plants and vegetable samples was to be carried out.

CHAPTER 2

2:0 LITERATURE REVIEW

2:1 A REVIEW ON THE METHODOLOGY OF FLUORIDE ANALYSIS IN FOODS AND PLANTS.

The recommended AOAC Methods for determining fluoride lack the sensitivity for accurately determining microgram levels of fluoride in foods. The main problem is the high contamination from furnaces which result in high blank values, the slowness and questionable accuracy of direct standard addition methods using fluoride selective electrode [Dabeka and Mckenzie 1981]. For a long time, determination of fluoride has proved to be one of the most difficult in analytical chemistry [Mclue 1933, Mcdonald 1965] and so far no one method has been recommended. However, the determination of small amounts of fluoride in biological materials has been facilitated by the increasing adoption of diffusion procedures for the separation of fluoride from the interfering ions [Hall 1968] together with the development of the ion selective electrode [Frant and Ross 1966].

2:2 THE TREATMENT OF THE SAMPLE BEFORE ANALYSIS.

2:2:1 WASHING:

Washing of the sample is necessary inorder to remove the fluoride on the surface, soil particles, dust, pesticides (e.g fluoroacetamides and dichlofluororamides) and other particulate matter. The washing is done for a short duration, 40-60 seconds, with either distilled or de-ionised water, EDTA,

or dilute mineral acids [Compton 1970]. However care should be taken using the latter two because they may leach fluoride.

2:2:2 DRYING:

Drying of the sample is very necessary especially when one is dealing with dry weights instead of fresh weights and can be a source of analytical error. Samples are dried using a lamp at temperatures of 80 -100^{o}C for 6-12 hours depending on the nature of the sample. Other workers have dried samples in an oven set at 60 -80^{o}C [Cooke et al 1976, Villa 1979], or set at 100-130^{o}C [Levaggi et al 1971, Baker 1972, McQuaker and Gurney 1977, Jacobsson and McCunne 1972] for 12-48 hours. High temperatures should be avoided in order to reduce losses of volatile fluoride.

2:2:3 GRINDING:

The sample is reduced into a fine powder to increase its surface area and to achieve a high degree of homogeneity. Loss of material during grinding should be avoided especially when small particles are blown away. Oelschläger (1968) investigated the errors caused by grinding. Samples of red clover were ground rather coarsely (approximately 1mm). The powder was shaken in glass bottle in circular motions in order to separate the coarse and fine particles. The coarse particles on analysis contained 6.1 µg g^{-1} fluoride while the fine particles contained 14.1 µg g^{-1} fluoride.

The ground particles should pass through a 60 mesh sieve (particles of 0.5nm and smaller) [Cooke et al 1976]. After grinding, the samples are mixed thoroughly and then stored in either plastic bottles which are tightly closed or sealed plastic paper bags to avoid any contamination.

2:3 SAMPLE PREPARATION:

This involves the decomposition of organic material and the conversion of the fluoride to inorganic forms for subsequent determination. It is important that, during preparation, there should be neither loss nor contamination of the sample. Several methods have been used namely, ashing and fusion, pyrohydrolysis, oxygen flask combustion and acid digestion.

2:3:1 ASHING METHOD:

Ashing of biological materials serve two important functions:-

(a) It destroys all inorganic matter which otherwise would partly distill or diffuse over and interfere with spectrophotometric methods or diffusion methods.

(b) It makes covalently bound organic fluoride available for isolation and subsequent determination.

Although some carbon-fluoride bonds are cleaved by acid digestion and some by alkaline digestion, it is found that, the carbon-fluoride bonds in most organic compounds are extremely strong and require more drastic measures such as combustion at elevated temperatures and fusion with alkali solution.

Fusion with alkali media. The method has been restricted to vegetation and biological samples where cations such as aluminium and iron are not present in sufficient concentrations that may interfere with the ion-selective electrode analysis. When alkaline solutions are used as fixatives, it is necessary to evaporate them to dryness and char the ash on a hot plate or under an infrared lamp before fusing. This method has been used widely by various workers employing temperatures in the range 450-600°C and ashing for 6-16 hours [Baker 1972, McQuaker and Gurner 1977, Eyde 1982]. At temperatures above 600°C, there is a possibility of:

(a) overflowing and thus reducing the fluoride content,

(b) converting the fluoride into volatile forms for example, into silicon tetrafluoride (SiF_4) or hydrogen fluoride (HF) which escape reducing the fluoride content.

Most of the furnaces used for ashing contain an appreciable amount of fluoride especially those furnaces which are made of clay. At high temperatures, fluoride escapes from the walls of the furnaces either as hydrogen fluoride or silicon tetrafluoride thus contaminating the sample. Analysis of the lining of an oven that had been in use for nine years was found to contain 0.42 $\mu g\ g^{-1}$ fluoride while the lining of a new muffle oven contained 93.3 $\mu g\ g^{-1}$ fluoride [Nömmic 1953]. Two parallel samples fused in these two ovens gave 3.1 $\mu g\ g^{-1}$ fluoride while the new oven had 19.8 $\mu g\ g^{-1}$ fluoride. This showed that, the fluoride diffused into the sample as gaseous

fluoride since the crucibles were completely covered during the ashing. Oelschläger (1968) found that a furnace, which had been in operation for 10 years, when used to ash roughage for periods 2,6,12,24 and 48 hours at 540°C gave consistently increasing values of fluoride i.e. 20, 21, 23, 28 and 37 μg g^{-1} fluoride. He also found that, the blank value in the oven increased with increasing ashing time.

Inorder to avoid contamination, it has been suggested that furnaces should be lined with gas tight nickel or platinum casings to prevent the fluoride diffusing from the clay furnaces to the sample.

The main fixing agent which has been used is sodium hydroxide. McQuaker and Gurney (1977) used 6 cm^3 of 17M sodium hydroxide followed by filtration at a pH of 8-9 to remove aluminium, iron and silicates. Other fixing agents which have been used are magnesium chloride [Taves 1983, Marier and Rose 1966], magnesium hydroxide [Roost and Sigg 1978] and lithium hydroxide followed by potassium hydroxide [Hall 1968]. Venkateswarlu (1983) has argued that, when samples rich in manganese are fused with sodium hydroxide, sodium manganate is produced during distillation with perchloric acid, manganate ion undergo internal disproportionation forming manganese dioxide and permanganate. The permanganate oxidises any chloride ions present in the sample into chlorine gas. The chlorine gas produced may interfere with subsequent volumetric, colorimetric or diffusion methods. The permanganate ions should therefore be reduced using hydrogen peroxide before distillation.

Another fixing agent, sodium carbonate - zinc oxide mixture [Louw and Richard 1972] has been found to give poor recovery [Edmond 1969].

2:3:2 PYROHYDROLYSIS

The sample, held in a platinum boat, is mixed with some reactive oxides such as tungstic oxide or vanadium pentoxide and placed in a tube heated to between 800-1200°C. A flow of nitrogen gas mixed with superheated steam is then passed over the heated sample. In some cases, superheated steam is replaced with moist oxygen. The procedure takes 15-20 minutes and hydrogen fluoride gas liberated is condensed and collected in sodium hydroxide. When elements such as sulfur and phosphorous are present in the sample, they are carried over with fluoride and will interfere with colorimetric and titrimetric methods of analysis for fluoride [Berns et al 1972, Venkateswarlu 1983]. Pyrohydrolysis has been applied in the analysis of some biological samples e.g. urine and organofluoride compounds [Van Gogh 1966], tea, tobacco, human teeth and bacteria [Newman 1968]. Other workers [Clement et al 1971, Peters and Ladd 1971, Van Leuven 1979] have also used the method for plant samples.

It is not certain how effective pyrohydrolysis is on releasing organic bound fluoride. According to Peter and Ladd (1971), the method does not give good recovery when applied to inorganic fluoride compounds especially alkaline earth elements. The method is also expensive especially when platinum tube has to be used. Due to the risk of explosion, skilled manpower has

to be employed. The materials used to construct less expensive units namely silica or ceramic materials normally react with fluoride to form complexes which interfere with the ion selective electrode and hence reduce the amount of fluoride.

2:3:3 SCHÖNIGER OXYGEN FLASK COMBUSTION METHOD

The method involves mainly combustion of the sample in a platinum wire gauze with oxygen in a sealed glass flask or Schöniger oxygen flask. The sample, folded in a strip of ashless filter paper is burnt in an atmosphere of oxygen confined in a flask containing few mili litres of either water, an alkaline solution or a buffer as an absorbing media [Moody et al 1976, Levaggi et al 1971, Vandeputte et al 1976]. Different amounts of sample have been used ranging from 25mg to 50 mg. Some researchers have used samples mixed with potassium chlorate to facilitate complete destruction.

Although the method is quick and many samples can be analysed per day, it suffers from two disadvantages, namely:-

a) when pyrex glass is used, the fluoride is found to be absorbed by the glass walls only to be released after a few analysis.

b) large volume of the absorbing media in comparison to the small weight of the sample used introduces some analytical errors during measurement, such that it is not possible to determine accurately the value of the blank or of samples with low fluoride.

2:3:4 DIRECT ACID DIGESTION

Acid digestion involves either (a) addition of acid to sample and allowing to digest at room temperature or (b) addition of acid to sample and then distilling. Villa (1979) used 0.1M perchloric acid to digest the samples for 25 minutes and analyse for fluoride using fluoride ion-selective electrode at pH 1. But his method has some setbacks in that, with 0.1M perchloric acid most of the sample may not be digested completely and at pH 1-4, the fluoride ion complexes with hydrogen ions and is not sensed by the fluoride selective electrode. According to Stuart (1970), the direct acid digestion is inaccurate and cannot be employed in organic samples because it does not give the total fluoride. Ke et al (1969) digested the sample with perchloric acid in the presence of silver perchlorate and distilled the mixture. The silver perchlorate in this case reacts with halogens to form insoluble halides.

$$AgClO_{4(aq)} + X^-_{(aq)} \longrightarrow AgX_{(s)} + ClO^-_{4(aq)}$$

X = Cl, Br, I.

Another disadvantage with perchloric acid distillation is that at high temperatures, explosion may occur when perchloric acid comes into contact with organic samples. The other acids used include:-

i) Nitric acid [Vandeputte et al 1976].

ii) Sulphuric acid buffered with 0.5M trisodium citrate.

iii) A mixture of concentrated nitric acid and perchloric acid then buffered with sodium acetate-CDTA buffer [Cooke et al 1976].

iv) Phosphoric acid [Bauman 1968]. Several different analytical reagent grades of phosphoric acid yield significant fluoride contamination which lowers precision especially with samples of low fluoride content [Villa 1979].

Weinstein et al (1965) used semi automated technicon autoanalysis to distil the plant samples. Acid base extraction at room temperature has been used [Jacobsson et al 1977, Johnson 1976, Gyoerkoes 1970].

However in comparison to other acids, perchloric acid is prefered because it has the lowest blank value.

2:4 EFFECT OF INTERFERING IONS IN THE FLUORIDE ANALYSIS.

The determination of fluoride using the fluoride ion-selective electrode is affected by three factors

1. Ionic strength
2. pH of the solution
3. The presence of complexing ions such as aluminium (III) and iron (III).

2:4:1 IONIC STRENGTH

In the determination of the fluoride ion using fluoride ion selective electrode, the potential is given by the equation

$$E = E^{o} - 0.059 \log [F^{-}] \qquad (2.1)$$

E = emf of the cell in volts

E^o = Standard emf

$[F^-]$ = Fluoride ion concentration

It has been shown that, as long as the ionic strength is kept constant (at 1M), the measured electrode potential is proportional to the fluoride concentration [Srinivasan and Rechnitz 1968]. The ionic strength is kept constant by the addition of either sodium chloride or potassium chloride [Shirashi et al 1973, Bagg 1976, Kauranen 1977].

2:4:2 THE EFFECT OF pH

The acidity or basicity of the measuring solution may affect the fluoride ion selective electrode in two ways [Frant and Ross 1966].

1. Negative Effect. This is produced when the solution has a pH<5. At this pH, the electrode records a decrease in the fluoride concentration. This decrease was attributed to the formation of hydrogen fluoride [Frant and Ross 1966], which is not sensed by the electrode. Srinivasan and Rechnitz (1968) has shown that, at low pH, there are two equilibria present

$$H^+ + F^- \rightleftharpoons HF \qquad (2:2)$$

$$HF + F^- \rightleftharpoons HF_2^- \qquad (2:3)$$

At high hydrogen ion concentration, undissociated hydrogen fluoride complex is formed as shown by the two equations. Studies carried out by Srinivasan and Rechnitz (1968) showed that, at a total fluoride concentration of 0.0040M, the amount

of free fluoride present varies with the hydrogen ion concentration. Fig. 2:1 shows that a tenfold decrease of the hydrogen ion concentration gives a tenfold increase of the free fluoride ion concentration. At high hydrogen ion and low fluoride ion concentration, equilibrium (2:2) is the predominant one but at low hydrogen concentration and high fluoride concentration both equilibria (2:2) and (2:3) are present with HF_2^- as the predominant complex. However, other workers have postulated the formation of additional polynuclear species $H(HF)_3^+$ but Srinivasan and Rechnitz (1968) found that, at low pH only F^-, HF and HF_2^- are present with HF_2^- as the highest complex.

The fluoride selective electrode will only sense the free fluoride ion but does not sense the bound fluoride (HF or HF_2^-). Hence the amount of fluoride sensed by the electrode will be lower than the actual fluoride concentration present and this produces the negative effect.

2. Positive Effect. This is produced by the hydroxyl ion. The hydroxyl ion will interfere with the electrode in that, the hydroxyl ion has got same charge and approximately equal ionic radii with the fluoride ion. Hence, the electrode will "see" the hydroxyl ion as if it were the fluoride ion and respond directly to its activity. The presence of the hydroxyl ion in the background will lower the limiting potential and causes an early deviation from linearity. As a result, at pH>8.0 the "apparent" fluoride concentration will be higher than the actual one in solution. Low fluoride concentrations cannot be measured accurately at pH>8.0 because the electrode responds

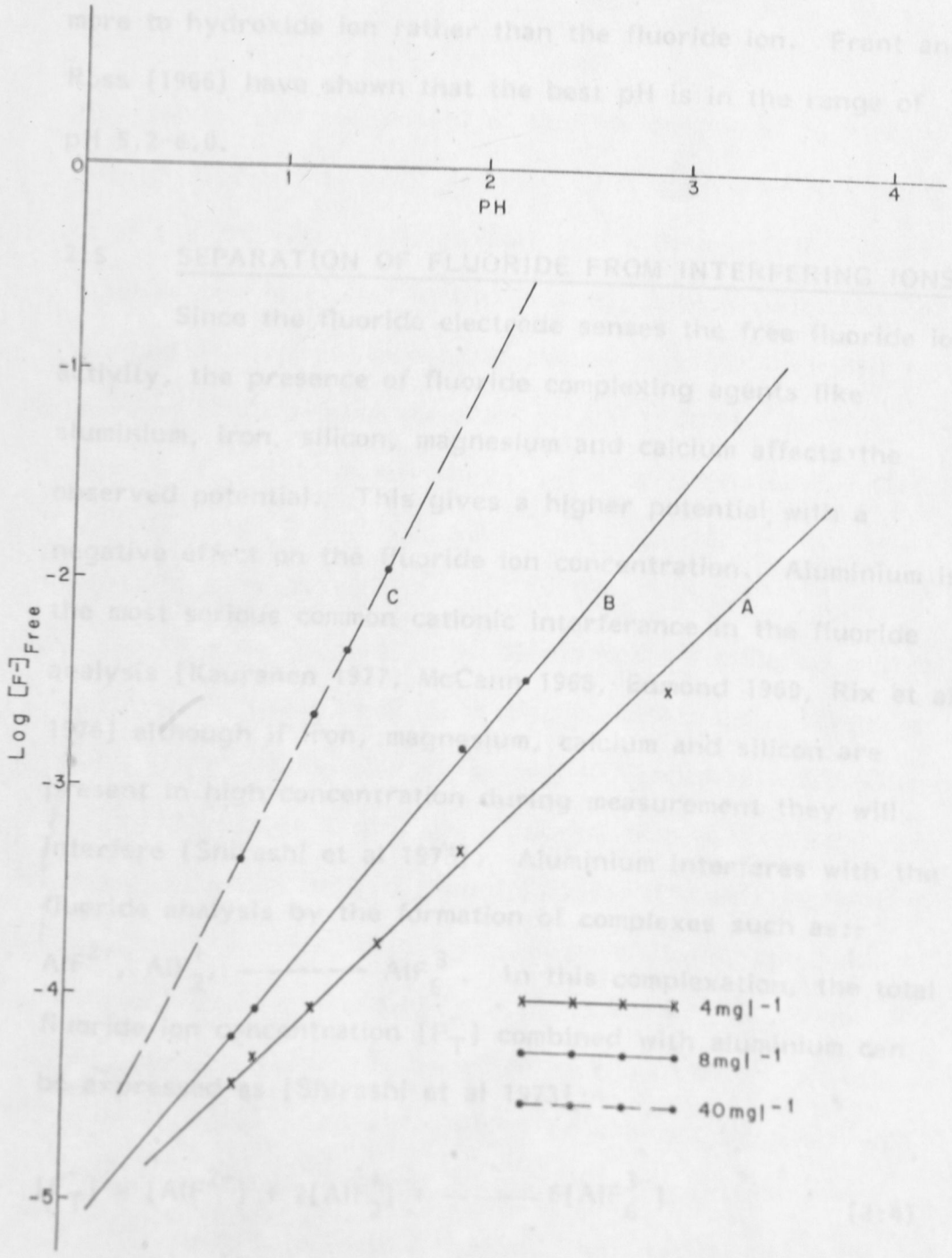

Fig. 2:1 Effect of pH on fluoride*.

*Data obtained from Srinivasan and Rechnitz (1968).

more to hydroxide ion rather than the fluoride ion. Frant and Ross (1966) have shown that the best pH is in the range of pH 5.2-6.0.

2:5 SEPARATION OF FLUORIDE FROM INTERFERING IONS

Since the fluoride electrode senses the free fluoride ion activity, the presence of fluoride complexing agents like aluminium, iron, silicon, magnesium and calcium affects the observed potential. This gives a higher potential with a negative effect on the fluoride ion concentration. Aluminium is the most serious common cationic interferance in the fluoride analysis [Kauranen 1977, McCann 1968, Edmond 1969, Rix et al 1976] although if iron, magnesium, calcium and silicon are present in high concentration during measurement they will interfere [Shirashi et al 1973]. Aluminium interferes with the fluoride analysis by the formation of complexes such as:- AlF^{2+}, AlF_2^{+}, --------- AlF_6^{3-}. In this complexation, the total fluoride ion concentration $[F_T^-]$ combined with aluminium can be expressed as [Shirashi et al 1973]:-

$$[F_T^-] = [AlF^{2+}] + 2[AlF_2^{+}] + -------6[AlF_6^{3-}] \qquad (2:4)$$

Inorder to analyse the fluoride ion with the electrode, the fluoride should be in free ionic forms and it has been suggested [Shirashi et al 1973] that, the ratio of complexed fluoride to free fluoride should be kept lower than 10^{-2} i.e.

$$\frac{[F^-]_{complexed}}{[F]_{free}} < 0.01 \qquad (2:5)$$

This implies that the amount of fluoride complexed with aluminium or any other interfering ions, should be less than 1% of free fluoride. The complexation with interfering ions is taken care of by (a) distillation, (b) adding decomplexing agents (which form stronger complexes with the interfering ions and hence release the fluoride), (c) precipitation at high pH, (d) ion exchange, (e) adsorption and (f) diffusion.

2:5:1 DISTILLATION

For a long time, the Willard-Winter distillation method has been used whereby fluoride is distilled either as fluorosilicic and/or hydrofluoric acid depending on concentration. Nömmic (1953) has reported that when fluoride is present in the sample in low concentration (0.5 µg fluoride), it distils as hydrofluoric acid but at a concentration of 3.60 µg fluoride, about 42% of the fluoride distils as fluorosilicic acid (H_2SiF_6). Perchloric acid and sulphuric acid have been used for distillation but due to low blank value, and the solubility of perchlorate compounds, perchloric acid is prefered. In addition, perchlorate ions do not interfere with colorimetric determinations unlike the sulphate ions. There are several disadvantages of the distillation method [Jacobsson and McCunne 1972] :

i) It is time consuming and careful control of temperature and special apparatus have to be used.

ii) Silicon in fluorosilicic acid will interfere with the ion-selective electrode analysis.

iii) A large volume has to be collected. This necessitates preconcentration of the distillate.

2:5:2 COMPLEXING AGENTS

The complexing agents form stronger complexes with the interfering ions and thus decomplex the fluoro complexes releasing free fluoride ions. The complexing agents include CDTA, EDTA, citrate and are contained in different types of buffers. The buffer solution also helps in:

a. providing a background of high and constant ionic strength.

b. adjusting the pH of the solution.

So far the buffers that have been used include:

1. TISAB I - an acetate-chloride solution containing sodium citrate.
2. TISAB II-which is an acetate-chloride solution containing CDTA.
3. TISAB III - it contains ammonium acetate, ammonium chloride, acetic acid and CDTA.
4. TISAB IV - which is made of concentrated hydrochloric acid, Tris(hydroxymethylaminomethane and sodium tartarate.
5. TISAB II-which is an acetate - chloride solution containing EDTA.
6. Citrate buffer - a mixture of sodium citrate and citric acid.
7. Tiron - A solution made with pyrocatechol-3,5-disulphonic acid disodium salt.

Citrate buffer is thought to be a better complexing agent for aluminium and has been recommended [Shirashi et al 1973, Kauranen 1977, Vickery and Vickery 1976, Duff and Stuart 1970]. The aluminium ions complex with citrate forming mainly aluminium citrate and trace amounts of aluminium hydrogen citrate and aluminium hydroxy citrate.

Interfering ions have also been removed by precipitating them as hydroxides at either pH 8-9 [McQuaker and Gurney 1977, Eyde 1982] or pH >9 [Baker 1972]. However, it has been shown that at pH>9, aluminium hydroxide and silicates are appreciably soluble and may pass into the filtrate. Phosphoric acid as a precipitating agent has been used [Baumann 1968, Louw and Richard 1972] and like in the above, there is a risk of fluoride co-precipitating together with silica.

2:5:3 ION EXCHANGE

Anion exchange resins have also been used. Fluoride will be less strongly absorbed among the univalent anions while polyvalent ions like sulphate and phosphate will be adsorbed strongly. Cation exchange resins have been used to remove cations such as aluminium and iron [McElfresh 1978].

2:5:4 ADSORPTION

In this method, solid calcium phosphate which is fluoride free is used as an adsorbing agent. The fluoride from the sample is adsorbed by calcium phosphate, separated by centrifuging and then subjected to microdiffusion [Venkateswarlu et al 1974]. The main advantage is that, even samples which contain large

bulk of interfering substances can be analysed. It is also possible to concentrate fluoride quickly from a large volume of sample. The calcium phosphate adsorption method has been widely used by Venkateswarlu et al (1983) to determine fluoride in a wide variety of biological samples for example, in milk, saliva and urine.

Adsorption on magnesium oxide has also been employed. The method involves boiling the solution containing fluoride and the interfering ion with magnesium oxide. Most of the fluoride is adsorbed onto the magnesium oxide almost instantaneously. It is possible to adsorb as much as 10µg fluoride per milligram of magnesium oxide [Venkateswarlu and Narayanarao 1957].

2:5:5 DIFFUSION

The method uses sulphuric acid or perchloric acid to separate fluoride as hydrogen fluoride which is trapped by a layer of sodium hydroxide in a closed system. The system is placed into an oven set at $60^{o}C$, for 16-24 hours. The hydrogen fluoride diffuses into the sodium hydroxide layer where it is trapped as sodium fluoride. Several variations of this method have been used [Singer and Armstrong 1954, 1959; Hall 1968; Marshall and Wood 1969].

Recently, diffusion using hexamethyl disiloxane (HMDS) at room temperature for six hours has been introduced. The method involves mixing HMDS and the acid before adding to the sample [Taves 1968, Sara and Wänninen 1975]. HMDS is found to increase the rate of diffusion by the formation of trimethylfluorosilane (TMFS) as shown below :

$$HF + (CH_3)_3SiOSi(CH_3)_3 \longrightarrow (CH_3)_3SiF + (CH_3)_3SiOH \qquad (2:6)$$

$$(CH_3)_3SiOH + HF \longrightarrow (CH_3)_3SiF + H_2O \qquad (2:7)$$

The TMFS is hydrophobic, very volatile and it readily diffuses into the sodium hydroxide layer where it is hydrolysed.

$$2(CH_3)_3SiF + 2OH^- \longrightarrow 2(CH_3)_3SiOH + 2F^- \qquad (2:8)$$

$$2(CH_3)_3SiOH \longrightarrow (CH_3)_3SiOSi(CH_3)_3 + H_2O \qquad (2:9)$$

The silanol condenses, returns to the acid, and the cycle is repeated. Taves (1968) found that when 19.7 µg of HMDS is present, 85.5 µg of fluoride diffused from a bone sample in one hour.

2:6 METHODS FOR FLUORIDE ANALYSIS IN SOIL

Soil samples have a very diverse mineral composition and some of the chemical constituents may hinder separation of fluoride. Therefore, special procedures for the sample preparation prior analysis of the total fluoride in the soil must be considered. Hall (1968) suggested that fluoride in the soil is found as:-

a) The inorganic fluoride which is water and dilute acid labile.

b) The fluorominerals which contain aluminium, calcium, magnesium, iron, silicon and phosphorous. These fluorominerals can only be diffused after fusion with strong alkali.

The fluoride is also found in organic form coming from dead animals and plants [Nõmmic 1953]. The total fluoride in soil is therefore the sum of the three types of fluoride.

Like in plants, total fluoride in the soil has been for along time carried out using the Willard-Winter distillation method followed by either titration or colorimetric analysis. Recent methods which use ion-selective electrode have also been applied to fluoride analysis in the soil. But unlike plants, soil contains high concentration of aluminium, iron and silicon which interfere with the fluoride analysis. McQuaker and Gurney (1977) suggested that 0.5g of digested soil sample diluted to 100 cm^3 may contain more than 2 mg l^{-1} aluminium together with iron levels in excess of 200 mg 1^{-1}. Bellack (1972) indicated that, at a level of 1 mg 1^{-1} fluoride, a suppression of 10% occurs if either 2 mg 1^{-1} aluminium or 200 mg 1^{-1} iron are present.

Different fusing agents have been used for fluoride analysis in the soil samples. These include:-

1. Sodium carbonate-zinc oxide [Shell and Craig 1954, Jagner and Pavlova 1972]. The method was introduced by Shell and Craig (1954) who pointed out that there was no loss of fluoride by volatilization. However it was found that, incomplete fusion of the soil sample can be a big cause for fluoride loss. Fusion with zinc oxide however results with the formation of insoluble zinc salts that hinder dissolution of soluble salts and possibly retard thorough decomposition. Hence, the fluoride will be trapped together with insoluble

salts and this tends to lower the fluoride recovery especially in soils containing high calcium and phosphorous content.

2. Sodium peroxide followed by zinc sulfate (Chu and Schafer 1956]. The sample was decomposed with sodium peroxide followed by the precipitation of silicates and aluminium ions using zinc sulfate. During the precipitation process, fluoride ions may be precipitated together with silicates and aluminium ions reducing the fluoride recovery.

3. Potassium hydroxide [Hall 1968] and sodium hydroxide [Oelschläger 1968, Eyde 1983]. The two fusing agents are mainly used in the samples which contain high silica content.

4. Direct distillation [Seel et al 1964]. The direct distillation method is mainly applied for rock phosphate samples. The method is slow and only 2-3 samples can be analysed per day. Perchloric-phosphoric acid mixture has been used for distillation especially for samples containing zirconium.

5. Pyrohydrolysis: The procedure is the same as that of plants but in soil, it is mainly applied to samples containing cryolite and rock phosphate [Peter and Ladd 1971].

6. Lithium tetraborate. This is used prior to distillation of the sample. Fusion with lithium tetraborate has been found to lower the fluoride recovery due to the formation of non-ionised fluoroborate which distils over during distillation [Bodkin 1977].

7. Sodium carbonate - potassium hydroxide [Crenshaw and Ward 1975, Hopkins 1977] in this method, sodium citrate and citric acid buffer is used to dissolve the fused sample, adjust ionic strength and inhibit any metal ion fluoride complex.

Labile fluoride in the soil is of great interest because it is the one that has an influence on the fluoride in the plants. Many methods using different reagents have been used to extract fluoride from the soil. For example:-

1. Water extraction:- This gives the most loosely bound fluoride. The amount of fluoride extracted is low because the fluoride in the soil is in form of compounds like calcium fluoride and magnesium fluoride which are sparingly soluble [Nömmic 1953, Brewer 1966]. Water extraction for fluoride has been used in many places. For example, extraction of water soluble fluoride in Belgium soils was found to range from 0.1-8.0 $\mu g\ g^{-1}$ fluoride while the soils in Sweden showed higher values [Smith 1983]. Eyde (1983) found that, from clay soils, more than one quarter of the fluoride extracted by 0.01M hydrochloric acid could be extracted by water. Peat soil was found to contain 12 $\mu g\ g^{-1}$ water soluble fluoride and 39 $\mu g\ g^{-1}$ of 0.001M hydrochloric acid extractable fluoride.

2. Calcium chloride 0.01M, [Larsen and Widdowson 1971]. Although calcium is known to form sparingly soluble calcium fluoride, Larsen and Widdowson (1971) found that there was no precipitation of calcium fluoride during the extraction process. Comparison of the results obtained by calcium chloride and potassium chloride media showed that the two media gave similar results.

3. Aluminium chloride [Larsen and Widdowson 1971].

Aluminium complexes with fluoride to form soluble complexes and in order to extract fluoride from aluminium complex, sodium hydroxide is added. This method reduces the fluoride recovery because the fluoride ion co-precipitate together with aluminium hydroxide.

4. Acid extraction: Different acids have been used for example, hydrochloric acid [Eyde 1983, 1985], perchloric acid or sulphuric acid [Hall 1968, Maclenahen and Schuiz 1976, Brewer 1966, Zimmerman and Bertland 1978].

5. Other extracting media which have been used are:- citrate solution [Nömmic 1953], acetic acid [Zimmerman and Bertland 1978] and sodium citrate - EDTA solution [Urumova et al 1982].

During the extraction of the labile fluoride, different ratios of the soil to the extracting media have been used. In water, extraction ratios such as 1:1, 2:1, 1:50 (w/v) have been used [Brewer 1966, Eyde 1983]. The extracting time has been found to be an important factor and different times ranging from 15 minutes to 16 hours have been used.

CHAPTER 3

3:0 EXPERIMENTAL PROCEDURES

3:1 REAGENTS:

All the reagents used in this work were of analytical grade. Solutions were made with either Milli-Q water (Distilled water further purified in a millipore Milli-Q system) or distilled water.

Sodium Fluoride Stock Solution: 1000 mg l^{-1} fluoride solution was made by dissolving 2.210g sodium fluoride (previously dried for 2 hours at 120°C) in water and diluted to one litre. The solution was stored in a plastic bottle.

Standard Fluoride Solution: The standard solutions in the range of 0.025-10 mg l^{-1} were prepared by serial dilution of the stock solution.

Acetate Buffer Solution: A mixture of 58.00g sodium chloride, 12.00 cm^3 glacial acetic acid, 107.00g sodium acetate trihydrate and 0.294g trisodium citrate dihydrate were dissolved in 500 cm^3 water. The pH was adjusted to between 5.2 to 5.5 with either 5M sodium hydroxide or acetic acid and the solution diluted to one litre.

1M Citrate buffer pH 6: This was prepared by dissolving 294.00g trisodium citrate dihydrate in 500 cm^3 water in one litre flask. The pH was adjusted to pH 6.0 using 1M citric

acid and the volume adjusted to the mark. This was stored in one litre plastic bottle.

TISAB II: To approximately 500 cm^3 of water, 58.0g of sodium chloride, 57.0 cm^3 glacial acetic acid and 4.0g CDTA were added. The solution was warmed slightly and stirred in order to dissolve the solids. The pH of the solution was adjusted to between 5.2 to 5.5 with 5M sodium hydroxide (approximately 150 cm^3 5M NaOH) and the solution diluted to one litre. The solution was stored in a plastic bottle.

0.5M Sodium hydroxide in methanol: Sodium hydroxide pellets (2.00g) were dissolved in 2.00 cm^3 water and diluted to 100 cm^3 with methanol.

Calcium Hydroxide-fluoride free: This was prepared by dissolving 147.0g calcium chloride in 900 cm^3 water in a one litre flask and 5.0g tertiary calcium phosphate were added to the solution. The suspension was repeatedly mixed by swirling and left to stand overnight. The mixture was filtered through a 125 mm folded filter paper. The first portion of the filtrate was returned back to the suspension to ensure that any fluoride from filter paper was also removed. All the filtrate was added in small portions to a solution of 100.0g sodium hydroxide in 900 cm^3 water in a two litre flask. The mixture was swirled thoroughly after each addition. The solution was then adjusted to the mark with water. The calcium hydroxide

precipitate was allowed to settle and the supernatant solution decanted. The precipitate was washed several times, each time using 200 cm^3 water, leaving for six hours to settle and then decanting. Six to eight times washings were found to be adequate. The calcium hydroxide suspension was transfered into a plastic bottle and diluted to one litre with water.

Sodium citrate - EDTA Solution: This mixture was prepared by dissolving 147.0g sodium citrate dihydrate and 2.50g EDTA in 500 cm^3 water in a one litre volumetric flask. The pH of the solution was adjusted to 6.0 using 1M citric acid and the volume made to the mark with water. This solution was 0.5M and 6.7×10^{-3}M with respect to sodium citrate and EDTA respectively.

Ammonium Lactate Solution: A mixture of 458.4 cm^3 of glacial acetic acid, 511.8 cm^3 lactic acid and 322 cm^3 of 25% ammonia solution in a two litre flask was mixed thoroughly. The solution was left standing overnight and then the volume adjusted to the mark using water. 1 cm^3 of this solution was diluted five times prior use.

Sodium Citrate - TISAB II Solution: A solution of sodium citrate - TISAB II was made by mixing 200 cm^3 water, 300 cm^3 sodium citrate and 500 cm^3 TISAB II. The solution was stored in a one litre plastic flask.

Aluminium Standard Solution: 1000 mg l^{-1} aluminium solution was made by dissolving 13.90g hydrated aluminium nitrate

($Al(NO_3)_3.9H_2O$) in water and diluted to one litre. The solution was stored in one litre plastic bottle.

Iron Standard Solution: 1000 mg l^{-1} iron solution was made by dissolving 8.63g hydrated ammonium Iron(III) sulphate [$NH_4Fe(SO_4)_2.12H_2O$] in water and diluted to one litre. The solution was stored in one litre plastic bottle.

Silicon Standard Solution: A solution containing 10 mg l^{-1} was made by mixing 1.30g of hydrated silicic acid powder ($SiO_2.nH_2O$) with 5 cm^3 water. 1.52g of sodium hydroxide pellets were added to the mixture and shaken gently. After complete dissolution 30 cm^3 water were added and allowed to cool. The solution was then diluted to 50 cm^3 with water and stored in a plastic bottle.

3:2 INSTRUMENTS:

1. Fluoride ion-selective electrode, metrohm E.A 306F.
2. Fluoride ion-selective electrode, orion model 94-09-00.
3. Double junction reference electrode metrohm 60724-140.
4. Single junction reference electrode Orion Model 90-01-00 with filling solution 90-00-01.
5. Radiometre PHM 85 pH metre.
6. Orion pH metre Model 407A.
7. Atomic absorption spectrophotometer 2380 Perkin-Elmer.
8. pH electrode metrohm AG 9100 Herisau.

3:3 APPARATUS:

Crucibles: Nickel (50 cm^3) and platinum (30 cm^3).

Muffle furnace Gallenkamp Model J. Din Serial No. 6909E134.

Meeker burners

Schöniger oxygen flask (Quartz)

Plastic beakers (2.5-10 cm^3)

Plastic bottles (50-1000 cm^3)

Plastic filter funnel (50 cm^3).

Polystyrene petri dishes (55mm diameter)

1 and 5 cm^3 Finn pipettes with polypropylene tips

1 cm^3 glass pipette

Volumetric flask 10-2000 cm^3

Oven Gallenkamp Cat. No. OV.050 AP. No. 3A 1636A.

Shaker GFL Model 3015

3:4 CALIBRATION CURVES:

(a) with TISAB II: 5.0 cm^3 of each fluoride standard solutions were placed in 10 cm^3 plastic beaker and 5 cm^3 of TISAB II added. These solutions were used to calibrate the instrument.

(b) with 1M citrate buffer and TISAB II: 2 cm^3 of each fluoride standard solutions were placed in 10 cm^3 plastic beaker and 3.0 cm^3 1M citrate buffer added. The solution was left standing for at least 2 hours and then 5.0 cm^3 TISAB II added.

3:5 RECOVERY EXPERIMENTS:

3:5:1 RECOVERY OF FLUORIDE AT DIFFERENT pH VALUES:

These experiments were carried out using 2.0 mg l^{-1} fluoride standard solutions diluted 1:1 with TISAB II using 10 cm^3 of each of these solutions. The pH was adjusted to cover the range pH 0.5 to pH 12 using little amounts of either concentrated hydrochloric acid or 5M sodium hydroxide. The volumes of acid or base required were in the range 0.02 cm^3 to 0.1 cm^3.

3:5:2 RECOVERY OF FLUORIDE AT DIFFERENT ALUMINIUM CONCENTRATIONS:

These experiments were carried out by mixing standard fluoride solutions with standard aluminium solutions. The final solution had a concentration of 2.0 mg l^{-1} with respect to fluoride but the aluminium concentration ranged from 0-300 mg l^{-1}. The fluoride concentration was determined by either TISAB II or both citrate buffer and TISAB II. For example, to prepare a solution that was 2.0 mg l^{-1} fluoride and 2.0 mg l^{-1} aluminium, 10.0 cm^3 of 4 mg l^{-1} fluoride standard solution and 10.0 cm^3 of 4 mg l^{-1} aluminium were mixed, divided into two equal portions. The portions were treated as follows:-

a. To one portion 10.0 cm^3 TISAB II was added (1:1 v/v dilution) and fluoride concentration determined.

b. To the other portion, 15.0 cm^3 of 1M citrate buffer was added and the mixture left standing for at least two hours. 25.0 cm^3 of TISAB II were then added and fluoride concentration determined.

3:5:3 RECOVERY EXPERIMENTS AT DIFFERENT IRON(III) CONCENTRATIONS:

The above experiments with aluminium were repeated with different iron concentrations ranging from 0-490 mg l^{-1} instead of aluminium.

3:6 SAMPLING:

The samples used included vegetables, plants and soils. These were collected from various parts of the country and put in plastic bags. In the laboratory the vegetable and plant samples were washed with de-ionised water for about two minutes and dried at 80°C for 48 hours. The dry samples were ground to a fine powder and stored in sealed plastic bags. The soil samples were also dried at 80°C for 12 hours, ground to a fine powder and stored in sealed plastic bags.

3:7 ASHING OF THE VEGETABLE AND PLANT SAMPLES:

(a) In the furnace.

About 0.2-0.5g ground sample were weighed accurately in a nickel or platinum crucible 2.0 cm^3 water and 2.5 cm^3 calcium hydroxide suspension were then added to the sample and then thoroughly mixed using a steel rod (1mm diameter). Evaporation to dryness and charring was done on a hot plate for 45 minutes. The crucibles with lose fitting lids were transfered into a furnace and the samples ashed for 6 hours at 600°C.

(b) On an open flame (using Meeker burner).

About 0.2-0.5g ground sample were weighed accurately in nickel crucible. 2.0 cm^3 water and 2.5 cm^3 calcium hydroxide suspension were then added. The mixture was thoroughly mixed using a steel rod. Evaporation to dryness and charring was done on a hot plate for 45 minutes. The sample was then ashed on a Meeker burner for 20 minutes as follows:-

i) 10 minutes at a slightly lower temperature with the crucibles half covered until the sample was completely carbonized.

ii) 5 minutes at a slightly higher temperature with the crucibles half covered until the ash turned white.

iii) 5 minutes at a fairly high temperature (about $\frac{1}{2}$ of the crucible red i.e. at approximately 800^{o}C) with the crucibles completely covered.

3:8 DIFFUSION:

After the crucibles with the ashed samples had cooled to room temperature, 2.5 cm^3 water were added followed by 4.0 cm^3 of 8M perchloric acid. The acid was added slowly with the crucibles about three quarters covered. The mixture was stirred well using a Finn pipette and allowed to cool. 5.0 cm^3 of the sample were pipetted and placed at the bottom part of a petri dish containing 0.2 cm^3 of 2M silver perchlorate. The petri dish was immediately covered with a petri dish cover. The petri dish cover used contained a thin layer of sodium hydroxide.

This layer had been prepared by placing 0.2 cm^3 of 0.5M sodium hydroxide in methanol on the cover, allowing it spread and then drying in an oven for three minutes at 60^oC. The petri dish contents were mixed carefully by slow swirling and the petri dish placed on a tray and put in an oven set at 60^oC. After 20 hours in the oven, the petri dish cover was removed and allowed to cool in a dessicator. The sodium hydroxide layer was dissolved in 2.5 cm^3 acetate buffer (1:1 v/v) and the solution analysed for fluoride. A blank having only calcium hydroxide and water was treated similarly. The value obtained for the sample was corrected using the value for blank. The set up for diffusion was as shown in Fig. 3:1 below.

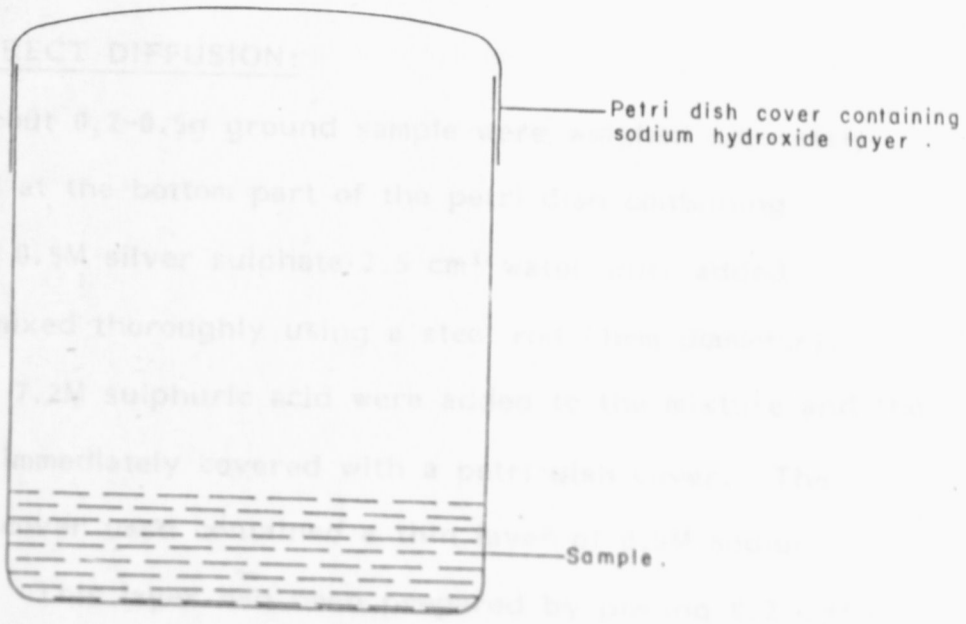

Fig. 3:1 A schematic diagram of diffusion apparatus.

The diffusion procedure was also adopted for:

i) Standard fluoride solutions in order to establish the efficiency.

ii) Ashed samples containing known amounts of added fluoride.

3:9 RECOVERY OF FLUORIDE AFTER DECOMPOSITION OF SODIUM CARBONATE WITH PERCHLORIC ACID:

About 1.0g sodium carbonate was weighed accurately in a plastic beaker. 2.0 cm^3 water and a known fluoride concentration were then added. The mixture was shaken gently and 8.0M perchloric acid added slowly with:

(a) No lid.

(b) The lid covering three quarters.

and the diffusion carried out.

3:10 DIRECT DIFFUSION:

About 0.2-0.5g ground sample were weighed accurately and placed at the bottom part of the petri dish containing 0.2 cm^3 of 0.5M silver sulphate. 2.5 cm^3 water were added and then mixed thoroughly using a steel rod (1mm diameter). 4.0 cm^3 of 7.2M sulphuric acid were added to the mixture and the petri dish immediately covered with a petri dish cover. The petri dish cover used contained a thin layer of 0.5M sodium hydroxide. This layer had been prepared by placing 0.2 cm^3 of 0.5M sodium hydroxide in methanol on the cover, allowing it spread and then drying in an oven for three minutes at 60°C.

The petri dish contents were mixed carefully by slow swirling, placed on a tray and put in an oven set at 60°C. After 20 hours in the oven, the petri dish cover was removed and allowed to cool in a dessicator. The sodium hydroxide layer was dissolved in 2.5 cm^3 acetate buffer (1:1 v/v) and the solution analysed for fluoride. A blank value having only sulphuric acid and water was treated similarly. The value obtained for the sample was corrected using the value for blank.

3:11 SCHÖNIGER OXYGEN FLASK COMBUSTION:

The procedure followed has been described by other workers [Moody et al 1980, Levaggi et al 1971, Thomas and Amtower 1969]. However, in this work, Quartz flasks were used in place of pyrex flasks. The absorbing media was citrate buffer (pH6) rather than TISAB II or sodium hydroxide. For samples low in fluoride, 1.0 cm^3 citrate buffer and 1.5 cm^3 water was used, while for samples with high fluoride, the volumes were doubled. The set up for combustion was as shown in Fig. 3:2 below.

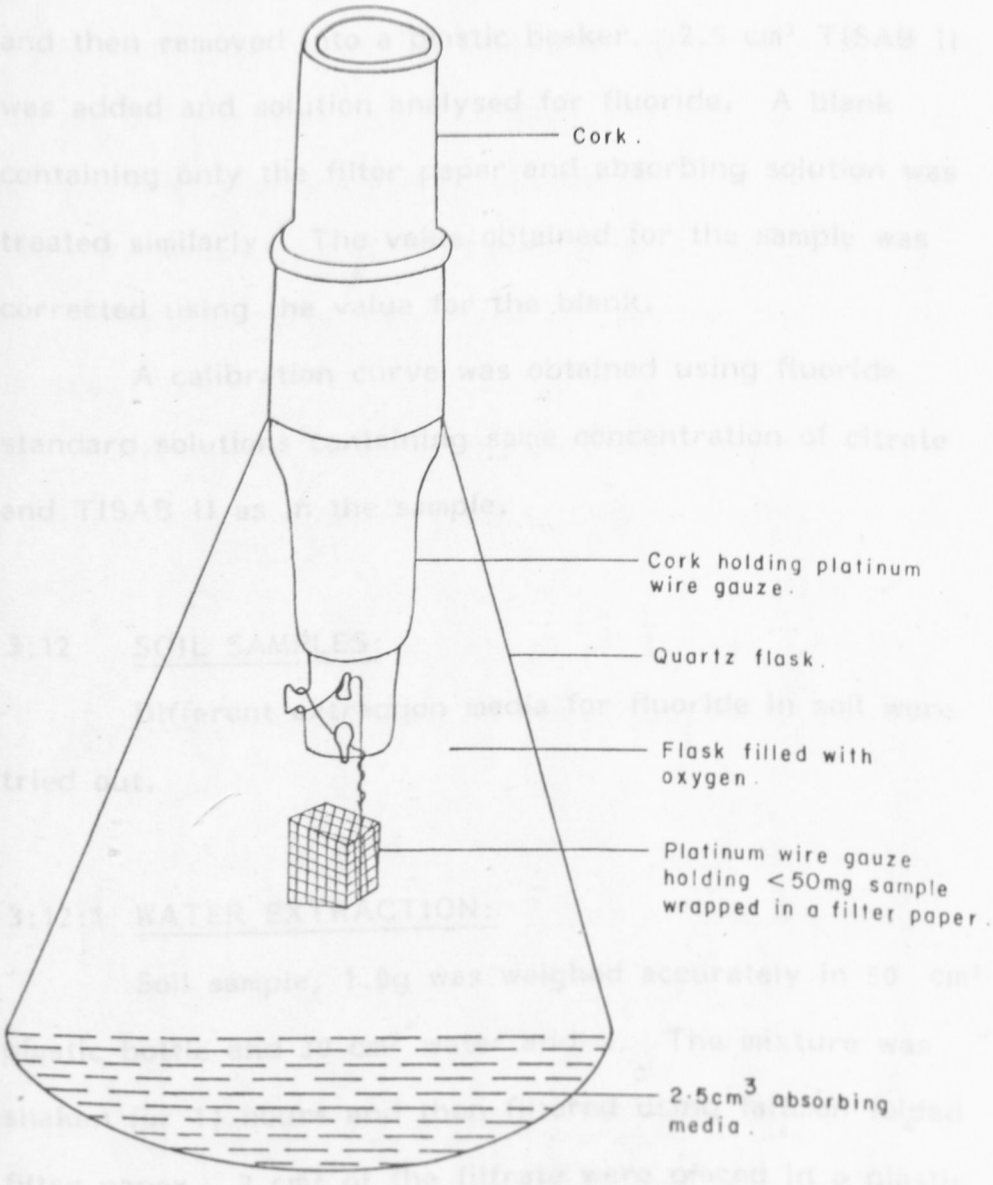

Fig. 3:2 A schematic diagram of the Schöniger oxygen flask.

After combusion, the clear colourless solution was allowed to stand in the flask for 30 minutes with occasional shaking and then removed into a plastic beaker. 2.5 cm^3 TISAB II was added and solution analysed for fluoride. A blank containing only the filter paper and absorbing solution was treated similarly. The value obtained for the sample was corrected using the value for the blank.

A calibration curve was obtained using fluoride standard solutions containing same concentration of citrate and TISAB II as in the sample.

3:12 SOIL SAMPLES:

Different extraction media for fluoride in soil were tried out.

3:12:1 WATER EXTRACTION:

Soil sample, 1.0g was weighed accurately in 50 cm^3 plastic bottle and 20 cm^3 water added. The mixture was shaken for $1\frac{1}{2}$ hours and then filtered using faltmen folded filter paper. 2 cm^3 of the filtrate were placed in a plastic beaker, 3 cm^3 of 1M citrate buffer added and left standing for at least 2 hours. After 2 hours, 5.0 cm^3 of TISAB II was added and the solution analysed for fluoride.

A calibration curve containing TISAB II and citrate buffer was prepared.

3:12:2 AMMONIUM LACTATE EXTRACTION:

(a) About 1.0g sample was weighed accurately in a 150 cm^3 plastic bottle and 100 cm^3 of ammonium lactate solution added. The mixture was shaken for 1½ hours and filtered. 2.0 cm^3 of the sample were placed in a plastic beaker, 3.0 cm^3 of 1M citrate buffer added and left standing for at least 2 hours. 5.0 cm^3 TISAB II was then added and solution analysed for fluoride.

(b) Inorder to establish the best conditions for the extraction, the ammonium lactate extraction was repeated as below:

i) Using different amounts of soil samples e.g. 2.0g, 3.0g, 4.0g but keeping other quantities as in (a) above.

ii) Varying the shaking time from ½ hour to 4 hours.

iii) Using 1.0g soil samples spiked with known amounts of fluoride.

3:12:3 SODIUM CITRATE - EDTA EXTRACTION:

(a) About 1.0g soil sample was weighed accurately into 100 cm^3 plastic bottle and 40 cm^3 of sodium citrate - EDTA reagent added. The mixture was shaken for 1½ hours, filtered and analysed for fluoride directly.

(b) The sodium citrate - EDTA was repeated

i) Using 20.0 cm^3 of sodium citrate - EDTA solution.

ii) Allowing to shake for 20 minutes and then leaving to stand for 2 hours.

iii) Allowing to shake for 20 minutes and then leaving to stand for 4 hours.

3:12:4 SODIUM CITRATE - TISAB II EXTRACTION:

The experiments above 3:12:3 using sodium citrate-EDTA were repeated using sodium citrate - TISAB II reagent as the extracting media.

3:13 TEA SAMPLES:

3:13:1 EXTRACTION OF WATER LABILE FLUORIDE:

About 1.0g of processed tea was weighed accurately, added to 250.0 cm^3 boiling water and allowed to boil for 30 minutes. The mixture was quickly filtered while hot. After cooling, the volume of the filtrate was adjusted to 250 cm^3. 2.5 cm^3 of the solution were placed in a plastic beaker, 2.5 cm^3 TISAB II added and solution analysed for fluoride.

3:13:2 Same procedure in 3:13:1 was repeated.

i) Using different amounts of processed tea - 0.2g, 0.5g, 1.0g, 1.5g and 2.5g.

ii) Using 10.0g (wet weight) unprocessed tea samples.

3:13:3 Inorder to establish the amount of water labile fluoride in tea, the following experiments were carried out:

i) About 2.5g of processed tea were weighed accurately and added to 250 cm^3 boiling water. It was allowed to boil for 15 minutes and filtered quickly while hot.

After cooling, the volume of the filtrate was adjusted to 250 cm^3 and analysed for fluoride as in 3:13:1 above. The residue tea leaves were then placed back into the beaker and 250 cm^3 boiling water added. This was again allowed to boil for 15 minutes, followed by filtration, volume adjustment and fluoride analysis. This treatment of residue tea leaves being reboiled for 15 minutes, filtered etc. was repeated until the final filtrate showed most of the fluoride had been extracted. A total duration of 2 hours was found necessary.

3:13:4 The procedure in 3:13:3 above was repeated using 10.0 g (wet weight) of unprocessed tea samples.

3:14 ANALYSIS OF TOTAL ALUMINIUM IN VEGETABLE, PLANT AND TEA SAMPLES:

WET DIGESTION USING H_2SO_4 + $HClO_4$ + HNO_3 MIXTURES:

A mixture containing 50.0 cm^3 of 65% nitric acid, 12.5 cm^3 of 70% perchloric acid and 5.0 cm^3 of 96% sulphuric acid was prepared in a 250 cm^3 flask.

Vegetable, plant or tea sample, 0.2-0.5g, was weighed accurately directly into 50.0 cm^3 Kjeldahl flask and 10.0 cm^3 of the acid mixture added. The mixture was heated to a gentle boiling until the brown fumes of nitrogen dioxide disappeared. The temperature was then increased to 230 -250^oC and retained at this value until all the white fumes over the

mixture had disappeared. The temperature was then again raised to 350-370^{o}C until a ring of condensed sulphuric acid was obtained on the lower part of the flask. This mixture was maintained at this temperature for 10 minutes and then allowed to cool. After cooling, 20 cm^{3} of water was added and the mixture reboiled for a further 20 minutes. The mixture was then diluted using water so as to obtain a solution approximately 0.3M sulphuric acid (pH>0.15). The aluminium concentration was then determined using an atomic absorption spectrophotometer.

Aluminium standard solutions used for the calibration curve were also prepared containing approximately 0.3M sulphuric acid.

3:15 ANALYSIS OF ALUMINIUM IN SOIL SAMPLES USING 10.5M NITRIC ACID:

About 1.0g soil sample was weighed accurately and 20.0 cm^{3} 10.5M nitric acid added. The mixture was left standing on a hot water bath for 8 hours and filtered while hot. After cooling to room temperature, the volume was adjusted to 50.0 cm^{3} with water. The aluminium concentration was then determined using an atomic absorption spectrophotometer.

Aluminium standard solutions used for the calibration curve were also prepared containing approximately 4.2M nitric acid.

3:16 ACCURACY OF THE RESULTS:

All the samples were analysed in duplicate and two determinations per sample carried out. For those samples where the duplicates did not agree, 2 to 5 determinations were carried out, while five determinations were performed for the blank samples.

The standard deviation for the measurements was found to vary from 0.0-1.9%. It was noticed that, for these determinations, the best reproducibility was obtained with finely ground sample. The complete decomposition of the sample and the removal of interfering ions also played a big role.

Since the analysis of fluoride was carried out using a Radiometre pH meter which gives potential and orion pH meter Model 407A which gives concentration, both potential (volts) and concentration (mg l^{-1}) readings were taken depending on the meter being used.

The time of response for the electrode was maintained between 2 to 5 minutes depending on the fluoride concentration of the sample and the fluoride concentration in the previous analysis. For samples with low fluoride concentration, the electrode took longer to stabilize and hence 5 minutes was taken for those samples.

In order to condition the electrode, it was normally stored in 4 mg l^{-1} fluoride solution. Also to get accurate readings especially for blanks or low fluoride samples, the electrode was put for two minutes in a mixture of water and

acetate buffer diluted 1:1 after thorough washings of the electrode with water.

Calibration curve using the fluoride standard solution was done every day before the sample analysis and frequent recalibration of the electrode (about 3 times per day) was found necessary in order to maintain the accuracy. This was also found necessary because of the drifting of the electrode after continuous analysis of the samples especially those with low fluoride content.

The variation between potential and log $[F^-]$ is not linear over the whole concentration range. At low fluoride concentration, the variation deviates from linearity hence it was prefered to use two calibration curves. One for the low fluoride concentration ranging from 0.025-0.25 mg l^{-1} with a correlation of 0.9556 and the other for high fluoride concentration ranging from 0.25-10.0 mg l^{-1} with a correlation of 0.9999.

CHAPTER 4

4:0 RESULTS AND DISCUSSION

4:1 REMOVAL OF INTERFERING IONS IN THE MEASURING SOLUTION.

4:1:1 pH EFFECT.

The pH of the solution was varied by addition of either acid or base. Figure 4:1 gives the results of the pH effect at a constant fluoride concentration of 2 mg l^{-1}. As mentioned earlier, hydrogen and hydroxyl ions interfere with the fluoride analysis. From fig. 4:1, it can be seen that, at a lower pH (pH<4.5) there is a negative deviation of the curve. At this pH most of the fluoride is complexed while at pH>8.20 a positive deviation occurs. Above pH>8.2 the electrode responds mainly to hydroxide ion. From fig. 4:1, it is apparent that fluoride analysis can only be carried out between pH 4.5 and pH 8.2. At pH 5.0-6.0, the curve shows that the electrode has sensed only 2 mg l^{-1} fluoride.

In the present work, measurements were carried out at pH 5.5-5.7. This pH was adopted because, the sodium hydroxide layer used for trapping hydrogen fluoride during diffusion when dissolved in a buffer (pH 5.2-5.3) was found to raise the pH to 5.5-5.7. Same effect on pH was produced when citrate buffer (pH 6.0) was mixed with the same buffer. A pH of 5.2-5.5 has been recommended [Frant and Ross 1966, Orion Manual 1983].

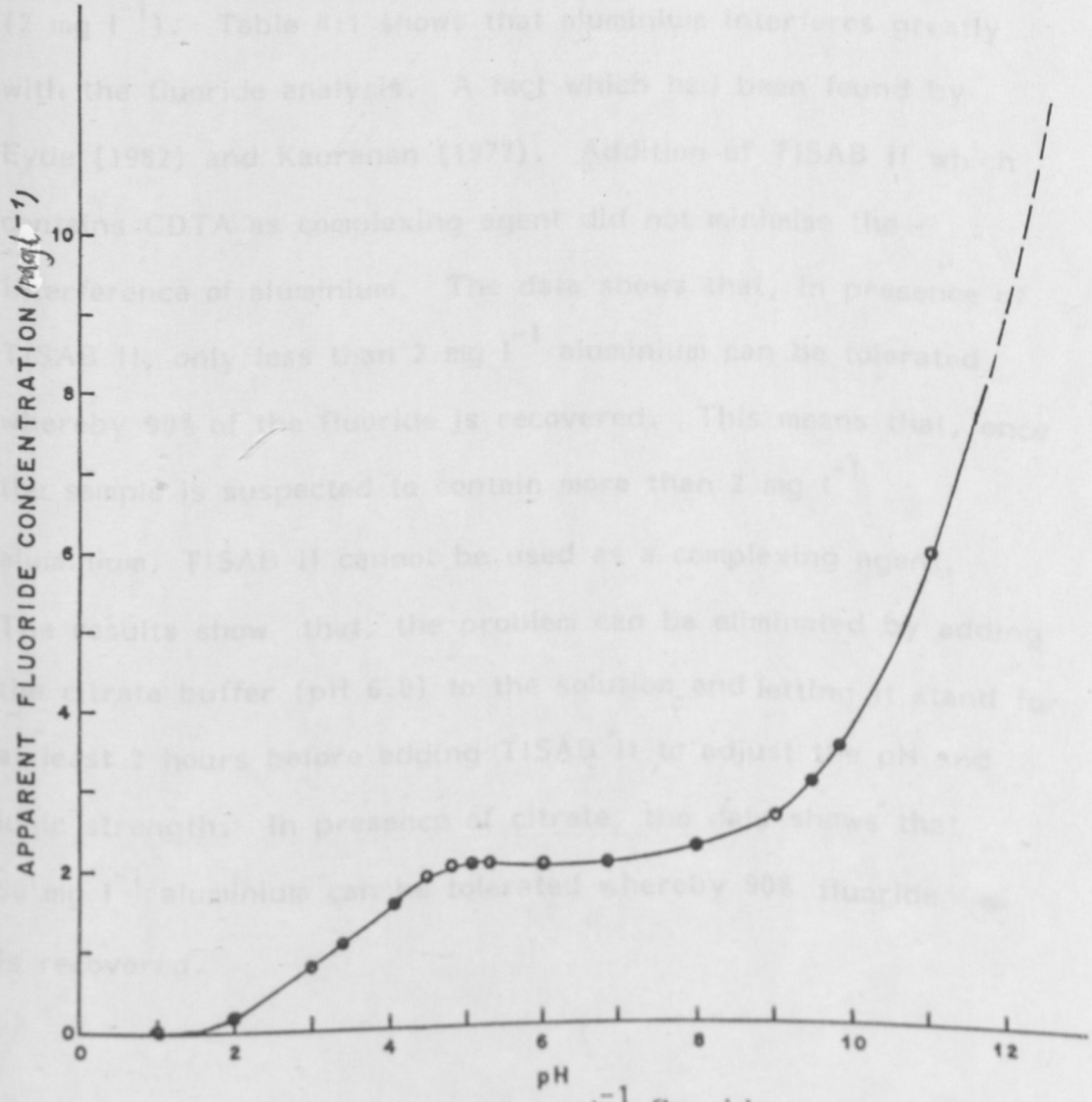

Fig. 4:1 Effect of pH on 2 mg l^{-1} fluoride concentration.

4:1:2 EFFECT OF ALUMINIUM.

As discussed earlier, aluminium interferes greatly with the fluoride measurement. In order to remove the interference, different complexing agents have been recommended. In this work, TISAB II (containing CDTA as a complexing agent) and citrate — TISAB II buffers are compared at different aluminium concentrations but at a constant fluoride concentration (2 mg l^{-1}). Table 4:1 shows that aluminium interferes greatly with the fluoride analysis. A fact which had been found by Eyde (1982) and Kauranen (1977). Addition of TISAB II which contains CDTA as complexing agent did not minimise the interference of aluminium. The data shows that, in presence of TISAB II, only less than 2 mg l^{-1} aluminium can be tolerated whereby 90% of the fluoride is recovered. This means that, once the sample is suspected to contain more than 2 mg l^{-1} aluminium, TISAB II cannot be used as a complexing agent. The results show that, the problem can be eliminated by adding the citrate buffer (pH 6.0) to the solution and letting it stand for at least 2 hours before adding TISAB II to adjust the pH and ionic strength. In presence of citrate, the data shows that 50 mg l^{-1} aluminium can be tolerated whereby 90% fluoride is recovered.

TABLE 4:1 EFFECT OF ALUMINIUM ON 2 mg l^{-1} FLUORIDE SOLUTION IN PRESENCE OF TISAB II AND CITRATE + TISAB II.

Aluminium (mg l^{-1}) added	Fluoride (mg l^{-1}) present	TISAB II	CITRATE + TISAB II
		% Recovery	% Recovery
0.0	2.0	100.0	100.0
1.0	2.0	95.0	100.0
2.0	2.0	90.0	99.5
5.0	2.0	81.0	99.5
10.0	2.0	70.0	98.0
20.0	2.0	50.5	93.5
50.0	2.0	32.0	90.0
100.0	2.0	20.0	73.5
150.0	2.0	16.5	61.0
200.0	2.0	5.0	14.0
250.0	2.0	3.5	9.0
300.0	2.0	3.0	8.0

Leaving the sample standing for at least 2 hours in the presence of citrate buffer helps in the decomplexation of fluoro-aluminate complexes and also the complexation of aluminium with the citrate buffer. Concentration of the citrate plays an important role. When less than 1M citrate buffer in the solution was used only 10mg l^{-1} aluminium could be tolerated and as a result less than 1M citrate was found not to be effective. This had been suggested by Shirashi et al (1973) and Kauranen (1977).

4:1:3 EFFECT OF IRON.

Iron has also been cited as a fluoride complexing agent. Unlike aluminium, iron does not interfere greatly with fluoride analysis as table 4:2 shows.

TABLE 4:2 EFFECT OF IRON ON 2mg l^{-1} FLUORIDE IN PRESENCE OF TISAB II AND CITRATE + TISAB II.

Iron(III) (mg l^{-1}) added	Fluoride (mg l^{-1}) present	TISAB II	CITRATE + TISAB II
		% Recovery	% Recovery
0.0	2.0	100.0	100.0
1.0	2.0	100.0	100.0
2.0	2.0	100.0	100.0
5.0	2.0	100.0	100.0
10.0	2.0	100.0	100.0
20.0	2.0	99.0	100.0
50.0	2.0	97.5	100.0
100.0	2.0	96.5	99.5
200.0	2.0	93.5	99.0
490.0	2.0	64.5	97.5

In the presence of TISAB II, it is possible to tolerate about 200mg l^{-1} Iron (III) whereby 93.5% fluoride is recovered. The effect of citrate as a complexing agent is very clear. 490 mg l^{-1} Iron(III) gave a recovery of 97.5% fluoride in presence of citrate buffer. This means 1M citrate buffer is a better complexing agent for both aluminium and Iron(III) than TISAB II. Apart from the complexing ability, a constant ionic strength was maintained and pH adjusted to pH 5.7 by the presence of TISAB II.

Unlike plants and foods which contain low amount of these interfering ions, soil samples have been quoted to contain high concentration of these interfering ions [McQuaker and Gurney 1977]. These interferences can be taken care by adding citrate buffer. It was also found not necessary to adjust the pH to 8.5 for precipitating out these cations so that they could be filtered off as reported by McQuaker and Gurney (1977), Eyde (1982) and Baker (1972). Since 1M citrate was capable of complexing the cations, it was found that at a fluoride concentration of 1mg l^{-1}, a suppression of 10% occurs if either 2mg l^{-1} aluminium or 200 mg l^{-1} iron are present [McQuaker and Gurney 1977]. In the presence of TISAB II at a concentration of 2mg l^{-1} fluoride, table 4:2 shows that a suppression of 10% occurred in the presence of 2 mg l^{-1} aluminium or 200 mg l^{-1} iron(III). After addition of citrate, a suppression of 10% occurred only above 50 mg l^{-1} aluminium while a suppression of 3% was produced in presence of 490 mg l^{-1} Iron (III).

4:2 METHODOLOGY FOR FLUORIDE ANALYSIS IN FOODS AND PLANTS.

4:2:1 DIFFUSION.

In the present work, various methods for diffusion found in the literature were considered. The method described by Marshal and Wood (1969) was chosen because preliminary experiments showed that, with minor modifications, it would be suitable for the present work as regards to its simplicity in

technique, reproducibility and accuracy of results. It is also not very expensive. Silver perchlorate (0.4 nmol) was added to the sample in order to suppress interference from other halogens especially chloride which would otherwise neutralise the alkaline layer, thus preventing the reaction with the diffused hydrogen fluoride. This would lower the fluoride recovery. Silver sulphate used by Stuart (1970) and Hall (1968) could not be used here on account of the high concentration of calcium from the fixing agent used in ashing. Sealed vessels have been used for diffusion but in this work in accordance with Marshall and Wood (1969), it was found that sealing of the dishes in order to avoid losses of hydrogen fluoride was unnecessary. Disposable polystyrene plastic petri-dishes were used. It was found that, depending on where the dishes were bought, sometimes, it was difficult to achieve a uniform layer of sodium hydroxide on the lid. To avoid this, the lids were wiped with acetone prior to the spreading of the sodium hydroxide.

The diffusion method was tested with fluoride standard solutions (1-17 mg l^{-1} fluoride) and gave a recovery of 97.6-102%. Table 4:3 gives the recovery values for standard solution using 4.8M perchloric acid.

The concentration of perchloric acid in the diffusion process is not critical as only a slight increase in recovery (1.6%) was noticed when the concentration was increased from 3.6 to 7.3M. In absence of interfering substances, it was not necessary to increase the time of diffusion to 24 hours as others [Hall 1968,

TABLE 4:3 EFFICIENCY OF DIFFUSION USING STANDARD FLUORIDE SOLUTION.

Fluoride ($mg\ l^{-1}$) added	Mean % Recovery
1.0	97.0 ± 0.5
2.0	99.0 ± 0.8
3.5	99.7 ± 1.0
5.0	98.0 ± 0.2
10.0	100.0 ± 0.5
17.0	102.9 ± 1.0

Thomas et al 1969] have done. The increase in yield with longer diffusion time in presence of silica and aluminium is discussed later otherwise 20 hours was found to be a good diffusion time. It has been suggested by Eyde (1982) that there is a chance of losing fluoride during the addition of the acid prior to diffusion due to effervescence. This was tested and table 4:4 shows values obtained when standard fluoride solutions were mixed with sodium carbonate and treated with perchloric acid. It was found that, there was no fluoride lost through splashing as long as the acid was added slowly with the crucibles three quarters covered. Preliminary results of this work have been discussed by Gustafsson and Njenga (1988).

TABLE 4:4 RECOVERY OF FLUORIDE AFTER DECOMPOSITION OF SODIUM CARBONATE WITH PERCHLORIC ACID

Lid	Fluoride ($mg\ l^{-1}$) added	Mean % Recovery
No lid	2.0	99.3 ± 1.5
No lid	5.0	95.5 ± 1.5
No lid	10.0	98.5 ± 1.0
Lid on	2.0	100.6 ± 0.5
Lid on	5.0	100.1 ± 1.0
Lid on	10.0	100.1 ± 0.5

4:2:2 INTERFERENCES DURING DIFFUSION.

Workers using diffusion for separating fluoride seem generally not to have considered the influence of even small amounts of aluminium and silica on the diffusion rate. Tables 4:5 and 4:6 show the effect of aluminium and silicon respectively on diffusion using perchloric acid and sulphuric acid media. It is apparent from the results that the interference from aluminium is larger in sulphuric acid than in perchloric acid and this also holds for silicon. The latter interference is a more serious problem because some plants can have a higher concentration of silicon while the aluminium content is low. Cannon (1960) showed that, most plants ashes have an average aluminium concentration of 8600 $\mu g\ g^{-1}$. Determination of

TABLE 4:5 EFFECT OF ALUMINIUM ON DIFFUSION.

Fluoride (µg)	Aluminium (µg) added	Mean % Recovery	
		4.4M H_2SO_4	4.8M $HClO_4$
10	100	99.3 ± 1.0	100.0 ± 0.5
10	200	91.7 ± 1.2	99.5 ± 1.0
10	500	86.7 ± 0.4	99.1 ± 0.8
10	1000	70.8 ± 0.3	99.0 ± 0.5
10	2000	59.7 ± 1.0	93.0 ± 0.2

TABLE 4:6 EFFECT OF SILICON ON DIFFUSION.

Fluoride (µg)	Silicon (µg) added	Mean % Recovery	
		4.4M H_2SO_4	4.8M $HClO_4$
2	2050	92.0 ± 1.0	99.4 ± 0.5
2	5100	87.0 ± 0.0	91.6 ± 1.0
2	10300	86.0 ± 1.0	83.2 ± 1.2
10	2050	85.2 ± 0.4	96.8 ± 1.8
10	5100	80.5 ± 1.0	95.0 ± 1.2
10	10300	76.1 ± 0.5	81.9 ± 0.2

aluminium in 20 vegetables and plants in the present work (table 5:12) gave aluminium concentration ranging from 180-1350 μg g^{-1}. This indicates that only <675 μg aluminium was present (since only 0.2-0.5g sample was used) during diffusion and this did not greatly interfere. At 60°C, the diffusion temperature, silica forms a viscous gel in both acids and this leads to trapping of hydrogen fluoride and water into the newly formed micellar spaces of silicic acid. This reduces the diffusion rate into the sodium hydroxide layer.

Increasing diffusion time from 20 to 40 hours in the presence of 10300 μg silicon increased the recovery of 10 μg fluoride from 81.9 to 92.2% in perchloric acid .
The interference of silica was stronger at higher concentration of perchloric acid. Thus values of 66.9, 80.8 and 85.0% were recovered for diffusion times 16, 20 and 40 hours respectively when the concentration of perchloric acid was 7.0M. As the silicon content of plants and vegetables was found to be lower than 10000 μg g^{-1} silicon, diffusion time was maintained at 20 hours for practical reasons. If a very viscous gel was noticed after diffusion (indicating a high concentration of silicon), the analysis was repeated using a smaller weight.

From tables 4:5 and 4:6, it is apparent that perchloric acid gave the best recovery. Also calcium hydroxide can be used as a fixative when ashing. Interferences from other halides was removed by the addition of silver perchlorate. Since perchloric acid gave the best recovery, and has the lowest blank value than sulphuric acid, it was used for the rest of the work.

4:3 FURNACE AND OPEN FLAME ASHING.

4:3:1 BLANKS:

It has been mentioned in chapter 2 that, some furnaces, depending on the lining material and time for ashing, emit fluoride and thus contaminate the samples. High blank values have also been reported. In the present work, a muffle furnace that had been in intermittent use for about 20 years was used for ashing and tested for blank values obtained during ashing in the furnace and open flame ashing.

TABLE 4:7 COMPARISON OF BLANK VALUES FROM FURNACE AND OPEN FLAME ASHING.

Ashing method	Time	Blank value Fluoride (µg)
Furnace at 600°C	6 hours	0.23 ± 0.03
"	8 hours	0.31 ± 0.01
"	16 hours	0.60 ± 0.02
Open flame	20 minutes	0.06 ± 0.02

From the table, it is seen that ashing in nickel crucibles for 20 minutes in an open flame gave the lowest blank values.

4:3:2 COMPARISON BETWEEN FURNACE AND OPEN FLAME ASHING.

The results obtained when samples were analysed using three different methods are presented in table 4:8. Most ashings in the furnace were done using 30 cm^3 platinum crucibles because ashing using nickel crucibles was often incomplete after 6 hours. It was found that after 30 minutes (the recommended time) and at 600°C, there was a black residue which showed incomplete ashing while at a temperature of 800°C recommended by Vickery and Vickery (1976), it was found that, there was alot of overflowing of the salt especially the tea samples and this introduced a big error.

From the values given (table 4:8), it can be noticed that in almost all the samples, the values increased significantly (20-80%) when ashing was performed on an open flame using Meeker burner instead of in the furnace. These samples were also found to contain a substantial concentration of silicon for example, pumpkin leaves (Cucurbita pepo) contained 10300 $\mu g\ g^{-1}$ silicon. It has been pointed out that, samples containing silica should be fused with sodium hydroxide [Oelschläger 1968] or potassium hydroxide [Hall 1968] after ashing. Ashing on a Meeker burner with a final short heating at a fairly high temperature (800-850°C) seems to result in a similar release of fluoride bound with silicate after ashing with calcium hydroxide and thus converting fluoride to acid labile form. Use of high temperature has been discouraged in that it may lead to loss of volatile fluoride [Oelschläger 1968, Stuart 1970] or formation of

TABLE 4:8 COMPARISON BETWEEN ASHING/DIFFUSION METHODS AND OXYGEN FLASK METHOD.

Sample	Fluoride ($\mu g\ g^{-1}$)		
	Ashing in the furnace (6 hours)	Ashing on Meeker Burner (20 minutes)	Oxygen flask Combustion
Curry powder	9.9 ± 0.3	9.4 ± 0.1	10.4 ± 0.4
Tea leaves * 1)	256.0 ± 1.2	-	265.0 ± 5.0
2)	349.6 ± 0	-	345.0 ± 5.0
Celery (Apium graveolens)	6.1 ± 0	6.3 ± 0.12	-
Pumpkin leaves (Cucurbita pepo)	10.6 ± 0.1	18.9 ± 0.5	26.3 ± 2.33
Cucurbita sps	4.5 ± 0.45	7.1 ± 0.4	-
Solanum nigrum	28.1 ± 1.0	30.0 ± 0.1	32.2 ± 3.0
Kale (Brassica intergrifolia)	14.6 ± 0.1	14.2 ± 0.1	23.0 ± 0.1
Cabbage (Brassica oleracea)	4.7 ± 0.1	5.5 ± 0.2	-
Sporabolus	51.6 ± 1.2	59.0 ± 0.1	59.6 ± 0.3
Amaranthus	9.2 ± 0.9	11.8 ± 0.16	12.0 ± 1.0
Lettuce (Lactuca sativa)	7.2 ± 0.01	9.1 ± 0.3	9.6 ± 0.5
Sesbania speciosa	95.6 ± 1.5	99.8 ± 0.2	-

* Tea leave samples from two factories.

silicium-oxy-fluoride which binds fluoride more strongly. However, this work shows that when using calcium hydroxide, which is a good fixing agent, and a moderate increase of temperature during the last 5-10 minutes resulted in an increase in yield rather than a loss. Samples with low silicon content gave similar results with both ashing methods.

Ashing 1.0g of a hay sample (containing 3400 μg g^{-1} silicon) alone or spiked with 10-80 μg fluoride gave yields which increased with increasing temperature (up to 850°C) during the last 10 minutes period. With this calcium hydroxide matrix, the yield of fluoride did not decrease when the heating was maintained at this higher temperature for 20 minutes or if an even higher temperature was applied with the whole nickel crucible red during the last 10 minutes. Heating too long at too high temperature might increase the blanks and would shorten the life of the nickel crucibles and therefore ashing was stopped after heating for only 5 minutes at about 850°C.

4:4 COMPARISON BETWEEN OPEN FLAME ASHING AND OXYGEN FLASK COMBUSTION.

Some fluoride values obtained by the oxygen flask combustion method are also shown in table 4:8. With regard to low sensitivity of this method caused by the low weight of the sample (30-50 mg) taken for analysis and larger volume at measurement, the agreement with the values found with open flame ashing is generally good. For two samples, however, the values are significantly lower (27 and 38%) with the ashing

method. So far this discrepancy remains unexplained but these samples may contain volatile organic compounds that escape during charring since the ashing temperature is high enough to break the carbon-fluoride bonds. The blank value using oxygen flask method was found to be 0.081 µg fluoride and this allows even low fluoride values to be determined using oxygen flask method.

4:5 COMPARISON BETWEEN OPEN FLAME ASHING AND DIRECT DIFFUSION METHOD:

Hall (1968) suggested that, with most plants, the total fluoride is also the diffusible acid-labile fluoride. Taves (1983) analysed 93 individual foods comparing :

i) an ashing method with magnesium chloride as a fixing agent followed by diffusion with hexamethyldisiloxane (HMDS) at room temperature with

ii) Direct diffusion without previous ashing.

After ashing,only three samples were found to have values more than 25% higher than those obtained by direct diffusion. Singer et al (1980) on the other hand found the ashing method to give higher values than direct diffusion. In the present work, a comparison was made with open flame ashing followed by diffusion and the direct diffusion. For both methods,diffusion was carried out at 60°C for 20 hours but in direct diffusion sulphuric acid (2.9M or 5.8M the latter giving higher values) was used instead of 4.8M perchloric acid. The values obtained by both methods

TABLE 4:9 COMPARISON OF OPEN FLAME ASHING AND DIRECT DIFFUSION.

Sample	Fluoride µg g^{-1}		% Fluoride direct diffusion/ ashing
	Ashing on Meeker Burner	Direct Diffusion 5.8M H_2SO_4	
*TN1	258.0 ± 1.5	251.0 ± 1.0	93.3
*TK1 (dust)	256.0 ± 1.0	250.8 ± 0.5	98.0
*TC2	289.0 ± 0	270.9 ± 0.2	93.7
Tea from Sweden market	204.5 ± 0.1	168.0 ± 0.1	82.2
Lettuce *Lactuca sativa*	9.1 ± 0.3	6.6 ± 0.1	72.5
Pumpkin leaves *Cucurbita pepo*	18.9 ± 0.5	4.4 ± 0.0	23.3
Solanum nigrum	30.0 ± 0.1	18.1 ± 0.5	60.3
Kale *Brassica intergrifolia*	14.2 ± 0.1	6.2 ± 0.4	43.7
Cabbage *Brassica oleracea*	5.5 ± 0.2	2.5 ± 0.4	45.5

* Tea samples from different factories.

are presented in table 4:9. The values found for five vegetable samples analysed by direct diffusion were in the range of 23-73% of the values obtained with the ashed method. Only for the tea samples did direct diffusion give nearly the same yield (82-98% of the ashing value). From the present results, it can be concluded that, most of the fluoride in tea leaves is acid labile and easily extracted while in other plants and vegetables, a large portion of the fluoride is covalently bonded to carbon and not accessible by direct diffusion. This fluoride which is covalently bonded to carbon needs to be fused with an alkali before diffusion in order to make it acid labile.

4:6 RECOVERY EXPERIMENTS USING OPEN FLAME ASHING.

In order to investigate the recovery, sodium fluoride standard was added to some samples before ashing. As seen from table 4:10 below, the recoveries ranged 74 to 103%. Hay and pumpkin leaves with high silicon content gave lower recovery (74-93%) than the other samples which gave recovery from 93-103%. The latter samples were low in silicon as indicated by the amount of gel noticed after diffusion. The losses of fluoride added to hay and especially pumpkin leaves are larger than would be expected from their content of aluminium and silicon. Tables 4:5 and 4:6 indicate that higher recovery should be achieved at those concentrations of aluminium and silicon found in the samples.

TABLE 4:10 RECOVERY OF FLUORIDE ADDED TO SOME SAMPLES PRIOR TO ASHING ON OPEN FLAME.

Sample	Fluoride in Sample ($\mu g\ g^{-1}$)	Fluoride added (μg)	Recovery[a] (%)
Hay[b]	1.25	5	81.2 ± 1.9
"	"	10	88.1 ± 2.0
"	"	20	91.2 ± 1.5
"	"	80	94.5 ± 1.0[c]
Pumpkin leaves[d] *Cucurbita pepo*	18.9	5	77.3 ± 1.7
"	"	10	74.0 ± 1.0
Amaranthus hybridus	11.8	10	97.0 ± 0.1
Lettuce *Lactuca sativa*	9.1	5	92.8 ± 1.5
"	"	10	102.7 ± 0.5
Kale *Brassica intergrifolia*	14.2	5	94.0 ± 1.0
"	"	10	99.6 ± 0.7

[a]Mean and standard deviation (n = 2). [b]Sample from Sweden containing 0.31% Si, 0.08% Al. [c]Half the amount of ashed sample taken for diffusion leading to less interference from Si. [d]Sample containing 1.03% Si, 0.12% Al.

To study if the fluoride was retained by the nickel crucibles, those crucibles used for ashing of the spiked hay samples in table 4:11 (5-80 µg fluoride added to 1g sample) were carefully washed with water after withdrawing the required 5 cm^3 of the sample solution for diffusion. About 0.2g of sodium hydroxide and a few drops of water were then fused in the covered crucibles by heating for 1.5-2.0 minutes on a small flame. The melt was then analysed by diffusion as a sample using only 1 cm^3 of acetate buffer diluted 1+1 to dissolve the alkaline layer on the lid. The amount found was 1.8% (mean of 8 analyses) of total fluoride present at ashing, independent of the quantity of fluoride added to the hay. Obviously, it is necessary to clean the crucibles between ashing. For cleaning, potassium hydroxide was prefered to sodium hydroxide because the melting point is lower and thus the attack on the crucibles will be less serious.

The expected sum of losses caused by the retention of fluoride in the crucibles and the presence of aluminium and silicon at diffusion amounts to about 7% for hay and 13% for pumpkin leaves. For hay, the negative errors in recovery do not differ much from the expected, except as regards the low amount of fluoride added (5 µg F). Otherwise the method gives good recovery for samples with low silicon content.

4:7 METHODOLOGY FOR THE ANALYSIS OF LABILE FLUORIDE IN SOIL.

In this section, methods for labile fluoride extraction are discussed. Water extraction was compared with ammonium lactate extraction. The latter method was studied more in detail and it was adopted since in some laboratories, it had been used to extract chloride, sulphate, phosphorous, calcium and iron available to plants. Urumova et al (1982) suggested that, sodium citrate-EDTA mixture gave the best results for the extraction of available fluoride. Therefore, the ammonium lactate method was compared with Urumova's method.

4:7:1 SOIL PROPERTIES:

Four different soils namely:- Medium clay, organic, loam silt and sandy loam were taken and some of the characteristics determined as shown in table 4:11. These soils were used to establish a technique for the extraction of labile fluoride using ammonium lactate solution. [Larsen and Widdowson 1971].

TABLE 4:11 SOME CHARACTERISTICS OF SOIL SAMPLES USED.

Soil Texture	pH	Organic Matter %	Clay Matter %	Ammonium Lactate ($\mu g\ g^{-1}$)					Nitric acid ($\mu g\ g^{-1}$)		
				P	K	Ca	Mg	Fe	Al	Fe	Si
Medium Clay	7.2	3.3	25-40	112	3850	2400	420	226	19.3×10^3	80.0×10^3	78
Organic	4.6	73	0	14	135	3420	252	5150	5.0×10^3	31.0×10^3	240
Loam silt	5.3	3.2	5-15	164	130	350	23	156	7.5×10^3	29.0×10^3	250
Sandy loam	6.0	1.9	2-5	73	125	660	24	176	6.5×10^3	12.0×10^3	130

These four soils were chosen because they have diversified properties especially the factors which influence the amount of fluoride released in the soil namely pH, organic matter and clay matter.

4:8 COMPARISON OF SODIUM CITRATE-EDTA AND SODIUM CITRATE-TISAB II AS EXTRACTING MEDIA.

Sodium citrate-EDTA has been cited by Urumova et al (1982) as the best extracting media for the labile fluoride. Both sodium citrate-EDTA and TISAB II have been widely used as buffering and complexing agents in fluoride analysis. Many workers have complimented sodium citrate to be the best complexing agent for aluminium which interferes with the fluoride analysis. In the experiments of soil analysis, sodium citrate is added to the extract and left standing for at least 2 hours. This is to allow its complexation with aluminium to take place. Since Urumova et al (1982) have quoted sodium citrate-EDTA to be the best extracting media, it was thought necessary to compare the extracting capacity with that of sodium citrate-TISAB II which has been used in these experiments as a complexing agent. Urumova et al (1982) recommended a shaking period of 20 minutes and then let it stand for 4 hours. This work showed that 1½ hours shaking time was better as compared to 20 minutes shaking plus 4 hours standing. The results obtained are shown in table 4:12.

From the data in table 4:12, it was found that, apart from the organic soil, the extracting time did not seem to play a big role. For organic soil, after 1½ hours shaking, the sodium citrate-EDTA extraction gave higher values than sodium citrate-TISAB II. The results obtained after shaking 1½ hours are very much comparable with those of 20 minutes shaking plus 2 hours standing.

TABLE 4:12 COMPARISON OF SODIUM CITRATE-EDTA AND SODIUM CITRATE-TISAB II (1:20 w/v).

Soil Type	Amount of Fluoride Extracted ($\mu g\ g^{-1}$) after				
	1½ hour Shaking		20 minute Shaking + 2hr standing		20 minute + 4hr standing
	Sodium Citrate-EDTA	Sodium Citrate-TISAB II	Sodium Citrate -TISAB II	Sodium Citrate -EDTA	Sodium Citrate -EDTA
Medium clay	11.4	11.2	11.7	11.4	10.2
Organic	53.6	39.8	48.2	39.6	42.4
Loam silt	11.7	11.9	12.3	12.0	10.8
Sandy loam	11.6	13.1	9.7	9.0	7.9

Extraction of the samples using 20 cm^3 of sodium citrate-EDTA for 4 hours 20 minutes gave a very viscous deep brown solution, an indication that some organic matter was digested and this might interfere with the electrode. Hence, it was not advisable to keep the solution standing for 4 hours. To reduce the viscosity and the brown colour, the volume of the extracting media was increased from 20 cm^3 to 40 cm^3. This also helped to extract more labile fluoride from the soil sample. The data obtained is given in table 4:13. Apart from the organic soil where the fluoride increased by 5.8% and sandy loam (15.7%), the amount of fluoride in the other two soils increased with a factor greater than 50%, showing that, most of the available fluoride is left in the soil when a low volume (20 cm^3) is used for extraction.

TABLE 4:13 THE AMOUNT OF FLUORIDE RELEASED USING 20 cm^3 AND 40 cm^3 SODIUM CITRATE-EDTA.

Soil Type	Amount of Fluoride Extracted ($\mu g\ g^{-1}$)		
	20 cm^3	40 cm^3	% Increase
Medium clay	11.4	18.2	60.9
Organic soil	53.6	56.7	5.8
Loam silt	11.7	19.6	67.5
Sandy loam	11.6	13.4	15.7

Therefore, 40 cm^3 of sodium citrate-EDTA and 1½ hours shaking was used in the other experiments. It was also concluded that, sodium citrate-TISAB II can be used as an extracting medium instead of sodium citrate-EDTA since they gave similar results.

4:9 EXTRACTION USING AMMONIUM LACTATE SOLUTION.

4:9:1 SOIL:SOLUTION RATIO.

Different soil:solution ratios were considered in which the volume was kept constant (100 cm^3) and the amount of soil varied accordingly. From fig. 4:2, it can be seen that, there is a progressive decrease of the amount of fluoride extracted in all the four soils used. As the amount of soil is increased from 1.0g to 5.0g, the total amount of fluoride extracted decreased. Although 0.5g sample would give high total fluoride content, it was thought not to be a good amount to use because the electrode took along time to stabilize during measurement. This may also give a big error since the readings fall in the non-linear region. 1.0g sample was used and thought to be the best amount because it gave suitable readings. In addition, since the soil contains high concentration of interfering ions, 1.0g sample will release low amount of these interfering ions.

4:9:2 SHAKING TIME.

The shaking time is a very important factor on the fluoride extraction. This is the time taken for an equilibrium

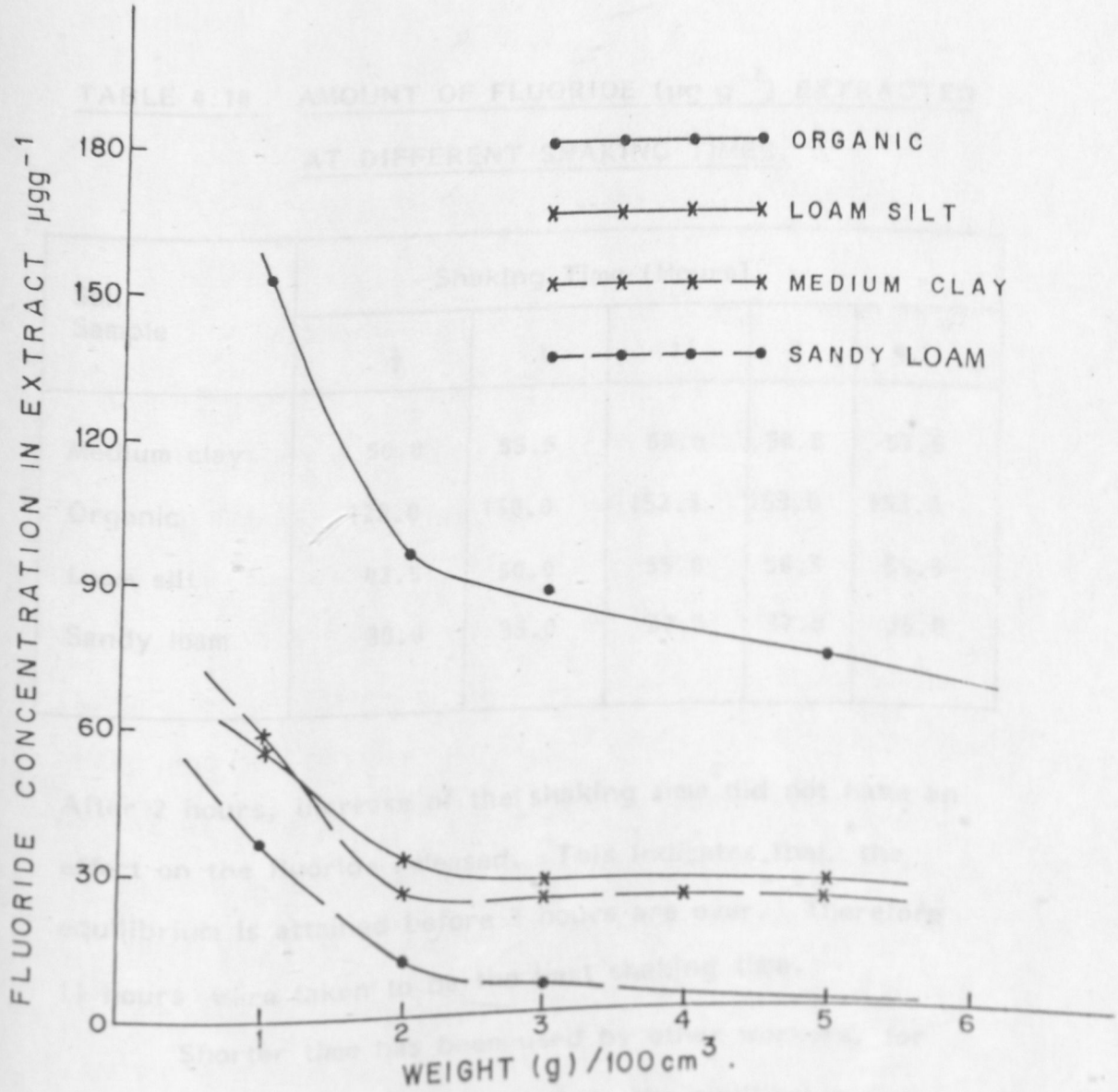

Fig. 4:2 Soil solution ratio .

to be established. The shorter the time, the better the method especially when many samples have to be analysed. It can be seen from table 4:14 below that, the amount of fluoride extracted increases as the shaking time increases up to 2 hours. But the increase is not very big.

TABLE 4:14 AMOUNT OF FLUORIDE ($\mu g\ g^{-1}$) EXTRACTED AT DIFFERENT SHAKING TIMES.

Soil Sample	Shaking Time (Hours)				
	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	4
Medium clay	50.0	55.5	58.0	58.5	57.5
Organic	128.0	150.0	152.5	153.0	153.0
Loam silt	43.5	50.0	55.0	56.5	55.5
Sandy loam	30.0	35.0	37.5	37.0	36.0

After 2 hours, increase of the shaking time did not have an effect on the fluoride released. This indicates that, the equilibrium is attained before 2 hours are over. Therefore $1\frac{1}{2}$ hours were taken to be the best shaking time.

Shorter time has been used by other workers, for example, Eyde (1982) suggested that, the equilibrium was reached within the first 15 minutes and varying the extraction time had little influence on the results. The 16 hours recommended by Larsen and Widdowson (1971) after

comparison with 5 day shaking was thought to be too long especially if one is dealing with many samples. The equilibrium is also attained very quickly before 2 hours are over and hence shaking for a longer time does not improve the results.

4:9:3 COMPARISON OF EXTRACTING MEDIA: HYDROCHLORIC ACID (1M), AMMONIUM LACTATE, SODIUM CITRATE-EDTA AND WATER.

The data showing the amount of fluoride extracted from 1.0g soil sample by different extracting media and shaking for $1\frac{1}{2}$ hours is given in table 4:15. From the table, it can be seen that, the amount of fluoride extracted increases with the increase in the acidity of the extracting media. Water gives the lowest amount of fluoride content and this is the most available fluoride for the plants in the soil. Comparison of ammonium lactate and sodium citrate-EDTA methods shows that, the amount of fluoride released increased by a factor greater than 2.7 in the ammonium lactate than in sodium citrate-EDTA solution. The difference may be attributed to the difference in pH of the two extracting media which are pH = 3.75 for ammonium lactate solution and pH = 6.0 for sodium citrate-EDTA solution. Previous work [Eyde 1982] with hydrochloric acid at different concentrations (0.01-4.0M) showed that, the more concentrated the acid, the higher the fluoride extracted. Apart from the organic soil, the other three types gave a fluoride concentration with 1M hydrochloric acid 2.5 times

TABLE 4:15 AMOUNT OF FLUORIDE($\mu g\ g^{-1}$)OBTAINED WITH DIFFERENT EXTRACTING MEDIA.

Soil Sample	1M HCl pH=O	Ammonium lactate pH= 3.75	Sodium citrate -EDTA pH = 6.0	Water
Medium clay	147.0	58.0	18.2	20.0
Organic	226.0	152.5	56.7	28.0
Loam silt	139.0	55.0	19.6	9.0
Sandy loam	114.6	37.5	13.4	3.0

higher than that found with ammonium lactate, which indicates that, the soils contain some fluoride bearing minerals which will release fluoride when extracted in 1M hydrochloric acid. For organic soil, the fluoride increased $1\frac{1}{2}$ times when extracted with hydrochloric acid.

Ammonium lactate as mentioned earlier, has been used in other laboratories to extract various ions from the soil in order to determine the amount of these ions available for plants. Analysis of labile fluoride in the soil using ammonium lactate therefore could be done in the same extract used in determining these other ions. From the previous experiments on interfering ions, it was found that 1M citrate buffer was capable of complexing more than 50 mg l^{-1} aluminium present in the soil. Analysis of total aluminium, iron and silicon using nitric acid digestion showed that medium clay contained 19.3×10^3 $\mu g\ g^{-1}$

aluminium as shown in table 4:11, while other soils had lower values. It is also apparent from the table 4:16 that, medium clay soil has the highest amount of iron but low levels of silicon. The amounts of these ions released in ammonium lactate solution during extraction are low and hence do not interfere with the fluoride analysis because they are complexed by the buffer.

Some Kenyan soils were also analysed for the labile fluoride and the data is presented in table 4:16. As observed from the previous soils, the fluoride extracted increases with the increase in the acidity of the extracting media. The data shows that, the amount of fluoride in 11 of the samples extracted with hydrochloric acid increases by a factor of two in comparison to the amount extracted with ammonium lactate solution. For the other three samples, the amount of fluoride increased three times. It has been previously pointed out that, most of the fluoride in volcanic soils is water and acid labile and this may explain why there is no big difference in the amount of fluoride extracted by both ammonium lactate and hydrochloric acid.

Both water and sodium citrate-EDTA media gave low fluoride concentration in comparison to the other two media but sodium citrate-EDTA gave higher values than water extraction. The soils were found to contain high calcium content than potassium as shown in table 4:16, while the percentage organic matter was below 10%. Apart from 5 soil samples with a pH<6.0, all other soil samples have a pH>6.0 with two soils S_7 and S_{12} with pH 8.0. The two soils also released the highest

TABLE 4:16 CHEMICAL COMPOSITION OF KENYAN SOIL SAMPLES.

	Soil Texture	Soil pH	Organic Matter %	Ammonium Lactate Extraction				Nitric Acid Extraction			Fluoride Extracted Using			
				P	K	Ca	Mg	Fe	Al	Si	Water	Ammonium Lactate	Sodium Citrate–EDTA	1M HCl
S_1	Clay Loam	6.4	4.5	30	1250	2020	420	4000	2900	120	12.6	91.7	16.3	147.3
S_2	Sandy Loam	6.8	3.2	80	1600	1840	226	2200	1900	85	13.8	82.0	16.1	136.7
S_3	Sandy Clay Loam	7.0	7.8	40	900	3200	580	1600	1400	105	14.7	86.0	15.6	135.0
S_4	Clay Loam	5.6	2.8	30	515	1960	360	3300	2400	125	9.7	92.7	18.9	153.7
S_5	Loam	6.4	5.2	60	1300	3600	268	3000	2400	115	12.4	88.3	19.0	153.0
S_6	Clay Loam	6.5	5.1	12	1000	3800	380	4000	2900	130	10.9	86.0	17.0	157.0
S_7	Sandy Loam	8.0	4.0	51	2200	20600	360	2800	2600	185	41.7	282.0	102.5	937.7
S_8	Sandy Clay Loam	5.9	4.5	30	700	1120	216	7700	10400	135	6.9	78.0	11.9	116.7
S_9	Sandy Clay Loam	5.8	6.9	34	1300	1380	340	8300	8200	185	6.6	74.0	11.6	115.0

TABLE 4:16 CONTINUED

	Soil Texture	Soil pH	Organic Matter %	Ammonium Lactate Extraction				Nitric Acid Extraction			Fluoride Extracted Using			
				P	K	Ca	Mg	Fe	Al	Si	Water	Ammonium Lactate	Sodium Citrate–EDTA	1M HCl
S_{10}	Sandy Loam	7.7	2.5	54	480	6400	420	2100	1900	150	18.1	83.7	20.1	222.3
S_{11}	Clay Loam	5.0	0.9	20	265	1120	252	2300	2300	110	7.4	86.0	18.4	144.3
S_{12}	Clay Loam	7.9	1.8	77	335	9800	460	3200	3600	155	38.9	143.0	47.7	394.5
S_{13}	Sandy Clay Loam	6.3	5.2	340	850	5000	480	6000	4200	220	7.9	89.0	17.9	158.5
S_{14}	Sandy Clay Loam	5.9	3.6	20	405	1820	250	8900	3600	120	6.9	84.0	15.5	156.7

All concentration values in $\mu g\ g^{-1}$

amount of fluoride with all the extracting media in comparison to the other soil samples.

4:10 RECOVERY EXPERIMENTS.

In order to investigate the recovery, sodium fluoride standard was added to some samples before extraction and the amount of fluoride recovered is shown in table 4:17 below.

TABLE 4:17 FLUORIDE (%) RECOVERED USING AMMONIUM LACTATE MEDIA.

Soil Sample	Fluoride ($\mu g\ g^{-1}$) present in the sample	Fluoride (μg) added to 1.0g sample		
		50	100	200
Medium clay	58.0	80.0	87.5	83.8
Organic	152.5	94.0	90.0	93.7
Loam silt	55.0	91.4	92.5	96.7
Sandy loam	37.5	100.5	97.0	95.0

As can be seen from the table above, apart from clay soil where less than 90% recovery was obtained, the other soils gave a recovery ranging from 90% to 100%. Medium clay gave low recoveries because of the high aluminium content present. In clay soils, aluminium exist as aluminium hydroxide and on addition of fluoride, an ion exchange mechanism between fluoride and hydroxyl ions takes place [Nömmic 1953, Omueti and Jones 1977, Njenga 1982]. This exchange mechanism may explain the

low recovery of fluoride in medium clay soil. It is also apparent that, the best fluoride recovery is achieved from the sandy soil. Nömmic (1953) proposed that, when fluoride is added into the soil, it is bound and hence not easily recovered. His poor recovery was due to the fact that, after adding the fluoride standard to the soil, the sample was left standing for a few days. During this time, complexation of fluoride by other ions may have taken place. Preliminary work carried out whereby fluoride was added into the soil as sodium fluoride solution mixed thoroughly and then dried at 37°C showed that, after leaving it for 1 day, less than 50% fluoride was recovered. Huang and Jackson (1965), Perrot et al (1976) also reported that, added fluoride in the soil becomes immobile and hence not easily extracted.

CHAPTER 5

5:0 FLUORIDE CONCENTRATION IN VEGETABLES, FOODS AND PLANTS.

5.1 FLUORIDE CONCENTRATION IN VEGETABLES:

In this section, values of fluoride in vegetables collected from various parts of the country are reported. The samples were analysed by open flame ashing followed by diffusion method. The values of the fluoride concentration are reported in table 5:1 as the range in brackets, mean and the overall mean while the actual fluoride concentration is reported in Appendix I.

5:1:1 TERERE (AMARANTHUS):

Amaranthus has fluoride concentration ranging from 4.21-16.25 $\mu g\ g^{-1}$ and an overall mean of 7.82 $\mu g\ g^{-1}$ except for two samples which had a fluoride concentration of 0.42 $\mu g\ g^{-1}$ and 0.89 $\mu g\ g^{-1}$. Samples from Meru were collected along Meru-Nanyuki highway where they had been interplanted with potatoes. Apart from the influence of volcanic soil on these samples (mainly from Mt. Kenya) the amaranthus might have accumulated fluoride from the fertilizers. This factor might also account for the high fluoride concentration of samples from Molo (15.19 $\mu g\ g^{-1}$). The highest fluoride concentration was recorded in samples from rift valley (16.25 $\mu g\ g^{-1}$). This may be attributed to the influence of volcanic soil.

TABLE 5:1 FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) IN VEGETABLES FROM DIFFERENT LOCALITIES.

Locality	Terere *Amaranthus*	Cabbage *Brassica oleracea*	Pumpkin leaves *Cucurbita pepo*	Kahurura *Cucurbita sps*
Kiambu	8.17 (7.90-8.50)	0.70 (0.42-0.92)	9.16	5.88 (3.21-7.33)
Thika	5.28 (4.72-5.83)	1.51 (1.29-1.79)	3.14 (2.49-3.78)	
Rift Valley	8.57 (0.89-16.25)	6.11 (5.04-7.80)	8.29 (1.14-18.98)	
Molo	15.19		11.19	
Njoro			12.17	
Murang'a	9.41 (8.32-10.50)	1.45 (0.91-1.83)	5.63 (5.23-6.14)	3.71 (3.11-4.31)
Njeri		0.98 (0.56-1.32)	3.00 (2.48-3.51)	
Kirinyaga	0.66 (0.42-0.89)	0.58 (0.50-0.71)	1.33 (1.21-1.45)	
Nairobi	7.37 (5.30-11.84)	1.68 (1.12-2.40)	9.61 (8.64-10.57)	7.87 (6.88-9.62)
Embu	9.20 (8.94-9.45)	7.03 (6.24-7.82)	4.64 (2.94-6.34)	3.99 (3.42-4.56)
Kisii	9.41		3.82	
Kisumu		15.10		
Meru	12.07 (11.68-12.45)		9.33 (8.98-9.67)	
Mombasa	6.54 (5.94-6.83)		5.89	
Nyahururu			7.04 (6.75-7.32)	7.16
OVERALL MEAN (n)	7.82 (25)	2.92 (23)	6.10 (26)	5.80 (11)

TABLE 5:1 CONTINUED

Locality	Kunde *Vigna unguiculata*	Lettuce *Lactuca sativa*	Managu *Solanum nigrum*	Spinach *Spinacea oleracea*	Kale *Brassica integrifolia*
Kiambu	1.74 (0.87-2.79)	6.03 (3.01-9.05)	3.82	4.29 (1.68-10.32)	5.28 (0.48-14.68)
Thika			2.94 (2.73-3.14)	4.33	1.29 (1.14-1.43)
Rift Valley	1.37 (0.84-1.74)	6.39	18.65 (7.32-29.98)	3.02 (1.68-5.36)	3.73 (1.30-5.69)
Molo			1.44	0.95	4.33
Njoro					1.73 (1.18-2.27)
Murang'a	4.65 (4.56-4.74)		3.88 (3.25-4.50)	4.38 (3.92-5.12)	2.68 (1.92-3.88)
Nyeri	2.70 (2.30-3.10)		2.86 (2.45-3.28)	3.73 (0.42-7.80)	1.77 (0.48-3.06)
Kirinyaga	6.42 (1.30-11.54)		0.42 (0.31-0.52)	0.67 (0.40-0.86)	1.49 (0.48-3.90)
Nairobi	8.01 (5.69-10.32)		14.69 (13.63-15.75)	5.89 (1.35-10.06)	1.11 (0.31-2.32)
Embu	8.22 (7.96-8.47)		4.59 (2.94-6.24)	2.19 (1.85-2.52)	2.83 (0.52-5.01)
Kisii				9.57	4.43
Kisumu					1.73 (1.53-1.93)
Meru	4.35 (3.95-4.75)		9.33 (8.98-9.67)	8.05 (7.48-8.62)	5.73
Mombasa	6.92 (5.33-10.24)		5.89		3.72 (2.54-5.30)
Kericho					1.85
Nyahururu					
OVERALL MEAN (n)	4.90 (23)	6.15 (3)	5.67 (17)	4.38 (27)	3.01 (41)

5:1:2 CABBAGE (BRASSICA OLERACEA):

Cabbage is a commonly used vegetable in most homesteads. The fluoride concentration ranges from 0.42-15.10 μg g^{-1} with an overall mean of 2.92 μg g^{-1}. The fluoride concentration of cabbage was found to be generally low except for the samples from Kisumu, Embu and rift valley with a fluoride concentration of 15.10, 6.24-7.82 and 5.04-7.8 μg g^{-1} respectively. The samples from rift valley were collected at Kaptombesi where it was irrigated by one of the seasonal springs draining into lake Bogoria. Fertilizers might also have contributed. Generally, cabbages have very low fluoride although a value of 18 μg g^{-1} has been reported from Kericho [Owuor 1985]. This value is thought to be on a higher level. Cabbage have been reported to be capable of accumulating up to 297 μg g^{-1} fluoride [Sherlock 1984] when grown near a fluoride emitting industry. A value of 9.6 μg g^{-1} fluoride was recorded [Waldbott 1978, Jones 1971].

5:1:3 PUMPKIN LEAVES (CUCURBITA PEPO):

In the past, pumpkin leaves have been widely used especially in Murang'a. They are normally used for mashing food together. Pumpkin leaves were found to contain a substantial amount of fluoride ranging from 1.14-18.98 μg g^{-1} with an overall mean of 6.10 μg g^{-1}. A concentration of 20-50 μg g^{-1} fluoride has been reported [Owuor 1985]. This value is very high in comparison to the values obtained in the

present work. The age and size of the leaves did not show a big difference. Analysis of young leaves and old leaves (picked from the same plant) gave 6.75 and 7.32 μg g^{-1} fluoride respectively. The sample with the highest fluoride (18.98 μg g^{-1}) was picked from Kijabe which is in the rift valley hence high fluoride in the soil.

5:1:4 KAHURURA (CUCURBITA SPS):

This plant belongs to the same family as the cucurbita pepo and recently it has gained a lot of popularity for mashing food replacing the pumpkin leaves. It gave a fluoride concentration ranging from 3.11-9.62 μg g^{-1} and an overall mean of 5.80 μg g^{-1}. The sample with the highest fluoride concentration (9.62 μg g^{-1}) was collected from Nairobi (*Kariobangi sewage) area where sewage sludge and effluent are used. This might have contributed to the high fluoride concentration present. Cucurbita sps was found to accumulate almost the same amount of fluoride as pumpkin leaves when grown in the same area. For example, the samples collected from Nyahururu gave:- Cucurbita sps 7.16 μg g^{-1} while cucurbita pepo gave 7.32 μg g^{-1} fluoride.

*Kariobangi sewage - This refers to the farms around Kariobangi Sewage Treatment Works which use sewage sludge.

5:1:5 KUNDE (VIGNA UNGUICULATA):

Kunde is also commonly used as a vegetable. Table 5:1 shows that, the fluoride concentration ranges from 1.30-11.54 μg g^{-1} with the mean ranging from 1.37-8.22 μg g^{-1} and the overall mean of 4.90 μg g^{-1} for twenty three samples collected. Two samples were exception with a fluoride concentration of 0.84 and 0.87 μg g^{-1} as shown in appendix 1. The sample with the highest concentration (11.54 μg g^{-1} fluoride) was collected from Kirinyaga in a rice farm. In this farm, the soils are highly irrigated and are kept constantly wet. It is possible that, this type of irrigation makes the inorganic soil fluoride to be readily available to plants. The next sample with 10.32 μg g^{-1} fluoride was collect from Nairobi area (Kariobangi sewage) where the sewage sludge and effluent might have contributed to high fluoride.

5:1:6 LETTUCE (LUCTUCA SATIVA):

Lettuce has been reported to be a good accumulator of fluoride and it is capable of accumulating from 0.1 μg g^{-1} fluoride (normal values) to 44 μg g^{-1} fluoride when grown near a fluoride emitting industry [Waldbott 1978]. Different values from different areas have been reported [Jones et al 1972]. The present work shows that, the fluoride concentration is low (3.01, 6.39, 9.05 μg g^{-1}) although it is thirty to ninty times higher than the reported normal value.

5:1:7 MANAGU (SOLANUM NIGRUM):

The fluoride concentration varies from 2.45-29.98 μg g^{-1} with an overall mean of 5.67 μg g^{-1} with the exception of two

samples, from Kirinyaga and Molo (rift valley) with a fluoride concentration of 0.31-0.52 μg g^{-1} and 1.44 μg g^{-1} respectively. The sample with the highest fluoride concentration of 29.98 μg g^{-1} was collected from lake Bogoria park. The lake has a fluoride concentration of 1200 mg l^{-1} [Njenga 1982] and this may explain the high fluoride content in the sample.

5:1:8 SPINACH (SPINACEA OLERACEA):

Spinach has been reported to be a good accumulator of fluoride when grown near fluoride emitting industries. The reported normal value for spinach is 2.82 μg g^{-1} fluoride [Kumpulainen and Koivistoinen 1970]. The present work shows that the fluoride concentration varies from 1.35-10.32 μg g^{-1} with the exception of three samples with fluoride concentration ranging from 0.40-0.95 μg g^{-1}. The sample with the highest fluoride concentration (10.32 μg g^{-1}) was collected from Kiambu (Thindigwa coffee estate) where the water used for irrigation had high fluoride concentration. Previous work showed the water to contain 10 mg l^{-1} fluoride [Njenga 1982]. The amount of fluoride accumulated by this sample is 3.66 times higher than the reported normal value. Samples from Kariobangi sewage also have high fluoride concentration (10.06 μg g^{-1}).

Unlike other vegetables, it was found that the young spinach leaf contained high fluoride in comparison to old leaf. Samples collected from Kariobangi sewage gave 10.06, 8.59 and 4.33 μg g^{-1} fluoride after being grown for 3 months, 1 year and 1½ years respectively.

5:1:9 KALE (BRASSICA INTEGRIFOLIA):

This is the most commonly eaten vegetable in Kenya. It is mainly eaten together with ugali. Fluoride values range from 0.31-14.68 μg g^{-1}. Values ranging from 7-55 μg g^{-1} fluoride have been reported [Owuor 1985] and this is thought to be on the higher side. Sample with the highest fluoride concentration (14.68 μg g^{-1}) was collected from Kiambu (Thindigwa coffee estate) and might have derived the fluoride from the water used for irrigation. Samples from Meru and Embu also had a high fluoride concentration of 5.73 and 5.01 μg g^{-1} respectively. Values for Kale have been quoted as 0.16-3.0 μg g^{-1} fluoride on a fresh weight basis [Jones et al 1972].

5:1:10 OTHER VEGETABLES:

These are rare vegetables and are available only at various places. The fluoride concentrations of these vegetables are presented in table 5.2.

The samples contain high fluoride concentration ranging from 2.29-32.83 μg g^{-1} fluoride. Borage (32.83 μg g^{-1} fluoride) from Mombasa has the highest fluoride concentration followed by fenugreek (18.69 μg g^{-1}), pakiri (11.21 μg g^{-1}) and corchorus oloitaris (13.99 μg g^{-1}).

TABLE 5:2 FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) OF OTHER VEGETABLES.

Locality	Sample	Fluoride ($\mu g\ g^{-1}$)
Rift Valley	Fenugreek *Trigonella foenum graecum*	18.65
Nairobi	Thageti *Gynadropsis gynandra*	10.05
Kisii	" "	2.29
Kiambu	Cerely *Apium graveolens*	6.30
Kisumu	Apoth *Corchorus oloitaris*	13.99
Embu	Borage *Borago officinaris*	3.51
Nyeri	" "	4.93
Nairobi	" "	6.45
Mombasa	" "	32.83
Nyeri	Togotia *Erucastrum arabicum*	3.29
Mombasa	Mchunga *Luctuca capensis*	6.73
Mombasa	Pakiri *Sechiniedule cucurbitaceae*	11.21
Mombasa	Cassava leaves *Manihot esculenium*	2.90

5:2 FLUORIDE CONCENTRATION IN FOODS:

Fluorine is found in all parts of the environment hence it is not surprising that all foods contain fluoride and to prepare a meal without fluoride is a real challenge. Table 5:3 shows the concentration of fluoride of some commonly eaten foods in the country.

5:2:1 WHEAT AND MAIZE FLOUR:

The fluoride concentration of wheat and maize flour is low with values of 2.02 $\mu g\ g^{-1}$ and 1.21 $\mu g\ g^{-1}$ respectively. This agrees with the previous work where wheat flour was reported to contain 0.2-1.8 $\mu g\ g^{-1}$ fluoride [Sherlock 1984] and maize flour approximately 0.7 $\mu g\ g^{-1}$ fluoride [Waldbott 1978].

5:2:2 BANANAS:

Bananas were found to contain 1.24-4.63 $\mu g\ g^{-1}$ fluoride. It was noted that banana pulps (without peels) contained less fluoride than those with peels. The fluoride concentration of the peels ranged from 0.13-2.52 $\mu g\ g^{-1}$ which is 10.5-54.4% of the total fluoride. This implies that alot of fluoride is concentrated in the peels. Dried banana peels have been reported to have accumulated as high as 51 $\mu g\ g^{-1}$ fluoride in contrast to only 3.8 $\mu g\ g^{-1}$ fluoride in the pulp [Kumpulainen and Koivistoinen 1977]. The bananas analysed in this work contain far less fluoride than that reported.

TABLE 5:3 FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) IN DIFFERENT TYPES OF FOOD.

Locality	Sample	Fluoride ($\mu g\ g^{-1}$)
Shops	Wheat flour	2.02
	Maize flour	1.21
Murang'a	Bananas (with peels)	1.24
	Bananas (without peels)	1.22
	Bananas (with peels)	4.63
	Bananas (without peels)	2.11
Kisii	Bananas (ripe)	3.60
Kisumu	Bananas	2.30
Kiambu	Sweet potatoes (with peels)	6.60
	Sweet potatoes (without peels)	4.06
Murang'a	Sweet potatoes (white)	0.75
	Sweet potatoes (brown)	2.48
Kisii	Sweet potatoes (white)	0.95
	Sweet potatoes (brown)	3.87
Kisumu	Sweet potatoes (white)	2.41
	Sweet potatoes (brown)	1.14
Nairobi market	Arrow roots	1.00
Nairobi market	Irish potatoes	0.75
Murang'a	Irish potatoes	2.05

5:2:3 SWEET POTATOES:

Sweet potatoes were found to contain 0.75-6.60 μg g^{-1} fluoride with the brown sweet potatoes recording higher values than white potatoes. Like bananas, sweet potatoes with peels contain high fluoride than without peels. That means the peels contain 2.54 μg g^{-1} fluoride which is 38.5% of the total fluoride. Again the level is lower than that reported in literature where 75% of the fluoride is found in the peels of sweet potatoes [Kumpulainen and Koivistoinen 1977].

5:2:4 IRISH POTATOES AND ARROW ROOTS:

These show low fluoride content than the sweet potatoes although, according to Taves (1983) Irish potatoes should contain higher fluoride (0.494 μg g^{-1}) than sweet potatoes (0.209 μg g^{-1} fluoride). The value of fluoride in the present work is higher than that reported in the literature by Taves (1983). The difference may be accounted for by the fact that, while the present work gives the total fluoride, Taves (1983) reported the acid labile fluoride. Arrow roots do not contain very high fluoride concentration.

Generally it can be said that, the values for the tubers are high since the tubers are thought to contain less than 0.5 μg g^{-1} fluoride [Kumpulainen and Koivistoinen 1977].

5:3 FLUORIDE CONCENTRATION IN BEVERAGES:

A few beverages were bought from the supermarkets and analysed for fluoride content. Table 5:4 shows that some

beverages like passion fruit and Hey-Ho which contain 9.98 and 7.93 mg l^{-1} fluoride respectively can contribute significantly to the total fluoride intake per day. Fruits have been reported to contain very low fluoride content [Phantumvanit et al 1987] as a result, it would be expected that fruit juices should contain low fluoride content. However, fluoride content may be increased in the juices by the fluoride content in the water used for processing.

TABLE 5:4. FLUORIDE CONCENTRATION IN BEVERAGES.

Beverages	Fluoride (mg l^{-1})
Ribena	0.84
Passion (fresh)	0.46
Passion (pep)	9.98
Hey-Ho	7.93
Tree top (orange)	<0.1
Supa squash	0.34
Lime (fresh)	1.08
Pepsi cola	2.06
Fanta	0.19
Coca cola	0.19
Sprite	0.19
Krest (lemon)	0.17
Mirinda	0.19
Schwop	0.18
Coffee	0.22
Black and white gin	0.42
Grants whisky	0.42
Guiness	0.70
Tusker	0.35
White cap	0.30

Lime juice (fresh) and pepsi cola also show high values of fluoride concentration while all other beverages analysed had lower than 1 mg l^{-1} fluoride. This is in agreement with what has been reported by Sherlock (1984) that beverages contain less than 1 mg l^{-1} fluoride. Becker and Bruce (1981), have reported that fruit juices contain less than 0.5 mg l^{-1} fluoride. The data shows that sodas contain less than 0.20 mg l^{-1} fluoride while the beers contain less than 0.5 mg l^{-1} except guiness. Most of the fluoride in the soda comes from the water used. The Nairobi City Council water was found to contain 0.13 mg l^{-1} fluoride.

5:4 FLUORIDE CONCENTRATION IN BABY FOODS:

Four different types of baby foods from supermarket were analysed for acid labile fluoride and table 5:5 shows the fluoride concentration.

TABLE 5:5 FLUORIDE CONCENTRATION (µg g^{-1}) IN BABY FOODS.

Sample	Fluoride
Lactogen	0.18
Promil	0.29
Cerelac	0.24
Nan	0.26

The data shows that, the fluoride concentration is lower than 0.30 μg g^{-1}. As a result, they do not contribute very significantly to the fluoride content taken per day. Baby foods have been reported to contain very low amount of fluoride (0.265 μg g^{-1}) [Phantumvanit et al 1987].

5:5 FLUORIDE CONCENTRATION IN PLANTS:

Some plants from Rift Valley lakes, Naivasha, Nakuru and Bogoria were sampled and analysed for fluoride concentration. The plant samples were collected from these lakes to find out whether they accumulate any fluoride especially when growing near or next to highly fluoridated water from the lakes.

TABLE 5:6 FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) IN PLANTS.

Locality	Sample	Fluoride Concentration ($\mu g\ g^{-1}$)	Fluoride Concentration* of the water ($mg\ l^{-1}$)
Lake Nakuru	Euperus laevigatus Cyperaceae	1049.66	140.00
Lake Nakuru	Piuchea Bequaerti robyns composite	32.06	140.00
Lake Nakuru	Sesbania speciosa	99.20	140.00
Lake Bogoria	Conyza	69.92	1200.00
Lake Bogoria	Sporabolus spicatus	51.60	1200.00
Lake Naivasha	Sphaeranthus Napierce ross	162.04	1.50
Lake Naivasha	Polygonum Polygona senegalense	24.75	1.50

*Reference Njenga (1982).

It can be noted from table 5:6 that, apart from Euperus laevigatus, all the other plant samples contained a fluoride concentration ranging from 24.75 $\mu g\ g^{-1}$ to 162.04 $\mu g\ g^{-1}$ Euperus laevigatus was picked from the shores of Lake Nakuru which contain a fluoride concentration of 140 $mg\ l^{-1}$ [Njenga 1982]. This plant which had a fluoride concentration of 1049.66 $\mu g\ g^{-1}$ might have accumulated the fluoride from the water.

Comparison of this sample with another sample (piuchea) collected approximately 100 metres from the lake shores showed that, it has accumulated fluoride 32.7 times higher than that found in piuchea. Other plants collected from lake Nakuru contain high fluoride. Sesbania speciosa was collected 100 metres from Lake Nakuru entrance towards the lake and it was found to contain 99.20 $\mu g\ g^{-1}$ fluoride. This plant might have accumulated the fluoride from the dust coming from the lake.

Samples from lake Bogoria did not show high fluoride as would have been expected. Lake Bogoria was found to have a fluoride concentration of 1200 $mg\ l^{-1}$ and this high fluoride would have been expected to have a high influence on the plants. Conyza plant with a fluoride concentration of 69.92 $\mu g\ g^{-1}$ was picked near a hot spring. The hot spring showed a fluoride concentration of 60 $mg\ l^{-1}$ [Njenga 1982]. Sporabolus spicatus (51.60 $\mu g\ g^{-1}$ fluoride) was picked at lake Bogoria park. Sporabolus was used for direct diffusion experiments and it was found that 87.9% fluoride was acid labile.

There was a big difference for the samples picked at lake Naivasha (1.50 $mg\ l^{-1}$ fluoride). Sphaeranthus has a fluoride concentration 6.55 times higher than that found in polygonum. Both plants were collected at the shores of the lake. This difference in fluoride concentration is an indication that although plants may be growing in the same area, they will accumulate different levels of fluoride if they do not belong to the same family.

In general, it can be said that most of the plants found growing in these lakes are good accumulators of fluoride and can tolerate very high fluoride content.

5:6 FLUORIDE CONCENTRATION IN TEA:

5:6:1 PROCESSED TEA.

Tea is commonly used as a beverage in the country. The tea infusion contains higher fluoride concentration than most drinking-water supplies or beverages hence it contributes significantly to the total fluoride intake per day. The amount of fluoride released when tea leaves (different weights) are boiled for 30 minutes is shown in table 5:7 below.

TABLE 5:7 FLUORIDE RELEASED WHEN DIFFERENT WEIGHTS OF TEA LEAVES WERE USED.

Weight of tea leaves (g)	Total fluoride (µg) in 250 cm^3
0.1	75
0.2	105
0.5	150
1.0	275
1.5	353
2.0	400
2.5	425

From the table, it can be seen that, the amount of fluoride released increased with the increase of weight. This means that those people who normally drink concentrated tea are likely to take 176.5-212.5 µg fluoride (taking 1 cup of tea 125 cm^3). Tea has been widely reported to be a good accumulator of fluoride and can accumulate up to 400 mg l^{-1} fluoride which is water extractable and this gives 0.5mg fluoride for any cup of tea consumed [Waldbolt 1978]. An experiment was carried out to check whether all the fluoride content in tea was released when 2.5g was boiled for 30 minutes. The amount of fluoride extracted after every 15 minutes is shown in Fig. 5:1. The histogram shows that 350 µg (which is 46.8% of total fluoride) is released in the first 15 minutes. When the same sample was boiled two times (15 minutes each), 475 µg fluoride was released instead of 425 µg fluoride released when 2.5g was boiled for 30 minutes. Total amount of fluoride extracted after boiling for 2 hours was 747.5 µg fluoride. This means that, it may be possible to extract all the labile fluoride present in tea with water.

When tea from the farms is taken to the factory it is first withered with hot air until it is left with approximately 20% moisture content and then cut into small pieces. It is then fermented for 90 minutes at varying temperatures of 70-85°F with cold air and then taken to the kilns for firing. This is followed by grinding and sorting out of different classes and grades. There are two classes, primary and secondary and in these two classes, different grades are found for example,

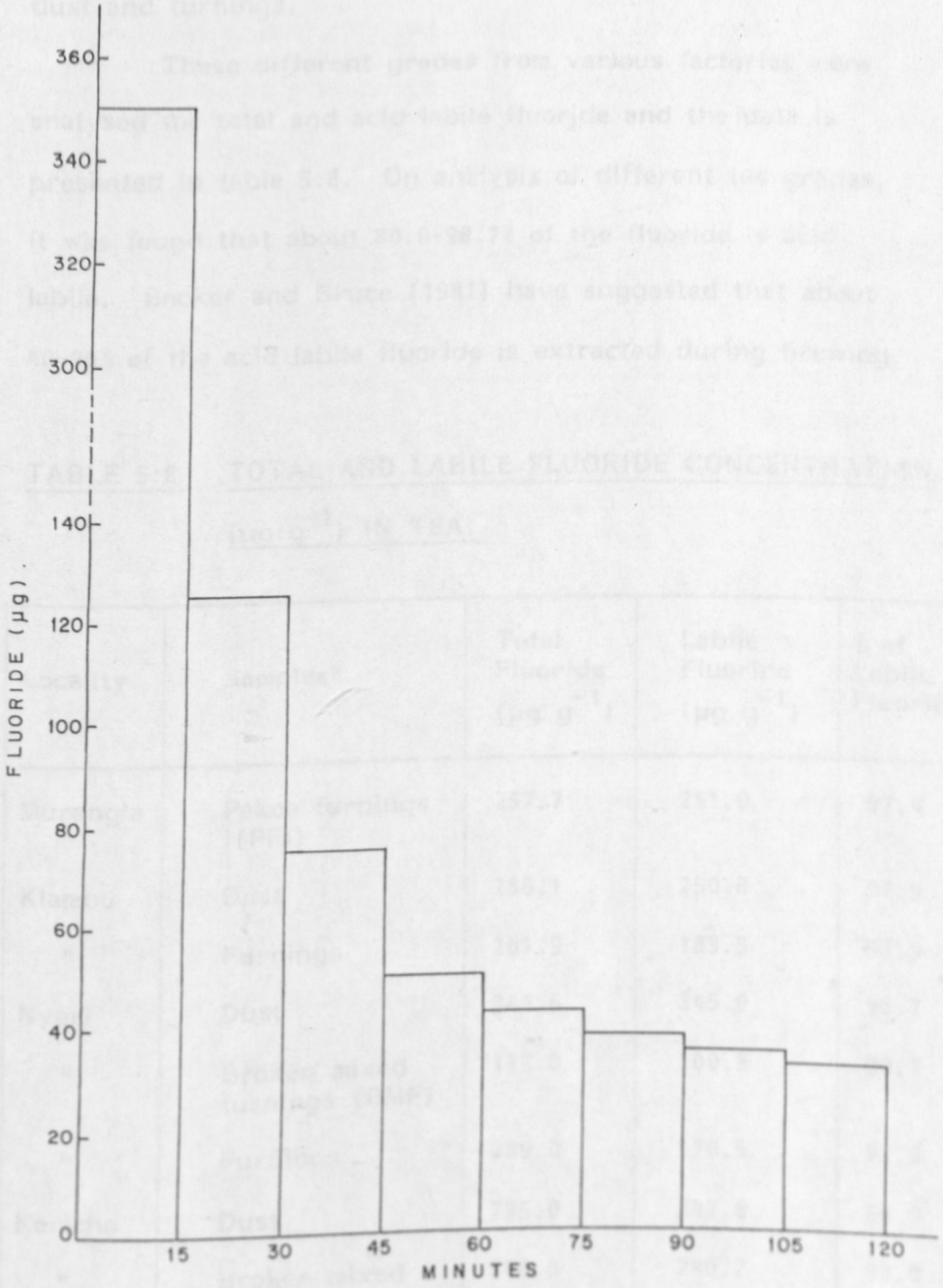

Fig. 5:1 Amount of fluoride (µg) released after every 15 minutes.

Broken pekoe one (BPI), Broken mixed furnings (BMF), dust and furnings.

These different grades from various factories were analysed for total and acid labile fluoride and the data is presented in table 5:8. On analysis of different tea grades, it was found that about 80.0-98.7% of the fluoride is acid labile. Becker and Bruce (1981) have suggested that about 40-90% of the acid labile fluoride is extracted during brewing.

TABLE 5:8 TOTAL AND LABILE FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) IN TEA.

Locality	Samples*	Total Fluoride ($\mu g\ g^{-1}$)	Labile Fluoride ($\mu g\ g^{-1}$)	% of Labile Fluoride
Murang'a	Pekoe furnings I(PFI)	257.7	251.0	97.4
Kiambu	Dust	256.1	250.8	97.9
"	Furnings	201.9	189.5	93.9
Nyeri	Dust	349.6	345.0	98.7
"	Broken mixed furnings (BMF)	112.0	100.5	89.7
"	Furnings	289.0	270.5	93.6
Kericho	Dust	295.0	247.8	84.0
"	Broken mixed furnings (BMF)	300.8	240.7	80.0
"	Furnings	354.3	318.6	89.9

* Tea samples from different factories.

Unprocessed samples from a farm were dried and analysed for comparison. The old leaves (4th and 5th leaves) were found to contain higher fluoride concentration than the young leaves (4.8 times higher). Table 5:9 shows the fluoride concentration in the two samples.

TABLE 5:9 FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) OF OLD AND YOUNG TEA LEAVES.

Sample	Total Fluoride ($\mu g\ g^{-1}$)	Labile Fluoride ($\mu g\ g^{-1}$)	% of labile fluoride
Old leaves (4th & 5th leaves)	578.9	550.0	95.0
2* leaves plus a bud	121.0	118.5	97.5

*2 leaves plus a bud: The first two leaves and the bud. These are the leaves picked for processing in the factories.

Comparison of the data from tables 5:8 and 5:9 shows that, fluoride content in the two leaves plus a bud is lower than that of the processed tea. This may be explained by the fact that:-

a. Tea from the factory is a mixture of many tea samples from different farms which may contain different levels of fluoride.

b. Although the samples are highly selected at the buying centres some farmers may sell 4th and 5th leaves. These leaves will increase the fluoride concentration.

c. Contamination of the sample from the picking up to when the sample is packed for selling after processing may increase the fluoride level.

d. Contamination also may occur in the factory where the air used for withering, fermentation and firing may contain some fluoride. The firing cabins are surrounded with clay and this may contribute in increasing the fluoride content.

5:6:2 UNPROCESSED TEA.

In this section, the fluoride concentration of the samples collected from various places is presented. Also fluoride concentration of different clones are compared. Comparison of 2 leaves plus a bud, 4th leaves, bottom leaves and the branches is also done mainly on a wet weight basis.

COMPARISON OF DIFFERENT CLONES

Four different clones were collected from a research station and water labile fluoride analysed as follows:

a) 2 leaves plus a bud

b) Fourth leaves

c) Bottom leaves

d) Branch

The samples were picked from four different clones which were less than 5 years old. The four clones were

a)	Clone STC	5/3	High pH resistant
b)	Clone	6/8	Low pH resistant
c)	Clone	31/11	Low pH resistant
d)	Clone TN	14/3	High pH resistant

The pH resistant indicates that the clone can only do well in either pH<7.0 or pH>7.0 for example clone STC 5/3 should be planted in areas where the soil has a pH>7.0 and that is why it is refered to as high pH resistant. The fluoride concentration in different clones is shown in Table 5:10 below. From the table, it can be seen that, apart from clone 6/8 (low pH resistant) with low fluoride concentration, all the others have accumulated almost equal amounts of fluoride concentration.

TABLE 5:10 FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) IN DIFFERENT CLONES (WATER LABILE).

Clones	2 leaves plus a bud	Fourth leaves	Bottom leaves	Branch
Clone STS 5/3 (High pH resistant)	28.4	121.1	285.3	25.0
Clone TN 14/3 (High pH resistant)	26.8	124.6	280.0	15.0
Clone 6/8 (Low pH resistant)	16.0	62.0	197.5	16.6
Clone 31/11 (Low pH resistant)	28.0	125.0	216.3	29.3

It has been pointed out that soils with high pH contain high fluoride which can be released to plant [Omueti and Jones 1977]. As a result, the high pH resistant should be able to tolerate and accumulate high fluoride content. This is clearly indicated by the old leaves. The fluoride concentration in these samples especially the shoot is low probably because the plants were young (<5 years old) and as a result they had not accumulated a lot of fluoride. These samples were also picked during the dry season when most of the ions from the soil were not labile and hence not readily taken up by the plants. But the main observation which can be made is that, the fluoride concentration increases with the increase of the age of the leaves and also the age of the plant. The bottom leaves which are normally hard and coarse have higher fluoride concentration than the fourth leaves which in turn have accumulated higher fluoride concentration than the young leaves. The shoot and the branch contained almost the same amount of fluoride.

The bottom leaves were also boiled for 15 minutes, decanted and the process repeated for $1\frac{3}{4}$ hours to find out whether all the fluoride was released in the first 30 minutes. Fig. 5:2 below gives the result.

From the histogram, high fluoride content (210 µg) was extracted in the first 15 minutes which is almost equal to the amount extracted in 30 minutes from the previous data of clone 31/11. A total of 519.5 µg fluoride was extracted after $1\frac{3}{4}$ hours.

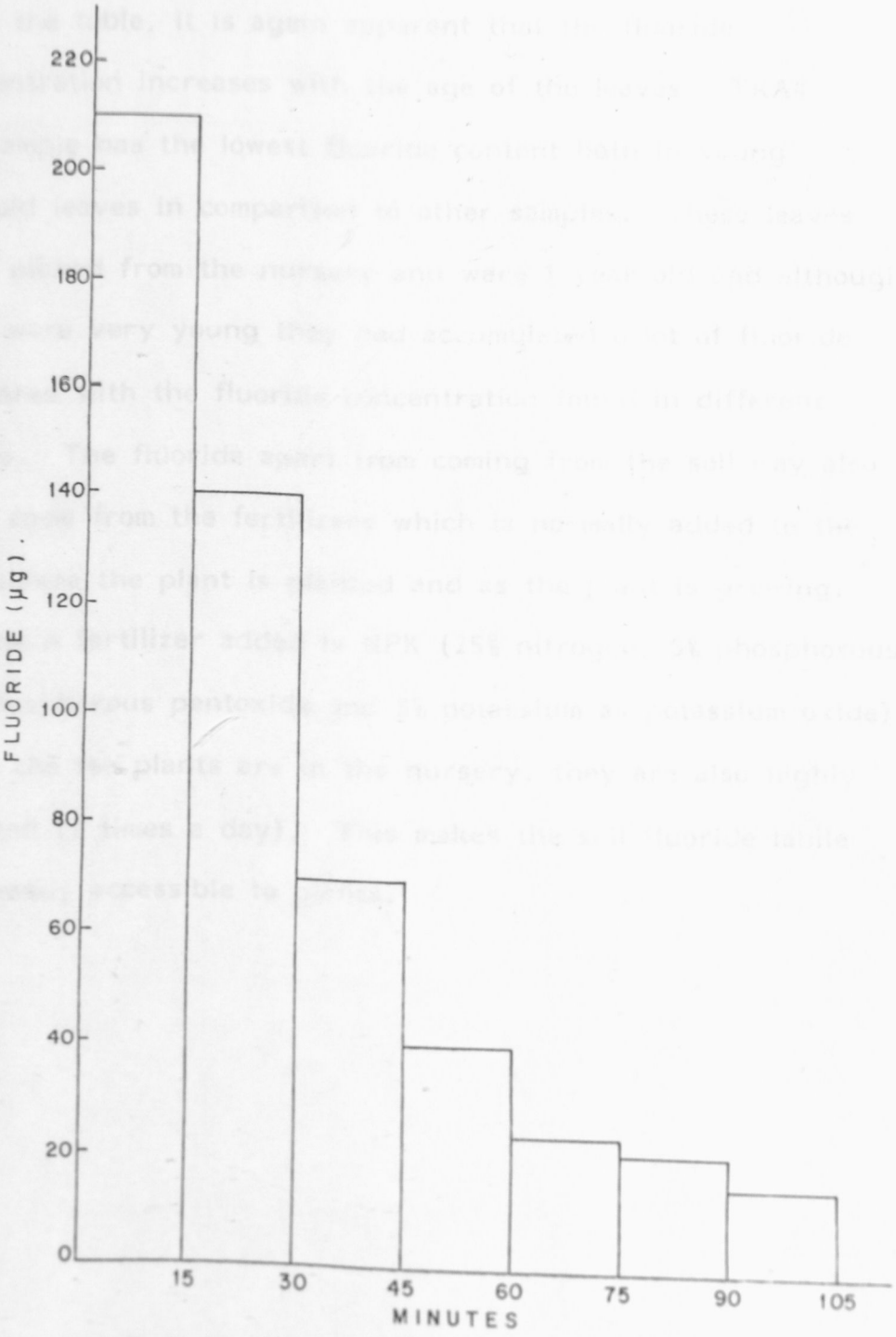

Fig. 5:2 Amount of labile fluoride (µg) extracted in $1\frac{3}{4}$ hours

Unprocessed tea samples from other areas were analysed for fluoride and data is presented in table 5:11. From the table, it is again apparent that the fluoride concentration increases with the age of the leaves. TKA4 tea sample has the lowest fluoride content both in young and old leaves in comparison to other samples. These leaves were picked from the nursery and were 1 year old and although they were very young they had accumulated a lot of fluoride compared with the fluoride concentration found in different clones. The fluoride apart from coming from the soil may also have come from the fertilizers which is normally added to the soil before the plant is planted and as the plant is growing. The main fertilizer added is NPK (25% nitrogen, 5% phosphorous as phosphorous pentoxide and 5% potassium as potassium oxide). When the tea plants are in the nursery, they are also highly watered (2 times a day). This makes the soil fluoride labile and easily accessible to plants.

TABLE 5:11 FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) OF TEA LEAVES FROM VARIOUS PLACES.

Locality *	2 leaves plus a bud	Bottom leaves
Murang'a		
TM_1	68.5	628.0
TM_2	70.0	665.3
TM_3	50.0	436.4
Nyeri		
TNY_1	42.5	483.4
TNY_2	60.0	559.8
Kiambu		
TKA_1	22.3	513.5
TKA_2	45.0	576.0
TKA_3	31.0	613.8
TKA_4	18.2	390.0

*Different tea samples (unprocessed) collected from different farms.

5:7 ALUMINIUM CONCENTRATION IN VEGETABLES.

Some vegetable samples were analysed for aluminium content and the data is presented in table 5:12 below. The aluminium concentration ranges from 180-1350 $\mu g\ g^{-1}$. As mentioned earlier, fluoride in plants is thought to exist as

aluminium hexafluoride (AlF_6^{3-}). The data shows that, high aluminium concentration is accompanied by high fluoride concentration except for one sample (Kale).

TABLE 5:12 ALUMINIUM AND FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) IN VEGETABLES.

Sample	Fluoride ($\mu g\ g^{-1}$)	Aluminium ($\mu g\ g^{-1}$)
Pumpkin leaves *Cucurbita pepo*	18.98	1180
Kale *Brassica integrifolia*	14.40	180
Terere *Amaranthus*	11.84	1350
Kahurura *Cucurbita sps*	7.16	680
Lettuce *Lactuca sativa*	9.05	1110
Cerely *Apium graveoleus*	6.30	364
Cabbage *Brassica oleracea*	5.04	394

The data shows that cerely and cabbage do not accumulate a lot of aluminium while pumpkin leaves, *amaranthus* and lettuce can be refered to as good accumulators of aluminium.

Two plants sampled namely, Eupherus laevigatus and piuchea were also analysed for aluminium and were found to contain 1095 and 1260 μg g^{-1} aluminium, respectively. Higher aluminium values of 8610 μg g^{-1} [Cannon 1960] in plants have been reported.

5:8 ALUMINIUM CONCENTRATION IN TEA.

Fluoride in tea is found in two forms:- organic and inorganic. The latter constitutes the highest amount of fluoride. The inorganic fluoride is thought to be in combination with either calcium, magnesium, aluminium, manganese, sulphur and iron or combined with some transition elements [Spiers 1983]. Although fluoride is thought to accumulate in plants in form of aluminium hexafluoride, aluminium in tea plays a very small role and analysis of aluminium in tea showed that, in comparison with vegetables, there is very low amount of the element in tea. The total aluminium content in tea is shown in table 5:13 and ranges from 640-940 μg g^{-1} aluminium. Values of aluminium in tea as high as 2050 μg g^{-1} have been reported [Vickery and Vickery 1976]. Analysis of tea infusion also showed that apart from fluoride, aluminium was also released. Some aluminium in tea has been reported to be organically bound [Spiers 1983] and not available for complexing with fluoride. Spiers (1983) also reported that, it was only small amount of fluoride which was complexed and has no relationship with the amount of aluminium present.

TABLE 5:13 ALUMINIUM CONCENTRATION ($\mu g\ g^{-1}$) IN TEA.

Sample*	Aluminium ($\mu g\ g^{-1}$)
TN_1 Pekoe furnings I (PFI)	660
TC_1 Dust	940
TC_3 Broken mixed furnings (BMF)	850
TK_1 Dust	640
TT_2 Furnings	736
TT_3 Broken mixed furnings (BMF)	704
4th and 5th leaves (unprocessed)	1153

* Tea from different factories.

When 4th and 5th leaves were analysed for aluminium, they were found to contain higher aluminium concentration (1153 $\mu g\ g^{-1}$) than in the processed leaves. Same trend was observed for fluoride concentration. This means that, the aluminium accumulation in tea also increases with the age of the leaves. Vickery and Vickery (1976) have also pointed out that high fluoride accumulation may be a secondary consequence for aluminium accumulation. There is no big variation of aluminium from one grade of tea to the other as the data shows.

Work done on interfering ions shows that, when 1M citrate buffer was used, it was possible to recover above 90% fluoride in presence of 50 mg l^{-1} aluminium in the measuring

solution. Vickery and Vickery (1976) reported that, with 0.25M sodium citrate buffer, it was possible to analyse 1.0g sample of commercial tea containing 2050 μg g^{-1} aluminium. In the samples analysed for aluminium, the maximum obtained was 1350 μg g^{-1}. This means that, this amount of aluminium will not interfere during analysis. Furthermore, it has also been shown that, when perchloric acid was used for diffusion, 93% fluoride was recovered in presence of 2000 μg aluminium. Since all the plant, vegetable and tea samples were ashed and diffused, the aluminium did not interfere with the fluoride analysis.

5:9 FLUORIDE CONCENTRATION IN THE SOIL:

Soil samples were collected from different parts of the country and analysed. Classification of the soils according to the soil texture showed that the soils can be classified into five categories as follows:

a. Clay soil

b. Clay loam soil

c. Sandy clay soil

d. Sandy loam soil

e. Loam soil

The pH of the soils was found to range from pH 5.0-8.9 as the data in appendix 2 shows. The soils were analysed for the labile fluoride using ammonium lactate media and the data in appendix 2 shows that the fluoride concentration ranges from 21.0-282.0 μg g^{-1}.

The fluoride concentration is also presented in fig. 5:3. Each histogram represents the labile fluoride in each soil texture. The fluoride concentration in clay soils ranges from 57.5-107.5 $\mu g\ g^{-1}$ with a mean fluoride concentration of 81.8 $\mu g\ g^{-1}$ while clay loam soils contain fluoride concentration ranging from 33.0 $\mu g\ g^{-1}$ - 282 $\mu g\ g^{-1}$. The mean fluoride concentration is 89.0 $\mu g\ g^{-1}$. Clay soils contain high aluminium content in form of aluminium hydroxide and aş a result, when fluoride ion comes into contact with clay soils, there may be ion exchange between fluoride and the hydroxide ion. Previous work done on defluoridation of water using clay pots showed that, clay pots removed the fluoride from the water. The pH of the water was found to increase and this proved that an exchange mechanism takes place when fluoride ions come into contact with hydroxide ions [Njenga 1982]. Clay soils have been reported to contain high fluoride content [Nömmic 1953, Omueti and Jones 1977].

Sandy soils were found to contain fluoride concentration ranging from 74.0 $\mu g\ g^{-1}$ - 86.0 $\mu g\ g^{-1}$, with an overall mean of 81.3 $\mu g\ g^{-1}$ fluoride while the sandy loam soils contained fluoride concentration ranging from 21.0 $\mu g\ g^{-1}$ - 71.5 $\mu g\ g^{-1}$ and a mean value of 42.2 $\mu g\ g^{-1}$ fluoride. Sandy soils normally contain very low clay and organic matter content hence, they are normally reported to contain lower fluoride content than clay or organic soils [Nömmic 1953, Murray 1983]. The data also shows that, the fluoride concentration of these sandy soils do not differ with the fluoride concentration found in clay soils.

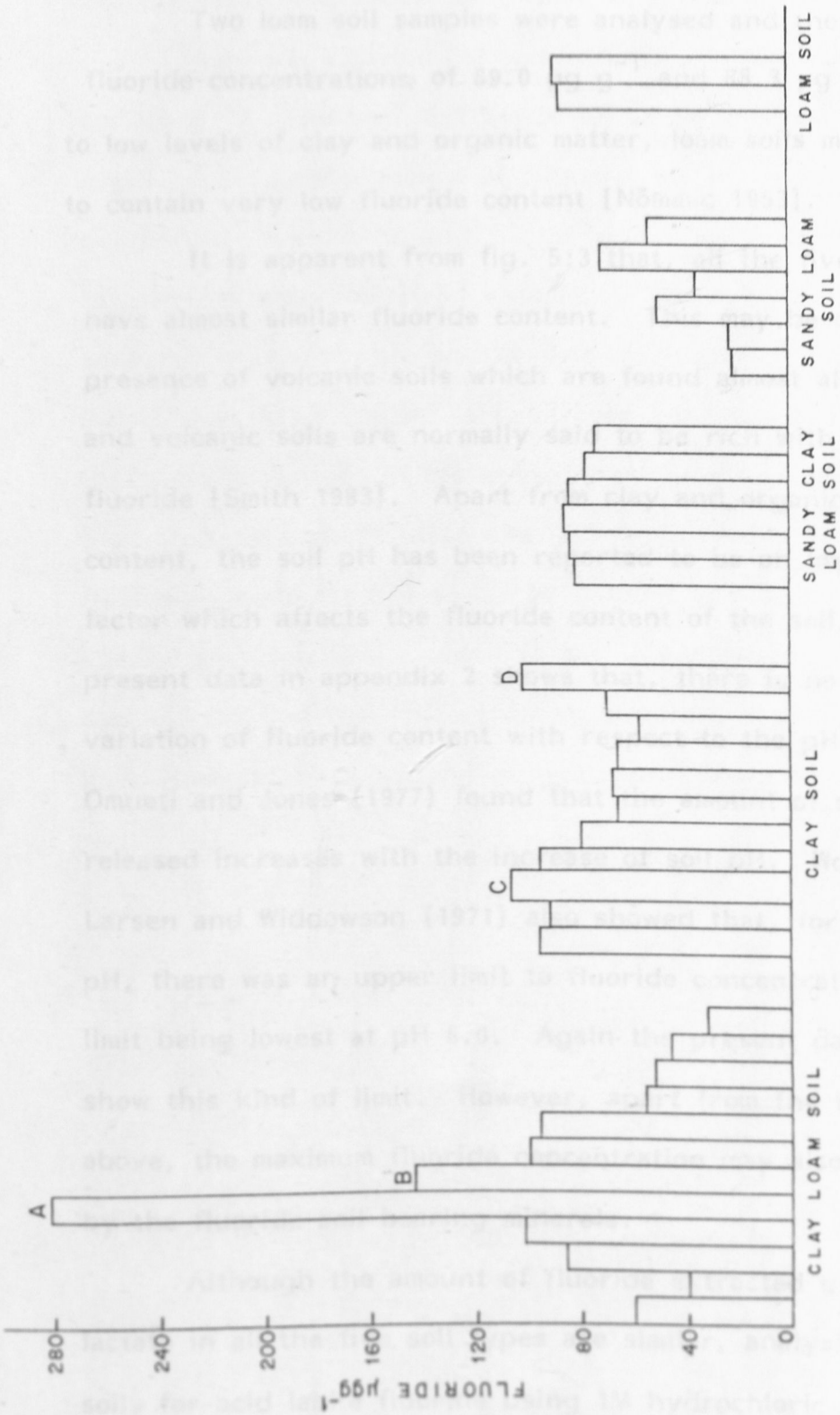

Fig. 5:3 Fluoride concentration in the soil.

Two loam soil samples were analysed and they had fluoride concentrations of 89.0 μg g^{-1} and 88.3 μg g^{-1}. Due to low levels of clay and organic matter, loam soils may be expected to contain very low fluoride content [Nömmic 1953].

It is apparent from fig. 5:3 that, all the five soil types have almost similar fluoride content. This may be due to the presence of volcanic soils which are found almost all over Kenya and volcanic soils are normally said to be rich with labile fluoride [Smith 1983]. Apart from clay and organic matter content, the soil pH has been reported to be an important factor which affects the fluoride content of the soil. The present data in appendix 2 shows that, there is no clear variation of fluoride content with respect to the pH of the soils. Omueti and Jones (1977) found that the amount of soil fluoride released increases with the increase of soil pH. Work done by Larsen and Widdowson (1971) also showed that, for any given pH, there was an upper limit to fluoride concentration with the limit being lowest at pH 6.0. Again the present data does not show this kind of limit. However, apart from the three factors above, the maximum fluoride concentration may also be determined by the fluoride-soil bearing minerals.

Although the amount of fluoride extracted using ammonium lactate in all the five soil types are similar, analysis of some soils for acid labile fluoride using 1M hydrochloric acid gave very different fluoride values. This indicates that, the fluoride-bearing soil minerals are different in these five soil types and as a result, the total fluoride is also not the same in all the samples.

The total fluoride should be higher than the labile fluoride.

Four soil samples had a fluoride concentration above 100 μg g^{-1}. The high extractable fluoride in these soils might be as a consequence of pollution by different types of pollutants. Samples A and B had high fluoride content due to pollution from industrial emission. The samples were collected near diatomite and cement industries, respectively. Fluoride emitting industries have been reported to increase the fluoride content in the environment [WHO 1970]. The high fluoride content in sample C may be coming from the fertilizers added to the soil. Work done by Oelschläger (1971) showed that fertilizers added to the soil increased the fluoride concentration of the soil. In sample D, the high fluoride may have come from the sewage effluent and sludge added to the soil.

CHAPTER 6

CONCLUSION

In this work, methods for fluoride analysis in plants foods and soils have been established. The results of Chapter 4 show that open flame ashing using a Meeker burner followed by diffusion can be used for fluoride analysis in foods and plants. The results also show that, diffusion method using perchloric acid instead of sulphuric acid was capable of removing interfering ions and can be carried out in presence of even 2000 µg aluminium and 5100 µg silicon whereby a recovery of above 93% fluoride is achieved. However, if high amounts of these interfering ions are indicated the analysis should be repeated with smaller weight of the sample. From the results of this work and the fact that, perchloric acid has lowest blank value than sulphuric acid, it is recommended for diffusion of the ashed samples.

The results of this work also show that open flame ashing using calcium hydroxide as a fixative gave higher fluoride values than ashing in the furnace. Open flame ashing is prefered because the ashing is more complete, the blanks are lower and interference from silicon is less, owing to the higher temperature. The direct diffusion method was found to give lower fluoride values than open flame ashing. However the results obtained with open flame ashing compared well with those obtained with Schöniger oxygen flask method. But while the oxygen flask method has the great advantage of simplicity and less danger of

losing volatile fluoride compounds, it is unsuitable for samples with low contents of fluoride because only a small amount (<50mg) of sample can be taken for analysis and because the method is only restricted to dry samples with sufficient homogeneity. Ashing over an open flame with the recommended fixative followed by diffusion, can be applied to wet samples. The recovery of the fluoride added to the sample and ashed with open flame followed by diffusion was good and above 90% fluoride was recovered. It was also found that, there was no fluoride lost during the addition of the acid to the ashed sample. Therefore from the results of this work, open flame ashing using calcium hydroxide as a fixative followed by diffusion was found to be a better method compared to ashing in the furnace and other methods. The result of this work also shows that, the method is cheap, accurate, reliable and reproducible. The plant and vegetable samples analysed were found to contain 180-1350 $\mu g\ g^{-1}$ aluminium and this amount of aluminium did not interfere with the diffusion method. Therefore open flame ashing followed by diffusion method can be adopted for the fluoride analysis in plant and vegetable samples and it can also be applied to low fluoride samples.

The use of 1M citrate buffer as a complexing agent was found to have an advantage over TISAB II buffer. 1M citrate buffer mixed with the sample (3:2 v/v) and left standing for at least 2 hours gave a fluoride recovery of above 90% in presence of 50 mg l^{-1} aluminium and 490 mg l^{-1} iron, while TISAB II buffer could tolerate only 2 mg l^{-1} aluminium. Therefore 1M

citrate buffer was found suitable for removing the interfering ions in the measuring solution and hence, it is recommended instead of TISAB II especially when dealing with samples containing high amount of interfering ions. The pH of the solution was found to have an effect on fluoride analysis. Below pH 4.5, there was a negative deviation while above pH 8.0 there was a positive deviation. Hence fluoride analysis could only be carried out at pH 4.5-8.0 although pH 5.7 is recommended since it gave 100% (±0.0) recovery.

In this work, it was also found that, ammonium lactate solution can be used for the extraction of labile fluoride. The amount of the sample, and shaking time were found to play an important role and therefore, 1.0 g sample in 100 cm^3 ammonium lactate gave the highest total fluoride in comparison with the higher weights. Also low amounts of interfering ions were released when 1.0 g sample was used. It was found that 1½ hour shaking time was enough for equilibrium to be established and hence recommended for the extraction of labile fluoride. Comparison of ammonium lactate method with water, sodium citrate-EDTA and 1M hydrochloric showed that, ammonium lactate gave higher fluoride values than either water or sodium citrate-EDTA methods but lower values than 1M hydrochloric acid. The results show that, apart from clay soils which gave a recovery of less than 90% fluoride, organic, loam silt and sandy loam soils gave a recovery of between 90-102% fluoride. This indicates that ammonium lactate method can be used to

estimate the amount of available fluoride from the soil to the plants.

Chapter 5 presents results on the application of the methods, the open flame ashing followed by diffusion was applied for the analysis of fluoride in vegetables, plants and food samples. The results show that, some vegetable samples can accumulate high fluoride depending on where they are grown. For example, solanum nigrum, amaranthus, spinach, cabbage and pumpkin leaves. The vegetables were also found to contain higher fluoride values than the reported normal values. Most of the foods contain low fluoride content except the tubers which were found to contain higher fluoride levels than the normal values. It was also found that, plants growing near the rift valley lakes contain high fluoride content.

In Chapter 5 it was found that, most of the fluoride content (82-98%) in tea is acid labile while about 40-90% is water labile and hence available for absorption. The fluoride content in tea was found to vary with the age of the leaves with the young leaves showing lower fluoride levels than the older leaves. Most of the beverages analysed contained less than 1.0 mg l^{-1} fluoride with the exception of four different beverages while the baby foods contained less than 0.3 µg g^{-1} fluoride.

A survey of the labile fluoride in soil from various parts of the country showed that most soils contained less than 100 µg g^{-1} fluoride except those soils which are contaminated. For example, soils collected near diatomite and cement industries. The results of this work showed that, the soil pH does not

play a major role on the fluoride accumulation.

It has been suggested that, the fluoride content in foods, beverages and water may be a contributor to fluorosis. While this may be true for beverages and water, foods may be contributing very little fluoride to the total fluoride intake per day. Not all the fluoride in food reaches the blood-stream because it is either of organic origin or complexed with the metal ions. Aluminium, magnesium, calcium and manganese when present inhibit the fluoride absorption and hence it is excreted. The vegetable samples were found to contain substantial amount of aluminium as the results show and this might reduce the fluoride absorption. However, it is not yet well established in which form fluoride occurs in plants and vegetables. And in order to establish the amount of fluoride consumed per day from foods, it is necessary to determine the occurrence of fluoride in plants.

The fluoride in tea could be a greater contributor to fluorosis since 82-98% fluoride was found to be acid labile and hence available for absorption. The result on tea shows that one cup of tea (125 cm^3) may contain between 176.5-212.5 µg fluoride. For a tea addict, who may take about four litres of tea per day, about 5648-6800 µg fluoride will be consumed and this may contribute significantly to the total fluoride taken per day. Most of the other beverages contribute very little fluoride per day. However it is necessary to carry out investigations on the effect of metal ions present in tea solution on the absorption

of fluoride in tea and whether the fluoride in tea can prevent dental caries. It is hoped that, with the method for fluoride analysis in food and plants available, it will be possible to determine the amount of fluoride taken per day from the foods.

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APPENDIX I: FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) IN VEGETABLES.

Locality	Terere (Amaranthus)	Cabbage (Brassica oleracea)	Pumpkin leaves (Cucurbita pepo)	Kahurura (Cucurbita sps)	Kunde (Vigna unguicu-lata)	Lettuce (Lactuca sativa)	Managu (Solanum nigrum)	Spinach (Spinacea oleracea)	Kale (Brassica integrifolia)
Kiambu	8.12 7.90 8.50	0.92 0.75 0.42	9.16	7.33 7.10 3.21	1.55 0.87 2.79	3.01 9.05	3.82	1.68 2.18 2.96 10.32	0.84 0.77 0.48 0.48 14.40 14.68
Thika	5.83 4.72	1.79 1.45 1.29	2.49 3.78				3.14 2.73		1.14 1.43
Rift Valley	16.25 0.89	5.50 7.80 5.04	2.38 1.14 18.98 10.59		1.74 1.53 0.84	6.39	7.32 29.98	5.36 1.68	5.15 5.69 4.88 1.30 1.63
Molo	15.19		11.19				1.44	0.95	4.33
Njoro			12.17						2.27 1.18

APPENDIX I CONTINUED:

Murang'a	8.75 10.50	1.83 1.62	5.53 6.14 5.23	4.31 3.11	4.74 4.56		3.25 4.50	4.01 3.95 5.12	3.88 2.24 1.92
Nyeri		0.56 1.07 1.37	2.48 3.51		2.30 3.10		2.45 3.26	0.42 2.96 7.80	0.48 3.06
Mombasa	6.83 6.57 6.83 5.92		4.33		5.20 10.24 5.33 7.67 6.18		5.89		4.29 3.54 2.73 5.30
Kirinyaga	0.42 0.89	0.50 0.52 0.71	1.21 1.45		1.30 11.54		0.31 0.52	0.86 0.76 0.40	0.48 0.90 0.52 3.90
Embu	8.94 9.45	6.24 7.82	2.94 6.34	4.56 3.42	7.96 8.47		6.24 2.94	2.52 1.85	1.89 1.82 4.91 5.01
Meru	12.45 11.68		3.72 6.85		4.75 3.95		9.67 8.98	8.62 7.48	5.73

APPENDIX I CONTINUED:

Nairobi	4.21 8.11 5.30 11.84	2.40 1.12 1.33	10.57 8.64	9.62 7.12 6.88	5.69 10.32			1.35 3.55 10.06 8.91 8.59 4.33	2.32 0.69 0.31
Nyahururu			6.75 7.32	7.16					
Kisii	9.41		3.82					9.57	4.43
Kericho									1.85
Kisumu		15.10							1.53

APPENDIX 2: FLUORIDE CONCENTRATION ($\mu g\ g^{-1}$) IN DIFFERENT SOILS COLLECTED FROM VARIOUS PARTS OF KENYA.

Soil Texture	pH	Fluoride ($\mu g\ g^{-1}$)	Mean
Clay soil	6.5	95.0	
	7.8	92.5	
	7.4	107.5	81.8
	7.5	95.0	
	6.5	79.0	
	7.3	66.5	
	5.6	69.0	
	6.9	57.5	
	7.0	66.5	
	7.4	70.0	
	5.0	101.5	
Clay loam	7.0	60.0	
	6.4	40.0	
	6.2	55.0	
	7.0	33.0	
	6.4	46.5	
	6.3	51.5	89.0
	6.4	91.7	
	5.6	92.7	
	7.0	86.0	
	8.0	282.0	
	6.5	86.0	
	7.9	143.0	
Sandy loam	7.6	21.0	
	7.8	21.5	
	8.4	50.0	42.2
	8.6	34.6	
	8.3	71.5	
	8.9	54.5	
Sandy clay loam	6.8	82.0	
	7.7	83.7	
	5.0	86.0	81.3
	5.9	78.0	
	5.8	74.0	
	5.9	84.0	
Loam	6.4	88.3	88.7
	6.3	89.0	