FLUORIDE CONTENT OF LEAFY VEGETABLES, IRRIGATION WATER, AND FARMLAND SOIL IN THE RIFT VALLEY AND IN NON-RIFT VALLEY AREAS OF ETHIOPIA

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ABSTRACT: The main objective of this study was to determine the fluoride content of leafy vegetables (lettuce, Swiss chard, cabbage, and Abyssinian cabbage), irrigation water, and farmland soil in the Rift Valley and in non-Rift Valley areas of Ethiopia. Fluoride contents of leafy vegetables cultivated in six farms were determined using alkali fusion – ISE technique and were found to be in the range (mg/kg dry weight): lettuce 2.95–5.76; Swiss chard 2.74–5.40; cabbage 2.12–2.70, and Abyssinian cabbage 2.08–2.59. The range of the fluoride levels in the irrigation water bodies in the study area was 0.43–7.66 mg/L. The ranges of the soluble and total fluoride levels in the studied farmland soil were: 4.30–23.4 and 133–802 mg/kg, respectively. Higher fluoride levels were observed in the vegetables cultivated in the Rift Valley farms compared to the non-Rift Valley farms. Similarly, higher fluoride levels were also observed in the irrigation water and the farmland soil in the Rift Valley area. Statistical analysis with ANOVA showed a significant difference between the mean fluoride contents of the vegetables at the $p<0.05$ confidence level. The Pearson correlation showed a variable (weak, moderate, or strong) relationship between the fluoride levels in the irrigation water and the vegetables, and between the fluoride levels in the soil and in the vegetables. In general, for those who regularly consumed these vegetables, the leafy vegetables were found to contribute a significant amount of fluoride to the total fluoride intake.

Keywords: Ethiopia; Farmland soil; Fluoride; Irrigation water; Leafy vegetables; Rift Valley.

INTRODUCTION

Ethiopia has a diverse range of altitude and climatic conditions which is conducive to various agricultural activities. The several lakes, perennial rivers, and about 2.6 billion cubic meters of ground water have a great potential for agricultural irrigation. The potential land area for irrigation is estimated at 10 million hectares, out of which only about 1% is currently under irrigation.[1]

Ethiopia's favorable weather, altitude, adequate water, availability of suitable soils, diversified agro-ecology, and potential to develop horticultural crops, such as fruits, vegetables, and root crops, mean that the country is well suited for horticultural production.[1,2]

Vegetables are important ingredients in the human diet since they contain carbohydrates, proteins, vitamins, minerals, trace elements, and dietary fiber, as well as having beneficial antioxidative effects. In the past, they were not considered as a major part of the Ethiopian diet except during the fasting period. Recently, however, the consumption of vegetables has increased in urban areas as a result of exposure to different cultures and a greater awareness of the food value of vegetables.[3]

Vegetable production in Ethiopia ranges from home gardening and smallholder farming to commercial farms owned by both public and private enterprises. This

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production is important for food security, human nutrition and health as a source of vitamins, minerals, antioxidants, dietary fiber, and compounds with anti-carcinogenic properties, raw material for agro-industries, and being a source of employment as it needs intensive labor and foreign currency. Vegetables serve as suitable crops for the diversification of farming systems and land intensification while maintaining ecological balance with the use of diverse species, particularly with recent increases in the establishment of small and medium scale irrigation schemes in the country. Ethiopia's diverse agro-ecologies are suitable for the production of vegetables in the tropical, sub-tropical, and temperate climatic zones.[2] Smallholder irrigation vegetable production in the Central Rift Valley region of Ethiopia is instrumental in ensuring an all-year-round availability of fresh vegetables in the country's local markets.[4]

In Ethiopia, vegetable cultivation took up about 1.18% (160,050 ha) of all the area under crops at a national level and the production of the vegetables lettuce, head cabbage, Ethiopian cabbage, tomatoes, green peppers, red peppers, and Swiss chard was 75,572 kg (7,557,282 quintal, 1 quintal = 100 kg). However, of the total estimated area under vegetable cultivation, the lion's share was used for growing red peppers and Ethiopian cabbage, 67.98% and 19.86%, respectively. Vegetable production contributed 2.78% of the total production of all crops, with red peppers and Ethiopian cabbage accounting about 31.69% and 42.76%, respectively, of the total vegetable production.[5] There has been a marked increase in vegetable and fruit production since 2010/2011 with land under cultivation for this purpose doubling.[1]

Lettuce (*Lactuca sativa*) belongs to the Asteraceae family (formally Compositae). Lettuce originated in a region occupying parts of Iran and Turkey and is likely a descendent of a wild lettuce (*Lactuca serricola*). Lettuce is one of the most important salad vegetables. Today, for organic producers, lettuce represents one of the most common and highest grossing products for fresh, local markets and the different classes of lettuce are distinguished by their morphologies and end use.[6]

Swiss chard (*Beta vulgaris var. cicla*) is basically a beet grown for its tops. It is probably the ancestor of our common beet. It is available in red, pink, yellow, and green leaf forms and is one of our winter garden staples. The green leaf forms tend to be hardier. Older leaves are always more strongly flavored. Swiss chard stands summer heat quite well if adequately watered. It can be harvested by pulling the outer leaves as needed or by cutting it off just above the crown and letting it resprout. The latter method usually gives more tender leaves and less stem. This can be a very ornamental plant and a good source of winter greens in mild climates.[7]

Cabbage (*Brassica oleracea, var. capitata*) has been cultivated at least 2,000 years. The cabbage started as a loose head in the Middle East, but moved to Europe and Asia thousands of years ago. It developed its firm head only in the 16th century. Ornamental cabbages look like giant roses. It can be stored in a root cellar environment for months if harvested with the roots attached. Red cabbages seem to be more insect and disease resistant. They are also very winter hardy. Over watering may increase club root. Cabbage is a heavy user of P, K, and S. Sometimes this whole family requires extra boron.[7] Cabbages have low carbohydrates, fats, and calories.

They are a good source of protein (balanced), minerals, vitamin A, vitamin C, and other vitamins. Cabbages are also known to contain compounds with anticancer properties including antioxidants, ascorbic acid, tocopherols, carotenoids, isothiocyanates, indoles, and flavanoids.

Abyssinian cabbage (*Brassica carinata*) belongs to the Brassicaceae family and is a fairly hardy, flavoursome, nutritious greens type vegetable originating from Ethiopian mustard. It is fast growing and popular for salad leaves if it is cut while young and tender. Texsel is particularly adapted to temperate climates. It is cultivated for its edible leaves in some areas and plants that are given some protection from the cold can supply edible leaves throughout all the winter. Leaves and young stems are used raw or cooked. The young growth has a mild and pleasant cabbage flavour and can be cut finely and used in mixed salads, whilst older leaves are cooked like cabbage leaves. When the immature flowering stems are cooked and used like broccoli, they make a nice vegetable. Edible oil is obtained from the seed. Oil from the wild species is high in erucic acid, which is toxic, though there are some cultivars that contain very little erucic acid and can be used as food.[8-10]

The major dietary sources of fluoride for most people are from drinking water, food, and beverages.[11] Fluoride exposure of human beings depends mainly on the water quality and the fluoride concentration in water depends on several contributing factors such as pH, total dissolved solids, alkalinity, and hardness.[12] The amount of fluoride occurring naturally in groundwater is governed principally by the climate, the composition of the host rock and hydrogeology, and some anthropogenic activities such as the use of phosphate fertilizers, pesticides, sewage and sludge for agriculture, depletion of groundwater, etc.[13] The prescribed norm for the safe fluoride limit in water is 0.8–1.5 mg/L.[14,15]

Fluoride is more soluble in acid soils and in these soils its uptake by plants is enhanced and it is mainly accumulated in the leaves. The excess accumulation of fluorides in vegetation leads to visible leaf injury, damage to fruit, and changes in the yield.[16] The fluoride levels of specific food vegetables depends upon the nature of the soil and the quality of the irrigation water, and so that the levels vary from place to place.[17,18]

The public health problem of fluorosis is prevalent in the areas with high water fluoride levels found in the Rift Valley region of Ethiopia, which is characterized by relatively high volcanic activity.[19] The excessive intake of fluoride in the Rift Valley of Ethiopia causes adverse human health effects with the chronic, endemic diseases of dental, skeletal, and non-skeletal fluorosis.[20] The food chain also makes a significant contribution to the total fluoride exposure of animals, as well as humans, particularly in areas with high concentrations of fluoride in water, soil, and biota.[21]

Vegetables and fruits normally contain fluoride at low concentrations (0.1–0.4 mg/kg). However, high fluoride concentrations in some leafy vegetables have been reported, for example in spinach, 25.70–29.15 mg/kg,[18,22,23] and in cabbage, 2–20 mg/kg.[24]

In the past, different scholars have suggested that the systemic ingestion of fluoride had both beneficial and detrimental effects on human health, particularly, for the formation and development of the dental and skeletal parts of the human body.[12,25-27] However, this scenario has now changed for several reasons: (i) the evidence that the systemic ingestion of fluoride via drinking water has a protective effect on dental caries is now less convincing, (ii) fluoride is no longer seen to be an essential element for human growth and development, (iii) the systemic exposure to fluoride is associated with an increased risk of dental and bone fluorosis, and (iv) although limited, there is evidence from epidemiological studies which points to other adverse heath effects occurring after systemic fluoride exposure, e.g., carcinogenicity, developmental neurotoxicity, and reproductive toxicity.[28] In contrast, it now considered that there is strong evidence that the topical application of fluoride to the teeth has a protective cariostatic effect, e.g., fluoridated toothpaste.[28]

Several studies have been done on water, beverages, and food stuffs in Ethiopia.[21,29-39] However, no study has been conducted on the fluoride content of leafy vegetables in Ethiopia. Hence it is worthwhile to determine the fluoride content of the common leafy vegetables cultivated and consumed in Ethiopia.

The main objective of this study was to determine the fluoride content in selected leafy vegetables cultivated in both the Rift Valley and in non-Rift Valley areas of Ethiopia: lettuce (*Lactuca sativa*), Swiss chard (*Beta vulgaris var. cicla*), cabbage (*Brassica oleracea, var. capitata*), and Abyssinian cabbage (*Brassica carinata*). The specific objectives were: (i) to determine, in both Rift Valley and in non-Rift Valley areas in Ethiopia, the fluoride contents of irrigation water, farmland soil, and selected leafy vegetables; (ii) to compare the fluoride content of selected leafy vegetables in the study areas; (iii) to compare the fluoride content of the leafy vegetables cultivated in Ethiopia with the values in the literature; and (iv) to correlate the levels of fluoride in the vegetables, irrigation water, and farmland soil.

MATERIALS AND METHODS

Study areas: The selection of the study areas in the Rift Valley was based upon observations of the severity of dental fluorosis through a literature review of related work, the accessibility of the areas for sampling, the availability of selected leafy vegetables during sampling, the vegetables being a common part of the diet for the communities, and the fluoride level in the nearby water bodies. The non-Rift Valley areas selected also had the same selection criteria, except that no dental fluorosis was observed in the communities in the areas selected. A total of six areas were selected for the study. The Rift Valley areas of Hawassa (Hawassa Zuria Woreda), Ziway (Dugda Woreda), and Wonji Shoa (Adama Woreda) and the non-Rift Valley areas of Akaki, Kera, and Pecock Park were selected from the Southern Nations, Nationalities, and Peoples' Region (SNNPR), the Oromia Regional State, and the Addis Ababa Administration.

Sample collection: The selected leafy vegetable samples (lettuce, Swiss chard, cabbage, and Abyssinian cabbage), the soil samples, and the irrigation water samples

were collected using random sampling technique from three sub sites nearly 2–3 km apart for each study area and thoroughly mixed for each sample type and size.

The leaves of the selected vegetable types were collected from five matured vegetables chosen randomly from the center and the four corners for each sub site and thoroughly mixed for each vegetable type and for each study area independently to get a sample size of approximately 500 g. The samples were collected in polyethylene bags and transported to the laboratory for further preparation and treatment to give a total of 24 vegetable samples from the six sampling sites.

Six composite farmland soil samples were also collected from the surface soil where the leafy vegetables samples grown and sampled. Five spots were collected from the center and the four corners for each sub site and thoroughly mixed on a plastic tray. These spots were taken from the root zone, i.e., 15–20 cm depth in a random fashion with a recommended auger. The quartering method was used to get homogeneous and representative 1 kg soil samples. The soil samples were stored in a polyethylene plastic bag and transported to the laboratory for further preparation.

A total of six irrigation water samples were collected from the main water body, i.e., the rivers and lakes in the respective study areas (the Hawassa and Ziway lakes, and Awash, Akaki, Kera, and Bulbula rivers) which were used for the irrigation of the leafy vegetables, at the point of diversion in the study area in pre-cleaned and labeled 500 mL polyethylene bottles and transported to the laboratory for further treatment and preparation.

Apparatus and instruments: Polyethylene plastic bags and 500 mL polyethylene plastic bottles were used for collecting the vegetable leaves, soil samples, and water samples. An oven (Digitheat, J.P. Selecta, Spain) was used to dry the samples and an electronic blending device (Geepas electric coffee grinder, Mainland, China) was used for grinding and homogenizing the samples. A weighing balance (Sartorius Group, Model VIC 303, USA) was used for weighing the vegetable and soil samples. A muffle furnace (Audiotronics, Wagtech International Ltd., UK) was used for the fusion of samples within nickel crucibles (50 mL). A pH/ISE meter (Orion model, EA 940 Expandable Ion Analyzer, USA) equipped with a combination fluoride ion selective electrode (Orion Model 96-09, USA) was employed for the determination of fluoride in the samples and in the standard solutions. A pH meter (HANNA instrument, HI 9025, Malaysia) equipped with a glass electrode was used to measure the pH values of the sample solutions. Borosilicate volumetric flasks (1000 mL) were used for preparation of 8 M NaOH solution. A hot plate with magnetic stirrer was used for the dissolution of soluble fluoride in the soil sample and fusion cake. Measuring cylinders (Duran, Germany), pipettes (Pyrex, USA), and micropipettes (0.5–10.5 µL, 1–100 µL, 100–1000 µL (Dragonmed, Shangai, China) were used during the measuring of the different volumes of the sample solutions and the fluoride standard solutions. 50 mL plastic centrifuge tubes were used for the storage of the sample solutions. Plastic funnels were also used for sample filtration. Different types of volumetric flasks (50, 100, 500, and 1,000 mL) and 50 mL plastic beakers were used for the sample and standard preparation during the determination of fluoride.

Chemicals and reagents: The reagents used in this study were all of analytical grade. De-ionized water was used throughout the experiment. Nitric acid (69%, Research Lab Fine Chemical Industries, Mumbai, India) was used for cleaning purposes and sodium fluoride (99%, Analar, NaF, BDH Chemicals Ltd, England) was used to prepare the standard solutions. The pH standard buffers (pH of 4, 7, and 10) were used for pH calibration purposes. Sodium chloride (Fisher Scientific UK), glacial acetic acid (100%, Sigma-Aldrich Laborchemikalien, Germany), trisodium citrate (BDH Laboratory Supplies, Poole, England), and EDTA (Scharlau Chemie S.A., Barcelona, Spain) were used to prepare the Total Ionic Strength Adjustment Buffer (TISAB) solution. Sodium hydroxide (Scharlau Chemie S.A., Sentmenat, Spain) solution was used to dissolve the vegetable samples homogeneously before the alkali fusion and were also used to adjust the pH of the TISAB solution to a pH of 5.3. Hydrochloric acid (36%, Fisher Scientific UK Limited) was used for neutralization of the dissolved fusion cake. TISAB was prepared by dissolving 58 g sodium chloride, 57 mL glacial acetic acid, 7 g of trisodium citrate, and 2 g EDTA in 500 mL deionized water into a 1000 mL beaker and its pH was adjusted to 5.3 with 5 M sodium hydroxide. The solution was then transferred to a 1000 mL volumetric flask and diluted to the mark with de-ionized water.

Sample preparation: The collected vegetable samples of lettuce, Swiss chard, cabbage, and Abyssinian cabbage were washed with tap water and then with distilled water, air dried for 20 days to a constant weight, chopped/ground with an electrical blender, sieved (1.4 mm sieve size), and stored in polyethylene bags for further preparation.

The soil samples, collected from the six sample sites, were air dried to a constant weight for ten days. The air dried samples were then ground using a pre-cleaned mortar and pestle and sieved through a 1.4 mm polyethylene sieve to make the sample uniform and remove materials which were not completely changed into soil, such as stones, gravel, plant materials, and other materials. The part of the sample which passed through the sieve was collected into a leveled plastic bag and stored for the fusion and analysis.

The six irrigation water samples which were collected were filtered with Whatman No. 42 filter paper (125 mm diameter) and stored in 500 mL polyethylene bottles for fluoride determination.

Fusion of vegetable samples: The fusions of the selected leafy vegetables were done by the reported method[29,30] in triplicate. An accurately weighed 0.5 g dry weight sample of each vegetable was placed in a 50 mL nickel crucible, 5 mL of 8 M NaOH was added, and the sample and the NaOH were thoroughly mixed. The crucible was then subjected to 150ºC in an oven until dryness was achieved and then transferred to a muffle furnace at 200ºC for 2 hr followed by the temperature being raised to 525ºC for about 3 hr. The fusion cake was then cooled to room temperature and 14 mL de-ionized water added. The crucible was kept on a hot plate in order to aid the dissolution of the fused cake. After dissolution was completed, the sample solution was transferred into a 50 mL plastic beaker. The sample solutions were neutralized using concentrated HCl drop wise to decrease the pH of the solution from

13–12 to 8–8.5 and then diluted HCl added up to the final pH of 7.0–7.4 with continuous stirring and pH control. The sample solutions were then transferred to a 50 mL plastic volumetric flask and diluted with de-ionized water to the volume by rinsing the beaker. The sample solutions were then filtered with Whatman No. 42 filter paper into a 50 mL volumetric flask and subjected to fluoride measurement.

Fusion of soil samples: Total soil fluoride was determined by the reported method,[40] in triplicate, with fusion for each soil sample. 0.50 g of prepared soil samples were weighed directly into 50 mL nickel crucibles and moistened with 1 mL de-ionized water. To the moistened sample, 6.0 mL of a 17 M sodium hydroxide solution was added and the contents placed in an oven (150ºC) for 2 hr until the sodium hydroxide had solidified. The crucible containing the dry sample was removed from the oven and transferred to a muffle furnace for fusion at 600ºC for 30 min. After cooling for 1 hr, 15 mL of de-ionized water was added to the sample and the contents heated on a hot plate for approximately 3 hr to facilitate the dissolution of the fusion cake. About 7 mL of concentrated hydrochloric acid were added drop wise to decrease the pH from 12.0–13.0 to 8.0–8.5 with continuous stirring and pH control. Subsequently, the sample was transferred to a 50 mL plastic volumetric flask. The crucible was rinsed successively with de-ionized water until the final volume reached 50 mL and all the washings were mixed and filtered with Whatman No. 42 filter paper (125 mm diameter) in a pre-cleaned and rinsed 50 mL plastic volumetric flask before being subjected to fluoride determination.

Calibration of the fluoride ion selective electrode: Different working standard solutions were prepared from 1000 mg/L NaF stock solution through serial dilution. Fluoride stock solution was prepared by dissolving 2.21 g of anhydrous sodium fluoride (99.0% NaF, BDH Chemicals, England) in 500 mL de-ionized water into 1000 mL volumetric flask and diluted to the mark with de-ionized water. The calibration curve was prepared using fluoride concentrations of 0.05, 0.5, 1, 5, and 10 mg/L for the fluoride determination in the vegetable samples. The calibration curve was also prepared using fluoride concentrations of 0.5, 1.0, 5.0, 10, and 20 mg/L for the determination of fluoride in the irrigation water and the soil samples. The slope and correlation coefficient values of –58 mV/decade and 0.9999, respectively, were obtained. The ISE was placed in a beaker containing 5 mL of standard solution, along with 5 mL of TISAB (1:1) at room temperature (25 ºC).

pH meter calibration: The pH meter was calibrated using pH 4, pH 7, and pH 9 buffer solutions before proceeding to the pH measurement of the reagent blank and sample solutions.

Fluoride determination: The levels of fluoride in the samples were determined by a fluoride ion selective electrode by using a direct measurement technique. All the determinations were made in triplicate at room temperature and the concentration of fluoride was recorded in the units of mg/L directly from the instrument reading for each solution.

Total fluoride determination in selected leafy vegetables: The total fluoride in the vegetables was determined through alkaline fusion by slightly modifying the reported

methods.[29,30] An equivalent amount of TISAB, i.e., 5 mL total ionic strength adjustment buffer (TISAB), was added to a 5 mL sample solution in a 50 mL plastic beaker and the concentration of fluoride was measured with an Orion ISE meter combined with an expandable electrode against the concentration of standard NaF standard in triplicate with continuous stirring.

Total fluoride in soil: The total soil fluoride in soil was determined through alkaline fusion by slightly modifying the reported methods.[40] An equivalent amount of TISAB, i.e., 10.0 mL total ionic strength adjusting buffer (TISAB), was added to 10.0 mL of the sample solution in a 50 mL plastic beaker and the concentration of fluoride was measured, with continuous stirring, against the concentration of standard NaF solutions in triplicate with an Orion ISE meter combined with an expandable electrode.

Soluble fluoride in soil: The soluble fluoride concentrations in the soil samples were determined after weighing 5 g soil in a 50 mL plastic beaker and mixing with 10 mL of de-ionized water. It was then stirred for about 1 hr to dissolve the fluoride in the soil and filtered with Whatman No. 42 filter paper (125 mm, diameter) into a 50 mL volumetric flask. After this, the filtrate was diluted to the volume with de-ionized water. A 10 mL aliquot of the filtrate solution was mixed with 10 mL TISAB solution in a 50 mL plastic beaker. The concentration of fluoride in the solution was measured using a fluoride ISE with continuous stirring against a standard NaF solution.

Fluoride determination in irrigation water: The fluoride contents of the water samples were determined by mixing an aliquot of 5 mL of each filtered water sample with 5 mL of TISAB solution into a 50 mL plastic beaker with continuous stirring for analysis using a ISE combined with a glass electrode. Each sample was analysed in triplicate.

Validation of analytical procedure: In this study, the validation of the analytical procedures for the determination of fluoride using the fluoride ISE was evaluated with a recovery test. In the recovery test, 25%, 50%, and 100% of the fluoride measured in the original vegetables were spiked into 0.5 g vegetable samples from a 20 mg/L fluoride standard solution in triplicate. Lettuce, Swiss chard, cabbage and Abyssinian cabbage were spiked with 36, 72, 144 µL; 33.8, 67.5, 135 µL; 16.4, 32.8, 65.5 µL; and 16.2, 32.4, 64.8 µL of 20 mg/L standard F^- solution, respectively.

The spiking experiments were also done with the irrigation water and farmland soil samples. A similar percentage of a standard fluoride solution, as was used in the validation test with the vegetables, was used for the spiking and calculated by considering the different volume of water and mass of soil in the recovery of water soluble fluoride. For the recovery of fluoride in water, soluble soil, and total soil, the samples were spiked with 478.8, 957.5, 1915 µL; 1.44, 2.88, 5.75 mL; and 5, 10, 20 mL of 20 mg/L standard fluoride solution, respectively.

RESULTS AND DISCUSSION

Recovery test for fluoride determination: In this study, the analytical procedure used for the determination of fluoride in vegetables, farmland soil, and irrigation water samples were validated by spiking experiments. The recovery test was

conducted for all the vegetable, soil, and water samples. The recovery test results of fluoride in the vegetable samples are given in Table 1. The percentage of recovered fluoride in vegetable sample ranged from 89–108% which is within the accepted range for percent of recovery. This recovery indicates that the method used for the fluoride determination in the vegetable samples was precise and reliable.

Table 1. Recovery test results for leafy vegetables

Vegetable	Concentration of F^- in unspiked sample (mg/kg)	Concentration of F^- added in unspiked sample (mg/kg)	Concentration of F^- in spiked sample (mg/kg)	Percent of recovery (%)
Lettuce	5.76 ± 0.03	1.44	7.05 ± 0.01	89 ± 9
	5.76 ± 0.03	2.88	8.53 ± 0.008	96 ± 3
	5.76 ± 0.03	5.76	11.2 ± 0.03	94 ± 5
Swiss chard	5.40 ± 0.03	1.35	6.81 ± 0.01	105 ± 10
	5.40 ± 0.03	2.70	7.85 ± 0.006	91 ± 2
	5.40 ± 0.03	5.40	10.4 ± 0.005	92 ± 1
Cabbage	2.62 ± 0.02	0.66	3.33 ± 0.004	108 ± 5
	2.62 ± 0.02	1.31	3.81 ± 0.007	91 ± 5
	2.62 ± 0.02	2.62	5.42 ± 0.03	107 ± 11
Abyssinian cabbage	2.59 ± 0.02	0.65	3.19 ± 0.003	92 ± 5
	2.59 ± 0.02	1.30	3.97 ± 0.01	106 ± 5
	2.59 ± 0.02	2.59	5.21 ± 0.009	101 ± 4

The recovery test results for the water samples are given in Table 2. The percentage recoveries of the irrigation water samples were 105 ± 8%, 108 ± 2%, and 97 ± 2% which are within the accepted range of percent recovery. This confirms that the method used for fluoride determination in the irrigation water samples was precise and reliable.

Table 2. Recovery test for water samples

Type of sample	Concentration of F^- in unspiked sample (mg/L)	Concentration of F^- added in unspiked sample (mg/L)	Concentration of F^- in spiked sample (mg/L)	Percent recovery (%)
Irrigation water	7.66 ± 0.18	1.92	9.67 ± 0.06	105 ± 8
	7.66 ± 0.18	3.83	11.8 ± 0.06	108 ± 2
	7.66 ± 0.18	7.66	15.1 ± 0.15	97 ±2

The recovery test results of the water soluble and total fluoride in soil are given in Table 3. The percentage recovery of both the water soluble and the total fluoride in soil ranged from 94–110% which is also within the accepted range of percent recovery and confirms that the method used for the fluoride determination in the soil samples was precise and reliable.

Table 3. Recovery test for soil samples

Type of sample	Concentration of F^- in unspiked sample (mg/kg)	Concentration of F^- added in unspiked sample (mg/kg)	Concentration of F^- in spiked sample (mg/kg)	Percent recovery (%)
Water soluble fluoride in soil	23.4 ± 0.1	5.85	28.7 ± 1	94 ± 4
	23.4 ± 0.1	11.7	34.8 ± 0.3	97 ± 2
	23.4 ± 0.1	23.4	45.7 ± 3	94 ± 3
Total fluoride in soil	802 ± 59	200	1062 ± 37	110 ± 5
	802 ± 59	401	1278 ± 37	107 ± 3
	802 ± 59	802	1572 ± 56	96 ± 7

Fluoride distribution in vegetable samples: The fluoride concentrations of the selected leafy vegetables cultivated in different parts of Ethiopia are given in Table 4. The distribution of fluoride in lettuce, Swiss chard, cabbage, and Abyssinian cabbage across the study area showed similar patterns. The highest and lowest fluoride concentrations in lettuce and Swiss chard were 5.76, and 5.40 mg/kg, and 2.95, and 2.74 mg/kg, recorded in Hawassa and Kera, respectively; in cabbage the highest was

2.70 mg/kg in Ziway and the lowest 2.12 mg/kg in Kera; in Abyssinian cabbage the highest was 2.59 mg/kg in Hawassa and the lowest was 2.08 mg/kg in Kera.

Table 4. Fluoride content in leafy vegetables grown in Ethiopia (values are mean ± SD, mg/kg dry weight, n = 9)

Sample site	F^- concentration in leafy vegetables (mg/kg dry weight)			
	Lettuce	Swiss chard	Cabbage	Abyssinian cabbage
Hawassa	5.76 ± 0.03	5.40 ± 0.03	2.62 ± 0.02	2.59 ± 0.02
Ziway	4.61 ± 0.05	3.18 ± 0.02	2.70 ± 0.03	2.40 ± 0.01
Wonji Shoa	4.96 ± 0.10	4.58 ± 0.02	2.48 ± 0.02	2.18 ± 0.03
Akaki	3.26 ± 0.02	3.03 ± 0.02	2.40 ± 0.01	2.23 ± 0.01
Kera	2.95 ± 0.01	2.74 ± 0.02	2.12 ± 0.01	2.08 ± 0.01
Pecock Park	3.21 ± 0.02	3.14 ± 0.01	2.47 ± 0.02	2.25 ± 0.01

The fluoride concentration of lettuce, Swiss chard, cabbage, and Abyssinian cabbage was higher in the Rift Valley compared with the non-Rift Valley areas in this study. However, the fluoride content of the Abyssinia cabbage in Wonji Shoa was comparable with the content in the non-Rift Valley areas. This might be arise from the nature of the vegetables and other micro factors or micro-nutrient interactions in the agro-ecological zone. Lettuce and Swiss chard had a higher attraction for fluoride accumulation compared with of cabbage and Abyssinian cabbage. When these vegetables were grown in a high fluoride area they accumulated a high fluoride concentration and when grown in a low fluoride area they accumulated a low fluoride concentration. In the cases of cabbage and Abyssinian cabbage, there were relatively smaller variations and the fluoride concentrations were comparable in all the study areas. This indicates that cabbage and Abyssinian cabbage have a lower attraction for fluoride.

Fluoride distribution in the irrigation water samples: The distribution of fluoride in the different irrigation water bodies is given in Table 5. The highest and lowest fluoride concentrations in the study areas were observed in Hawassa lake and Kera river, respectively. In increasing order, the concentrations of fluoride in the water bodies used for the cultivation of the leafy vegetables were 0.43 mg/L in Kera river, 0.78 mg/L in Akaki river, 1.48 mg/L in Bulbula river, 2.06 mg/L in Ziway lake, 2.77 mg/L in Awash river, and 7.66 mg/L in Hawassa lake. The water bodies found in the Rift Valley have high fluoride concentrations compared with the non-Rift Valley rivers. The fluoride levels in the water were higher than the fluoride concentrations in both the water soluble fluoride and the total fluoride in the soil. This might be due to the movement of fluoride from the soil and the environment to the water bodies

through different natural and anthropogenic factors adding fluoride to the water bodies such as runoff, rain, industrial waste disposal, and the use of fluoridated toothpaste.

Table 5. Fluoride distribution in selected irrigation water bodies
(values are mean ± SD, mg/L, n = 3)

Sample site	Water body	F^- concentration (mg/L)
Hawassa	Hawassa lake	7.66 ± 0.18
Ziway	Ziway lake	2.06 ± 0.08
Wonji Shoa	Awash river	2.77 ± 0.08
Akaki	Akaki river	0.78 ± 0.02
Kera	Kera river	0.43 ± 0.04
Pecock Park	Bulbula river	1.48 ± 0.03

Soluble fluoride distribution in soil samples: The water soluble fluoride distribution in the soil is given in Table 6. The order of the water soluble fluoride distribution in the soil samples in the study area was comparable with the fluoride distributions observed in the irrigation water. The highest soluble fluoride distribution was 23.4 mg/kg in Hawassa soil and the lowest fluoride was 4.30 mg/kg in Kera soil. These extreme values and the order of distribution of fluoride in the other soil samples confirm the relationship between the soluble fluoride concentration in soil and that in water. The highest water soluble fluoride distribution in Hawassa might result from the presence of water soluble fluoride complex compounds, and the application of phosphate fertilizers and other agricultural pesticides in the area. On the other hand, the lowest values in Kera soil might be from the presence of insoluble fluoride complex compounds in the area, the absence of a fluoride source in the soil, and low anthropogenic effects.

Table 6. Water soluble and total fluoride distribution in soil
(values are mean ± SD, mg/kg, n = 9)

No.	Sample site	F^- concentration in water soluble soil (mg/kg)	F^- concentration in total soil (mg/kg)
1	Hawassa	23.4 ± 0.1	802 ± 59
2	Ziway	15.1 ± 0.5	595 ± 33
3	Wonji Shoa	19.7 ± 0.5	553 ± 44
4	Akaki	8.77 ± 0.5	133 ± 8
5	Kera	4.30 ± 0.3	263 ± 12
6	Pecock Park	7.53 ± 0.05	336 ± 13

Total fluoride distribution in soil samples: The total fluoride distribution in the soil is given in Table 6. The highest fluoride concentration was 802 mg/kg recorded in Hawassa and the lowest was 133 mg/kg in Akaki soil. The order of the fluoride distribution pattern was 802 mg/kg in Hawassa > 595 mg/kg in Ziway > 553 mg/kg in Wonji Shoa > 336 mg/kg in Pecock Park > 263 mg/kg in Kera > 133 mg/kg in Akaki. The total fluoride in Ziway was higher than in Wonji Shoa although the soluble fluoride in the soil and water fluoride in Wonji Shoa was higher than in Ziway. This anomaly may be due to differences between the two areas in the availability of soluble and insoluble fluoride complex compounds, the presence or absence of different anthropogenic activities such as the production of industrial wastes and sewage disposal, and the application of phosphate fertilizers, agricultural pesticides, insecticides, and fungicides.

In general, the fluoride distributions in vegetables, soil, and water were high in Hawassa, because Hawassa is one of the high fluorosis risk areas in the main Ethiopian Rift Valley region which are characterized by a high fluoride distribution in the water bodies, the rocks, and the soils.

Fluoride distribution in fresh vegetables: All the vegetables selected for this study (lettuce, Swiss chard, cabbage, and Abyssinian cabbage) are sold in Ethiopia on a fresh weight basis. The vegetable fluoride levels in this study were also determined on a fresh weight basis. The fresh weight fluoride level in each vegetable can be calculated from the fluoride level on a dry weight basis with consideration of the percentage of moisture content. The percentage moisture content of each vegetable was calculated using the following formula:

$$\% \text{ moisture} = \frac{F_w - D_w}{F_w} \times 100$$

where F_w = fresh weight of the vegetable samples and D_w = dry weight of the vegetable samples

In this study, 500 g fresh weight samples of lettuce, Swiss chard, cabbage, and Abyssinian cabbage were air dried for 20 days until a constant weight was obtained for all the samples. The measured values for the vegetables, dry weight and % moisture, from the different areas were: (i) FROM HAWASSA: lettuce: 57.2 g, 88.6%; Swiss chard: 73.6 g, 84.7%; cabbage: 65.2 g, 87.0%; and Abyssinian cabbage: 93.9 g, 81.2%; (ii) FROM ZIWAY: lettuce 52.0 g, 89.5%; Swiss chard 67.7 g, 86.4%; cabbage 93.3 g, 81.3%; and Abyssinian cabbage 88.0 g, 82.4%; (iii) FROM WONJI SHOA: lettuce 48.6 g, 90.2%; Swiss chard 56.6 g, 88.6%; cabbage 82.5 g, 83.4%; and Abyssinian cabbage 70.2, 85.9%; (iv) FROM AKAKI: lettuce 50.1 g, 89.9%; Swiss chard 87.8 g, 82.4%; cabbage 58.0 g, 88.4%; and Abyssinian cabbage 70.1 g, 86.0%; (v) FROM KERA: lettuce 45.5 g, 90.8%; Swiss chard 72.1 g, 85.5%; cabbage 65.0 g, 87.0%; and Abyssinian cabbage 67.2 g, 86.5%; and (vi) FROM PECOCK PARK: lettuce 44.8 g, 91.0%; Swiss chard 72.2 g, 85.6%; cabbage 60.0 g, 88.0%; and Abyssinian cabbage 61.5 g, 87.6%.

The fluoride levels on a fresh weight basis in lettuce, Swiss chard, cabbage, and Abyssinian cabbage were calculated and are reported in Table 7. The conversion was performed using the following formula.

$$\text{Fresh weight fluoride} = \text{Dry weight fluoride} \times \frac{100 - \%\ \text{moisture}}{100}$$

Table 7. Fluoride concentration in fresh vegetables

Sample site	F^- concentration in fresh vegetables (mg/kg)			
	Lettuce	Swiss chard	Cabbage	Abyssinian cabbage
Hawassa	0.657	0.826	0.341	0.487
Ziway	0.484	0.432	0.504	0.422
Wonji Shoa	0.486	0.522	0.412	0.307
Akaki	0.329	0.533	0.278	0.312
Kera	0.271	0.397	0.275	0.289
Pecock Park	0.289	0.452	0.296	0.279

Comparison of fluoride levels in vegetable samples in the study areas: The fluoride distribution in the vegetables across the sampling sites and within a particular site is shown in the Figure. The fluoride concentrations in lettuce and Swiss chard in increasing order in the six study areas were: FOR LETTUCE: Kera < Pecock Park < Akaki < Ziway < Wonji Shoa < Hawassa, and for SWISS CHARD: Kera < Akaki < Pecock Park < Ziway < Wonji Shoa < Hawassa, respectively. The distribution pattern of fluoride in lettuce and Swiss chard had almost identical trends across the study areas with exception being that the fluoride concentration of lettuce in Pecock Park was lower than in Akaki and vice versa for the Swiss chard.

The fluoride concentrations in the cabbage and Abyssinian cabbage in the Rift Valley and non-Rift Valley areas were closer to one another. In decreasing order, the fluoride levels of the cabbage and Abyssinian cabbage were: FOR CABBAGE: 2.70 mg/kg in Ziway, 2.62 mg/kg in Hawassa, 2.48 mg/kg in Wonji Shoa, 2.47 mg/kg in Pecock Park, 2.40 mg/kg in Akaki, and 2.12 mg/kg in Kera, and FOR ABYSSINIAN CABBAGE: 2.59 mg/kg in Hawassa, 2.40 mg/kg in Ziway, 2.25 mg/kg in Pecock Park, 2.23 mg/kg in Akaki, 2.18 mg/kg in Wonji Shoa, and 2.08 mg/kg in Kera, respectively. The small differences in the accumulation of fluoride in the Rift Valley and non-Rift Valley areas may depend on the nature of the vegetables and the agro-ecological zone.

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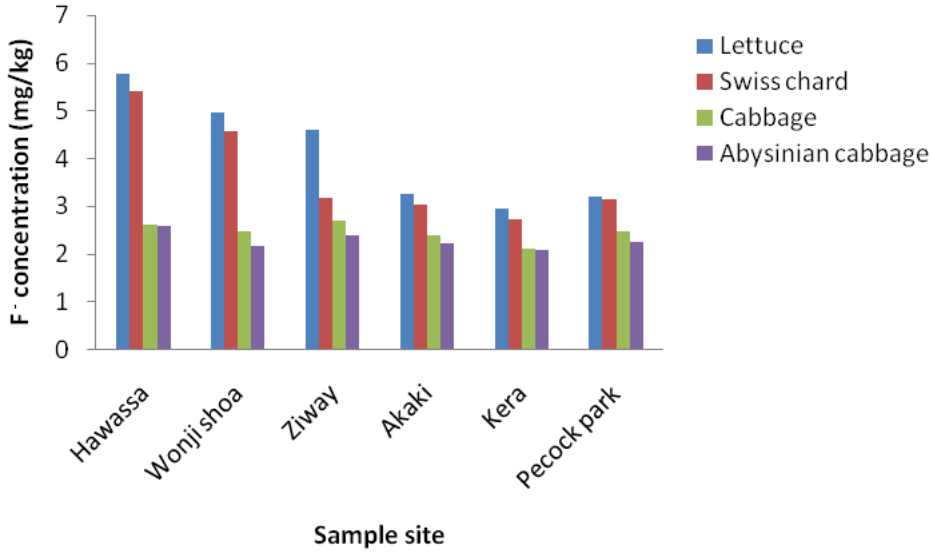

Figure. Comparison of the fluoride levels in lettuce, Swiss chard, cabbage and Abyssinian cabbage in the study areas.

Comparison of the fluoride concentrations in this study with the literature values: Many researchers have determined the concentration of fluoride in selected leafy vegetables in different parts of the world. The comparison of the results of this study with the literature values is given in Table 8. The fluoride concentration of lettuce reported in India, in 2015[18] was 5.6 mg/kg which is comparable to the fluoride content of lettuce in Hawassa and higher than the other sites examined in the present study.

The fluoride concentrations of cabbage have been reported in Nigeria[41] in 2011 and in India in 2009,[22] 2012,[13] and 2015.[18] The range of the fluoride concentration in this study was from 2.70–2.12 mg/kg which is higher than the values reported in Nigeria in 2011[41] and in India in 2012,[13] lower than the values reported in India in 2009,[22] and within the range reported in India in 2015.[18]

We have not found any reports on the concentration of fluoride in Swiss chard and Abyssinian cabbage in the literature. Accordingly, the fluoride contents found in the present study for Swiss chard and Abyssinian cabbage could not compared with values from the literature.

Table 8. Comparison of the fluoride concentrations in the leafy vegetable samples with values in the literature

Vegetable type	F^- concentration (mg/kg dry weight)	Origin	Reference
Lettuce	5.7	India	18
	2.95–5.76	Ethiopia	Present study
Swiss chard	2.74–5.40	Ethiopia	Present study
Cabbage	0.022–0.047	Nigeria	41
	3.91–11.30	India	22
	1.28–11.30	India	18
	1.25 ± 0.07	India	13
	2.12–2.70	Ethiopia	Present study
Abyssinian cabbage	2.08–2.59	Ethiopia	Present study

Analysis of variance: All the measurements were done in triplicate on triplicate analysis ($n = 3 \times 3 = 9$) and reported as mean ± SD. The statistical analysis was done using SPSS version 22 soft ware. The mean concentration variability of fluoride in the vegetables was analyzed using one way analysis of variance (ANOVA) which is the most common and popular statistical method for comparisons between and within sample means at the 95% confidence level for the significance difference test.[42] This statistical tool indicates whether the variation comes from measurement variability or sampling heterogeneity. The magnitude and direction of correlation of the fluoride concentration in vegetables with the fluoride concentrations in the water and soil samples were also tested using the Pearson correlation.

A one-way ANOVA between and within groups analysis of variance (Table 9) was conducted to explore the impact of the sample site on the fluoride concentration of the vegetables and the impact of the vegetable types. The sample sites were Hawassa, Ziway, Wonji Shoa, Akaki, Kera, and Pecock Park. There was a statistically significant difference in the mean fluoride concentrations of the leafy vegetable samples between and within the groups at the $p<0.05$ level.

Table 9. Analysis of variance (ANOVA) between and within leafy vegetable samples at the 95% confidence level (Df = degree of freedom, F_{cal} = F calculated, F_{crit} = F critical)

Vegetable	Comparison	Df	F_{cal}	F_{crit}	Remark
Lettuce	Between samples	5	36.9	2.41	Significant difference between sample means
	Within samples	48			
Swiss chard	Between samples	5	182.4	2.41	Significant difference between sample means
	Within samples	48			
Cabbage	Between samples	5	9.38	2.41	Significant difference between sample means
	Within samples	48			
Abyssinian cabbage	Between samples	5	9.68	2.41	Significant difference between sample means
	Within samples	48			

Pearson correlation: The Pearson correlation showed a weak, moderate, or strong relationship among the variables in a positive or a negative direction.[42] The correlations of the fluoride concentration in the vegetables with the fluoride concentrations in the soil and water are given in Tables 10 and 11. The concentrations of fluoride in lettuce had a very weak positive correlation in all irrigation water bodies except for the Awash and Bulbula irrigation water samples, where strong

negative and positive correlations were observed, respectively. For Swiss chard, strong or moderate positive correlations were observed except for the Akaki irrigation water sample which had a moderate negative correlation. For cabbage, there was no correlation with the Ziway lake irrigation water sample. For Abyssinian cabbage, strong positive and negative correlations were observed in Ziway lake, Bulbula, Akaki, and Kera rivers. Moderate negative and positive correlations were observed for Abyssinian cabbage in the Hawassa lake and Awash river water samples. The fluoride concentrations in Ziway lake and Bulbula river showed positive correlations with all vegetable types. In general, the correlation of the fluoride concentration in the vegetable and irrigation water samples was variable and in both directions.

Table 10. Correlation of fluoride in irrigation water and vegetable samples

Vegetable	Irrigation water					
	Hawassa lake	Ziway lake	Awash river	Akaki river	Kera river	Bulbula river
Lettuce	0.135	0.145	–0.897	0.112	0.165	0.957
Swiss chard	0.921	0.641	0.616	–0.377	0.486	0.451
Cabbage	–0.329	0.031	0.185	–0.795	0.839	0.450
Abyssinian cabbage	–0.505	1.00	0.422	–0.988	–0.922	0.720

Table 11. Correlation of soluble fluoride in farmland soil and vegetables

Vegetable	Farmland soil					
	Hawassa	Ziway	Wonji Shoa	Akaki	Kera	Pecock Park
Lettuce	0.771	0.950	–0.440	–0.568	–0.785	0.621
Swiss chard	0.413	-0.400	0.008	0.323	–0.531	0.880
Cabbage	–0.882	0.908	0.746	–0.202	–0.074	0.879
Abyssinian cabbage	0.214	0.436	–0.218	–0.646	–0.104	0.195

A variable relationship was present between the fluoride levels in the vegetables and the soluble fluoride levels in the farmland soil, similar to that present between the vegetable fluoride levels and the fluoride levels in the irrigation water samples. The concentration of fluoride in lettuce was strongly correlated, either positively or negatively, with the soluble fluoride level in Hawassa, Ziway, Kera, and Pecock Park

farmland soil and moderately negatively correlated in Wonji Shoa and Akaki farmland. The fluoride concentration in the Swiss chard showed a positive strong correlation with the Pecock Park farmland soluble fluoride and no correlation with the Wonji Shoa farmland soluble fluoride. In cabbage, a very weak negative correlation was observed between the vegetable fluoride level and the soluble fluoride in the Akaki and Kera farmland soils while a strong correlation, either positive or negative, was observed with the other sample sites. In Abyssinian cabbage, a strong negative correlation was observed in Akaki and weak or moderate correlations were observed in the other sites. From another perspective, across all vegetable samples, positive correlations were observed in the Pecock Park farmland soils and negative correlations in the Kera farmland soils.

CONCLUSIONS

The ranges for the fluoride contents of the leafy vegetables (mg/kg) from the six sites in the Rift Valley and in non-Rift Valley areas of Ethiopia were: 2.95–5.76 in lettuce, 2.74–5.40 in Swiss chard, 2.12–2.70 in cabbage, and 2.08–2.59 in Abyssinian cabbage. The ranges for the corresponding fluoride levels in the water (mg/L) and soil (both soluble and total, mg/kg) samples were: 7.66 ± 0.18, 23.4 ± 0.1, 802 ± 59 in Hawassa; 2.06 ± 0.08, 15.1 ± 0.5, 595 ± 33 in Ziway; 2.77 ± 0.08, 19.7 ± 0.5, 553 ± 44 in Wonji Shoa; 0.78 ± 0.02, 8.77 ± 0.5, 133 ± 8 in Akaki; 0.43 ± 0.04, 4.30 ± 0.3, 263 ± 12 in Kera, and 1.48 ± 0.03, 7.53 ± 0.05, 336 ± 13 in Pecock Park

One way analysis of variance showed that there was a significant difference in the mean fluoride concentrations of the vegetables at the $p<0.05$ confidence level. This means a single vegetable type across the six sampling site had different fluoride concentrations. This may be due to variations of the fluoride sources in the soil and irrigation water. Secondly, the one way ANOVA results indicate that the vegetables on the same site had different concentrations of fluoride. This also confirms that the variability of fluoride in a vegetable within a site arises from natural variations in the vegetable for the absorption, accumulation, and translocation of fluoride. The Pearson correlation showed a variable relationship between the fluoride levels in the water and the vegetables, and between the fluoride levels in the soil and the vegetables.

The fluoride concentrations in the lettuce and cabbage in this study were found to be comparable to the values reported in the literature but no literature values for comparison were found for Swiss chard and Abyssinian cabbage. In general, for those who regularly consume these vegetables, we found that the fluoride from leafy vegetables makes a significant contribution to the total fluoride intake.

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