FLUORIDE IN WATER AND FISH FROM KENYAN RIFT VALLEY LAKES

JOSEPH KANGANGI GIKUNJU, B. V. M.,
University of Nairobi

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Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi.

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DECLARATION

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

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J. K. Gikunju

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

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PROF. P. LØKKEN, D. D. S, M. Sc., Ph. D.

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DR. T. E. MAITHO, B. V. M., M. Sc., Ph. D.

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" Wisdom is better than weapons of war, but one sinner destroys much good." Ecc. 9: 18.

This work is dedicated to my father and mother,

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Abstract

FLUORIDE IN WATER AND FISH FROM KENYAN RIFT VALLEY LAKES

Although fish is an important source of food in Kenya, limited information is available on fluoride in Kenyan fish. Fluoride is accumulated by aquatic organisms living in high fluoride environment. It was therefore found relevant to undertake a more extensive investigation of the fluoride levels in Kenyan lake water and fish, and also to study various factors related to the accumulation of fluoride in fish.

Water samples were collected from Lakes Nakuru, Bogoria, Baringo, Naivasha, Elementaita and Magadi and the pH as well as fluoride concentrations measured with a pH meter and a fluoride ion specific electrode respectively. Water from all the lakes were alkaline with pH ranging from 8.4 to 10.7. The fluoride levels of water from Lakes Naivasha, Magadi, Nakuru, Elementaita, Bogoria, and Baringo were 2.4, 84, 344, 463, 738, and 5.4 mg F/l respectively.

After addition of decomplexing agents, TISAB (Total ionic strength adjustment buffer - Orion) II or TISAB III, the pH of water samples from lakes Naivasha, and Baringo were within the recommended pH range for fluoride analysis (5.0-5.5), while water samples from lakes Magadi, Bogoria and Elementaita had pH values above 5.5. It was therefore found relevant to study how dilution and addition of decomplexing solutions, affect the fluoride measurements in water from Lake Magadi.

Dilution of the water did not alter the pH before addition of buffer hence interfering ions could be responsible for the problem encountered in fluoride analysis in the alkaline water. Hence the commonly used methods for fluoride analysis in water, might give erroneous or unreliable results when used in the analyses of water with high pH as found in some of the lakes of the Rift valley.

A total of 320 fish samples from Lakes Naivasha, Baringo and Magadi were analysed for fluoride concentration in fillet, skin, gills and bones using fluoride ion selective electrode methodology after extraction from tissues.

The recovery of fluoride at various spiking levels were as follows (mean ± SEM %): blanks 99.6 ± 2 %, fillet 87.4 ± 4 %, skin 101 ± 16 %, gills 105 ± 6 % and Bones 109 ± 3%. Fluoride concentration in fish tissues were as follows (mean ± SEM mg F/kg): Lake Magadi fish; fillet 68 ± 12, skin 809 ± 140, gills 1366 ± 40 and 1661 ± 49 for bones. Lake Naivasha fish:*Oreochromis leucostictus*, fillet 11 ± 1, skin 18.2 ± 2, gills 571 ± 36, and 608 ± 22 in bones; *Tilapia zilii*, fillet 10 ± 0.4, skin 18 ± 1, gills 435 ± 24 and 455 ± 27 for the bones; *Micropterus salmoides* (Black bass), fillet 7 ± 0.6, skin 83 ± 24, gills 251 ± 23 and 338 ± 24 in bones. Lake Baringo: *Tilapia nilotica*, fillet 7 ± 1, skin 10 ± 1.6, gills 241 ± 20.8 and 268 ± 36 in bone tissues. All the values are on dry weight basis. Concentration of fluoride in tissues varied according to fish species ($p < 0.05$) and perhaps the feeding habit and fish growth rate.

Lake Magadi Tilapia fish were surviving under a fluoride concentration of 84 mg F/l in the water. Therefore the Lake Magadi Tilapia seems to be adapted to living in high fluoride environment possibly through an excretion mechanism.

Lake Magadi fish may be unsuitable for human consumption due to the high fluoride content in the tissue which could amount to a health risk. Fish from Lakes Naivasha and Baringo provide an important source of protein. In preparation of the fish, no part is removed except the scales and perhaps the fins. The amount of fluoride released by bones, gills and skin during the process of cooking is an area which requires further research.

There is no current concensus on the level of fluoride acceptable in various foods and beverages consumed on a daily basis and therefore intensive studies are necessary with regard to fluoride levels in food and beverages as well as bioavailability of fluoride. Accordingly, the present findings indicate that there is a need to carry out fluoride bioavailability studies in food substances like soups which are prepared using fillet, skin, gills and bones which may contain high concentrations of fluoride.

Chapter 1

Introduction

Fluoride is usually classified with the trace elements, which is reasonable since fluorides are biologically active in very small amounts, and concentrations in biological samples are generally in the parts per million (ppm) range or less.

Fluorides are widely distributed in the environment, and rank 13th among the elements in order of abundance in the earth's crust. In several European and American countries the domestic water is fluoridated to approximately 1 mg F/l, since fluoride exerts a remarkable caries prophylactic effect at this concentration. In some parts of the world, high fluoride concentrations occur naturally, but high fluoride concentration also occur as industrial pollution in association with aluminium smelters and phosphate plants. Fluoride is accumulated by aquatic organisms living in high fluoride environments, particularly in osseous tissues (Sigler and Neuhold, 1972).

The geology of Kenya renders it one of the places in the world, where fluorides occur in the highest concentrations, not only in rocks and soils but also in surface and ground waters (Manji and Kapila, 1984). Accordingly, the amounts of fluoride to which parts of the Kenyan population are exposed, rank among the highest in the world (Williamson, 1953). High fluoride concentration have also been found in potential

raw materials of animal feed, mostly of marine origin (Trautner and Siebert 1985). This fluoride might be further concentrated in the food chain and pass into products for human consumption. This also applies to livestock and wildlife as well as fish in Kenyan lakes. The main route of exposure to fluoride is ingestion of water, beverages, foods and feeds with high fluoride levels.

Fluoride intoxication affects human beings, domestic animals as well as wildlife. However, the tolerance to ingested fluoride varies markedly among species (Shupe and Olson, 1971). The pathogenic mechanism underlying skeletal and dental fluorosis is not known in detail, but may be caused either by an effect on the formation of the organic matrix or on the process of mineral deposition (Ammitzbøll *et al.*, 1988).

It is well known from dietary studies that fish accumulate fluoride in the hard tissues (Ke *et al.*,1970; Zipkin *et al.*, 1970) and in Japan and some parts of South East Asia this has resulted in some human population having a high fluoride diet (Minogushi, 1970). In several countries fish and marine organisms contribute significantly to the total daily intake of fluoride (Trautner and Siebert , 1985). So far there seems to be only one published study which deals with fluoride in Kenyan fish (Bergh and Haug, 1971). It was therefore found relevant to undertake a more extensive investigation of the levels of fluoride in Kenyan lake fish, and further to study various factors related to the accumulation of fluoride in fish.

The main objectives of the study were:

*to evaluate the reliability of the commonly used methodology in determining fluoride concentration in very alkaline lake water with high salt concentration.

*to investigate the fluoride levels in water from the Kenyan lakes.

*to set up an appropriate method for quantitative determination of fluoride in fish tissues (fillet, skin, gills, and bone) and obtain a quality assurance for the performances of the investigator and the method.

*to collect various species of fish from Kenyan lakes with different fluoride concentration in the water and investigate:

- the relationship between fluoride concentration in various tissues.
- the relationship between fluoride levels in water and fluoride levels in the fish.
- the differences between various fish species in their tendencies to accumulate fluoride.
- the accumulation of fluoride as related to age or weight of the fish.

*to provide basic data for further research on bioavailability of fluoride in foods beverages and feeds.

*to evaluate if fluoride in fish or fish products from Kenyan lakes may represent a health hazard.

Chapter 2

Literature review

2.1.0 Fluoride in the environment

Fluorine is the most electronegative of all elements, being so violently reactive chemically that it is rarely or never encountered in nature as elemental fluorine. Combined chemically in the form of fluorides, fluorine is thirteenth in the order of abundance of the elements in the earth's crust (Bell *et al.*, 1970). Fluoride occurs universally and may be either beneficial or harmful, depending on the dose. Fluoride is present in varying amounts in soil, air, water and in plant and animal tissues (Fig. 1).

2.1.1 Rocks and soils

At an estimated 0.065 % of the earth crust, fluorine is roughly as plentiful as carbon, nitrogen, or chlorine, and much more plentiful than copper or lead, though much less abundant than iron, aluminium or magnesium. Compounds whose molecules contain atoms of fluorine are widely distributed in nature. Many minerals contain small amounts of the element, and it is found in both sedimentary rocks and igneous rocks. Deposits of fluorspar (calcium fluoride), the chief ore, are found on the surface in many parts of the world. Cryolite, Na_3AlF_6, which is used both in the ceramic industries and the metallurgy of aluminium, is found in quantities in only

a few places. Fluoroapatite, $Ca_5F(PO4)_3$, frequently called rock phosphate, is the most plentiful mineral.

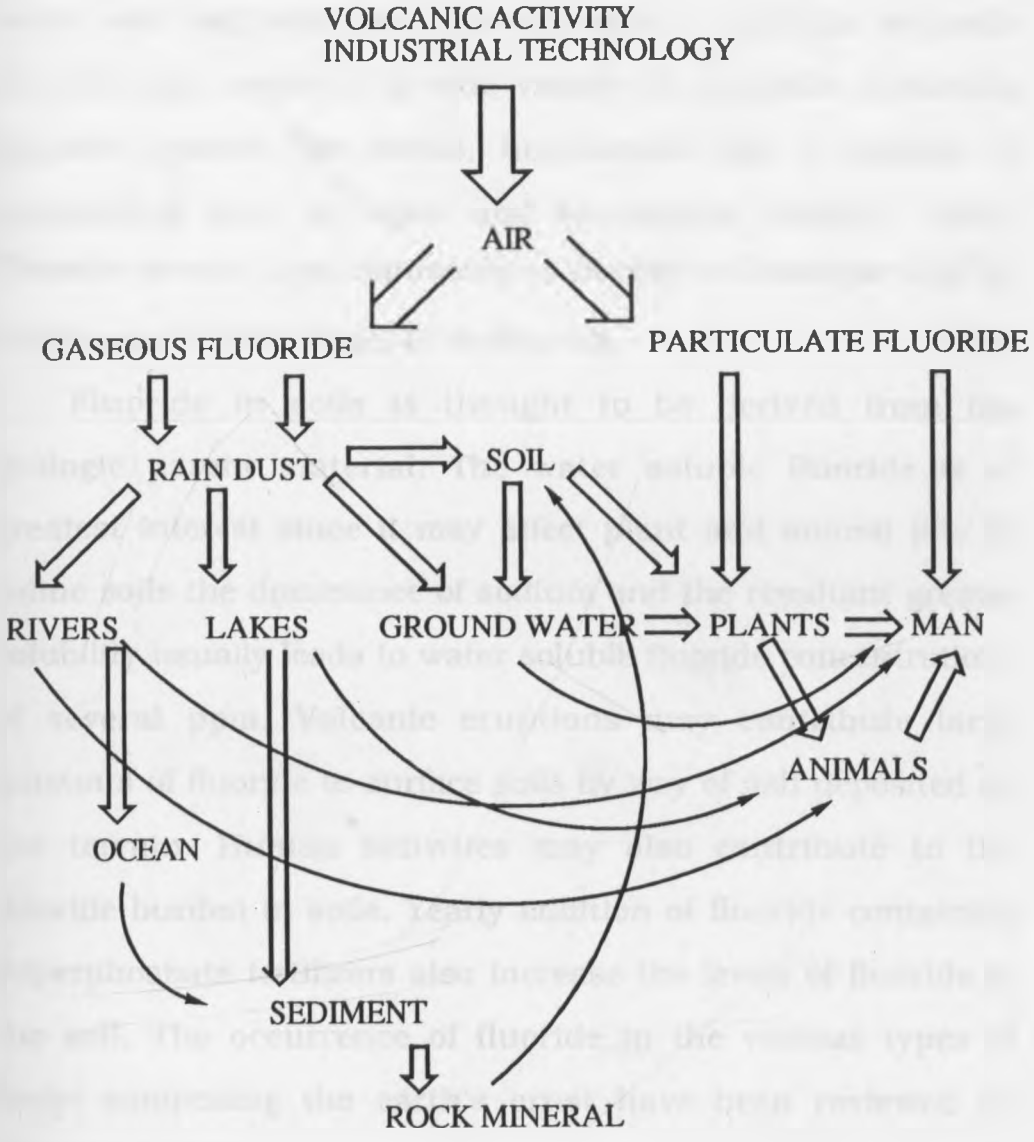


Fig.1. Dispersion of fluoride in the environment. (modified from Smith and Ekstrand, 1988)

The geology of East Africa is characterised by a landscape formed by vaulting and volcanic activity which resulted in volcanic rocks known to contain large quantities of fluoride in

a number of minerals such as fluorite, cryolite, apatite, topaz, and tourmaline. This is the ultimate source of fluoride in water, soil, vegetation and the atmosphere. In rocks and soils fluoride may occur in a wide variety of minerals, including fluorite, apatite, the micas, hornblende and a number of pegmatites such as topaz and tourmaline (Møller, 1982). Fluoride occurs most commonly as fluorite or fluorspar (CaF_2), which may contain upto 49 % fluoride.

Fluoride in soils is thought to be derived from the geologic parent material. The water soluble fluoride is of greatest interest since it may affect plant and animal life. In saline soils the dominance of sodium and the resultant greater solubility usually leads to water soluble fluoride concentrations of several ppm. Volcanic eruptions may contribute large amounts of fluoride to surface soils by way of ash deposited on the terrain. Human activities may also contribute to the fluoride burden in soils. Yearly addition of fluoride containing superphoshate fertilizers also increase the levels of fluoride in the soil. The occurrence of fluoride in the various types of rocks composing the earth's crust have been reviewed by Correns (1956).

2.1.2 Water

Fluoride enters the water cycle by leaching from soils and minerals into ground water and surface water. Fluoride concentration in water is affected by factors such as availability and solubility of fluoride containing minerals, porosity of the rocks or soil through which the water passes, temperature,

pH and the presence of other elements which may complex with fluoride (Fleisher, 1963). In river water most of the fluoride exists as free fluoride ions, however, salinity increases the complexed fraction. Sea water contains significant quantities of fluoride, levels having been variously recorded as 0.8-1.4 ppm.

The first attempt to study the distribution of fluoride in the ground water in Kenya was probably that by Williamson (1953), however no definite conclusion could be made regarding distribution of fluoride in the whole country. The reported fluoride concentrations of lakes ranged from 0.5-2800 ppm and those of rivers and springs from 0.45-49 ppm. Williamson also observed that many of the waters have high pH values, sometimes as high as pH 8 to pH 10. It would seem, however, that the other chemical characteristics of the waters - and possibly climatic conditions - play an important role in determining the very high fluoride levels in some Kenyan waters. Muriithi (1971) studied the distribution of fluoride in the Nairobi conservation area. Studies carried out by international organisations on water resources in Kenya all mentioned the fluoride problem. Among these were "Ground Water Resources in Kenya, WHO, 1973" and "Ground water survey, SWECO 1975". All researchers indicated that ground water in Kenya has very high fluoride concentration in general.

Table 1. Fluoride levels in natural waters in different countries. Data from WHO (1970).

Continent	Country	Range of fluoride levels (ppm)
Africa	Ethiopia	0 - 0.9
	Kenya	**0 - 2800**
	Nigeria	0 - 6.2
	South Africa	0 - 53.0
	Tanzania	0 - 95.0
Americas	Argentina	0 - 1.6
	Brazil	0 - 0.6
	Canada	0 - 1.2
	Chile	0 - 1.5
	Cuba	0 - 0.4
	Ecuador	0 - 1.5
	Peru	0 - 1.4
	USA	0 - 16
Asia	China	0 - 13.0
	India	0 - 6.4
	Iran	0 - 1.0
	Israel	0.3 - 1.5
	Japan	0 - 20.0
	Korea	0.8 - 10.0
	Taiwan	0 -1.5
	Thailand	0 - 1.5
Australasia	Australia	0 -13.5
	New Zealand	0 - 0.9
	Papua and New Guinea	0 -0.6
Europe	Austria	0.4 - 0.8
	Belgium	0 - 1.7
	Cyprus	0 - 3.6
	Czechoslovakia	0 - 28.0
	Denmark	0 - 3.3
	England	0 - 5.8
	Finland	0 - 5.0
	France	0 - 7.0
	Germany	0 - 4.9
	Ireland	0 - 0.2
	Italy and Sicily	0 - 7.1
	Luxembourg	0 - 1.2

Table 1. continued

Netherlands	0 - 2.0
Norway	0 - 2.7
Poland	0 - 1.1
Portugal	0 - 22.8
Sardinia	0 - 5.0
Spain	0 - 6.3
Sweden	0 - 10.0
Switzerland	0 - 1.4
USSR	0 - 7.0
Yugoslavia	0 - 4.2

The reported fluoride levels in Kenyan water is higher than that reported from any other country in the world (Table 1). The highest concentrations of fluoride in Kenyan water occurs in water from some springs, bore holes and in some of the lakes in Rift Valley (Manji and Kapila, 1984).

Table 2. Fluoride content of some lakes of Kenya. Data from Gitonga and Nair (1982).

Lake	Fluoride (ppm)
Victoria	0.6
Naivasha	8.0-15
Baringo	15-18
Turkana	100
Bogoria	1000-2800
Nakuru	1800-2800

Table 3. Fluoride concentration in some lakes of Kenya. Data from Kariuki *et al.* (1984)

Lake	Fluoride (ppm)
Naivasha	1.3-1.5
Victoria	0.7
Elementaita	480-1000
Nakuru	140-145
Bogoria	1200
Baringo	4.8-5.4
Magadi	1480
Turkana	13

Table 4. Fluoride levels in boreholes in Kenya, Data from Gitonga and Nair (1982)

Range of F- conc. ppm	No. of boreholes in range	% of total no. of boreholes
0.0-0.6	332	26
0.7-1.7	359	28
1.8-3.0	217	17
3.1-10.0	285	22
≥ 10.0	98	8

If the upper limit of 1.7 ppm of fluoride level in drinking water is to be complied with, it then becomes apparent that about half of our boreholes produce water whose fluoride levels are too high. It can be observed from the summary that 46.5 % of the boreholes have more than 1.7 ppm fluoride. The upper limit of fluoride concentration in drinking water depends on the temperature of a place which plays an important part in determining the amount of water intake.

The 1.7 ppm mentioned above is for those areas where the annual average of maximum daily temperature is 10-12^{o}C. Although the climate of Kenya varies widely from place to place, the average maximum temperatures are generally above 24^{o}C. This would therefore imply that the upper limit of fluoride concentration in drinking water for most parts of the country should be 1.0 ppm. With this upper limit, it is observed that 62.5 % of all the boreholes examined had concentration higher than the optimum (Gitonga and Nair, 1982). The distribution of fluoride in Kenya is a complex situation because even those areas which could be regarded as high fluoride areas, boreholes with negligible fluoride concentration could be found, at times only a short distance from another with very high content. However no definite conclusion could be made regarding distribution of fluoride in the whole country.

Natural compounds containing fluorides are only sparingly soluble, therefore surface waters do not usually have high fluoride levels.

The recommended levels of fluoride in water are 0.7 and 1 ppm for a hot and temperate climate respectively (McCLure, 1970). However dental fluorosis has been reported in communities supplied with fluorinated and non fluorinated water in U. S. A.. Similarly, Dental fluorosis has been reported in Kenya in areas of low fluoride in water (Manji *et al.*, 1986). This implies that sources of fluoride other than drinking water might be of importance in contributing to the total daily

fluoride intake particularly in such areas with low fluoride in water.

Chemistry of fluoride in water

When a fluoride compound is dissolved in water, the element fluorine will be present mainly as fluoride ion, F-. However, depending on the ionic concentration and on the pH of the solution, the fluoride will also be present in solution as HF^-_2 and undissociated HF. In dilute solutions and at neutral pH, virtually all the fluoride will be present as fluoride ion, F-. However, as the pH of the solution decreases, the proportion of F^- present decreases while the proportion of HF^-_2 and undissociated HF increases (Borei, 1945).

Defluoridation

Defluoridation of water is a relatively poorly developed treatment process when compared to the other water treatment processes like filtration, coagulation, flocculation, sedimentation and disinfection. This is probably due to the absence of the excessively high fluoride levels in waters in most of the developed countries. Worse still the adverse effects associated with ingestion of water containing high fluoride levels are appreciated only gradually and their nature is such that Public Health authorities tend to give them little considerations when deciding their priority for Public Health requirements. Consequently very little has been done on defluoridatioin of public water supplies with an effective economical reliable and universally accepted method (Gitonga and Nair, 1982).

2.1.3 Atmosphere

Fluoride enters the atmosphere by volcanic action and by entrainment of soil and water due to the action of wind on the surfaces. It is returned to the earths surface by deposition as dust or in rain. Additional fluorides are widely distributed in the atmosphere originating from the dusts of fluoride-containing soils (Williamson, 1953), from gaseous industrial wastes, from the burning of coal fires in populated areas (Cholak, 1959), and from the gases emitted in areas of volcanic activity. All these sources tend to increase the fluoride level of rain or precipitation. Hazards to crops, animals and human health caused by atmospheric fluorides are well documented (Largent, 1961; Hodge and Smith, 1965; Thomas and Alther, 1966; Agricultural research council, 1967; Vostal, 1971). Amongst the chief sources of atmospheric fluoride are steel and aluminium smelters; elemental phosphorus, phosphate fertilizers and wet-process phosphoric acid plants ; bricks and ceramic works.

2.1.4 Vegetation

The widespread prevalence of fluoride in soil, water and rocks result in the presence of fluoride in many plant tissues. However, it is generally accepted that the fluoride content of most plants, with the exception of the roots, is not readily affected by the amount of fluoride in the soil in which they grow. Exception to this general rule are the tea plant and the Camelia; figures for the former have been reported upto 150 ppm and for the later, upto 2000 ppm (Allcroft, 1965). Plants

generally have limited ability to accumulate fluoride from soils, although acidic soils can enhance uptake (Underwood, 1977). Grain is generally lower in fluoride concentration, about 1 to 3 ppm (Underwood, 1977). Fluorine from the atmosphere combines with water and particles in air eventually settling on vegetation consumed by livestock. Forages and silage are most at risk of being contaminated by fluoride from aluminium smelters, steel mills, or fertilizer plants.

Organic forms of fluoride (fluoroacetate and fluorocitrate) may be formed in some forage and grain crops grown in areas contaminated with atmospheric fluoride.

2.2.0 Fluoride metabolism in humans and animals

Fluoride occurs universally and may be either beneficial or harmful, depending on the dose. The principal sources of fluoride supply to man and animals are: (1) water; (2) some species of vegetation; (3) certain edible marine organisms; (4) dusts in certain parts of the world; and (5) certain industrial processes (Bell *et al.*, 1970). There is no current concensus on the level of fluoride acceptable in various foods and beverages consumed on a daily basis (Rao, 1984).

The absorption, soft tissue distribution, calcified tissue uptake and renal excretion of fluoride are, in principle, all simultaneous events. A pharmacokinetic analysis of the plasma fluoride concentration curve defines various metabolic processes and kinetics of fluoride. Three phases are readily distinguished: absorption, distribution (alpha phase) and elimination (beta-phase), which can be described

quantitatively by using pharmacokinetic models. Fluoride is rapidly distributed to well perfused tissues such as the heart, kidney and liver. It is more slowly distributed to poorly perfused tissues like skeletal muscles and adipose tissues. In summary, the pharmacokinetics of fluoride can be described by a first order process, where the rate of elimination is directly proportional to the plasma fluoride concentration (Ekstrand and Whitford 1988).

2.2.1 Absorption

Fluorides are absorbed from the gastrointestinal tract, the lungs, and the skin. Absorption of fluoride from the gut appears to be governed by an interplay of anatomical, physiological and biochemical factors. The greater gastrointestinal absorption of fluoride in the rabbit than in the rat is attributed to the longer gastrointestinal tract of the rabbit. The gastrointestinal tract is the major site of absorption. In ruminants fluoride absorption is mainly confined to the rumen (Perkinson *et al.*,1955).The degree of absorption of a fluoride compound is correlated with its solubility. The relatively soluble compounds, such as sodium fluoride, are almost completely absorbed, whereas relatively insoluble compounds, such as cryolite (Na_3AlF_6) and the fluoride found in bone meal (fluoroapatite), are poorly absorbed. Certain cations (e.g. aluminium, calcium and iron) retard the absorption of the fluoride ion by forming low-

solubility complexes in the gastrointestinal tract and faecal excretion increases(Hodge and Smith, 1965).

The second most important route of absorption is through the lungs. Pulmonary inhalation of fluoride present in dusts and gases constitute the major route of industrial exposure. A third, and relatively rare, route of exposure, is through the skin. Fluoride from various sources may be absorbed at different rates as indicated on Table 5.

Table 5. Fluoride absorption from food and water by five young men. Data from McClure *et al.* (1945)

Fluoride added	Daily intake F^-(mg)	F^- absorbed %
Natural fluoride in water	3.77	89.7
Sodium fluoride in water	3.69	86.5
Sodium fluoride in food	4.76	83.3
Calcium fluoride in water	4.24	82.5
Calcium fluoride in food	5.34	68.9
Cryolite in food	5.34	68.2
Bone meal	4.18	53.6

Soluble fluoride compounds added to normal human diets are as readily absorbed as when then they are added to water, whereas the absorption of less soluble fluorides included in food may be reduced by 20 %.

Table 6. Percentage availability of F from various substances (Relative values: F from NaF 100 %) Data from Trautner and Siebert (1985).

Substance	Mean % availability of fluoride
Bone meal tablets	4
Fish bone meal	12
Seaweed flour	22
Canned sardines	23
Chicken bone meal	24
Tea and sugar	76
Krill	79
NaF and milk	80
Mineral water	85
Tea	89
NaF and sugar	100

The poor availability of fluoride from bone meal and sardines seems to be due to their high calcium content. Only part of the fluoride chemically bound to calcium is set free by digestive processes. Jowsey and Riggs (1978) reported a less increase in plasma fluoride values after concurrent administration of calcium carbonate and sodium fluoride than after sodium fluoride alone. Patz, Henschler and Fickenscher (1977), as well as Afseth, Ekstrand and Hagelid (1985), found no increase in plasma fluoride level following ingestion of calcium fluoride tablets despite their high solubility *in vivo* 0.1 M HCL. In contrast, Spencer *et al.* (1981), observed no change in fluoride absorption in man when several doses of calcium gluconate were given (total amount 200 - 2000 mg calcium daily). However, the intake of fluoride was separate

from that of calcium gluconate. The influence of concurrent administration of fluoride and calcium in the absorption of fluoride was shown by Quaassdorff (1985), who fed rats a normal diet with 1.1 % calcium and 30 - 80 mg F/kg diet and observed fluoride absorption of only 36 %. This absorption rate increased to 85 - 94 % when fluoride was given separately from the calcium containing diet, by use of a feeding machine.

The delay in fluoride uptake from milk cannot be fully explained by the formation of calcium fluoride with milk calcium (Ekstrand and Ehrnebo, 1979). There are several mechanisms that may influence fluoride release and absorption from food. Greater food intake leads to increased acid production in the stomach which might increase fluoride absorption (Whitford and Pasley, 1984). The quantity of food influences the speed of emptying of the stomach and thus the period of fluoride absorption in the upper part of the gastrointestinal tract. Concurrent intake of food influences affects fluoride absorption although the mechanism of this is not fully understood (Trautner and Siebert, 1986).

2.2.2 Distribution

Fluoride has been detected in all organs and tissues examined; however there is no evidence that it is concentrated in any tissues except hard tissues, and to a lesser extent in the thyroid, aorta and kidney. Fluoride is deposited in the skeleton and teeth, and the degree of skeletal storage is related to intake and age. This is thought to be a function of the turnover rate of skeletal components, with growing bone showing a greater deposition than bone in mature animals. Prolonged periods of time are required for mobilization of fluoride from bone. Fluoride is accumulated by the aorta, and concentrations increase with age, probably reflecting the calcification that occurs in this artery. Approximately 99 % of all the fluoride in the body is found in calcified tissues (Ekstrand and Whitfold, 1988). The selective affinity of fluoride for mineralised tissue is due to uptake on the surface of bone by the process of iso-ionic and heteroionic exchange and finally it is incorporated into the bone crystal lattice structure as fluoroapatite or fluorohydroxyapatite. Fluoride is not irreversibly bound to bone. In the foetus fluoride is readily taken up by the calcifying foetal bones and teeth. Normal plasma levels range from 0.037 to 0.126 ppm. Plasma fluoride concentration are not homeostatically regulated (Whitford and Williams, 1988), but instead they rise and fall according to the pattern of fluoride intake, and other factors such as the rate of bone resolution and the fluoride renal

clearance. There is a direct relationship between the fluoride concentration in bone and plasma.

2.2.3 Excretion

The major route of fluoride excretion is by the kidneys; however fluoride, is also excreted in small amounts by the sweat glands, the lactating breasts, and the gastrointestinal tract. Under conditions of excessive sweating, the fraction of total fluoride excretion contributed by sweating can reach nearly one half (Crosby and Shepherd, 1957). About 90 % of the fluoride filtered by the glomerulus is reabsorbed by the renal tubules. The percentage of the filtered fluoride that is reabsorbed from the renal tubules can range from about 10 % to 90 % (Ekstrand and Whitford, 1988). Whether tubular secretion of fluoride occurs is unknown. Ionic fluoride is not plasma protein bound, hence most of the fluoride is eliminated through the kidneys. Urinary fluoride excretion reflects the ingestion of fluoride (Toth and Sugar,1976; Vandeputte *et al.*, 1977). Excess of fluoride leads to increased urinary fluoride excretion. Renal damage may cause excessive high fluoride levels in plasma and bone. A wide variety of factors can influence urinary pH including the composition of the diet, certain drugs, respiratory and metabolic diseases. Any of these factors significantly influences the overall metabolism of fluoride through modification of pH. A vegetarian diet causes a less positive fluoride balance compared with a meat diet due to the alkaline pH which promotes fluoride excretion. Approximately one half of the

2.3.0 Fluoride toxicity

A clear distinction must be made between acute toxic effects, which result from a single massive dose, and the chronic toxic effect of large doses spread over a number of years. Chronic fluoride poisoning (fluorosis) is more common than acute fluoride poisoning. A number of adverse effects have been ascribed to fluorides. Although many reports of these claims are unsubstantiated, several have been studied sufficiently to deserve attention, including potential effects on kidney, thyroid, neurological functions and growth in general. Table 7 summarizes some of the proven or postulated effects at various concentrations.

Table 7. Biological effects of fluoride at various concentrations.

Data from Bhussry *et al.*, (1970)

Concentration or dose of fluoride	Medium	Effect
2 ppb	Air	Injury to vegetation
1 ppm	Water	Dental caries reduction
2 ppm or more	Water	Mottled enamel
5 ppm	urine	No osteosclerosis
8 ppm	Water	10 % osteosclerosis
20 -80 mg/day or more	Water or air	Crippling fluorosis
50 ppm	Food or water	Thyroid changes
100 ppm	Food or water	Growth retardation
More than 125 ppm	Food or water	Kidney changes
2.5-5.0 g	Acute dose	Death

2.3.1 Acute toxicity

Acute fluoride poisoning as distinct from the chronic fluorosis may result from the ingestion of large quantities of soluble forms of fluoride compounds. Acute fluoride poisoning usually results from the accidental ingestion of insecticides or rodenticides containing fluoride salts. Initial symptoms are secondary to the local action of fluoride on the mucosa of the gastrointestinal tract. Salivation, nausea, abdominal pain, vomiting and diarrhoea are frequent. The patient shows signs of increased irritability of the nervous system including hyperactive reflexes, tonic and clonic convulsions. These signs are related to the calcium binding effects of fluoride.The signs may be delayed for several hours. The blood pressure falls, presumably due to central vasomotor depression as well as direct toxic action on cardiac muscle. The respiratory center is first stimulated and later depressed. The lethal dose of sodium fluoride in man is about 5 g; however, recovery has been reported in patients ingesting much larger doses, whereas a dose as low as 2 g has been fatal. A dose as low as 5 mg F/kg may be fatal for some children (Whitford and Ekstrand,1988).

The acute effects of inhaling fluorine was observed chiefly in experimental animals and were described by Stokinger 1949, Table 8. When fluorine contacts directly the skin of animals (or man) it reacts so violently that it produces a thermal type of burn. Inhalation of fluorine at 300 ppm was fatal to all animals exposed for 3 hours or longer.

Table 8. Results of inhalation of gaseous fluorine (Stokinger 1949)

Concentration of F2	Duration		Effects on animals [a]
	days	hours	
5 ppm or more	18-29	95-160	Large numbers died (180 of 357)
2 ppm or less	31	176-178	Very few died (10 of 148)

[a] Rabbits,dogs, mice, rats, guinea-pigs, hamsters.

Table 9. Results of inhalation of gaseous hydrogen fluoride (Stokinger 1949)

Concentration of HF	Duration		Effects on animals
	days	hours	
33	26	166	All (47 of 47)rats and mice died; no(0 of 44) guinea-pigs, rabbits or dogs died
8.6	26	166	None (o of 60) of the same five species died

In domestic animals acute poisoning is commonly seen in pigs and is nearly always due to accidental ingestion of too much sodium fluoride which is used as an acaricide in swine. Poisoning following the use of insecticide powders is uncommon, but has been described in the dog (Holmes, 1946). The dusting powder responsible contained 40 % sodium fluoride. The main signs were diarrhoea and fall in milk yield. Volcanic eruptions can cause acute fluorine

poisoning in sheep, as the ash may contain upto 2000 ppm of fluoride.

The frequency of the symptoms reported in connection with 34 fatal cases of acute fluoride poisoning were described by Roholm (1937), Table 10.

Table 10. Frequency with which symptoms were observed in 34 fatal cases of acute fluoride poisoning (Roholm 1937)

Symptoms	No. of cases
Vomiting	31
Pain in the abdomen	17
Diarrhoea	13
Convulsions, spasms	11
General weakness and muscular weakness and collapse	8
Pain or paraesthesia in extremities	7
Paresis, paralysis	5
Difficulty in speech and articulation	5
Thirst	5
Perspiration	5
Weak pulse	5
Change in facial colour	5
Nausea	4
Unconsciousness	4
Salivation	3
Impaired swallowing	3
Motorial restlessness	2
High temperature	2

2.3.2 Chronic toxicity

Chronic fluoride toxicosis has been of interest in Humans domestic animals and in some wild animals. Chronic fluorosis usually develops gradually on continued ingestion of excess fluoride levels in food and water. Chronic toxicity is characterised by dental fluorosis, skeletal fluorosis as well as elevated levels of fluoride in bone urine and blood. Factors influencing the expression or severity of chronic fluoride intoxication include amount of fluoride ingested, species, age at the time of ingestion, nutritional status (malnutrition intensifies fluorosis), duration of ingestion, solubility of ingested fluoride, health status, stress factors and individual biological responses.

2.3.3 Mechanism of fluoride-induced pathological lesions in hard tissues

The primary effect of fluorine is thought to be a delaying and alteration of normal mineralisation of the preenamel, predentine, precementum, and preskeletal matrices. Excessive fluoride intake produces dental fluorosis by affecting the teeth during development. Specific ameloblastic and odontoblastic damage seems to be caused by high fluoride intake and varies directly with the levels consumed. Faulty mineralisation results when the matrix laid down by damaged ameloblasts and odontoblasts fails to accept minerals normally. Once a tooth is fully formed, the ameloblasts have lost their constructive ability and the enamel lesions can not be repaired. Odontoblasts can produce secondary dentine to

compensate for fluorotic deficiencies. The dental lesions of chronic fluorosis are accentuated by the rapid wear of the affected cheek teeth, especially if course feeds are fed. Oxidation of organic material in the teeth involved result in brown or black discolouration, which is observed in dental fluorosis.

There are two schools of thought concerning the pathogenesis of fluorotic bone lesions. One theory relates high fluoride levels to osteoblastic activity which leads to inadequate matrix and defective, irregular mineralisation. Others believe that bone lesions are related to the replacement by fluoride ion of hydroxyl radicals in the hydroxyapatite crystal structure of bone substance. This results in a decrease in crystal lattice dimensions. The pathologic results of skeletal fluorosis include dissociation of normal sequences of osteogenesis, acceleration of bone remodeling, production of abnormal bone (exostosis, sclerosis), and in some cases accelerated resorption (osteoporosis) (Osweiler *et al.*,1985).

Chronic toxicity in humans

In man, the main manifestations of chronic ingestion of excessive amounts of fluoride are osteosclerosis and mottled enamel. Chronic exposure to excess fluoride causes increased osteoblastic activity. Osteosclerosis is a phenomenon wherein the density and calcification of bone are increased. In chronic fluorosis, it is thought to represent the replacement of hydroxyapatite by the denser fluoroapatite. However, the

details of the mechanism of its development remains unknown (Ammitzbøll *et al.*, 1988). The degree of skeletal involvement varies from changes that are barely detectable radiologically to marked thickening of the cortex of long bones, numerous exostoses scattered throughout the skeleton, and calcification of ligaments, tendons, and muscle attachment to bone. In its severest form it is a disabling disease and is designated crippling fluorosis. Mottled enamel or dental fluorosis is a well recognised entity. The gross changes in very mild mottling consist in small, opaque, paper-white areas scattered irregularly over the tooth surface. In severe cases, discrete or confluent, deep brown to black stained pits give the tooth a corroded appearance. Mottled anamel is probably the result of a partial failure of the enamel-forming cells to elaborate and lay down enamel. It is a non specific response to a variety of stimuli, one of which is the ingestion of excessive amounts of fluoride.

Since mottled enamel is a developmental injury, the ingestion of fluoride following eruption of the tooth has no effect. Mottling is one of the first visible signs of an excessive intake of fluoride during childhood (Haynes and Murad, 1985). Food and water are the main sources from which animals usually acquire fluoride. Water should not contain more than about 1 ppm fluoride.

Fluoride ions play a significant role in the prevention of dental caries. Lack of fluoride in the diet may also lead to less solid bone structures and predispose for fractures. The border between the beneficial effects of fluoride and the toxic

amounts of fluoride is narrow. What is a recommendable daily intake is uncertain. Not least in Kenya, more research is needed to establish the optimal intake and the levels that can be accepted in the drinking water.

Fluorosis affects human beings as well as domestic animals and wildlife. The tolerance to ingested fluoride varies markedly among species (Shupe *et al.*, 1971). There is no substance known that completely prevents the adverse effects of excessive amounts of fluoride. Some products, however, can counteract and lessen the potential danger of ingested fluoride (Shupe, *et al.*, 1971; Said *et al.*, 1977). The high prevalence of fluorosis in Kenya has triggered investigations on the levels of fluoride in water and various foods and beverages. Most of work is geared towards prevention of ingestion of excessive amounts of fluoride.

The greater part of the fluoride intake in man originate from food and water ingested each day. Although the dominating fluoride source is the drinking water, the widespread use of fluoride-containing dentifrices may contribute significantly to the total daily intake. This also holds true for fish, tea and food items containing considerable amounts of fluoride. There is no current consensus of the levels of fluoride acceptable in various foods and beverages consumed on a daily basis (Rao, 1984). High fluoride levels upto 15 ppm in some commercially marketed fruit juices and carbonated soft beverages in Kenya present a health risk especially to children (Opinya *et al.*, 1989). There is some indication that house hold detergents, pesticides, and

indication that house hold detergents, pesticides, and fertilizers containing fluoride may be additional sources of fluoride exposure (Oelschlager, 1971; Kitner, 1971).

Chronic toxicity in domestic animals

Excessive fluoride ingestion can cause specific dental and skeletal lesions and in severe cases adversely influence the health and productivity performance of domestic animals (Shupe *et al.*, 1971; Suttie, 1983; Bunce; 1985). Fluoride intake exceeding 5 ppm has adverse effects on reproduction in cows (Rensburg and Vos, 1966). In Kenya fluorosis in farm livestock is likely to be a problem in volcanic areas especially in animals drinking from deep boreholes (Said, 1981). This applies mainly to the arid zones in the Kenyan Rift Valley. Fluoride intoxication caused loss of body condition and a fall in milk production in a herd of dairy cattle in Machakos, Kenya (Murray, 1967). Commercial feed concentrate and mineral mix with excessive amounts of fluoride caused a drastic decrease in milk production; as much as 1.5 million kg of milk in a dairy herd of 52 to 120 milking cows over a period of 6 years, in U.S.A. (Eckerlin *et al.*, 1986). In China,long term administration of fluoride at 2.15 ppm in drinking water led to fluorosis in dairy cows on one farm, but not on another.The milk production during the first and the second lactation of the cows from the two farms were not different but milk yield in the third and fourth lactation were lower in cows with fluorosis (Xiao and Zhu, 1987). This is of some importance especially in zero grazed daily animals which depend on

animals reared in the arid zones of Kenya where fluoride levels in water are usually above 2 ppm.

Long term dietary fluoride tolerance for various livestock are as follows (United Stated States NRC, 1974; NRC, 1980):

Table 11. Dietary fluoride tolerance for various species of livestock

Animal	ppm dietary fluoride
Dairy or beef heifer	30-40
Mature dairy cattle	40
Mature beef cattle	40-50
Finishing cattle	100
Feeder lambs	150
Breeding ewes	60
Horses	40-60
Swine	150
Turkeys	150
Chickens	200

Fluoride tolerances are usually expressed as the concentration in the diet assumed to be a constant exposure. Work by Suttie *et al.*, (1972) indicated that intermittent exposure to levels in excess of the tolerances may cause increased severity of bone and tooth lesions, even if the yearly average is within tolerance limits. This is particularly important since under field conditions exposure is rarely constant. Eckerlin *et al.*, (1986) recently postulated that the tolerance levels set by the National Academy of Sciences in USA for fluoride ingestion are too high.

Calves born by fluoride intoxicated cows show congenital fluorosis manifested by brown discolouration of enamel,

enamel hypoplasia, brown mottling of bones, severe retardation of cartilage cell differentiation, atrophy of osteoblasts, osteopenia, atrophy of bone marrow cells, serous atrophy of bone marrow fat and severely stunted growth, (Maylin *et al.*, 1986).

Fluoride intoxication in sheep was apparently noted almost 1000 years ago in Iceland, where its occurrence was correlated with volcanic eruptions (Roholm, 1937). Research in Kenya showed that growth rate was significantly reduced in sheep fed high fluoride water, (Said *et al.*, 1977). In Britain pregnant ewes fed 30 mg F/litre experimentally, resulted to reduced birth weight of lambs and a reduction of about 18 % in wool production (Wheeler *et al.*, 1985)

Dog and cat commercial bone meal products with fluoride levels of 1046 mg fluoride/kg dry weight are marketed in Kenya, (Mburu *et al.*, 1989). Cat food containing fish has been reported to contain high levels of fluoride (Mumma *et al.*, 1986).

In the horse fluorosis is characterised by unthriftness, poor skin and hair coat, dental fluorosis, diffuse hyperostosis and lameness (Shupe and Olson, 1971).

Wild ungulates are susceptible to adverse effects of ingestion of excessive amounts of fluoride with primary characteristics of bone and teeth lesions, (Shupe *et al.*, 1984). Chronic ingestion of excessive amounts of fluoride was associated with agalactiae in commercial fox herds (Eckerlin *et al.*, 1986). In Kenya, fluoride intoxication of the wild life has not yet been reported.

2.4. 0 Studies on fluoride in fish and marine animals

In both vertebrates and invertebrates, fluoride is largely accumulated in skeletal structures. There is little or no accumulation in soft, edible tissues, with the exception of fish skin. If fish is consumed with skin, the fluoride intake is increased. The process of fluoride deposition in fish is likely to be similar to that in higher vertebrates, where the F^--ions exchange with OH^- groups in the hydroxyapatite complex. The displacement of phosphate by fluoride is thought only to occur in very concentrated fluoride media. In crustaceans (and probably molluscs), where the ultrastructure of the skeleton is more amorphous than the well-defined crystalline lattice of the vertebrate, there is probably a much greater proportion of simple CaF precipitation (Wright and Davison, 1975). Although *Notothenia rossii marmorata*, an antarctic fish, feed mainly on fluoride-containing krill (*Euphausia superba Dana*), crustaceans, polychaets and salps; the tissues of the fish have a low levels of fluoride (Oehlenschlager and Rehbein, 1982)

Fluoride is accumulated by aquatic organisms living in high fluoride environments, especially in osseous tissues. Arctic char *(Salvinus alpinus)*, a fish found in the temperate regions, accumulate fluoride in muscles (16.6 mgF/kg) and bones (1150 mgF/kg) when living in water with 2-20 ppm F (Christensen, 1987).

The toxicity of fluoride to fertilised eggs of a fresh water fish *Catla catla* has been demonstrated experimentally in India (Pillai and Mane, 1984). Eggs of rainbow trout *(Salmo*

gairdneri) exposed to 1.5 ppm fluoride showed delayed hatching while the LC_{50} for the rainbow trout was between 2.7 and 4.7 ppm fluoride in water. However the LC_{50} for carp was between 75 and 91 ppm in water (Neuhold and Sigler, 1960). In 1969 Moore, claimed that a fluoride level of 32 ppm in water could kill oyster populations. After five days in water of about 7 ppm fluoride *Perna perna*, a mussel showed toxic effects as a result of exposture to the fluoride (Hemens and Warwicks 1972).

Krill (*Meganyctiphanes norvegica* and *Euphausia superba*), contain high amounts of fluoride (1300-2400 mg F/kg dry matter); (Søvik and Brœkkan, 1979). In spite of these high levels, feeding of Atlantic salmon and rainbow trout with frozen krill or krill meal, did not markedly increase the fluoride level in the fish flesh (Grave, 1981; Tiews *et al.*, 1982). The fluoride content of the fish bones, however, increased considerably. Although krill contain valuable nutrients, e.g. protein and essential fatty acid, the high fluoride content makes krill not recommendable for humans (Siebert *et al.*, 1982), but krill might be used in fish feed.

High fluoride levels not only reduce growth rate of oyster spats, but also render oyster meat unsuitable for human consumption (Nell, 1986). Oysters are cold blooded estuarine animals.

In several countries fish and marine organisms contribute significantly to the total daily intake of fluoride (Trautner and Siebert 1985). Fish is an important source of food in Kenya. So far, there seems to be only one published study which deals

with fluoride in Kenyan fish (Bergh and Haug, 1971). In Kenya the actual effects of high fluoride levels to the growth rate, survival and reproduction of fish is not established. Certain foodstuff contain relatively high concentrations of fluoride. These include fish which generally contain more fluoride than other meats. (Table 12).

Table 12 Fluoride contents of various foodstuffs. Adopted from Bell *et al.*, 1970

Food	Fluoride content (ppm)
Meats:	
Chicken	1.4
Beef	2.0
Round steak	1.3
Pork	< 0.2
Pork chops	1.0
pork shoulder	1.2
Frankfurters	1.7
Lamb	1.2
Veal	0.9
Mutton	< 0.2
Fish:	
Fish fillets	1.5
Mackerel	
boned	< 0.2
with bones	3.9
Fresh	26.9
Dried	84.5
canned	12.1
salmon	
canned	4.5
fresh	5.8
dried	19.3
Sardines	
canned	7.3
in olive oil	16.1
Shrimps	
canned	4.4
edible portion	0.9
Codfish	
fresh	7.0
salted	5.0
Oysters	
fresh	0.7
Crab meat canned	2.0
Herring, smoked	3.5
Tuna fish flakes, canned	0.1

Table 13. Fluoride content of meat and fish. Data from Anon (1965)

Fish from Lake Turkana	Fluoride (ppm)
Tilapia bones	828
Tilapia flesh	15
Nile perch bones	1006
Nile perch flesh	7
Distichodus flesh	35

2.5.0 Methods for fluoride analysis

Several methods and techniques are available for fluoride analysis in biological samples:

- direct potentiometric determination.
- microdiffusion technique
- gas-liquid chromatographic technique.
- high pressure liquid chromatography.
- colorimetric analysis.

Most current methods in use for analysis of fluoride in food utilise the fluoride ion specific electrode for determination of fluoride after the isolation of fluoride by:

- perchloric acid diffusion from washed samples (Dabeka *et al.*,1979).
- similar acid diffusion from samples ashed in open crucibles (Singer *et al.*, 1979).
- silanol extraction after ashing in closed oxygen bomb (Venkateswarlu,1975).

Colorimetric methods for fluoride analysis of foods were routinely used in the 1960s and earlier, before the general

availability of the fluoride ion specific electrode. There is now evidence that these colorimetric methods may have over estimated fluoride content due to the presence of interfering substances in some of the samples analysed (Singer and Ophaug, 1979; Taves, 1983). Thus, development of a generally accepted method for accurate and precise estimation of fluoride in food and beverages should be a high priority for future research (Rao, 1984). Proton activation analysis measures total fluoride content in food and the method is not perturbed by the chemical form of fluoride (Shroy *et al.*, 1982). Improved analytical procedures show that many earlier values for fluoride concentration in human food are too high (Oelschlager *et al.*, 1982, 1983.) yet unexpectedly high fluoride values in some food and meat products have been reported (Trautner and Siebert, 1985). To minimise loss of fluoride Birkeland (1970) designed a method that involves fluoride dissolution in a closed double tube chamber.

Fluoride ion specific electrode The basic principle of the ion selective electrode methodology is that there is an instantaneous electromotive force of the measuring cell, which is characteristic of the instantaneous concentration or activity of a solution contacting the electrode. When the electrode is sensitive to the material to be determined; the methods measure the primary ion and are called direct methods. If the material to be determined is allowed to react with other substances before analysis, then the method is

termed reagent measuring method or indirect analytical method (Covington 1979).

The main advantage of applying ion-selective electrodes, beside the possibility of selective measurements, is the simplicity of the technique, provided there are no complicating factors affecting the electromotive force established in the measuring cell (Covington 1979). Ion selective electrode method also offers a wider applicability in determination of fluoride in water, beverages, foods, feeds, biological samples, fertilizers, soils etc.

The electromotive force values are more rapidly stabilized in a stirred solution, especially buffered solutions. The response time for the electrode can change in the course of examination. It is therefore necessary to allow the potential to come to a steady state.

The fluoride electrode consists of a sensing element bonded into an epoxy body. When the sensing element (a lanthanum fluoride membrane) is in contact with a solution containing fluoride ions, an electrode potential develops across the sensing element. This potential, which depends on the level of free fluoride ions in solution, is measured against a constant reference potential with a pH/mV meter. The measured potential corresponds to the level of fluoride ions in solution (Orion,1987).

The method for analyzing fluoride in water and beverages is relatively simple. It is performed by potentiometric measurements with the aid of a fluoride ion specific electrode (Frant and Ross, 1966). Measurements of fluoride in solution

can be made directly after addition of an appropriate buffer solution.

While in drinking water, fluoride is in ionic form (free), fluoride in food is present in both ionic and bound forms (Duff, 1981; Spak *et al* ., 1982). Preferably, analysis of foods to determine fluoride content should be done using a method that determines both free (F^-) and bound fluoride. A convenient and sensitive method for determining the amount of free and bound fluoride in foods, has not yet been found. Such a method would be of help in the development of profiles for the bioavailability of fluoride in various foods (Rao, 1984).

Methods used to hydrolyse food samples are generally cumbersome procedures that lead to either loss or acquisition of fluoride from the environment due to lability and ubiquitousness of fluoride.

Foods may be ashed prior to analysis for fluoride to convert some or all non-ionic fluoride to the ionic form which is detected with the fluoride ion specific electrode

Dry ashing of untreated tissue was found to cause variable losses of fluoride (Hemens and Warwicks, 1972). Some fluorocarbon may be lost during open ashing because of their volatility (Venkateswarlu, 1975) leading to an underestimation of total fluoride. Fluoride from most foods is diffusible from acidic solutions (Taves, 1983), but some foods because of the possible presence of non ionic fluoride, should be ashed prior to determination of their total fluoride content. However, the separation of fluoride from unashed samples by acidic

diffusion and determination of the isolated fluoride with some colorimetric methods may give erroneous results (Dabeka *et al.*,1979) as compared to determinations using fluoride ion specific electrode. The basic problems encountered in the open ashing of samples containing submicrograms of fluoride are loss of fluoride and extraneous fluoride contamination.

Widely employed preparatory methods for converting the fluoride content of foods into the ionic form for determination with the fluoride specific ion electrode, have been tested. On dry ashing in a muffle furnace, substantial and irregular losses of fluoride occur over 500^{0}C, even in the presence of ashing aids and fixatives. Low temperature plasma oxygen ashing resulted in 60 -100 % loss, presumably as elemental fluoride. Analyses of numerous sodium oxide (NaO_2) batches showed contamination of the reagent at 0.2 ppm fluoride, precluding meaningful tests using the peroxide. Alkali ashing is satisfactory for a range of food matrices, but not for fatty materials (Shamschula *et al.*,1979).

Chapter 3

Fluoride in water

3.1 Introduction

East Africa's Great Rift Valley contains numerous shallow, closed system lakes lying at low depression points. Since the catchment basins are composed primarily of basic volcanic rocks and have no visible outlets, these lakes are to varying degrees alkaline, with sodium carbonate as the principal solute.

The six lakes selected for the present study, Lakes Naivasha, Magadi, Baringo, Bogoria, Elementaita and Nakuru all are located in the Rift Valley. None of them has a river outlet. Lakes Naivasha and Baringo are fairly fresh, possibly due to a subterranean outlets. Lakes Bogoria, Elementaita, Nakuru and Magadi are strongly alkaline, due to evaporation leaving salts brought in by inflowing rivers and springs. The ecological and biological characteristics of Lake Naivasha and Lake Baringo are mainly based on Lincer *et al.*, 1981.

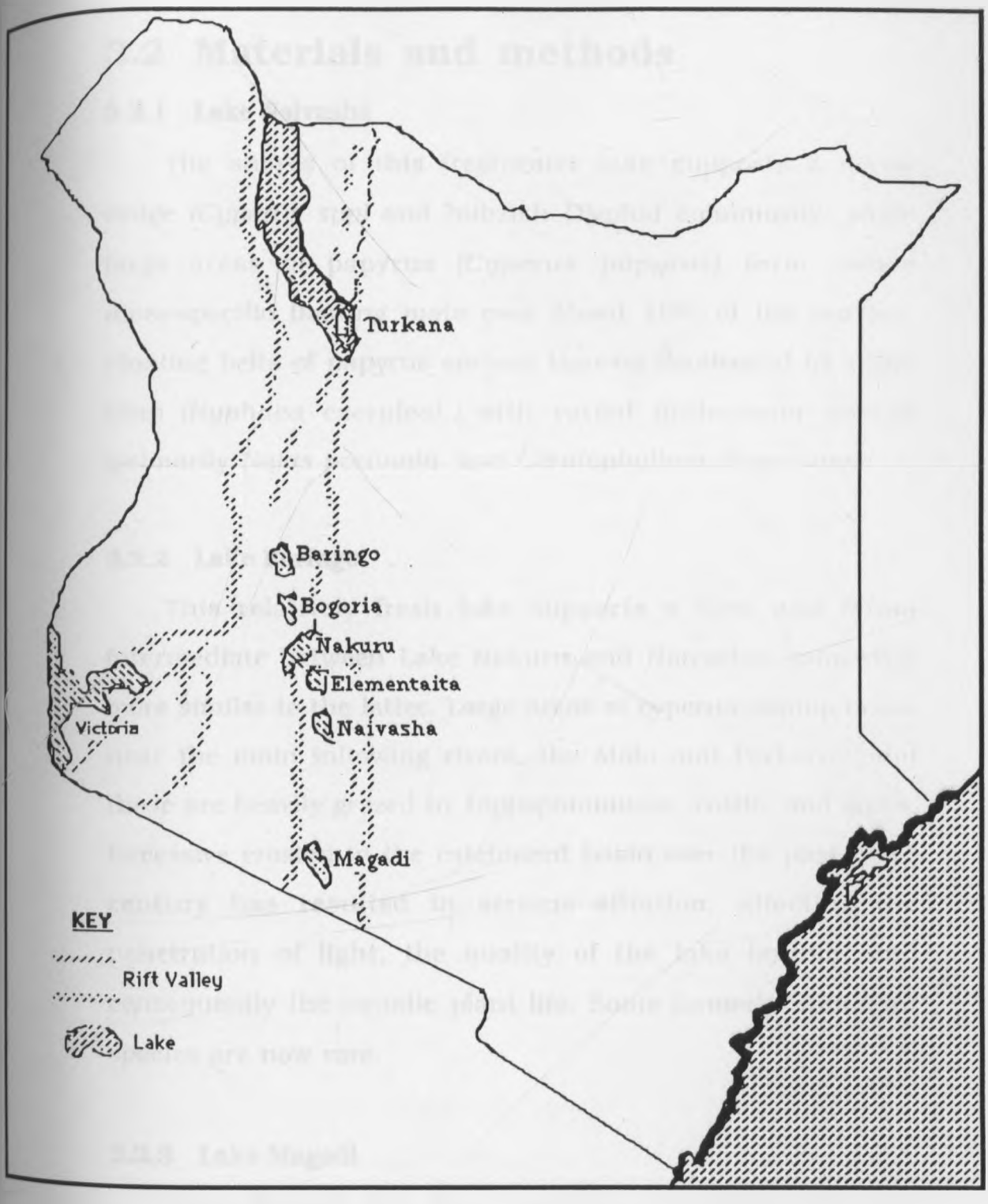


Fig.2. Map of Kenya indicating the Great Rift Valley and the lakes in the Rift Valley.

3.2 Materials and methods

3.2.1 Lake Naivasha

The shores of this freshwater lake supports a dense sedge *(Cyperus spp)* and bulrush (Typha) community, while large areas of papyrus *(Cyperus papyrus)* form dense monospecific floating mats over about 10% of the surface. Floating belts of papyrus enclose lagoons dominated by water lilies *(Nyphaea caerulea).*, with varied underwater growth (primarily *Najas pectinata* and *Ceratophyllum demersum).*

3.2.2 Lake Baringo

This relatively fresh lake supports a flora and fauna intermediate between Lake Nakuru and Naivasha, somewhat more similar to the latter. Large areas of cyperus swamp occur near the main inflowing rivers, the Molo and Perkerra, and these are heavily grazed by hippopotamuses, cattle, and goats. Excessive erosion in the catchment basin over the past half century has resulted in serious siltation, affecting the penetration of light, the quality of the lake bottom, and consequently the aquatic plant life. Some formerly abundant species are now rare.

3.2.3 Lake Magadi

Lake Magadi also has lagoons and alkaline volcanic springs. The water from Lake magadi is warmer than water from most of the other lakes in the Rift valley and it is also saline with high pH values.

Table 14. Physical characteristics of lakes under study Adapted from Lincer *et al.*, (1981).

	Naivasha	Baringo	Magadi
Altitude (m)	2237	1187	580
pH	7.3	8.9	10.1
Surface area*(km^2)	170	180	100
Catchment area (km^2)	2130	6920	-
Usage %:			
Cultivation	50	10	-
Range ranching	40	60	-
Forest/bush	10	30	-
Town	0	0	-

*Lake surface area are subject to marked fluctuations as the lake levels are constantly rising and falling

3.2.4 Collection and handling of water samples

Water samples were obtained from Lakes Naivasha, Baringo, Nakuru, Elementaita, Bogoria and Magadi. The samples were collected in clean 500 ml polyethylene bottles. Six bottles were obtained from each Lake. The samples were transported to the laboratory in a cool box and then stored at - 20 ^{0} C awaiting further preparation for analysis.

3.2.5 Instruments and equipment.

Fluoride combination electrode (96-09,Orion Research Incorporated, Cambridge Mass, USA). Digital pH meter (3020

Orion®). Automatic voltage regulator (model CVR 500 AX, Samlex®, England). Electrode filling solution (Orion, 90-00-01). pH electrode storage solution (91-00-01, Orion®). Magnetic stirrer and a teflon coated bar, 5 mm x 11 mm. Polyethylene tubes (15 ml). Twenty millilitre plastic cups (NDD/TL, Norsk Dental Depot, Oslo, Norway). Pipette tips (1000 µl, 9604, Treff, Degershein, Switzerland). Half- litre Plastic bottle for holding deionised water. Ten ml plastic disposable straight pipettes. 500 µl digital transfer pipette (Transferpette ® W. Germany).

Fluoride ion activity electrode. To determine the fluoride concentration in water, a combination fluoride electrode and a digital pH meter were used. The size of the electrode permits analysis of small sample volumes. The following factors have to be considered regarding the final solution to be analysed: the fluoride ion concentration should be above 10^{-6} M (0.02 ppm F), the pH of the water sample should be between 5 and 7 so as to give maximum fluoride concentration and minimum interference from the hydroxyl ions, the ionic strength should be kept constant and fluoride complexing agents should be inactivated.

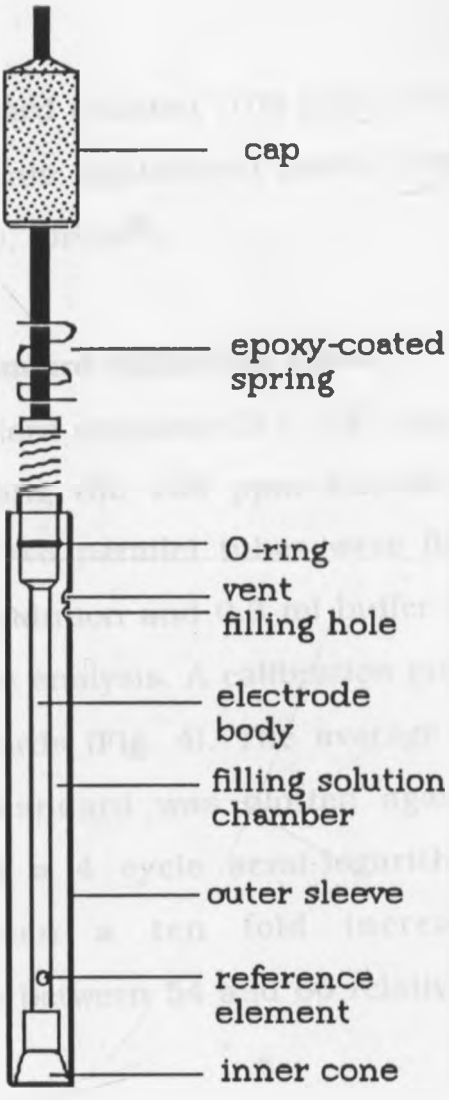


Fig. 3. Model 96-09 Combination fluoride electrode

In order to determine fluoride concentration in water standards and samples, the solutions must be adjusted to the same pH and ionic strength.

3.2.6 Reagents

Fluoride standard solution (100 ppm, 94-09-07, Orion®). TISAB III (Total ionic adjustment buffer),(940911, Orion®). TISAB II (94-09-09, Orion®).

3.2.7 Fluoride standard-calibration curve

Fluoride standard solutions (0.1, 1.0, and 10.0 ppm) were prepared by diluting the 100 ppm standard solution with deionised water. Two parallel tubes were filled with 3.0 ml standard fluoride solution and 0.3 ml buffer (TISAB III) added to each tube before analysis. A calibration curve was prepared from these standards (Fig. 4). The average relative millivolt value for each standard was plotted against the fluoride concentration on a 4 cycle semi-logarithmic paper. The difference between a ten fold increase in fluoride concentration was between 54 and 60 relative millivolts.

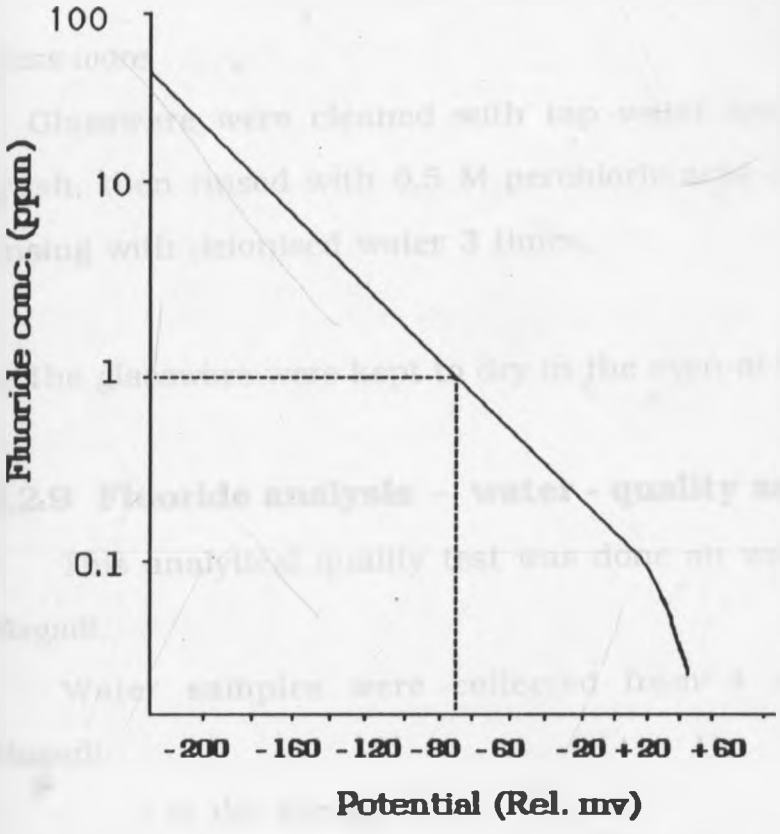


Fig. 4. Fluoride concentration calibration curve

3.2.8 Cleaning procedure

Plastic tubes

1. The tubes were cleaned with tap water, soap and a test tube brush and soaked in a soapy solution for 2 to 3 hours. The tubes were then rinsed in deionized water and allowed to dry.

2. The tubes were thereafter soaked in 7.8 M sodium hydroxide for 24 hours after which they were soaked in 35 % nitric acid for 24 hours.

3. Finally the tubes were rinsed in deionised water and then dried in the oven at about 60 ^{o}C.

Glass ware

1. Glassware were cleaned with tap water and a test tube brush, then rinsed with 0.5 M perchloric acid 3 times before rinsing with deionised water 3 times.

2. The glassware were kept to dry in the oven at 60°C.

3.2.9 Fluoride analysis - water - quality assurance

This analytical quality test was done on water from Lake Magadi.

Water samples were collected from 4 sites at Lake Magadi:

* at the spring.
* 3 metres from Lake Magadi spring (the pond gate.)
* about 100 metres from the spring.
* 400 metres from Lake Magadi spring (next to the entrance of Lake Magadi.)

Preparation of standard solutions with TISAB II.

One ml of standard fluoride solution was added to 1ml of TISAB II.

Each water sample was prepared and analysed in two parallels; first without any dilution then after a dilution with deionized water: 1:10, 1:25 1:50, 1:75, 1:100, 1:150 and 1:200. As for the standards, one ml of TISAB II was added to 1 ml volumes of the water samples.

Preparation of standard solutions with TISAB III.

Of the standard fluoride solution, 3 ml was added to 0.1ml of TISAB III solution. Each water sample was prepared and analysed in two parallels; first without any dilution then after a dilution of 1:10, 1:25 1:50, 1:75, 1:100, and 1:150. Deionised water was used for diluting the samples. As for the standards, 0.3 ml of TISAB III was added to 3 ml of the water samples.

3.3 Results and discussion

Table 15 pH and fluoride concentration in some Lakes of the Rift Valley (six parallels were analysed for each Lake).

Lake	pH		Fluoride (ppm)	
	Mean	(range)	**Mean**	(range)
Naivasha	**8.5**	(8.4-8.9)	**2.4**	(2.4-2.6)
Magadi	**10.1**	(10.0-10.2)	**84**	(70-90)
Nakuru	**10.3**	(10.0-10.6)	**344**	(300-350)
Elementaita	**10. 1**	(10.0-10.3)	**463**	(440-480)
Bogoria	**10.3**	(10.0-10.5)	**738**	(710-750)
Baringo	**10.4**	(10.1-10.7)	**5.4**	(5.3-5.6)

All lakes were alkaline with pH ranging from 8.4 to 10.7 on the extreme limits (Table 15). Lake Naivasha is the least alkaline of all the waters analysed due to the presence of an underground drainage. Lake Naivasha, being relatively fresh also contains low levels of fluoride compared to other lakes. Among the six lakes considered in this study Lake Bogoria had the highest levels of fluoride ranging from 710 to 750 ppm. There are considerable variations in the fluoride concentrations reported for the lakes in Rift Valley. The values for lake Naivasha range from 2.0 to 30.0 ppm fluoride, for Lake Baringo from 6.0 to 18.0 ppm (Williamson 1953; Bergh and Haug 1971; Gitonga and Nair 1982; Kariuki *et al.* 1984). Our fluoride levels for Lake Naivasha (2.4-2.6) and Lake

Baringo (5.5-6.0) agree fairly well with the values of Kariuki *et al.* (1984) for Lake Naivasha (1.3 to 1.5) and Lake Baringo (4.8-5.4). However their recording of 1480 ppm fluoride in Lake Magadi exceeds our results by about 10 times.

Table 16. pH of water samples from Lake Magadi.

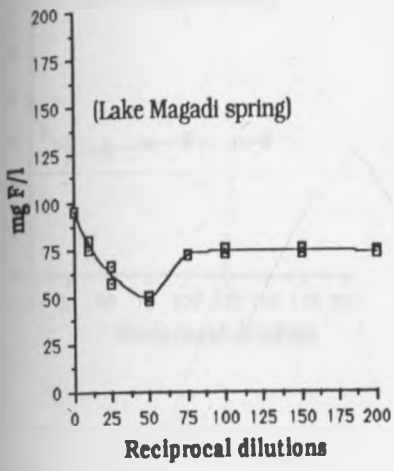
Site of water sample collection	pH	
	parallel 1	parallel 2
Spring	10.1	10.1
Pond gate (3 m from the spring)	10.1	10.1
100 m. from the spring	10.2	10.1
Entrance to Lake Magadi (400 m from the spring)	10.2	10.1

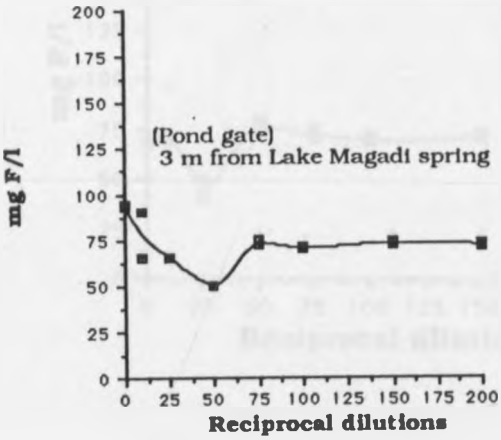
The pH of all samples were measured and found to vary from 10.0 to 10.2. The pH of all undiluted samples from Lake Magadi, even after addition of decomplexing agents, TISAB II or TISAB III were higher than the acceptable maximum of 5.5 stated in the specifications for the fluoride electrode, and accordingly measurements made with the fluoride electrode were unreliable. The samples were then diluted so as to lower the pH towards the specified range, and the effects on fluoride determination observed.

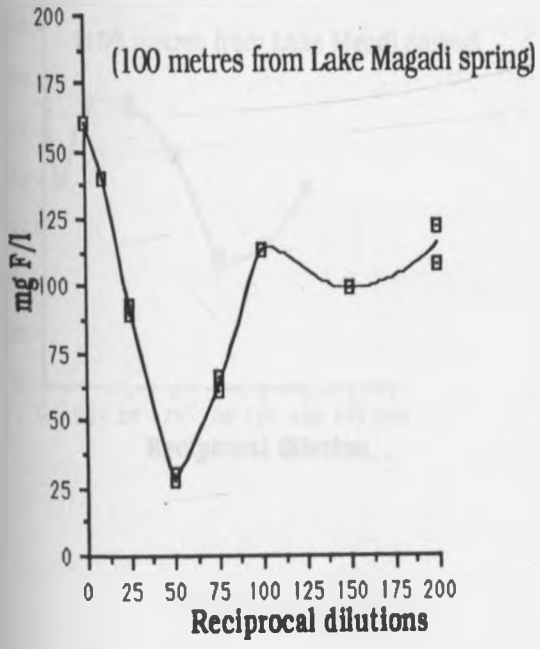
Figs. 5 and 6 portray a general trend that as the water is diluted the fluoride concentration tends to falls to a minimum and then the fluoride concentration rises slightly and then comes to an almost steady value.There are several possibilities for this observed phenomenon. This is probably due to the fact that initially only free unbound fluoride is detected and then

at a certain dilution, bound fluoride is released by the TISAB solution and this is indicated by a sudden rise in fluoride measured. Another possibility is the presence of interfering ions in the water.

Fig. 5 Effects of dilution and buffering with TISAB II on fluoride measurement of water from various sites of lake Magadi.







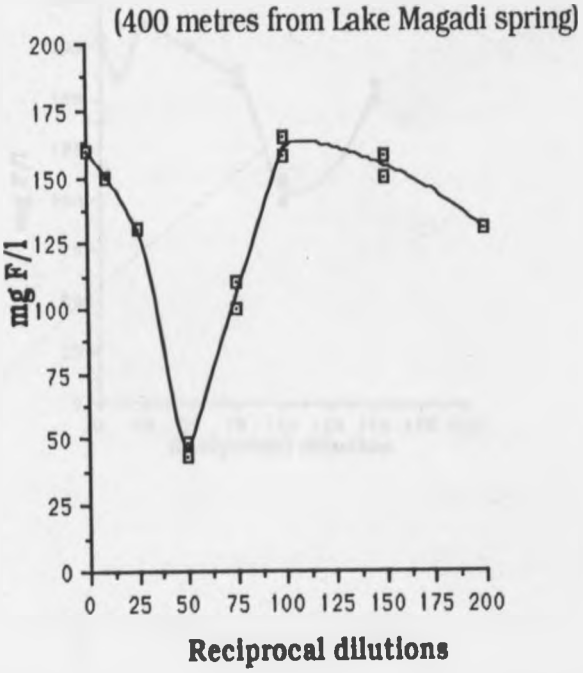
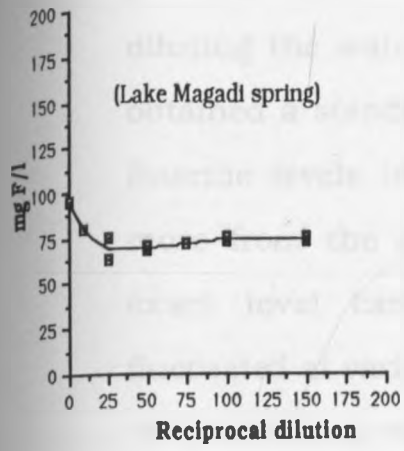
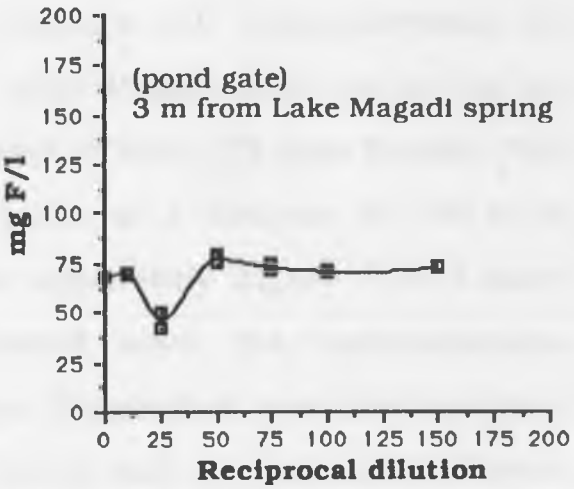
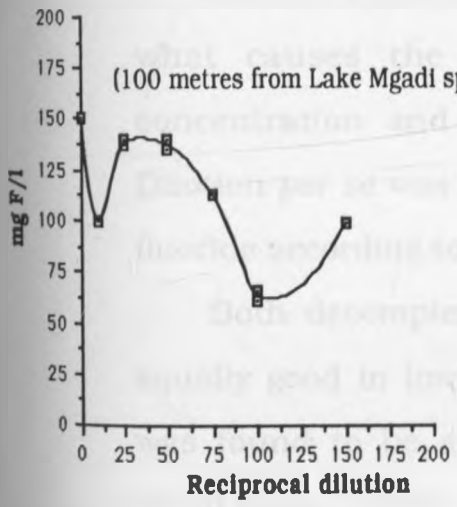
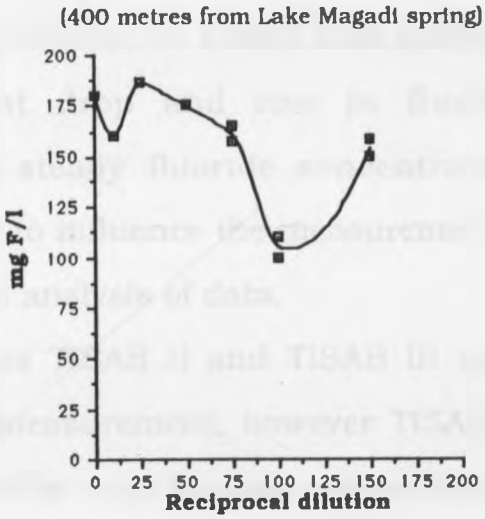


Fig. 6 Effects of dilution and buffering with TISAB III on fluoride measurement of water from various sites of Lake Magadi.









There are several possible explanations for the variations, for instance the site of collection, seasonal differences and variations in the degree of evaporation, and the analytical problems caused by the extreme salt concentrations. By diluting the water entering Lake Magadi from the spring, we obtained a steady plateau level of about 73 ppm fluoride. The fluoride levels in samples taken at a distance of 100 m or more from the spring were apparently higher, but a more exact level cannot be stated since the concentrations fluctuated at various dilutions. Evaporation may have increased the fluoride as well as the general salt concentrations to levels at which the presently used and common methods for fluoride analyses become unreliable.

Although the dilution of samples of alkaline water helps in obtaining appropriate pH it cannot be stated with certainty what causes the consistent drop and rise in fluoride concentration and finally a steady fluoride concentration. Dilution *per se* was found not to influence the measurement of fluoride according to statistical analysis of data.

Both decomplexing agents TISAB II and TISAB III seem equally good in low fluoride measurement, however TISAB III was found to be a better buffer and fluoride decomplexing agent than TISAB II because it caused release of bound fluoride between dilutions 1: 20 and 1: 25 while TISAB II decomplexes fluoride between dilutions 1: 50 and 1: 80. TISAB III can therefore be used in analysis of fluoride in alkaline water in preference to TISAB II.

According to the Technical Director of Orion, Dr. M. Frant (1989 personal communication, see Appendix 16 and 17) TISAB solutions were designed for analysis of fluoride in drinking water. Therefore it is likely that TISAB II and TISAB III solutions are not suitable for analysis of alkaline water. Therefore, highly alkaline water may give false levels of fluoride in water samples. This could explain the reporting of low and high fluoride in some of the water from alkaline lakes. The results also show that fluoride concentration of water from Lake Magadi varies according to the site of collection possibly due to evaporation factor. Analysis of water from lakes Bogoria, Nakuru and Elementaita revealed a similar picture to that potrayed by Lake Magadi.

Analysis of fluoride concentration in water is usually regarded as simple and easy. However several workers, have come up with fluoride concentration in lake waters of Kenya with large variation, therefore caution should be taken particularly when analysing alkaline water. Consequently, more investigation in measurement of fluoride in alkaline water is required.

Chapter 4

Fluoride in fish

4.1 Introduction

A wide variety of environmental and genetic factors influence the fluoride concentration in fish. Some of the variations in fluoride concentration can also be explained by postulating a chloride-fluoride excretion mechanism over the epithelial tissues (Sigler and Neuhold, 1972). Fish can accumulate fluoride in muscle skin and bone tissues when exposed to high fluoride environment. In several countries fish and marine organisms contribute significantly to the total daily intake of fluoride (Siebert and Trautner 1985). Fish is an important source of food in Kenya. So far, there seems to be only one published study which deals with fluoride in Kenyan fish (Bergh and Haug, 1971). Hence this study was designed so as to investigate the fluoride levels in fish living in high fluoride environment. Lakes Magadi, Baringo and Naivasha were chosen for this investigation.

4.2 Materials and methods.

4.2.1 Description of the lakes

Lake Naivasha

Fish in Lake Naivasha are mostly exotics, which have largely replaced the small indigenous minnow *Aplocheilichths antinorii*. The present dorminant vegetarian species is a hybrid between *Tilapia spirulus nigra* and *T. leucosticta*. In the 1920's large mouth black bass (*Micropterus salmoides*) were introduced to prey on the Tilapia and provide sport fishing: they too are abundant and widespread in Lake Naivasha.

With a shoreline of about 80 km, Lake Naivasha supports a much more diverse population of fish eating and other water birds than Lakes Nakuru Baringo or Elementaita. Bird species include three grebe species (*Podicipedidae*): two cormorant species (*Phalacrocolax*): the darter (*Anhinga rufa*): two pelican species (*Pelecanus*): at least twelve resident and migrant ducks and geese (*Anatidae*): ten species of herons and egrets (*Ardeidae*): storks, spoonbills and ibises (*Ciconidae* and *Tresklornithidae*); and many rails, crakes, and Jacanus (*Rallidae* and *Jacanidae*). During the cold season, the resident species are augmented by numerus waders (*Charadriidae* and *Scolopacidae*) and four species of Kingfisher (*Alcedinidae*). About 90 pairs of fish eagles exist here, forming one of the most dense populations of this species. Of about 350 species recorded in the catchment area, 60 to 80 species are basically aquatic at some times of the year.

Lake Baringo

Four species of fish *Labeo cylindricus* a small bottomfeeder, *Clarius mosambicus* a cat fish, and two predators, *Barbus gregori* and *Tilapia nilotica*, make up most of the fish fauna of Lake Baringo. Other species of fish include *Protopterus aethopicus.*

About 300 bird species occur in this lake basin, the most common aquatic species being the darter and several herons, notably the goliath heron (*Ardea goliath*) and the night heron (*Nycticorax nysticorax*). About 35 pairs of fish eagles breed on Lake Baringo. While the fish eating bird fauna is less varied than that of lake Naivasha, it is more varied than that of lake Nakuru was prior to 1961, when the *Tilapia grahami* (or *Oreochromis alcalicus grahami)* were introduced.

Lake Magadi

There are several species of fish (Tilapia) isolated in the alkaline lakes of the Great Rift Valley of Africa, living in extreme conditions of temperature salinity and pH (Reite *et al.*,1974). One of the fish, the Lake Magadi Tilapia (*T. grahami*), lives in the lagoons and alkaline volcanic springs around the margins of Lake Magadi. The ability of *T. grahami* to survive in alkaline water is superior to that known for any other fish. The fish is also thermal tolerant and can withstand low levels of dissolved oxygen.

4.2.2 Collection of fish samples

Lake Magadi

Fish samples from Lake Magadi were obtained by trapping the fish using a net which could catch a variety of fish sizes from the lagoon adjacent to the Lake. After being caught the fish were grouped together and eviscerated to slow down decomposition process. They were then packed in plastic papers and transported to the laboratory in a cool box. In the laboratory, all the fish were weighed and fish of approximately the same weight kept in one bag. All the bags were stored at - 20 ^{o} C ready for the next preparation process. A total of 118 fish *Oreochromis alcalicus grahami* (Tilapia) weighing between 3.9 and 19 g were caught and their fillet, skin, gills and bones prepared for analysis of fluoride.

Lake Naivasha

Some of the fish samples from Lake Naivasha were obtained by catching the fish using a fish net, while some of the fish were bought from fishermen at the landing bay of Lake Naivasha. Evisceration and grouping of the fish was done followed by packing in plastic bags and transportation in a cool box to the laboratory. In the laboratory, the fish samples were weighed and stored at - 20 ^{o} C awaiting further preparation.

90 fish, *(Oreochromis leucostictus)* Tilapia with a weight ranging from 33 g to 250 g were prepared and their fillet,

skin, gills and bone analysed to determine the fluoride concentration.

A total of 47 fish *Tilapia zilli* (Tilapia) weighing between 40 and 169 g were caught and their fillet, skin, gills and bones prepared for analysis of fluoride.

15 fish *(Micropterus salmoides)* Black bass weighing between 5.8 g and 1448 g were obtained and their muscles, skin gills and bones prepared for determination of fluoride.

Lake Baringo

A total of 50 fish from Lake Baringo *Oreochromis nilotica* (Tilapia) weighing between 156 and 198 g were caught and their fillet, skin, gills and bones prepared for analysis of fluoride. The fish samples from Lake Baringo were obtained and treated in a similar manner to the samples from Lake Naivasha.

Lakes Nakuru, Elementaita and Bogoria were reported to have no fish and therefore no samples were available for determination of fluoride.

Table 17 Fish samples collected for fluoride analysis from the three lakes in the Rift Valley

Lake	Fish species	No. of fish
Naivasha	*Oreochromis leucostictus*	90
	Tilapia zilli	47
	Micropterus salmoides	15
Baringo	*Oreochromis niloticus*	50
Magadi	*Oreochromis alcalicus grahami*	118
Total		320

4.2.3 Fish samples - preparation in the laboratory.

The head and the scales of the fish were removed first as the fish were resting on a clean plastic sheet. Each fish was dissected carefully using a surgical blade to separate fillet, skin, gills and bone tissues. Fillet was just peeled of from the bone to avoid scraping of bone chips on to the fillet. Tissues from the same group of fish were then pooled together and kept in clean petridishes which had been weighed previously. The petridishes were then weighed to get the wet weight of the sample in the petridish after which they were put in an oven at 100^{0} C for 24 hours. The petridishes were allowed to cool to room temperature and then the dry weight was obtained and recorded for each tissue.

Each dry tissue was ground in a mortar using a pestle to fine homogeneous particles. Care was taken to avoid

contamination of samples by using different mortars and pestles for a particular tissue from a given group. The ground samples were then stored in plastic tubes which were screwed tightly to avoid absorption of moisture.

4.2.4 Instruments and equipment.

Fluoride combination electrode (96-09,Orion Research Incorporated, Cambridge Mass, USA). Digital pH meter (3020 Orion). Automatic voltage regulator (model CVR 500 AX, Samlex, England). Electrode filling solution (Orion, 90-00-01). pH eletrode storage solution (91-00-01, Orion). Magnetic stirrer and a teflon coated bar, 5 mm x 11 mm. Outer polypropylene tubes (15 ml), Inner polypropylene tubes. Twenty millilitre plastic cups (NDD/TL, Norsk Dental Depot, Oslo, Norway). Pipette tips (1000 µl, 9604, Treff, Degershein, Switzerland), forceps. Half- litre Plastic bottle for holding deionised water. Ten ml plastic disposable straight pipettes. 500 µl digital transfer pipette (Transferpette ® W. Germany).

4.2.5 Reagents

11.6 M perchloric acid (Riedel-de Haen AG, Hannover, West Germany.) 14.3 M nitric acid (Riedel-de Haen AG). *Acid mixture:* Equal parts of 11.6 M perchloric acid and 14.3 M nitric acid. *Base mixture*: 7.8 M sodium hydroxide and 1.0 M *tri*-sodium citrate in a ratio of 3:10. *Blank II solution*: a mixture of 150 ml of 7.8 M sodium hydroxide, 500ml of 1M *tri*-sodium citrate and 100 ml of acid mixture. Sodium hydroxide pellets (May and Baker Limited, Dagenham,

England). Tri-sodium citrate (BDH Limited Poole England). All reagents were of analytical grade.

4.2.6. Fluoride standards - calibration curve.

Fluoride standards were prepared as described in 3.2.4 but Blank II solution was use as the background solution instead of deionised water. A calibration curve was prepared similar to that in Fig.4.

4.2.7 Cleaning procedure

The cleaning procedure outlined in 3.2.8 (pp 48) also applies in this section.

4.2.8 Dissolution of fish samples by closed chamber method

The closed double tube arrangement is presented in Fig. 4. The outer tubes were prepared by warming the bottom third of the tube and then pressing hard to create a depression that goes up to about halfway the diameter of the tube.

The inner tube was handled with clean forceps to avoid any contamination.

Digestion tube for fluoride samples

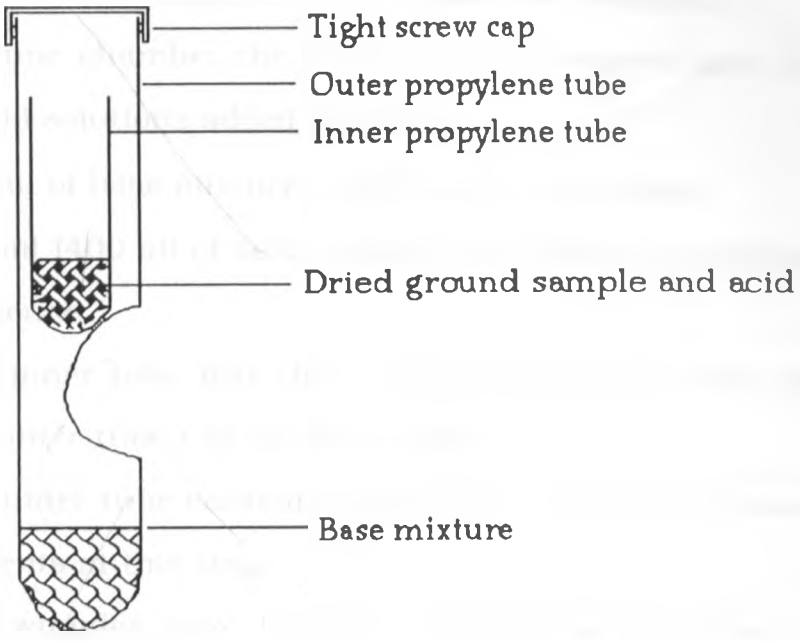


Figure 7. Closed double tube arrangement used for dissolution of samples and buffering.

With a balance (Mettler AE 163), about 45 milligrams (30 - 50 mg) of dry sample were weighed using a clean spatula to scoop the dry sample. A small plastic beaker (25 ml Azlon Labplex, England) was used to hold the inner tube during the weighing. The plastic beaker was placed on the balance and then the inner tube carefully picked from a container and placed in the beaker.The balance windows were then closed and the balance tared or zeroed.

About 45 mg of the dry sample was carefully transfered into the small propylene tube. The inner tube was then picked with a pair of forceps and inserted into the outer tube which thereafter was capped tightly. After weighing each sample the spatula was cleaned in deionized water and wiped using paper

tissue ("Kleenex"). From each sample 5 or 6 parallels were analyzed. The weight of each sample was recorded.

In a fume chamber the tube cap was removed and the base and acid solutions added as follows:

* 2.6 ml of base mixture added in the outer tube
* 0.4 ml (400 µl) of acid mixture was added to the inner tube contents.
* The inner tube was then replaced into the outer tube and the outer tube cap screwed tightly.
* The inner tube contents should not mix with the outer tube contents at this stage.
* The samples were kept at room temperature for 1 hour. Thereafter they were placed in a plastic beaker (100 ml Azlon,Labplex, England) and kept in an oven at 60 ^{o}C for 30 -60 minutes.
* The samples are then kept on the bench for a few minutes to cool after which they were kept at -20 ^{o} C for 20 minutes so that hydrofluoric acid (HF) fumes could settle down.
* After thawing at room temperature, the tubes were then turned upside down to mix the sample dissolved in acid with base.

Concentrated acid dissolves the fluoride bound in the fish tissues and forms hydrofluoric acid which then forms sodium fluoride , while the base mixture acts as a buffer and stabilises the solution's ionic strength. The pH of each digested sample was checked, and proved for all the samples to be between 5.0 to 5.5. Fourteen ml plastic tubes suitable for fluoride electrode analysis were used to hold sample solutions for

for analysis of fluoride with a combination fluoride electrode (Fig. 3).

4.2.9 Analytical quality assurance

Preparation of fish tissues

Tilapia fish (*Oreochromis leucostictus*) weighing between 153 to 232 grams were dissected carefully and the fillet, skin, gills and bone tissues separated. Fillet was just peeled from the bone to avoid scraping of bone chips onto the fillet. Four dry petridishes were weighed individually when empty and then weighed together with some fresh tissue of fillet, skin, gills, or bones.

Preparation of blank and spiked samples

Spiking is the addition of known fluoride concentration to a sample with a known fluoride concentration in order to check the percentage of added fluoride that can be detected after extraction by the method used. The spiking procedure is also referred to as recovery studies.

Recovery studies were carried out with blanks, standards and test samples (fillet, skin, gills and bone tissues).

Recovery of fluoride in blank tubes

The main aim of this test was to check whether there was contamination of blank II solution during the preparation.

The pH range of all the samples were between 5.0 and 5.2 , which is within the recommended operational pH range of 5.0 to 5.5 (Orion 1987).

The total volume of the solution analysed in each tube was either 3.1 or 3.3 ml. This volume of sample allows proper stirring and enhances rapid attainment of the steady potential as well as protecting the sensitive element of the electrode.

Preparation of fish tissues for recovery studies.

From the ground dry tissue, 6 parallel samples with a weight range of 43.6 mg to 47.8 mg were digested with acid to extract bound fluoride at an oven temperature of 60 ^{o}C for 30 minutes. A base solution was used for neutralization and buffering of the acidic digested sample (fig.7) The contents of the digestion tube were then emptied into a tube ready for checking the pH of the solution. The pH of all the samples were between 5.0 and 5.5.

4.3 Results and discussion

4.3.1 Analytical quality assurance

Table 18. Recoveries of fluoride (F) at various spiking levels of blanks and fish tissues.

Sample	No. of samples	Original F- (ug F) Mean	Range	Spiking F (ug F)	% of spiking F- recovered Mean	Range	SD
Blank	6	-	-	**0.5**	**98.3**	86 - 108	8.1
"	6	-	-	**1.5**	**100.8**	96 - 105	4.0
Fillet	5	**0.4**	0.3 - 0.5	**0.5**	**92.8**	70 - 118	17.7
"	5	**0.4**	0.3 - 0.5	**2.5**	**76.7**	68 - 93	9.6
"	6	**0.4**	0.3 - 0.5	**10.0**	**91.8**	79 - 114	12.1
Skin	5	**3.2**	2.9 - 3.8	**2.5**	**104.8**	77 - 125	15.9
"	6	**3.2**	2.9 - 3.8	**10.0**	**97.1**	85 - 111	8.3
Gills	6	**26.4**	22.5 - 29.1	**10.0**	**92.7**	77 - 108	13.9
"		**26.4**	22.5 - 29.1	**30.0**	**118**	88 - 138	18.0
Bones	6	**26.4**	24.0 - 30.0	**10.0**	**102**	92 - 108	7.1
"	5	**26.4**	24.0 - 30.0	**30.0**	**118.4**	110- 121	15.0

S.D.Standard deviation from the mean

The recovery of added quantities of fluoride was satisfactory (Table 18.) for blanks and tissue samples. This was an indication that the method is suitable for analysis of fluoride and possibly other food substances. The average recovery percentage for blanks was 99.6 %, this indicates that approximately 0.4 % of fluoride was lost during the dissolution process. The average recovery for fillet tissues was

87.4 %, this was the lowest recovery obtained, possibly because fillet has the lowest content of fluoride among the tissues analysed. Spiked skin samples gave an average recovery of 101 %, while gill tissues gave a mean recovery of 105.4 %. Bone tissues gave a recovery of 110 %.

4.4.1 Fluoride levels in fish from Lake Naivasha

Oreochromis leucostictus (Tilapia from Lake Naivasha)

The average fluoride concentration in fillet ranged from 6.0-18.3 µg F/g (dry weight basis) for all the fish with extreme values of 4.9 and 24 µg F/g. In skin, the average fluoride concentration ranged from 11.0-29.0 µg F/g in the samples of fish analysed though the extreme values of fluoride concentration were 8.0-38.8 µg F/g. The average fluoride concentration in gills tissues ranged from 309-688 µg F/g. However, the lowest fluoride concentration was 119 µg F/g while the highest fluoride concentration was 987 µg F/g. Bone average fluoride concentration ranged from 471-671 µg F/g. However, the lowest fluoride concentration in bone tissues was 322 µg F/g while the highest fluoride concentration was 823 µg F/g.

At a given weight of fish, bones have the highest fluoride concentration followed by gills, skin, and fillet respectively. Overall the fluoride concentration in the fillet of *Oreochromis leucostictus* is higher than the fluoride concentration in water from Lake Naivasha which is 2.6 mg F/l.

Fig. 8 Relationship between fluoride levels in fillet and skin of *Oreochromis leucostictus* and the fish weight

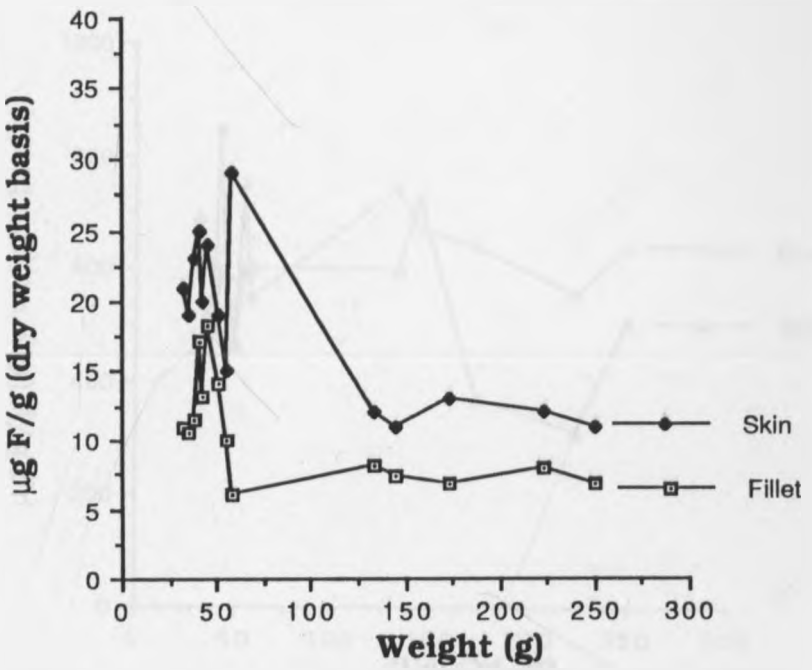
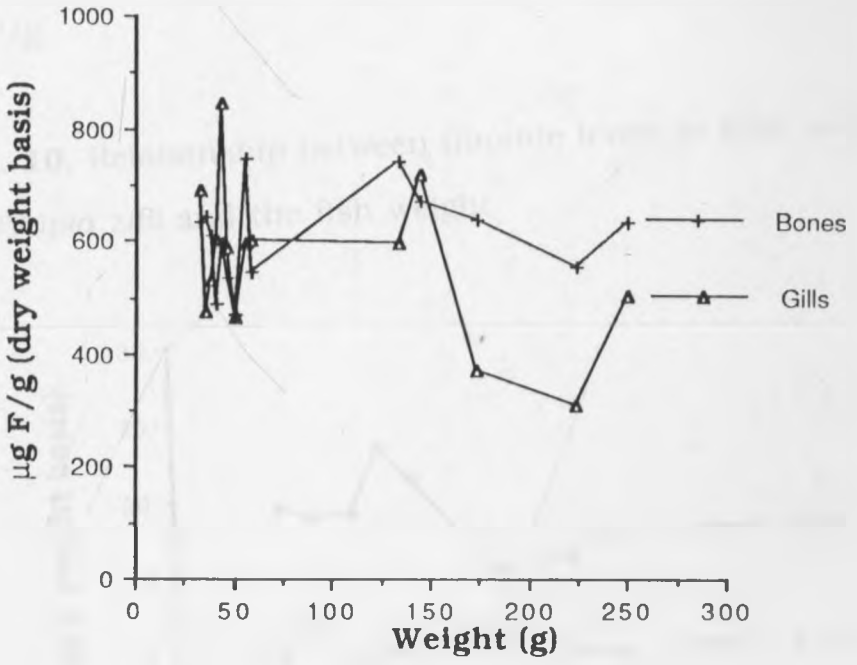


Fig. 8 indicates that the fluoride content in fillet is lower than fluoride concentration in skin. In both tissues the fluoride concentration fluctuates but there is no apparent increase in fluoride concentration with increase in fish weight.

Fig.9. Relationship between fluoride levels in gills and bones of *Oreochromis leucostictus* and the fish weight



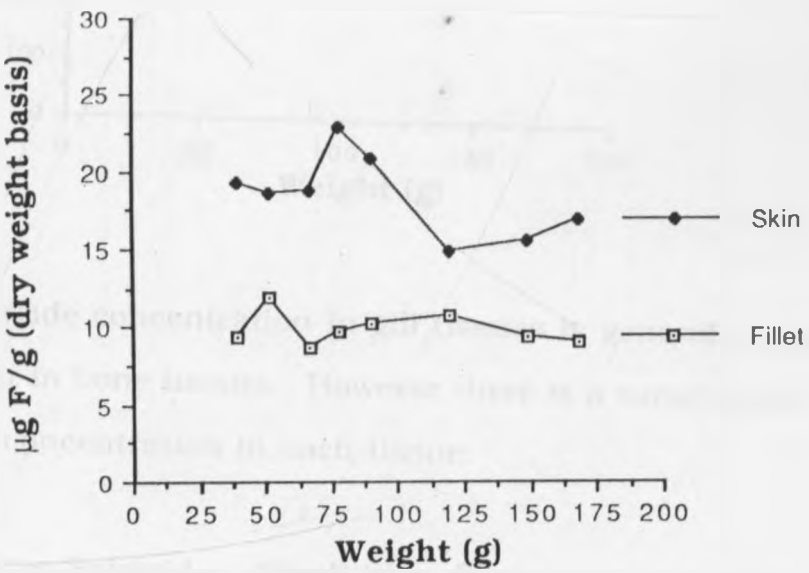
Fluoride concentration in gill tissues is generally lower than that in bone tissues . However there is variation of fluoride concentration in each tissue.

Tilapia zilii (Lake Naivasha)

The average fluoride concentration in fillet tissues ranged from 8.7-11.9 µg F/g. However, the fluoride concentration varied from 6.9-15.6 µg F/g. The average fluoride concentration in skin tissues ranged from 14.8-22.9 µg F/g. However, the lowest fluoride concentration in skin was 13.1 µg F/g while the highest fluoride concentration was 25.9 µg F/g. The average fluoride concentration in gill tissues ranged from 334-521 µg F/g. However, the fluoride concentration in

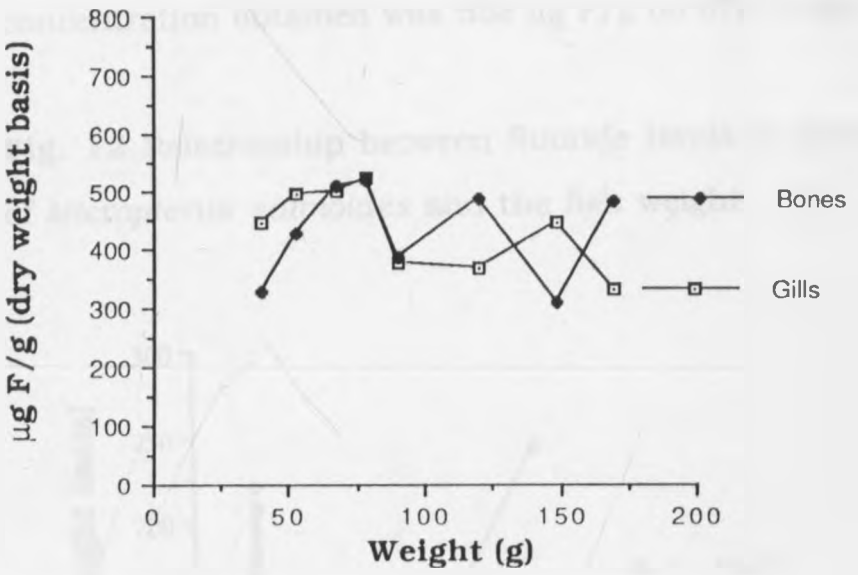
gills varied from 269-570 µg F/g. Bone tissues had an average fluoride concentration ranging from 309-519 µg F/g. However, the lowest fluoride concentration in bone was 280 while the highest fluoride concentration encountered was 608 µg F/g.

Fig. 10. Relationship between fluoride levels in fillet and skin of *Tilapia zilii* and the fish weight



In *Tilapia zilii* the fluoride concentration in the skin is approximately twice that of the fillet tissues.

Fig. 11. Relationship between fluoride levels in bones and gills of *Tilapia zilii* and the fish weight



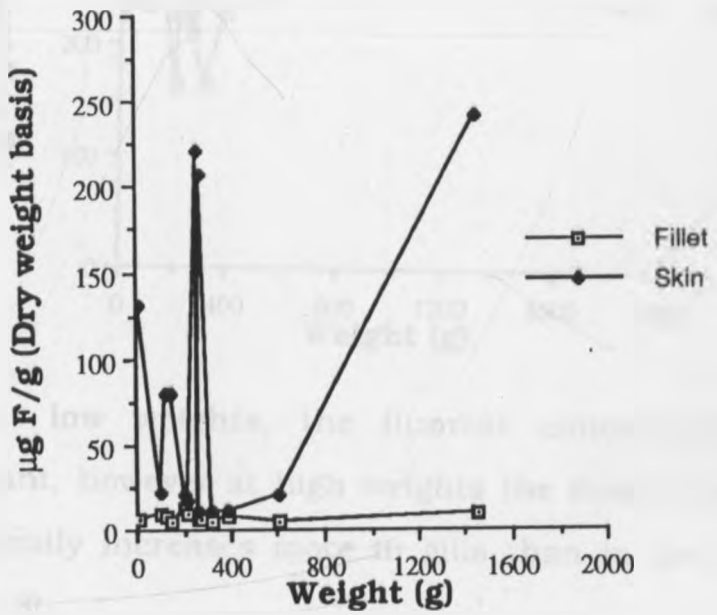
Fluoride concentration in gill tissues is generally lower than that in bone tissues . However there is a variation of fluoride concentration in each tissue.

Micropterus Salmoides (Black bass from Lake Naivasha)

The average fluoride concentration in fillet tissues ranged from 5.2-8.6 µg F/g of dry weight matter. However, the lowest value was 4 µg F/g while the highest value was 18 µg F/g of dry matter. The average fluoride concentration in skin tissues ranged from 11-221 µg F/g but the lowest value was 8 µg F/g while the highest value obtained was 286 µg F/g of dry matter. The average fluoride concentration in gill tissues ranged from 162-486 µg F/g. The lowest value of fluoride concentration was 150 µg F/g while the highest value obtained was 564 µg

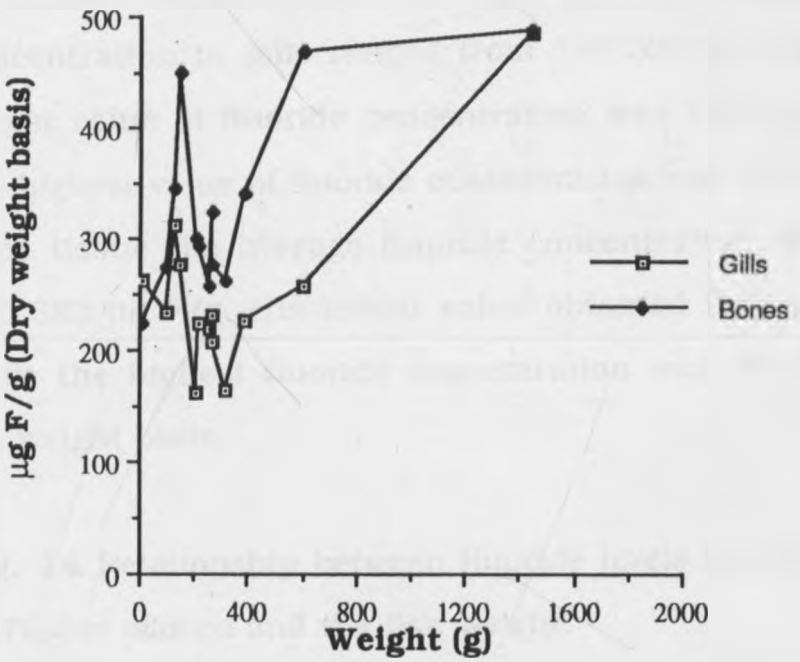
F/g. In bone tissues the average fluoride concentration ranged from 223-489 µg F/g, however the lowest fluoride concentration was 186 µg F/g while the highest fluoride concentration obtained was 558 µg F/g on dry weight basis.

Fig. 12 Relationship between fluoride levels in fillet and skin of *Micropterus salmoides* and the fish weight.



Fluoride concentration in fillet of *Micropterus salmoides* is fairy constant as compared to fluctuating fluoride concentration in skin tissues. Weight does not appear to affect fluoride concentration but from figure 12 it is hard to give a definite conclusion as to whether weight influences fluoride concentration in skin and fillet tissues of Black bass.

Fig. 13.Relationship between fluoride levels in gills and bones of *Micropterus salmoides* and the fish weight.



At low weights, the fluoride concentration is fairy constant, however at high weights the fluoride concentration apparently increases more in gills than in the bone tissues (Fig. 13).

4.4.2 Fluoride levels in fish from Lake Baringo

Oreochromis nilotica

50 fish from Lake Baringo *Oreochromis nilotica* (Tilapia) weighing between 156 g and 198 g were obtained and their muscles, skin, gills and bones prepared for analysis of fluoride. The average fluoride concentration in fillet ranged from 4.1-12.6 µg F/g. The lowest value of fluoride concentration was 2.9 µg F/g while the highest value obtained was 19.3 µg F/g of

dry weight tissue. In skin the average fluoride concentration ranged from 4.7-16.5 µg F/g.The lowest fluoride concentration was 4.4 µg F/g while the highest concentration of fluoride obtained was 45.1 µg F/g. The average fluoride concentration in gills ranged from 148-328 µg F/g. but the lowest value of fluoride concentration was 116 µg F/g while the highest value of fluoride concentration was 364 µg F/g. In bone tissue the average fluoride concentration ranged from 160-383 µg F/g, the lowest value obtained was 150 µg F/g while the highest fluoride concentration was 434 µg F/g on dry weight basis.

Fig. 14 Relationship between fluoride levels in fillet and skin of *Tilapia nilotica* and the fish weight.

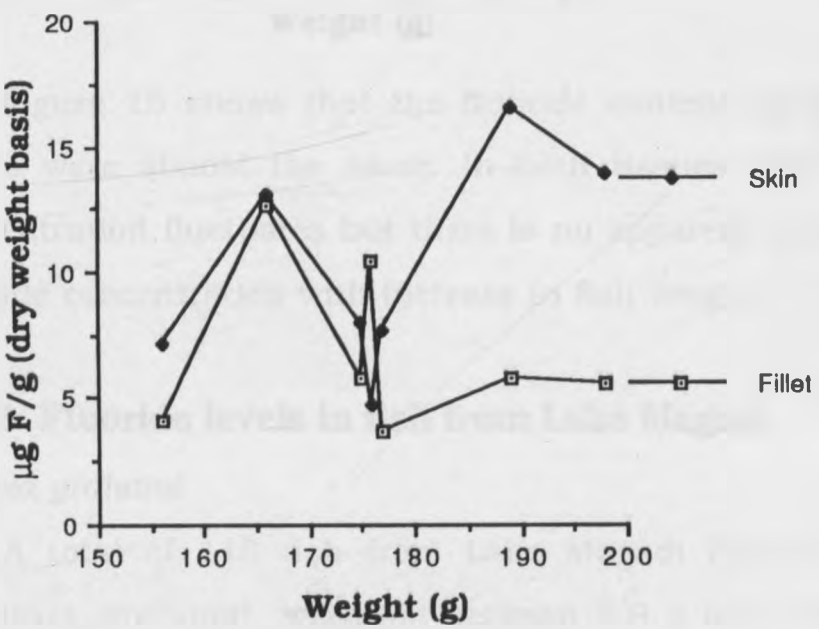


Figure 14. indicates that fluoride content in fillet is generally lower than fluoride concentration in skin. In both tissues the fluoride concentration fluctuates but there is no

apparent increase in fluoride concentration with increase in fish weight.

Fig. 15 Relationship between fluoride levels in gills and bones of *Tilapia nilotica* and the fish weight.

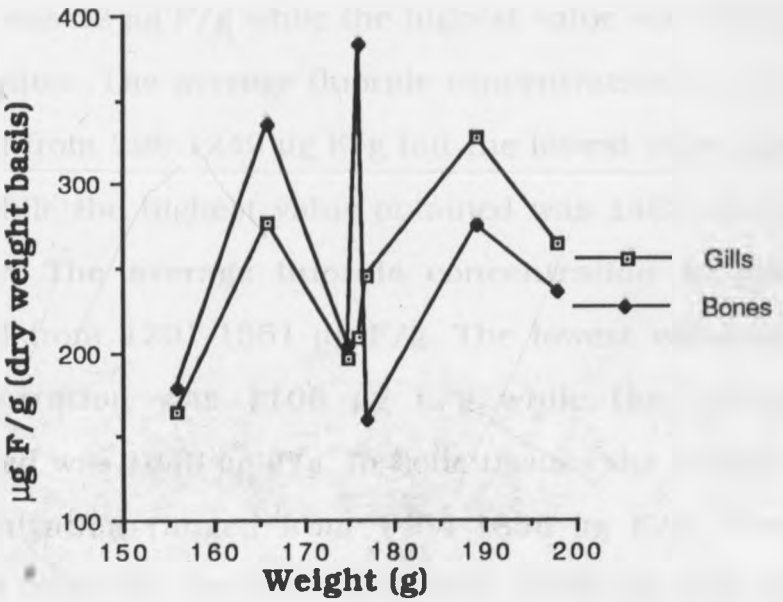


Figure 15 shows that the fluoride content in gills and bones were almost the same. In both tissues the fluoride concentration fluctuates but there is no apparent increase in fluoride concentration with increase in fish weight.

4.4.3 Fluoride levels in fish from Lake Magadi

Tilapia grahami

A total of 118 fish from Lake Magadi *(Oreochromis alcalicus grahami)* weighing between 3.9 g and 19 g were obtained and their muscles, skin gills and bones prepared for determination of fluoride as described in chapter 3. The water apparently contained about 84 ppm fluoride where the Tilapia

was caught in Lake Magadi. This exceeds by far the fluoride concentrations, other fish species are able to tolerate (Christensen 1987), and illustrates the remarkable ability of tilapia to survive under extreme conditions.

The average fluoride concentration in fillet tissues ranged from 24-142 µg F/g on dry weight basis. However the lowest value was 22 µg F/g while the highest value was 155 µg F/g of dry matter. The average fluoride concentration in skin tissues ranged from 298-1249 µg F/g but the lowest value was 275 µg F/g while the highest value obtained was 1482 µg F/g of dry matter. The average fluoride concentration in gill tissues ranged from 1201-1551 µg F/g. The lowest value of fluoride concentration was 1106 µg F/g while the highest value obtained was 1648 µg F/g. In bone tissues the average fluoride concentration ranged from 1454-1856 µg F/g, however the lowest fluoride concentration was 1290 µg F/g while the highest fluoride concentration obtained was 1904 µg F/g on dry weight basis.

Fig. 16. Relationship between fluoride levels in fillet and skin of *Oreochromis alcalicus grahami* and the fish weight.

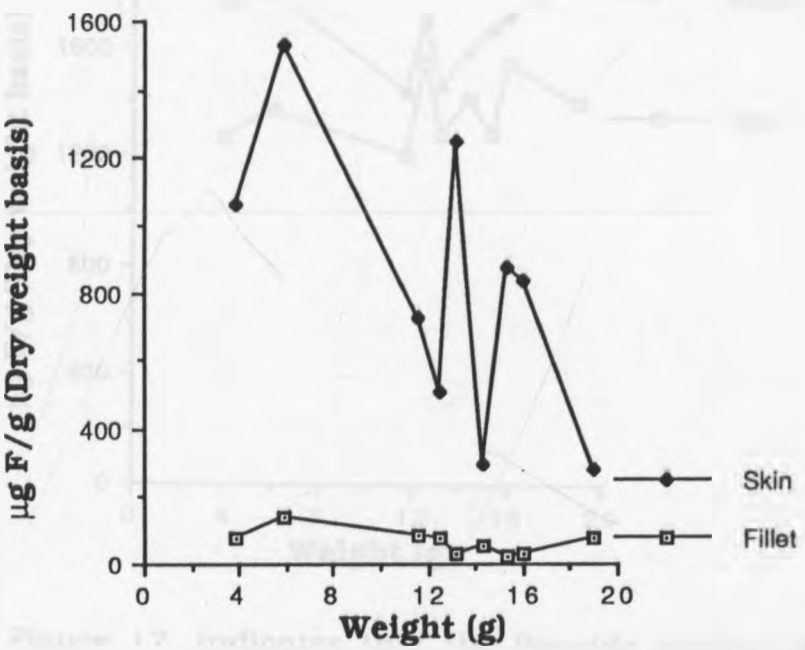


Figure 16. indicates that the fluoride content in fillet is lower than fluoride concentration in skin. In both tissues the fluoride concentration fluctuates but there is no increase in fluoride concentration with increase in fish weight. Apparently the fluoride concentration in skin tissue decreases as the weight of the fish increases while the fluoride concentration of the fillet is more or less constant.

Fig. 17. Relationship between fluoride levels in bones and gills of *Oreochromis alcalicus grahami* and the fish weight.

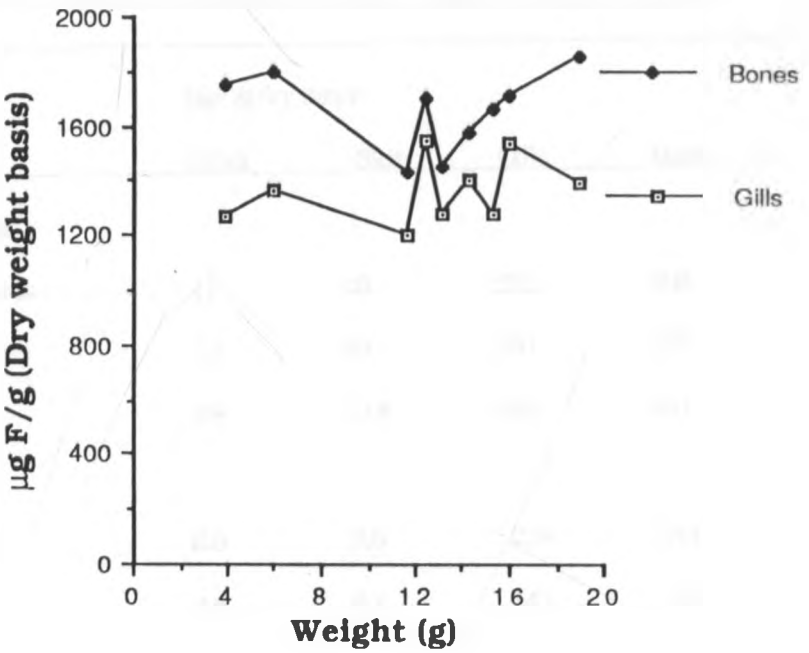


Figure 17. indicates that the fluoride content in gills is slightly lower than fluoride concentration in bones. In both tissues the fluoride concentration fluctuates but there is no apparent increase in fluoride concentration with increase in fish weight. Possibly there is a saturation level about which fluoride concentration fluctuates in each of these tissues.

4.4.4 Comparison of fluoride concentration in fish from lakes Naivasha, Baringo and Magadi.

Table 19 Relationship between the concentration of fluoride in various tissues in fish from Kenyan Lakes.

Lake	*µgF/g dry tissue			
Species	Fillet	Skin	Gills	Bones
L.Naivasha				
O.leucostictus	11	18	570	608
M.salmoides	7.3	83	251	330
T.zilli	9.9	18.4	435	431
L.Baringo				
Tilapia	6.6	9.6	229	244
Barbus	4.6	5.7	143	178
L.Magadi				
T.grahami	68.2	819	1366	1661

*The values given are means and detailed data can be found in the Appendix.

Arctic char accumulates fluoride when living in the high fluoride water environment especially in osseous tissues (Christensen, 1987).This applies also to fish from Lakes Magadi, Naivasha and Baringo. Fish from Lake Magadi tend to accumulate fluoride particularly in skin, gills, and bones. The fluoride concentration in skin is 12 times that of the fillet tissues of *Tilapia grahami* possibly because the skin is externally exposed to fluoride and also receives fluoride from the blood circulation.

The concentration of fluoride in fillet of fish from Lake Naivasha ranges from 7.3-11 µg F/g; *Micropterus salmoides* (Black Bass) having the lowest value (7.3 µg F/g) while *Oreochromis leucostictus* has the highest value (11 µg F/g). These values of fluoride concentration in the fillet closely agree with the observation from Wright and Davison, 1975 that fluoride is largely accumulated in skeletal structures with little or no accumulation in soft, edible tissues with the exception of fish skin.

Fish from Lake Magadi *Oreochromis alcalcus grahami* (Tilapia) contain more fluoride in their tissues than fish from Lakes Naivasha and Baringo. Lake Naivasha fish have higher fluoride levels than those from Lake Baringo.

The fluoride concentration in fillet, skin, gills and bones of Barbus fish from Lake Baringo are lower than those of *Tilapia nilotica* from the same lake. This could be due to species variation and possibly due to different feeding habits and growth rate.

The concentration of fluoride in water from Lake Magadi where the fish were living was 84 ppm, while Lake Naivasha water fluoride concentration was 2.6 ppm and Lake Baringo fluoride concentration was 5.4 ppm. It was surprising to find that though the water fluoride concentration in Lake Naivasha was lower than that of Lake Baringo, the fluoride level in tissues from Lake Baringo were lower than the fluoride concentration in fish tissues from Lake Naivasha on general consideration. This was probably due to the variation of the species.

Among chicken, beef, mutton, veal, pork and oysters, fish and fish products contained high levels of fluoride - 85 ppm in dry mackerel fish meat (Bhussry *et al.*, 1970). In Kenya, Anon 1965 reported fluoride concentration as high as 35 ppm in Distichodus flesh and 15 ppm in Tilapia flesh. All the fish were from Lake Turkana. Bergh and Haug, 1971, analysed Nile perch fish samples and found an increase in fluoride content with increase in size, contrary to our current finding.

It is therefore necessary to know the fluoride content of the individual food substances and water consumed by both human and livestock inorder to avoid food and water with high fluoride levels which could precipitate fluorosis. The highest muscle fluoride concentration was 68 ppm from *Tilapia grahami* found in Lake Magadi. The fluoride concentration in fillet from the rest of the fish from Lake Naivasha and Lake Baringo ranged from 5 to 11 ppm. Fluoride concentration of skin from *Tilapia grahami* was 809 ppm while skin from other fish was between 6 and 18 ppm. Skin tissue contained more fluoride than fillet for each particular species. Normally the fillet and the skin are consumed as food especially in fish from Lake Naivasha and Lake Baringo. The fluoride concentration in gills of *Tilapia grahami* was 1366 ppm while fluoride concentration in the other fish was between 143 and 570 ppm. The fluoride concentration of bone from *Tilapia grahami* was 1661 ppm while the fluoride concentration in bones from other lakes range from 178 to 606 ppm. Fish caught in water with high fluoride levels also

had high amounts of fluoride, which agrees with the finding of Milhaud *et al.*, 1981.

Though no evidence was found to indicate that fish from Lake Magadi is consumed by human beings, the fish is eaten by cormorant (*Phalacrocolax*) and pelican (*Pelecanus*) species of birds which are found in Lake Magadi. It is difficult to predict the effect of the high fluoride in the water and fish on the predator birds. However, the fish is unsuitable for human consumption due to the high fluoride content in the tissue which could amount to a health risk. Fish from Lakes Naivasha and Baringo are consumed locally and provide an important source of protein. Fish soup is very popular and it is usually combined with other starch food to make a meal. In preparation of fish, no part is removed except the scales and perhaps the fins. Therefore, fillet, skin, gills and bones are cooked together in most cases. It is not clear as to the amount of fluoride released by bones, gills and skin during the process of cooking. However, the fluoride released from tissues would probably depend on the fluoride concentration in the fish tissues, the duration of heating and the material used to make the sufuria or pot. Though the fish could contribute to high level of fluoride intake it is difficult to establish the fraction of the absorbed fluoride from the ingested fluoride.

The fluoride level in fillet of tilapia from Lake Magadi averaged 15 mg fluoride/kg (wet weight basis), being nearly the same as the concentrations (8-14) mg Fluoride/kg fillet) reported in four species of antarctic fish living in sea water (Manthey 1980). Two of the species examined by Manthey had

bone levels as high as 1,371 and 1,841 mg fluoride/kg, and accordingly exceeded the 641 mg fluoride/kg bones in tilapia from Lake Magadi. While Christensen (1987), in studies on char, found a correlation between bone fluoride concentration and the size of char living in water with 2-20 ppm fluoride, we were unable to demonstrate such a relationship.

Uptake of fluoride in fish may take place from water or from food. Sea water contains about 1.3 mg fluoride/L. The high levels in antarctic fish are ascribed to consumption of krill which concentrates and accumulates fluoride by several orders of magnitude. It may be concluded from our experiments, as well as from studies on antarctic fish, that even at a very high exposure to fluoride, will the fluoride level in the fillet remains relatively low.

On average the three species from Lake Naivasha contained 1.7-2.0 mg fluoride/kg (wet weight basis) fillet, and the tilapia from Lake Baringo 1.3 mg/kg. These values are somewhat higher than the 0.2-0.3 mg/kg reported in muscles of various species of fresh water and sea fish by Oelschlager *et al.* (1982) and Demmel *et al.* (1988).

4.4.5 Toxicological aspects In adults, a total daily intake of 1.5 to 4.0 mg fluoride is regarded as safe and adequate (United States National Research Council 1980). Assuming that all fluoride is bioavailable, consumption of 200 g fillet with 2 mg F /kg will provide 0.4 mg fluoride. The intake of fluoride might be considerably larger if, for example, fish soup is prepared by cooking fish with skin and bones, which is

common in the district. The skin contains markedly higher levels of fluoride than the muscles and the ingestion of muscles and the skin can increase the intake of fluoride attributable to fish, a similar conclusion was arrived at by Milhaud *et al.*, 1981. Particularly if consumed by children, the amounts provided by such soup might cause or contribute to the development of dental and skeletal fluorosis.

Chapter 5

conclusion

Highly alkaline water can give false levels of fluoride in water samples. This could explain the reporting of low and high fluoride in some of the water from alkaline lakes in Kenya. Hence the commonly used methods for analysing fluoride in water, might give erroneous or unreliable results when used in the analyses of water with such a high salt concentration and alkalinity, as found in some of the lakes of the Rift valley. More investigation in measurement of fluoride in alkaline water is required especially with regard to identification of the interfering ions.

Tests on the double-tube arrangement technique of fluoride extraction showed that fluoride concentration in the fish fillet, skin, gills and bones could be adequately quantified and hence the technique can be adopted for analysis of fluoride in fish and possibly other food substances. The recovery of added quantities of fluoride at various spiking levels were as follows (mean ± SEM %): blanks 99.6 ± 2 %, fillet 87.4 ± 4 %, skin 101 ± 16 %, gills 105 ± 6 % and Bones 109 ± 3%.

Fluoride concentration in fish tissues varied according to the species, and perhaps the feeding habit and fish growth rate. For instance in Lake Naivasha *Tilapia zilii* and *Oreochromis leucostictus* feed primarily on plant materials

while *Micropterus salmoides*, a carnivorous fish, feeds on frogs smaller fish and crabs.

Fish living in high fluoride environment also had high levels of fluoride in the tissues particularly *Tilapia grahami* from Lake Magadi. Lake Magadi Tilapia seems to be well adapted to living in high fluoride environment either by having a certain fluoride excretory mechanism or through a tissue fluoride saturation limit.

It may be concluded from our experiments, as well as from studies on antarctic fish, that even at a very high exposure to fluoride, the fluoride level in the fillet remain relatively low. However, the intake of fluoride might be considerably larger if, for example, fish soup is prepared by cooking fish with skin and bones. The skin contains markedly higher levels of fluoride than the muscles and the ingestion of muscles and the skin can increase the intake of fluoride from fish. Particularly if consumed by children, the amounts provided by such soup might cause or contribute to the development of dental and skeletal fluorosis.

Accordingly the present findings indicate that there is a need to carry out fluoride bioavailability studies in foodstuffs like soup which are prepared using fillet, skin, gills and bones which may contain high concentration of fluoride.

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Appendix 1 Per cent recovery of fluoride in blanks and fish tissues.

(5 or 6 parallel analyses were used in each spiking level.)

Sample	µg F- measured (µg F)	µg F- added (µg F)	recovery (%)
Blank	0.47	0.50	94.0
	0.43	0.50	86.0
	0.50	0.50	100.0
	0.53	0.50	106.0
	0.48	0.50	96.0
	0.54	0.50	108.0
Blank	1.58	1.50	105.3
	1.52	1.50	101.3
	1.58	1.50	105.3
	1.45	1.50	96.7
	1.49	1.50	99.3
	1.45	1.50	96.7
Fillet	0.78	0.50	70.0
	0.87	0.50	88.0
	0.87	0.50	88.0
	1.02	0.50	118.0
	0.93	0.50	100.0
Fillet	2.14	2.50	68.4
	2.29	2.50	74.4
	2.23	2.50	72.0
	2.32	2.50	75.6
	2.76	2.50	93.2

Appendix 1 (continued)

Sample	µg F- measured (µg F)	µg F- added (µg F)	recovery (%)
Fillet	9.30	10.0	88.7
	8.68	10.0	82.5
	9.61	10.0	91.8
	8.37	10.0	79.4
	9.92	10.0	94.9
	11.79	10.0	113.5
Skin	5.10	2.50	76.8
	5.85	2.50	106.8
	6.00	2.50	112.8
	6.30	2.50	124.8
	5.85	2.50	106.8
	5.70	2.50	100.8
Skin	12.40	10.0	92.0
	13.02	10.0	98.2
	13.02	10.0	99.2
	11.76	10.0	85.6
	13.02	10.0	98.2
	14.26	10.0	110.6
Gills	37.20	10.0	108.0
	34.10	10.0	77.0
	37.20	10.0	108.0
	35.70	10.0	93.0
	35.70	10.0	93.0
	34.10	10.0	77.0

Appendix 1 (continued)

Sample	µg F- measured (µg F)	µg F- added (µg F)	recovery (%)
Gills	62.70	30.0	121.0
	62.70	30.0	121.0
	66.00	30.0	132.0
	52.80	30.0	88.0
	67.70	30.0	138.0
	59.40	30.0	110.0
Bones	37.20	10.0	108.0
	37.20	10.0	108.0
	36.90	10.0	105.0
	35.90	10.0	93.0
	36.90	10.0	105.0
	35.70	10.0	93.0
Bones	62.70	30.0	121.0
	62.70	30.0	121.0
	62.70	30.0	121.0
	62.00	30.0	119.0
	59.40	30.0	110.0

Appendix 2 Recovery studies in potentiometric determination of fluoride in blank parallel tubes.

Sample	pH	F^-/ppm	F^-/μg	%Rec	X	S.D.	Range
1	5.2	-	-	-	-	-	-
2	5.0	-	-	-	-	-	-
3	5.0	0.150	0.47	94.0			
4	5.0	0.140	0.43	86.0			
5	5.1	0.160	0.50	100.0	98.3	8.1	86-108
6	5.1	0.170	0.53	106.0			
7	5.0	0.155	0.48	96.0			
8	5.0	0.175	0.54	108.0			
9	5.0	0.480	1.54	105.3			
10	5.1	0.460	1.52	101.3			
11	5.0	0.480	1.58	105.3	101	4	96-105
12	5.0	0.440	1.45	96.7			
13	5.0	0.450	1.49	99.3			
14	5.0	0.440	1.45	96.7			

X = mean

Appendix 3 pH and Fluoride concentration of lake Magadi spring water before and after buffering with TISAB II.

Dilution	pH before buffering , **Mean** Parallel(1, 2)	pH after buffering , **Mean** Parallel (1, 2)	F*- conc. (ppm) **Mean** Parallel (1,2)
0	**10.1** (10.0, 10.1)	**5.6** (5.6, 5.6)	**95** (95,95)
1:10	**10.3** (10.3, 10.3)	**5.3** (5.3, 5.3)	**77** (75,79)
1:25	**10.0** (10.0,10.0)	**5.3** (5.2, 5.3)	**61** (57,65)
1:50	**10.0** (10.0, 10.0)	**5.2** (5.2, 5.2)	**49** (48,50)
1:75	**10.1** (10.1, 10.1)	**5.3** (5.3, 5.3)	**72** (71,72)
1:100	**10.1** 10.1, 10.1)	**5.3** (5.3, 5.3)	**74** (72,75)
1:150	**9.6** (9.6, 9.6)	**5.3** (5.3, 5.3)	**74** (72,75)
1:200	**10.0** (10.0,10.0)	**5.3** (5.3, 5.3)	**72** (73,72)

Appendix 4 pH and fluoride concentration of water from the pond gate, before and after buffering with TISAB II.

Dilution	pH before buffering , **Mean** Parallel (1,2)	pH after buffering **mean** Parallel (1,2)	F*- conc. (ppm) **Mean** Parallel (1,2)
0	**9.8** (9.8,9.8)	**6.3** (6.4,6.3)	**93** (94,93)
1:10	**10.0** (10.0,10.0)	**5.0** (5.0,5.0)	**65** (90,90)
1:25	**10.0** (10.0,10.0)	**4.9** (4.9,4.9)	**65** (65,65)
1:50	**10.0** (10.0,10.0)	**4.9** (4.9,4.9)	**50** (50,50)
1:75	**9.5** (9.5,9.5)	**5.3** (5.3,5.3)	**73** (75,71)
1:100	**9.4** (9.4,9.4)	**5.3** (5.3,5.3)	**70** (71,70)
1:150	**9.8** (9.8,9.8)	**5.2** (5.2,5.2)	**73** (74,72)
1:200	**10.0** (10.0,10.0)	**5.3** (5.3,5.3)	**72** (71,73)

Appendix 5 pH and fluoride concentration of water from 100 m from the spring, before and after buffering with TISAB II.

Dilution	pH before buffering . **mean** Parallel (1,2)		pH after buffering **Mean** Parallel (1,2)		F*- conc. (ppm) **Mean** Parallel (1,2)	
0	**9.8**	(9.8,9.8)	**6.5**	(6.4,6.6)	**160**	(160,160)
1:10	**10.1**	(10.1,10.1)	**5.0**	(5.0,5.0)	**140**	(140,140)
1:25	**10.2**	(10.2,10.2)	**4.8**	(4.8,4.8)	**92**	(93,91)
1:50	**10.0**	(10.0,10.0)	**4.8**	(4.8,4.8)	**29**	(28,30)
1:75	**9.8**	(9.8,9.8)	**5.3**	(5.3,5.3)	**63**	(66,61)
1:100	**10.1**	(10.1,10.1)	**5.3**	(5.3,5.3)	**113**	(113,113)
1:150	**9.8**	(9.8,9.8)	**5.3**	(5.3,5.3)	**99**	(99,99)
1:200	**9.4**	(9.4,9.4)	**5.2**	(5.2,5.2)	**115**	(122,108)

Appendix 6 pH and fluoride concentration of water from near Lake Magadi entrance, before and after buffering with TISAB II.

Dilution	pH before buffering . **mean** Parallel (1,2)		pH after buffering **mean** Parallel (1,2)		F*- conc. (ppm) **mean** Parallel (1,2)	
0	**9.9**	(9.9,9.9)	**6.8**	(6.8,6.8)	**160**	(160,160)
1:10	**10.2**	(10.2,10.2)	**5.0**	(5.0,5.0)	**150**	(150,150)
1:25	**10.3**	(10.3,10.3)	**5.0**	(5.0,5.0)	**130**	(130,130)
1:50	**10.2**	(10.2,10.2)	**4.9**	(4.9,5.0)	**46**	(48,43)
1:75	**9.7**	(9.7,9.7)	**5.3**	(5.3,5.3)	**105**	(110,100)
1:100	**9.8**	(9.8,9.8)	**5.3**	(5.3,5.3)	**162**	(165,158)
1:150	**9.9**	(9.8,9.8)	**5.3**	(5.3,5.3)	**154**	(158,150)
1:200	**9.5**	(9.5,9.5)	**5.2**	(5.2,5.2)	**130**	(130,130)

Appendix 7 pH and fluoride concentration of water from lake Magadi spring , before and after buffering with TISAB III.

Dilution	pH before buffering , **mean**	Parallel (1,2)	pH after buffering **mean**	Parallel (1,2)	F*- conc. (ppm) **mean**	Parallel (1,2)
0	**9.7**	(9.7,9.7)	**6.1**	(6.2,6.1)	**95**	(95,95)
1:10	**10.0**	(10.0,10.0)	**5.0**	(5.0,5.0)	**80**	(80,80)
1:25	**10.0**	(10.0,10.0)	**4.9**	(4.9,4.9)	**64**	(75,63)
1:50	**10.0**	10.0,10.0)	**4.9**	(4.9,4.9)	**70**	(71,70)
1:75	**10.1**	(10.1,10.1)	**4.9**	(4.9,4.9)	**71**	(71,71)
1:100	**10.0**	(10.0,10.0)	**4.9**	(4.9,4.9)	**73**	(75,72)
1:150	**10.1**	(10.1,10.1)	**4.9**	(4.9,4.9)	**73**	(75,72)

Appendix 8 pH and fluoride concentration of water from the pond gate, before and after buffering with TISAB III.

Dilution	pH before buffering , **mean**	Parallel (1,2)	pH after buffering **mean**	Parallel (1,2)	F*- conc. (ppm) **mean**	Parallel (1,2)
0	**9.8**	(9.8,9.8)	**6.8**	(6.4,6.8)	**68**	(68,68)
1:10	**10.0**	(10.0,10.0)	**5.0**	(5.0,5.0)	**70**	(70,69)
1:25	**10.0**	(10.0,10.0)	**4.9**	(4.9,4.9)	**47**	(43,50)
1:50	**10.0**	(10.0,10.0)	**4.9**	(4.9,4.9)	**78**	(75,80)
1:75	**10.1**	(10.1,10.1)	**5.2**	(5.2,5.2)	**73**	(75,71)
1:100	**10.1**	(10.1,10.1)	**4.9**	(4.9,4.9)	**70**	(71,70)
1:150	**10.0**	(10.0,10.0)	**4.9**	(4.9,4.9)	**72**	(74,72)

Appendix 9 pH and fluoride concentration of water 100 m from the lake Magadi spring, before and after buffering with TISAB III.

Dilution	pH before buffering, mean	pH before buffering, Parallel (1,2)	pH after buffering, mean	pH after buffering, Parallel (1,2)	F*- conc. (ppm), mean	F*- conc. (ppm), Parallel (1,2)
0	**9.8**	(9.8,09.8)	**6.5**	(6.4,6.6)	**150**	(150,150)
1:10	**10.1**	(10.1,10.1)	**5.0**	(5.0,5.0)	**100**	(100,100)
1:25	**10.2**	(10.2,10.2)	**4.8**	(4.8,4.8)	**138**	(140,139)
1:50	**10.0**	(10.0,10.0)	**4.8**	(4.8,4.8)	**138**	(140,135)
1:75	**10.4**	(10.4,10.4)	**5.2**	(5.2,5.2)	**118**	(118,118)
1:100	**10.2**	(10.2,10.2)	**4.9**	(4.9,4.9)	**64**	(66,61)
1:150	**10.1**	(10.1,10.1)	**4.9**	(4.9,4.9)	**99**	(99,99)

Appendix 10 pH and fluoride concentration of water near Lake Magadi entrance, before and after buffering with TISAB III.

Dilution	pH before buffering, mean	pH before buffering, Parallel (1,2)	pH after buffering, mean	pH after buffering, Parallel (1,2)	F*- conc. (ppm), mean	F*- conc. (ppm), Parallel (1,2)
0	**9.9**	(9.9,9.9)	**6.8**	(6.8,6.8)	**180**	(180,180)
1:10	**10.2**	(10.2,10.2)	**5.0**	(5.0,5.0)	**160**	(160,160)
1:25	**10.3**	(10.3,10.3)	**5.0**	(5.0,5.0)	**187**	(187,187)
1:50	**10.2**	(10.2,10.2)	**4.9**	(4.9,4.9)	**175**	(175,175)
1:75	**10.5**	(10.5,10.5)	**5.2**	(5.2,5.2)	**161**	(165,157)
1:100	**10.2**	(10.2,10.2)	**4.9**	(4.9,4.9)	**105**	(110,100)
1:150	**10.1**	(10.1,10.1)	**4.9**	(4.9,4.9)	**150**	(158,150)

* Fluoride concentration adjusted for the dilution.

Appendix 11 Fluoride levels in various tissues of *Oreochromis leucostictus* of different weights from Lake Naivasha.

Weight	µg F/g of dry tissue, **mean** (range).			
(g)	Fillet	Skin	Gills	Bones
33	**10.9** (7.1-14.3)	**21.4** (14.0-23.2)	**688** (590-782)	**633** (589-721)
36	**10.6** (8.6-12.6)	**18.5** (16.4-20.8)	**573** (491-610)	**642** (517-705)
38	**11.6** (7.8-13.9)	**22.5** (16.0-25.8)	**531**(465-591)	**608** (518-664)
41	**17.2** (16.4-18.4)	**24.6** (19.0-28.7)	**600** (525-662)	**490** (457-516)
43	**13.3** (11.3-16.8)	**20.4** (15.8-27.5)	**843** (756-987)	**594** (564-635)
46	**18.3** (16.0-24.0)	**23.8** (22.5-25.3)	**588** (556-700)	**537** (450-577)
51	**14.1** (9.3-17.3)	**19.3** (14.3-25.1)	**467** (383-536)	**471** (412-519)
56	**10.0** (9.1-13.0)	**15.4** (12.8-21.0)	**594** (551-657)	**747** (662-823)
59	**6.0** (4.9-6.9)	**29.0** (24.6-38.8)	**603** (119-164)	**546** (445-595)
133	**8.1** (7.4-9.0)	**12.3** (11.3-14.4)	**597** (544-649)	**744** (656-784)
145	**7.4** (6.3-8.7)	**11.0** (8.0-13.0)	**717** (642-784)	**671** (591-729)
173	**6.7**(5.9-7.5)	**13.0** (9.9-16.3)	**370** (331-429)	**637** (575-699)
223	**7.9** (7.1-9.4)	**11.8** (7.5-13.5)	**309** (297-333)	**556** (502-586)
250	**6.8** (6.0-7.6)	**11.4** (11.1-13.2)	**504** (306-724)	**633** (603-693)

Appendix 12 Fluoride levels in various tissues of *Tilapia zilli* of different weights from Lake Naivasha.

Weight	µg F/g of dry tissue, **mean** (range)							
(g)	Fillet		Skin		Gills		Bones	
40	**9.3**	(7.7-10.0)	**19.3**	(15.1-22.6)	**444**	(386-502)	**329**	(303-378)
52	**11.9**	(8.9-15.6)	**18.6**	(15.0-24.0)	**494**	(417-541)	**427**	(348-460)
67	**8.7**	(7.9-9.2)	**18.7**	(14.9-25.8)	**498**	(431-558)	**508**	(422-606)
78	**9.8**	(8.5-11.8)	**22.9**	(19.7-25.9)	**521**	(460-570)	**519**	(354-608)
90	**10.3**	(6.9-12.4)	**20.8**	(19.0-22.6)	**377**	(299-409)	**388**	(347-411)
120	**10.8**	(9.0-13.0)	**14.8**	(13.4-16.3)	**368**	(348-412)	**486**	(421-547)
149	**9.3**	(7.8-9.8)	**15.5**	(13.1-18.5)	**447**	(343-630)	**309**	(280-364)
169	**9.1**	(8.4-10.3)	**16.9**	(15.4-18.8)	**334**	(269-379)	**481**	(455-506)

Appendix 13 Fluoride levels in tissues of Tilapia fish of different weight from Lake Baringo.

Weight	µg F/g of dry tissue, **mean** (range)							
(g)	Fillet		Skin		Gills		Bones	
156	**4.1**	(3.7-4.3)	**7.2**	(5.2-8.9)	**165**	(156-174)	**179**	(160-203)
166	**12.6**	(10.0-19.3)	**13.0**	(10.0-14.0)	**277**	(223-308)	**336**	(294-363)
175	**5.8**	(4.9-6.1)	**8.0**	(6.6-10.0)	**197**	(171-235)	**205**	(171-215)
176	**10.4**	(8.9-13.6)	**4.7**	(4.5-45.1)	**209**	(188-224)	**383**	(355-434)
177	**3.7**	(2.9-4.3)	**7.7**	(4.4-12.7)	**246**	(225-272)	**160**	(150-169)
189	**5.8**	(5.2-6.7)	**16.5**	(11.8-23)	**328**	(305-364)	**275**	(249-294)
198	**5.6**	(5.0-5.7)	**13.9**	(11.8-16.7)	**264**	(224-276)	**235**	(227-243)
159*	**4.6**	(3.1-6.4)	**5.7**	(4.4-6.6)	**143**	(116-153)	**178**	(150-197)

*Barbus fish from Lake Baringo.

Appendix 14 Fluoride content in *Micropterus salmoides* (Black bass) fish from Lake Naivasha

Weight	µg F/g dry tissue **Mean** (range)							
(g)	Fillet		Skin		Gills		Bones	
5.8	**6.2**	(6-7)	**130**	-	**263**	-	**223**	-
103	**9.4**	(6.8-12.)	**22**	(18-25)	**234**	(198-247)	**273**	(225-317)
127	**7.2**	(7-8)	**80**	(50-90)	**313**	(276-333)	**345**	(264-401)
147	**5.2**	(3-13)	**80**	(58-117)	**277**	(249-317)	**449**	(389-464)
207	**13**	(12-17)	**25**	(22-31)	**162**	(155-167)	**299**	(287-315)
221	**9.6**	(9.8-11)	**17**	(13-20)	**224**	(161-183)	**292**	(247-362)
261	**6.8**	(4-11)	**221**	(184-250)	**220**	(184-250)	**257**	(194-313)
264	**5.9**	(5-7)	**11**	(8-13)	**232**	(185-260)	**324**	(304-354)
269	**5.8**	(5-7)	**207**	(189-265)	**207**	(189-265)	**275**	(259-296)
316	**5.4**	(4-7)	**10**	(8-13)	**165**	(150-186)	**262**	(186-326)
386	**7.2**	(6-8)	**12**	(9-13)	**227**	(182-245)	**340**	(278-392)
604	**5.2**	(4-7)	**20**	(14-25)	**256**	(191-316)	**468**	(347-527)
1448	**8.6**	(7-18)	**241**	(188-286)	**486**	(408-564)	**489**	(428-558)

Appendix 15 Fluoride levels in various tissues of *Oreochromis alcalicus grahami* of different weight from Lake Magadi.

Weight	µg F/g of dry tissue, mean (range)							
(g)	Fillet		Skin		Gills		Bones	
3.9	**80**	(59-114)	**1064**	(901-1194)	**1273**	(1151-1382)	**1754**	(1659-1849)
5.9	**142**	(120-155)	**1527**	(1469-1613)	**1367**	(1340-1373)	**1801**	(1630-1891)
11.6	**84**	(77-98)	**728**	(629-905)	**1201**	(1106-1285)	**1431**	(1290-1606)
12.5	**81**	(62-97)	**510**	(438-625)	**1551**	(1506-1648)	**1704**	(1666-1798)
13.2	**35**	(31-43)	**1249**	(1116-1482)	**1283**	(1210-1382)	**1454**	(1329-1607)
14.3	**54**	(49-60)	**293**	(275-308)	**1401**	(1279-1444)	**1579**	(1452-1689)
15.3	**24**	(22-28)	**874**	(806-1002)	**1280**	(1173-1458)	**1661**	(1521-1737)
16.0	**33**	(27-43)	**839**	(683-1012)	**1541**	(1517-1637)	**1710**	(1652-1751)
19.0	**81**	(71-88)	**284**	(268-312)	**1395**	(1290-1481)	**1858**	(1821-1904)

Appendix 16 Letter to Orion

Dr. J. K. Gikunju
Department of Public Health
Pharmacology and Toxicology.
University of Nairobi
P.O. Box 29053 Kabete
Nairobi
KENYA

Orion Research AG
Fahnlibrunnenstrasse 3
CH-8700 Kusnacht
SWITZERLAND

Dear Sirs,

I am a postgraduate student working on a M. Sc. project in the University of Nairobi. As part of my project I am studying fluoride concentrations in various tissues of fish from Kenyan lakes, as well as the fluoride concentrations in the water of certain lakes. Some of the lakes in the Rift Valley region are very alkaline and have a high salt content.

There are some published data on the fluoride concentrations in these lakes (Williamson, *E. Afr. Med. J.*, 1953, 30: 217-233; Bergh & Haug, E. *Afr. Agr. Forest J.*, 1971, 36: 392-400: Gitonga & Nair, Technical Report, IDRC/Univ. Nairobi, 1982; Kariuki *et al.*, 1984 pp. 32-37 in Fluorosis Research Strategies, Afr. Med. Res. Found.,). The reported values varies considerably, e.g. for Lake Nakuru from 140 to 2,800 ppm F; Lake Turkana from 10.2 to 100 ppm F. There might be several explanations for the variations in the reported values; e.g. the time of the year when the water was collected (how much the lake was dried up). Another possibility is that the analyses have not been quite properly carried out.

During my measurements I have observed a phenomena, which I would be most grateful if your company would be willing to comment on, since it might be related to the proper use of the fluoride ion electrode and complexing buffers.

I have used the following equipment and chemicals: Digital ionalyzer (701A, Orion), Digital pH meter (3020 Orion), fluoride combination electrode (96-09 Orion), automatic voltage stabilizer (Samlex, UK), magnetic stirrer and a teflon coated bar, plastic beakers and tubes, electrode filling solution 90-00-01 Orion), pH electrode storage solution (91-00-01 Orion), fluoride standard solution , 100 ppm (94-09-07 Orion), TISAB II (94-09-09 Orion), TISAB III (940911 Orion).

Standards of 0.1, 1.0 and 10.0 ppm were prepared by diluting with deionized water. According to the recommendations 1 ml of each standard was added to 1 ml TISAB II and 3 ml of each standard added to 0.3 ml of TISAB III. When in the same way samples of water from the more alkaline lakes were added to the TISAB's, the pH ranged from 5.6 and 7.8. To obtain an appropriate pH it was decided to dilute the original water samples with deionized water before adding the TISAB complexing and buffering solutions.

Lake Magadi is one of the lakes I have studied. Kariuki *et al.* reported 1480 ppm F in water from this lake. The pH of the lake is about 10.1, and it has a very high salt content. Never-the-less, there is fish in the lake.

Water samples were collected from five different sites of the lake, and diluted as indicated in Fig. 1-4 (TISAB II) and Figs. 5-8 (TISAB III). The fluoride concentration after correction of the dilution factor are illustrated in fig.1 -8.

Samples 1, 3, 4, and 5 were collected at different sites of Lake Magadi. You will notice that the apparent fluoride concentrations after correction for the dilutions varies considerably. The variations are most marked for the samples with the highest fluoride concentrations. For example as illustrated in Fig. 3, at a dilution of 1:50 the fluoride concentration is apparently 29 ppm (actual reading 0.58 ppm F), while at 1:100 the concentration is apparently 113 ppm F (actual reading 1.13 ppm F).

I will be most grateful if you have time and are willing to offer me an explanation.

Yours sincerely,

Dr. Joseph K. Gikunju

Appendix 17 Photocopy of a reply letter from Orion.

October 30, 1989

Dr. J.K. Gikunju
Department of Public Health
Pharmacology and Toxicology
University of Nairobi
PO Box 29053 Kabete
Nairobi
Kenya

Dear Dr. Gikunju,

This is in reply to the letter and very detailed data which you sent to our European office on the problems you were having with fluoride measurements.

I believe I know what the problem may be. When I designed TISAB, it was intended solely for use in measuring fluoride in drinking water. We found that there were discrepancies in measured fluoride in drinking water depending on nature of the water treatment process. A very common procedure is to add alum or some form of aluminum sulfate, and then to precipitate it by adding lime. This results in a gelatinous aluminum hydroxide precipitate which carries off algae and many other visible inpurities. In some drinking water plants there was an excess of aluminum or iron present, and this resulted in some of the fluoride being complexed. Since the electrode responds to free fluoride activity, erroneous concentration results were obtained.

We attempted to decomplex the fluoride by adding other complexing agents (such as CDTA or tartrate) which would more tightly complex aluminum or iron and thus free the fluoride. The level of complexing agents was based on levels which might be expected in drinking water. I believe that in your situation there are interactions and various complexes being formed which interfere with your measurement.

Orion Research Incorporated
THE SCHRAFFT CENTER
529 MAIN STREET
BOSTON, MA 02129 USA
TELEPHONE 617 242 3900 / TELEX 4430019

The evidence from your data which suggests this is the case is that as the samples are diluted, the apparent fluoride level actually increases. This would be expected if weak complexes were present. Without knowing the detailed composition of the lake waters, it is difficult to anticipate what complexes might be present. One has to consider not only the strength of the complexes, but how quickly they would be decomplexed in the presence of other agents or on dilutions.

My suggestions would be as follows:

1. Work with as high a dilution as you can, consistent with remaining on the straight line portion of the curve.

2. You can check for the formation of the complexes by doing known additions. That is, if you double the concentration of fluoride in a diluted sample, you should see close to a 18 mV change.

3. I would experiment with the use of citrate or tartrate as decomplexing agents and I would work as far on the alkaline side as the fluoride level permits.

The work you are doing sounds extremely interesting. I hope you will send us a reprint when it is published.

Sincerely yours,

Dr. Martin Frant
Technical Director

cc: Orion AG

MF/jb
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